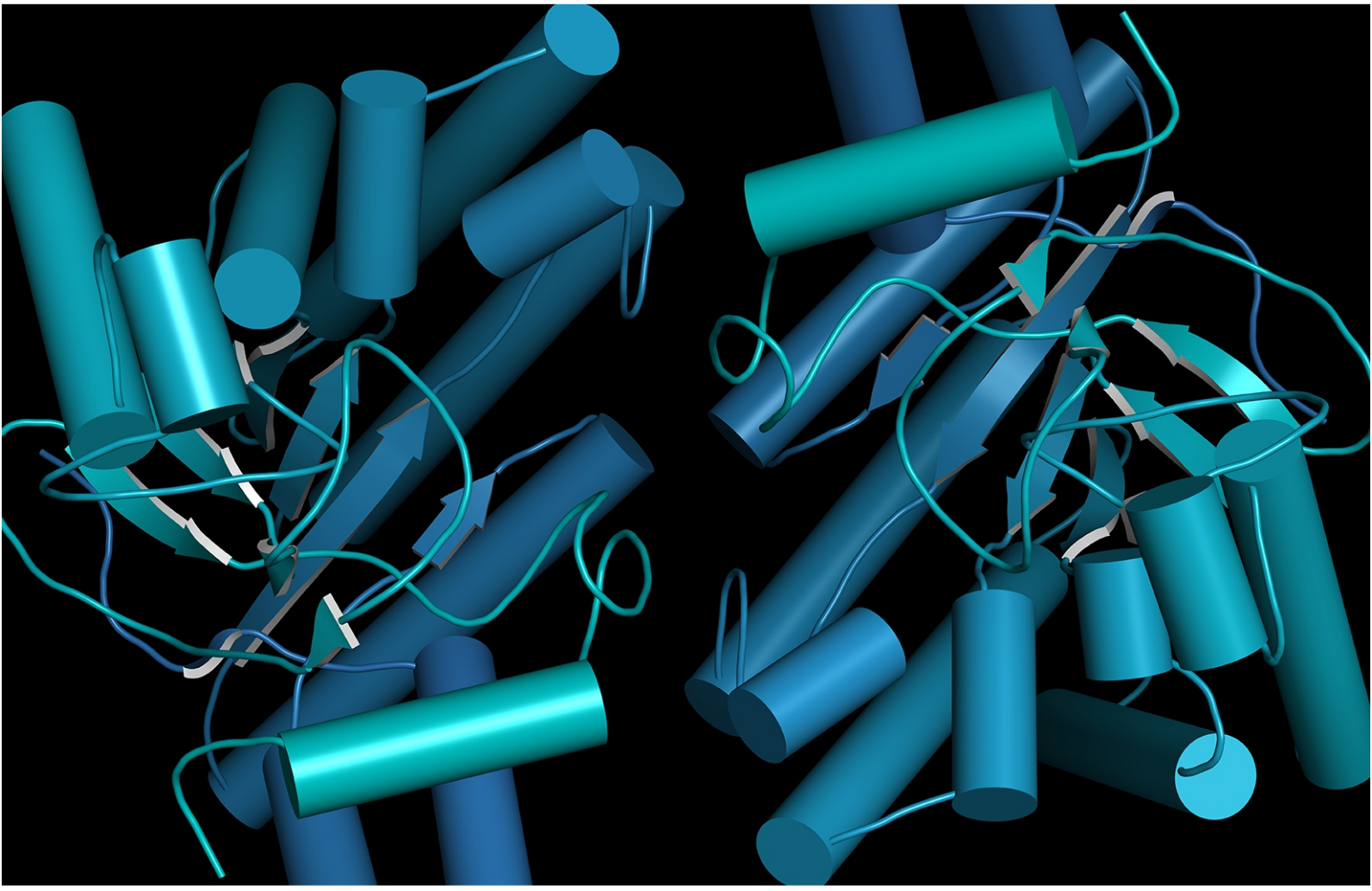
A TEXTBOOK OF ENZYMOLOGY



Shashikant Patil

A TEXTBOOK OF ENZYMOLOGY

A TEXTBOOK OF ENZYMOLOGY

Shashikant Patil

ALEXIS PRESS

Published by: Alexis Press, LLC, Jersey City, USA
www.alexispress.us

First Published 2023

A catalogue record for this publication is available from the British Library

Library of Congress Cataloguing in Publication Data

Includes bibliographical references and index.

A Textbook of Enzymology by *Shashikant Patil*

ISBN 979-8-89161-375-1

CONTENTS

CHAPTER 1

EXPLORING THE PROBIOTICS' ABILITY FOR PRODUCING ENZYMES AND THE CREATION OF COMPOUND ENZYMES

Shashikant Patil, Professor
Department of uGDX, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- shashikant.patil@atlasuniversity.edu.in

ABSTRACT:

Probiotics are a class of living, active microorganisms that alter and colonize the host's flora to its advantage. It is crucial to control host mucosal and systemic immune activity as well as the balance of intestinal flora in order to support the development of human gastrointestinal nutrition and health. The goal of this study is to assess the enzyme's biological properties and safety as a preventative medication, investigate its manufacturing activity, and give references for future research on enzyme synthesis and compound enzyme creation by probiotics. The drinking water control group, the Lactobacillus plantarum experimental group, and the Bacillus licheniformis experimental group were the four probiotic groups used in this investigation. To further investigate the impact on the digestive system, several complicated enzyme tests as well as single factor experiments were set up. The findings demonstrated that intestinal digestibility might be greatly enhanced by probiotics and treatments containing compound enzymes. The weight of the chicken was over 1 Jin more when probiotics were present compared to the control group, and the average digestibility was 4.3% higher. Although the enzyme's impact on digestibility is greater than that of probiotics, the overall effect is usually steady.

KEYWORDS:

Enzymes, Medication, Microorganisms, Probiotics, Treatments.

INTRODUCTION

The development of green feed additive solutions that may substitute antibiotics has become a worldwide research priority owing to the major concerns of antibiotic resistance and antibiotic residues. The balance of intestinal microbes may be improved, maintained, and supplemented by microbial agents, which can be employed for productivity improvement, health promotion, and illness prevention and treatment. China's mushroom technology has advanced quickly in recent years, but large-scale industrial production has evolved gradually. After harvest, the amount of leftover substrate mushroom bran quickly rose. The mycelium and nutrients in the fungal bran were abundant. The manufacture of probiotics from mushroom bran offered a solution to the pressing issue of the industry's sensible exploitation of edible fungus bran. Probiotics have been extensively shown to reduce gastrointestinal inflammation and prevent colorectal cancer. However, the mechanism behind probiotics' immunomodulatory impact on the development of extraintestinal cancers is still unknown. Li investigated the effectiveness of probiotic feeding in preventing liver cancer and the probable mechanism of tumor progression inhibition using a mouse model and genome sequencing. In comparison to the control group, Prohep, a novel probiotic combination, may dramatically slow down tumor development and lower tumor volume and weight by 40%. According to the mechanism, probiotics feeding significantly reduced the levels of IL-17 cytokines and their major producer Th17 cells, which had a significant impact on the reduction of tumor size. Cell staining revealed that the reduced frequency of Th17 cell migration in intestinal and peripheral blood was the primary cause of the decline in Th17 cells in the probiotic therapy

group [1], [2]. Tanaka developed an improved fluorescence in situ hybridization technique to find and count the host carried probiotics in abalone intestines in order to analyze the probiotic colonies. The samples were hybridized with a TAMRA-labeled probe at 40 °C for 3 hours after being fixed with 4% paraformaldehyde. Enterococcus and Lactobacillus are two types of control bacteria that strain Ab1 clearly differs from. RPT positive bacteria's fluorescence signal may be clearly differentiated from any audible background noise. RPT positive bacteria were found in both the probiotic-treated and control abalone, but the probiotic-treated abalone had two orders of magnitude more RPT positive bacteria than the control. Additionally, the average number of RPT-positive bacteria was two orders of magnitude larger in the intestinal attachment zone than it was in the free living region. Both the biochemical approach and the molecular biological method were used to identify a single colony from each group. Both techniques work to detect candida. In a simulated gastrointestinal environment, the famata strain Y5's cytotoxicity, adhesion, surface characteristics, hemolytic activity, and survival rate were investigated.

Balasingham collected 63 isolates from Yorkshire pigs in order to isolate probiotics from pig intestines. The isolate was morphologically identified after being injected in Man Rogosa Sharpe broth for 48 hours at 37°C and 5% CO2. For further physiological and biochemical identification tests, the colony of gram-positive bacilli was chosen. Each chosen isolate underwent two and three rounds of testing using the recommended procedure. Through a series of experiments relating to pH tolerance, bile tolerance, and antibiotic activity, the probiotic properties of the discovered species were ascertained. The number of gram positive bacilli strains discovered was just 23 according to morphological identification. Four of these 23 strains did not exhibit any growth under any of the examined circumstances, according to subsequent results of physiological testing on them. Biochemical techniques were used to find the remaining 19 strains. This paper studies the -amylase, acid protease, and neutral protease that have good effects in industry today and their roles in reducing sugar content and digestibility of digestive tract, etc., and roughly determines the best kind and dosage of enzymes using the method of single factor experiment. The chosen enzymes were then optimized using response surface methods to create a new composite enzyme [3], [4].

DISCUSSION

A compound enzyme preparation is made up of one or more preparations with a single enzyme as the major component that are combined or fermented to create one or more microorganisms. The ability of some nutrients to be absorbed is influenced by a variety of circumstances. They relate to food composition and processing methods in addition to animal types, ages, and average life spans. Different cattle and poultry may have customized enzyme preparations made for them depending on their digesting characteristics and feed content. Additionally, various foods may produce different sorts of particular enzymes. A range of food substrates may be broken down using a particular complex enzyme preparation, and several enzyme types can have synergistic effects that can increase the nutritional value of food.

Function of Enzymes and Probiotics

According to their functional properties, the enzyme feed preparations currently used in livestock and poultry production can be classified into the following groups: compound feed enzymes, which are primarily made of protein and amylase and are primarily used to make up for congenital enzyme deficiencies. A mixture of xylanase and -glucanase found in feed enzymes. In order to counteract the antinutritional effects of NSP, this enzyme preparation is mostly utilized in feed that uses grains as the primary raw material, such as barley, oats,

wheat, and rye. The two primary enzymes in complex foods are pectinase and cellulose. Aspergillus, penicillium, and trichoderma are the principal producers of this enzyme. Its primary purposes include destroying plant cell walls, releasing nutrients to cells, making contact with digestive enzymes readily, removing antinutritional components from the meal, reducing gastrointestinal viscosity, and promoting animal digestion and absorption. The ingredients in feed enzyme include cellulose, pectinase, amylase, glucoamylase, and protease. This compound enzyme preparation combines the traits that different enzyme systems have in common and has a positive nutritional impact.

Probiotics

Milk that has been fermented may have been the first meal to include living creatures since the dawn of human civilization. The word probiotic, which means "life," has Latin roots. It is created by organic compounds, and those chemicals have the power to encourage the growth of further substances. It was later identified as a kind of sustainable ingredient that could be appropriately applied, was safe for animals, and could guarantee animal health following extensive examination by specialists and researchers. They must first be able to survive in the intestinal transit in order to qualify as probiotics. They need to have the ability to stick to the animal's skin, mucous membrane, and colonization of the stomach. They must fight against the outside environment in order to survive. Animals must not be harmed by them. It ends up being beneficial for animals. Genera, species, and strains underwent taxonomic identification. Yeast, Bifidobacterium, and Bacillus are the conclusions of the existing needs [5]–[7].

Research Techniques for Probiotics' Enzyme Production Activity

Probiotics have a strong antioxidant impact, according to several in vitro and in vivo studies that have been conducted recently. Reactive oxygen species and free root scanning, metal chelating ions, oxygen reduction control system in preventive agents, activation of antioxidant system, decrease in metabolic capacity after ingestion by the body, and lipid lowering effect are some of probiotics' antioxidant effects. The scanning ability of free roots and the identification of antioxidant enzyme activity are now the major areas of attention in research on the antioxidant activity of disease prevention medications. The action of antioxidant enzymes generated from probiotics is the subject of the most in-depth study among them. For instance, aniline peroxide may create water whereas peroxide disulfide can remove free roots from the body. An iron-containing porphyrin-binding enzyme is called catalase. Its primary job is to scavenge hydrogen peroxide created during metabolism and take part in the active metabolism of oxygen. SOD, CAT, and glutathione peroxidase are the three primary enzyme systems in vivo.

Probiotics do various enzyme-related tasks in addition to generating enzymes that are connected to antioxidants. In direct relation to the creation of bile salt hydrolase, probiotics may lower cholesterol. To lower serum cholesterol, bile salt hydrolase may be used with the cholate solution. A strain of Lactobacillus casei has the capacity to manufacture cholesterol hydrolase, which has a 35.74% cholesterol degradation efficiency. Bile salt hydrolase is produced by the bile salt lyase gene found in Lactobacillus plantarum. Furthermore, research has shown that several prebiotics, like Lactobacillus reuteri and Bacillus, have the ability to generate amylase. Additionally, prophylactic medications may also result in the production of enzymes involved in lactose reduction, such as -lactosidase, which can hydrolyze nonreducing lactose glycosidase compounds linked to bonds and catalyze the breakdown of lactose into glucose and lactose molecules to produce lactose cleavage.

The maximal enzyme activity of Lactobacillus fermentans, which may create -lactose, is 21.38 u/ml. -lactose may be produced by Lactobacillus fermentans and Bifidobacterium longum, and the enzyme activity can reach 22.41 u/ml. numerous investigations have shown the ability of Lactobacillus bulgaricus to generate lactose. Finally, a high-yield lactose strain that was identified as Lactobacillus brevis was isolated from Yunnan Tempe samples. In summary, probiotics may generate enzymes associated to antioxidants during development and reproduction. SOD has been the subject of several studies, and the activity of SOD generated by various preventative medications varies greatly. Probiotics may also create additional enzymes necessary for appropriate function in addition to those linked to antioxidants.

The purpose of feed enzymes

Wheat diet contains polysaccharides without starch that are high in protein, fats, and other nutrients. These compounds are connected by a variety of chemical connections, including peptide bonds. Without starch, the digestive enzymes in the digestive track of monogastric animals are unable to break down polysaccharides. The production of nonstarch polysaccharide enzymes, which disassemble the structure of the plant cell wall and take part in the body's regular metabolism, allows for the release of protein and starch from cells. Cellulose may considerably enhance the pace at which minerals dissolve, the digestibility of cell wall components, the efficiency of chicken production, and the rate at which nutrients are used.

Endogenous enzymes are unable to hydrolyze a large number of feed compounds. Animals exposed to these metabolites have antinutritional effects that inhibit the digestion and absorption of nutrients such protein, fat, and carbohydrate. Enzyme preparations may reduce or totally remove the antinutritional effects of antinutrients in feeds that have antinutritional effects on animals, such as nonstarch polysaccharides, phytic acid, phytosanitary hemolysin, and protease inhibitors. This increases feed consumption. Nonstarch polysaccharide enzyme, protein, and enzyme preparation added to the meal may considerably increase the rate at which nutrients are used, increase the effectiveness of cattle and poultry production, lower environmental pollution, and increase economic advantages [8], [9].

Probiotics were divided into four groups: CG, the drinking water control group; KJT, *Lactobacillus plantarum*; DSSJ, *Clostridium butyricum*, and DB, *Bacillus licheniformis*. A farm in Zhejiang Province provides the lactating hens, while a vegetable market in the same province provides the feed. The hens were famished for eight hours before to the experiment. They must be mounted on a board before being murdered, having their heads severed, and having their gastrointestinal tracts swiftly removed. The digestive system then uses scissors to separate the two intestines in the stomach. The tissue samples were sliced using scissors, and a portion of the connective tissue was removed. The remaining material was then rinsed away with cold saline water. The water on the surface is absorbed using filter paper, and then 5 times as much cold brine solution is weighed and applied. It is employed and centrifuged on an ultralow temperature high-speed freezing centrifuge at a speed of 1200 R/min after homogenization. The original enzyme solution was what was left after the supernatant was removed; it was frozen and kept at 30°C. To avoid altering the enzyme activity, frozen samples should be avoided.

Investigation of In Vitro Digestion

Prior to being weighed, the weighing dish was dried in an oven at 530°C to the weight of the solid. A feed sample of around 2 g was weighed, placed in the weighing dish, dried for three hours, moved to the dryer to cool for half an hour, and then weighed again. The feed sample

was also placed in the oven for 45 minutes, dried for 45 minutes, and then moved to the dryer to chill for 20 minutes. Repeated drying was done till the weight didn't change. The weight of water represents the mass lost after drying. A 100 ml conical flask containing a dry feed sample was filled with 10 ml of a homogenous raw stomach enzyme solution, followed by 10 ml of a PBS adjustable solution. HCI was used to bring the pH to about 3, and 2 ml of penicillin at a concentration of 60 u/ml was added once per hour. After the rubber cover was added, the rubber cover was shaken for two hours at a rate of 60 times per minute in a shaker with a constant temperature of 25°C. The second stage involved removing the conical flask from the shaking table, adding sodium hydroxide solution, adjusting the pH to about 6.5, adding 20 ml of intestinal homogenate enzyme solution, 15 ml of PBS adjustable solution, and finally adding 2 ml of 60 u/ml streptomycin once every two hours. The rubber cover was then inserted, and the mixture was then heated to 25°C and stirred at a rate of 60 times per minute for eight hours. The third step included adding 1 ml of 35% trichloroacetic acid to the cone and pausing the enzyme process for 20 minutes. The residue was then collected, dried, and weighed after being cleaned with ethanol and acetone, respectively, using quantitative filter paper of recognized quality [10], [11].

The reducing sugar curve was created by absorbing 3 ml of acetic acid and 20 ml of distilled water to create a standard blank sample. Next, 2 ml of ldns reagent was added, heated in boiling water for 8 minutes, cooled with tap water at room temperature, and then added. Using sodium acetate and acetic acid, respectively, glucose solutions from 1, 2, 3, 4, and 5 ml pipettes were diluted to 50 ml to create a glucose standard solution with concentrations of 0.3-0.5 mg/ml. Two parallel 100 ml volumes of the aforementioned standard glucose solutions were added to the calibration tube, along with 1 ml each of acetic acid, sodium acetate, and 4 ml of the ldns reagent. Three seconds of electromagnetic oscillation and five minutes of boiling water heating were completed. The samples were then cooled with tap water at room temperature, 30 ml of distilled water was added, and the OD absorption value at 380 nm was calculated. The conventional curve was drawn.

Following enzymatic processing, the material was vacuum-filtered into an 80 ml volumetric flask and 3 ml of acetic acid were applied to 1 ml of the solid volume filter. The reagent 4 ml LDNS and sodium acetate adjustable solution are combined, and then they are cooked in boiling water for 10 minutes. After that, distilled water was used to dilute it to 30 ml. The color was contrasted with 380 nm after mixing. It was determined how much reducing sugar was present. 1 ml of water, 3 ml of acetic acid, and 4 ml of sodium acetate-regulating solution were added to the blank control group. The quantity of reducing sugar generated in the enzyme solution minus the amount in the control solution is the net yield of reducing sugar. After the feed sample has undergone enzymatic hydrolysis, the compound enzyme is measured. Measurement procedure: The residue was filtered, digested, and then dried to a consistent weight in a 100°C oven using nitrogen-free filter paper of known weight. The residue's energy value was then weighed and calculated.

At birth, chickens' digestive tracts are aseptic. Following birth, foreign bacteria progressively enter the gut via environmental interaction with the breastfeeding environment and the delivery clinic. The intestinal flora is developed to maintain its own intestinal microecological equilibrium after attachment and colonization. Consequently, Lactobacillus and other helpful bacteria are used to finish infant breastfeeding so that the helpful bacteria occupy the effective position of the intestinal tract earlier. This has an absolute advantage in quantity and action intensity, effectively inhibits the attachment of other pathogenic bacteria to the site, and effectively prevents the generation and invasion of harmful bacteria. Probiotics are a crucial antimicrobial substance. The generation of lactic acid may lower

intestinal pH levels and stop the development of harmful bacteria like Salmonella and Escherichia coli. By consuming oxygen, Bacillus fosters the growth of anaerobes, or anaerobic bacteria, such as lactic acid bacteria and Bifidobacteria, stops the reproduction of Escherichia coli and Salmonella, and maintains the balance of intestinal flora to ward off disease. Rich digestive enzymes, organic acids, and vitamins may be produced by the bacteria and lactic acid bacteria included in the chemical formulation [12], [13].

A form of secure, dependable, and clean green feed additive is enzyme preparation. It has the capacity for biocatalysis reactions, which may decrease environmental pollution, increase the productivity of cattle and poultry, and save feed resources. By breaking down the antinutritional feed additives and altering the digestion and metabolism of animals, enzyme preparation may increase the pace at which feed and raw materials are used. Because it has a direct impact on metabolism, immune system control, and the health of host illnesses, the flora in the digestive tract is regarded as a novel nutrient. They enable the host to boost nutrition absorption and modify calorie intake. According to studies, a variety of variables, including changes in the environment, the seasons, and the presence of food additives, have an impact on the distribution and stability of intestinal microflora, which in turn has an impact on how animals grow to some degree. Probiotics function as a kind of dietary supplement that is advantageous to the gut flora. Probiotics may assist the body manage dangerous bacteria and have positive effects on the flora in the gut. Probiotics may also control the immune system to defend against different illnesses and develop disease resistance.

CONCLUSION

The in vivo approach and the in vitro method are the two major ways for evaluating the nutritional value of enzymes in compound feed. The in vivo approach, often called the biological method, involves testing an animal's diet and metabolism. Although it requires a lot of time and labor, this way of testing is reasonable and scientific. It is significantly impacted by outside elements temperature, environment, human function, etc., and evaluating a big quantity of feed or food in a short length of time is challenging. To assess the nutritional content of food, the in vitro approach was utilized to replicate the digestive tract of monogastric animals.

The process is quick, easy, labor and time-saving. Using this technique, the effects of complex enzymes and probiotics on the chicken digestive system were investigated in this investigation. The total energy of the digestive tract residue and the net output of reducing sugar were used as indicators. The results demonstrated the significance of probiotics and compound enzyme production in the growth and development of the chicken.

REFERENCES:

[1] J. Chapman, A. E. Ismail, and C. Z. Dinu, “Industrial applications of enzymes: Recent advances, techniques, and outlooks,” *Catalysts*. 2018. doi: 10.3390/catal8060238.

[2] A. M. Egorov, M. M. Ulyashova, and M. Y. Rubtsova, “Bacterial enzymes and antibiotic resistance,” *Acta Naturae*. 2018. doi: 10.32607/20758251-2018-10-4-33-48.

[3] S. Raveendran *et al.*, “Applications of microbial enzymes in food industry,” *Food Technology and Biotechnology*. 2018. doi: 10.17113/ftb.56.01.18.5491.

[4] D. Petrovic, V. A. Risso, S. C. L. Kamerlin, and J. M. Sanchez-Ruiz, “Conformational dynamics and enzyme evolution,” *Journal of the Royal Society Interface*. 2018. doi: 10.1098/rsif.2018.0330.

[5] O. Khersonsky *et al.*, "Automated Design of Efficient and Functionally Diverse Enzyme Repertoires," *Mol. Cell*, 2018, doi: 10.1016/j.molcel.2018.08.033.

[6] B. Sharma, A. K. Dangi, and P. Shukla, "Contemporary enzyme based technologies for bioremediation: A review," *Journal of Environmental Management*. 2018. doi: 10.1016/j.jenvman.2017.12.075.

[7] S. Sakamoto *et al.*, "Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites," *Journal of Natural Medicines*. 2018. doi: 10.1007/s11418-017-1144-z.

[8] L. Noda-Garcia, W. Liebermeister, and D. S. Tawfik, "Metabolite-Enzyme Coevolution: From Single Enzymes to Metabolic Pathways and Networks," *Annual Review of Biochemistry*. 2018. doi: 10.1146/annurev-biochem-062917-01.

[9] A. Y. Jee, Y. K. Cho, S. Granick, and T. Tlusty, "Catalytic enzymes are active matter," *Proc. Natl. Acad. Sci. U. S. A.*, 2018, doi: 10.1073/pnas.1814180115.

[10] H. Yamaguchi, Y. Kiyota, and M. Miyazaki, "Techniques for preparation of cross-linked enzyme aggregates and their applications in bioconversions," *Catalysts*. 2018. doi: 10.3390/catal8050174.

[11] M. Winkler, M. Geier, S. P. Hanlon, B. Nidetzky, and A. Glieder, "Human Enzymes for Organic Synthesis," *Angewandte Chemie - International Edition*. 2018. doi: 10.1002/anie.201800678.

[12] D. Concolino, F. Deodato, and R. Parini, "Enzyme replacement therapy: Efficacy and limitations," *Ital. J. Pediatr.*, 2018, doi: 10.1186/s13052-018-0562-1.

[13] M. Andlar, T. Rezić, N. Marđetko, D. Kracher, R. Ludwig, and B. Šantek, "Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation," *Engineering in Life Sciences*. 2018. doi: 10.1002/elsc.201800039.

CHAPTER 2

A COMPREHENSIVE REVIEW ON CURRENT ISSUES AND FUTURE PROSPECTS IN ENZYME THERAPY

Raj Kumar, Assistant Professor
Department of uGDX, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- raj.kumar@atlasuniversity.edu.in

ABSTRACT:

Enzymes have emerged as prospective therapeutic agents for a variety of pathologies in recent years, from metabolic inadequacies, such as fibrosis disorders, ocular pathologies, or joint issues, to cancer or cardiovascular illnesses. A broad variety of target compounds may be converted by therapies based on the catalytic activity of enzymes to reestablish the proper physiological metabolism. Due to their affinity and specificity qualities, these therapies have significant benefits over traditional therapeutic methods. Enzymes do, however, come with significant drawbacks, including limited in vivo half-lives, a lack of focused activity, and, particularly, immune system reactions from patients. It is crucial to keep an eye on the serum immune response while receiving therapy because of this. This may be done using established methods, as well as brand-new, exciting instruments like microarrays. These assays have become more well-liked because of their ability for high-throughput analysis, their ease of use, and their potential to track patients' immune responses while receiving enzyme treatments. Current health issues are continually being researched in this expanding discipline.

KEYWORDS:

Biotechnology, Enzyme Therapy, Enzymes, Encapsulation, Molecular.

INTRODUCTION

Chemical catalysts for biological systems are enzymes. They enable organisms to self-replicate and catalyze crucial metabolic events in a selective and effective way. Except for ribozymes, which are a tiny category of RNA molecules having catalytic activity, all enzymes are proteins. These proteins can distinguish between substrates with different architectures thanks to their remarkable specificity. Additionally, they have a remarkable catalytic power that speeds up the targeted chemical processes. In aqueous solutions, biological processes are catalyzed at very mild pH and temperature conditions. For biological reactions to occur, enzymes are necessary. By storing and changing chemical energy and creating biological macromolecules from substrates, they catalyze hundreds of sequential metabolic steps. The integrity of their natural protein structure is necessary for their catalytic activity. In this context, mutations cause the activity of one or more enzymes to be reduced in many disorders. Numerous medications have been created with the goal of targeting malfunctioning enzymes due to the importance of the proper operation of the enzymes. Using enzymes as medicinal medicines directly is an alternate strategy. When enzymes like pepsin were first employed to treat dyspepsia around the end of the 19th century, they were initially utilized [1], [2].

Plasminogen activator Alteplase, the first recombinant enzyme medication for acute ischemic stroke, was authorized by the Food and Drug Administration in 1987. This medication's ability to break clots and reestablish tissue perfusion led to its prescription for the treatment of acute ischemic stroke. Major efforts are being made to enable the commercial production

of these enzymatic therapies, employing recombinant expression of these molecules in plants, mammalian systems, and microbial systems. Some enzyme medications, such as snake venom, are derived straight from nature, however.

In the years 2016 to 2018, the industrial market for enzyme-based medications is anticipated to develop at a compound yearly growth rate of 6.8%. Markets for carbohydrases and proteases are anticipated to reach 2 and 2.5 billion USD, respectively, in 2024. The rise of enzyme medications that have received authorization in recent years is a result of these market indicators. Along with this economic expansion, a rise in enzyme treatment papers has been seen, demonstrating the field's expanding popularity and potential. The observed rise in research papers and patents to date underlines the efforts made in this area, which are primarily motivated by the enzymes' exciting medicinal potential. Enzymes are now being utilized and researched for many various pathologies, including cancer and cardiovascular illnesses, in addition to the therapy of metabolic deficits [3], [4].

Regarding some aspects, enzyme-based medications' potential may be enhanced. First, the molecules' in vivo half-life has to be increased; second, the targeted activity is not always precise; and third, reliable techniques are required to manage the patient's immune response during enzyme therapies.

In this context, innovative methods of immune response monitoring, including microarrays, continue to be of interest for customized therapy. The potential advantage of enzyme treatment is further highlighted by the study of emerging strategies based on enzymes to treat infections like SARS-CoV-2 and its related pathology. The purpose of this review is to approach enzyme therapy from a different angle, including not just treatment examples but also contemporary issues and fresh approaches to problem-solving. This work gives the field of enzyme therapies a fresh, wider perspective.

DISCUSSION

A thorough search of current literature was done for the present review. The following databases were utilized: PubMed, Google Scholar, Web of Science, and ScienceDirect, with a focus on recent articles.

The terms "enzyme therapy," "enzyme drug," and "enzyme treatment" were included in the search that was conducted. The explanation of the primary illnesses treated with enzymes, the targeted pathways and the employed enzymes, as well as the key drawbacks and benefits of these kinds of therapy, were the main areas of attention. The EMA's medication database was searched using the anatomical therapeutic chemical index to find information on the many therapeutic enzyme usage. In the References section, there are ATC numbers.

Enzyme Treatments for Various Pathologies

Enzymes have been used extensively to treat enzymatic deficits and a variety of medical conditions since they were originally used as medications. Enzyme-based treatments may be systemic or non-systemic, and they can be administered orally, topically, respiratoryly, intravenously, orally. According to the kind of sickness, we categorized the primary diseases treated with enzymes.

Enzyme replacement therapy primarily tackles pathologies brought on by an enzyme deficit or absence. These medical procedures are used to attempt to recover the enzymatic activity that has been lost or changed. The enzyme is typically administered via an intravenous solution. Lysosomal storage disorders are the primary metabolic deficits addressed with ERT.

Lysosomal storage diseases

Lysosomal storage disorders are a diverse set of uncommon hereditary metabolic illnesses. They come from a buildup of noncatalyzed glycosaminoglycans that is brought on by a lack of lysosomal enzymes or by modifications in molecular transport. The LSD group of illnesses includes, among others, Gaucher's disease, Hunter's syndrome, Fabry's disease, Hurler's syndrome, Morquio syndrome type A, Maroteaux-Lamy syndrome, Sly syndrome, mannosidosis, Batten disease, and Pompe's disease. There are now various initiatives looking for biomarkers to help with LSD diagnosis. The characteristics of the aforementioned illnesses make ERT seem like a feasible therapy option.

The loss of the glucocerebrosidase enzyme, which causes a buildup of lipids like glucocerebroside, particularly in the bone marrow, spleen, and liver, is the cause of Gaucher's disease. As a result, afflicted individuals may also have anemia, thrombocytopenia, swelling liver and/or spleen, and skeletal deformities. With the use of intravenous injections of a recombinant form of the enzyme, ERT is able to balance the low levels of glucocerebrosidase in this situation. The uncommon and genetic condition known as Hunter's syndrome, also called mucopolysaccharidosis type II, is brought on by a lack of iduronate 2-sulfatase , an enzyme that catalyzes the breakdown of the glycosaminoglycans dermatan- and heparan-sulfate. As a result of molecule accumulation in organs and tissues in the absence of these enzymes, normal homeostasis becomes unbalanced, which may affect a person's physical and mental development. Recombinant I2S is administered intravenously as an ERT in these circumstances, which improves the clinical parameters.

The uncommon and genetic disorder known as Fabry's disease is brought on by a lack of the lysosomal enzyme -galactosidase A. As a result, a gradual buildup of an incompletely metabolized lipid substrate is seen in a variety of cell types, altering vascular reactivity and increasing the risk of thrombo-embolic illness. Renal and cardiac failure are the two primary factors contributing to morbidity, and it is thought that these abnormalities contribute to an elevated risk for specific issues. A recombinant version of AGAL administered intravenously as ERT may alter the course of the illness. Another example of LSD is Hurler's syndrome, which is characterized by deficiencies in -L-iduronidase, N-acetylgalactosamine-6-sulfate sulfatase, arylsulfatase B, -glucuronidase, -D-mannosidase, tripeptidyl peptidase 1, and acid -glucosidase, among other enzymes. ERT is the most effective therapy strategy for treating these disorders [5]–[7].

Additional Metabolic Deficits

There are various additional metabolic deficits that should be taken into account in addition to LSD. Pancreatic enzyme and bicarbonate secretion is compromised in exocrine pancreatic insufficiency. Upper gastrointestinal and pancreatic surgeries, as well as certain pancreatic disorders such cystic fibrosis, may all result in EPI. Multiple nutritional deficits are caused by the resulting maldigestion and malabsorption of nutrients. Pancreatic ERT is a legitimate strategy to enhance patients' quality of life. Although analogous experimental investigations have shown encouraging outcomes for the use of pancreatic ERT in the treatment of this illness, nutritional malabsorption has also been reported in AIDS.The phenylalanine hydroxylase gene may become mutated, leading to the inborn condition phenylketonuria. These modifications result in an enzyme shortage, which results in hyperphenylalaninemia. Using a PHA ERT is one method of regulating phenylalanine levels; unmodified PHA and phenylalanine ammonia-lyase PHA may be administered for this purpose.

A set of uncommon illnesses known as severe combined immunodeficiency is characterized by mutations in the genes that are essential for the growth and operation of immune cells. The

absence of the adenosine deaminase enzyme is a hallmark of one subtype of SCID. This enzyme's activity is required for the metabolism of nucleic acids in tissues and the breakdown of adenosine ingested from meals. Due to its shortage, harmful purine breakdown products build up and mostly attack lymphocytes, resulting in immunodeficiency. The quality of life is enhanced by ERT based on polyethylene glycol-conjugated adenosine deaminase. PEG alterations lessen the enzyme's immunogenicity, cellular uptake, and proteolysis in comparison to the enzyme's unmodified form, which lowers the enzyme's plasma clearance. As a result, the therapeutic enzyme's circulating levels and in vivo half-life are increased.

The following list includes several different metabolic illnesses where ERT might be very important. LAL might be given as an ERT to treat Wolman illness, which is characterized by the lack of the lysosomal acid lipase enzyme. An ERT based on HMBS/PBGD might be used to treat acute intermittent porphyria patients who lack the enzyme hydroxymethylbilane synthase, also known as porphobilinogen deaminase. Additionally, a decrease or absence of the SI enzyme leads to congenital sucrase-isomaltase deficiency, which may be treated with an ERT by giving Sucraid. TNSALP ERT is an effective therapy for instances of hypophosphatasia, a condition marked by a deficit in the tissue-nonspecific isoenzyme of alkaline phosphatase. By giving the protein, ERT can also cure protein C deficiency. Finally, microbial recombinant lactase may be administered as part of ERT to treat lactase insufficiency.

Conditions for fibrosis

The ability of peptidase enzymes to break down protein deposits in many kinds of tissues has led to an increase in interest in these enzymes. It is being researched how to treat many diseases using metalloprotease endopeptidases, which include collagenases and gelatinases. A summary of the many fibrosis problems that are treated with enzymes. A whole or partial blockage of the coronary arteries is known as chronic total occlusion. The buildup of a collagen plaque in a coronary artery causes the obstruction, which may impede blood flow to the heart. Collagenase Clostridium histolyticum, a bacterial collagenase formulation derived from Clostridium histolyticum, is capable of degrading the collagen plaques, and local administration via catheter of this enzyme is one of the current treatments. Additionally, CCH is used in Dupuytren's disease for fasciotomy, an enzymatic excision of the fibrotic fascia. The fascia, the fibrous layer of tissue beneath the skin of the palm and fingers, thickens, which is a characteristic feature of this condition. Hands display certain deformations as a consequence of this condition.

The enzymatic digestion of fiber plaques and tissue seen in Peyronie's disease and uterine fibroids, respectively, is also carried out using CCH [8], [9].

Enzymes may also be used to treat other fibrosis-related disorders such keloids, lung CF, and glaucoma. After surgery, keloids, which are fibroproliferative skin tumors with profuse extracellular matrix buildup, may develop. It has been shown that collagenases and matrix metallopeptidases are both safe and effective in reducing keloids. Furthermore, the condition known as lung CF is brought on by the development of thicker mucus in the lungs. To dissolve the secretions, dornase, a recombinant version of deoxyribonuclease I, might be administered. A series of eye disorders collectively known as glaucoma harm the optic nerve, which results in a variety of visual issues and has the potential to result in blindness. It is well known that extracellular matrix buildup in the trabecular meshwork in the anterior region of the eye and in the lamina cribrosa at the optic nerve head often leads to fibrosis. Purified collagenase is administered into a patient's eyes as part of a unique technique to alleviate fibrosis.

Enzyme Casualization

By boosting target specificity, lowering immunogenicity, and minimizing clearance, enzyme encapsulation has been used to carry the enzyme payload more precisely. This has led to notable decreases in dosage levels, off-target interactions, and toxicity. Nanoparticles, virosomes, liposomes, extracellular vesicles, and erythrocytes are a few examples of encapsulation vehicles. On the one hand, nanoparticles, both biological and inorganic, are multifunctional scaffolds with features that enhance their usefulness as delivery mechanisms. The precise and regulated release of pharmaceuticals is made possible by the structural, chemical, mechanical, magnetic, electrical, and biological features of NPs. For instance, NPs containing pyruvate dehydrogenase are being researched as a treatment for illnesses caused by Pseudomonas aeruginosa biofilms. Nanoparticles produced from vaults are one intriguing kind of NPs. Vaults are human internal ribonucleoprotein particle complexes that exist spontaneously and take the form of large hollow barrel-shaped nanocapsules. For instance, the biodegradation of organic pollutants using manganese peroxidase has been investigated and has been encapsulated in vault NPs. Enzymes may be encased inside these structures, improving their stability, and they can be effectively transported to the intended area when combined with molecules that drive molecules toward their targets, such as monoclonal antibodies. However, virosomes are created based on certain characteristics of viruses to enhance medicine distribution during enzyme therapies. Similar to viruses, virosomes attach to particular cell types and enter their cytoplasm. Their main obstacle is the patient's immunological reaction to the virosomes.

Although virosomes have not yet been employed for the delivery of enzymes, they offer an intriguing potential as vehicles for the administration of anti-cancer drugs, antigens, and adjuvants for vaccinations. Lipid vesicles having one or more bilayers are called liposomes. Due to their capacity to reach the cytoplasm, they are often used as delivery platforms. In juvenile brain ceroid lipofuscinosis, for instance, liposomes are being investigated for the delivery of palmitoyl-protein thioesterase-1, resulting in restored levels of enzymatic activity in patients' fibroblasts. EVs, which operate similarly to synthetic liposomes and have intriguing properties, are proteoliposomes that are discharged from the cell membrane. In vivo research using EVs for enzyme delivery is under underway. Gectosomes, programmable, highly fusogenic EVs, have been used to load and transport Cre recombinase and -lactamase. Finally, because of their low immunogenicity, long in vivo circulation time from a reduced clearance, theoretical lack of need for chemical modifications of the enzyme, and protection provided by the membrane, which keeps the enzyme active, erythrocytes are used as drug delivery systems. The exterior red-cell membrane may be combined with an enzyme to improve its fibrinolytic profile, as in in vivo investigations where tissue plasminogen activator was used. The enzyme might, however, also be enclosed inside the erythrocyte. Following this method, erythrocytes are being used in several publications as enzyme delivery vehicles. For instance, erythrocyte-containing asparaginase , when paired with chemotherapy, is shown encouraging outcomes in phase III clinical studies as a treatment for various malignancies [10]–[12].

When combined with erythrocytes, other enzymes like arginine deiminase or methionine gamma lyase are being investigated for cancer treatment. As a therapeutic substitute for ERT in PKU, phenylalanine ammonia lyase is authorized, and the encapsulation of this enzyme in erythrocytes is being researched as a potential solution to the problems with the existing ERT regimen. Erythrocytes are also employed to provide thymidine phosphorylase to patients with mitochondrial neurogastrointestinalencephalomyopathy to make up for the enzyme's lack. Erythrocyte-encapsulated thymidine phosphorylase has been given the designation of Orphan

Drug by the FDA and EMA, and phase II clinical studies are now being planned. Promising preclinical experiments for alcohol detoxification are also being conducted on erythrocytes that contain alcohol oxidase. EryDel focuses on encasing medicinal enzymes and other tiny and big molecules in the red blood cells of patients. This company is conducting preclinical studies with other enzymes coupled with red blood cells, such as PAL for PKU, uricase for refractory gout, guanidinoacetate N-methyltransferase for GAM deficiency, and cocaine esterase for cocaine addiction, as well as phase III clinical trials with erythrocyte coupled with thymidine phosphorylase. Allogenic erythrocytes are the vehicles used by Erytech in return. This firm primarily focuses on cancer treatments, and eryaspase is their top medication for the management of various cancers [13], [14].

Even if employing erythrocytes as carriers for enzyme delivery has shown promising outcomes, there are certain disadvantages to take into account. The issues associated with transfusing blood products, such as rejection or the spread of infections, first appear when employing allogenic red blood cells. Additionally, it is challenging to produce cell products due to their vast size and the extensive sterile effort required. Erythrocytes may disintegrate when administered if their quality is insufficient, releasing the enzyme uncontrolled and resulting in harmful side effects. Furthermore, the erythrocyte is readily left behind by low-molecular-weight substances, which makes it challenging to produce long-term deposits of the enzyme. To get around this, the enzyme may be changed to decrease the release, but doing so requires changing the activation of the enzyme within the cell, which causes people to react differently and makes it impossible to get consistent results. Alternately, the erythrocyte's membrane may be altered, but doing so causes it to be easier for reticuloendothelial system cells to recognize and remove from the circulation. Additionally, this issue may be solved by using certain shuttles, endocytosis, or exocytosis mechanisms. As a result, there are certain drawbacks to using erythrocytes as drug transporters, necessitating additional advancements in experimental techniques.

CONCLUSION

Enzyme therapy is a growing approach for treating a variety of diseases, including metabolic disorders, fibrosis, cancer, cardiovascular disease, and SARS-CoV-2 infections, among others. To maximize the therapeutic potential of therapeutic enzymes, however, their short functional in vivo half-lives must be increased owing to their exposure to endogenous degrading processes, undesired side effects and toxicity, inadequate tissue selectivity, as well as the activation of immune responses.

These restrictions are being removed as a result of advancements in biotechnology. Liposomes, membrane vesicles, nanoparticles, and erythrocytes are examples of encapsulating techniques for enzymes that enhance in vivo half-life, tissue selectivity, and lower immunogenicity. Additionally, functional bioavailability is improved and immunogenicity is decreased through targeted enzyme modification techniques, such as PEG conjugation. Last but not least, keeping track of patients' immunological responses may greatly enhance patient care and maintain the effectiveness and safety of medication. In this setting, microarray technology is becoming a useful tool for better tracking anti-enzyme immune reactions in patients receiving ERT. Because enzymes have such a high therapeutic potential, further study is still required to expand their application to a broader range of illnesses.

REFERENCES:

[1] D. Concolino, F. Deodato, and R. Parini, “Enzyme replacement therapy: Efficacy and limitations,” *Ital. J. Pediatr.*, 2018, doi: 10.1186/s13052-018-0562-1.

[2] G. Fuhrmann *et al.*, "Engineering Extracellular Vesicles with the Tools of Enzyme Prodrug Therapy," *Adv. Mater.*, 2018, doi: 10.1002/adma.201706616.

[3] A. Safary, M. A. Khiavi, R. Mousavi, J. Barar, and M. A. Rafi, "Enzyme replacement therapies: What is the best option?," *BioImpacts*, 2018, doi: 10.15171/bi.2018.17.

[4] H. L. Levy, C. N. Sarkissian, and C. R. Scriver, "Phenylalanine ammonia lyase (PAL): From discovery to enzyme substitution therapy for phenylketonuria," *Molecular Genetics and Metabolism*. 2018. doi: 10.1016/j.ymgme.2018.06.002.

[5] H. Sakuraba, T. Togawa, T. Tsukimura, and H. Kato, "Plasma lyso-Gb3: a biomarker for monitoring fabry patients during enzyme replacement therapy," *Clin. Exp. Nephrol.*, 2018, doi: 10.1007/s10157-017-1525-3.

[6] A. Giannakopoulos, A. Katelaris, M. Noni, T. Karakonstantakis, C. Kanaka-Gantenbein, and S. Doudounakis, "Hyperthyrotropinemia in newly diagnosed cystic fibrosis patients with pancreatic insufficiency reversed by enzyme therapy," *Eur. J. Pediatr.*, 2018, doi: 10.1007/s00431-018-3120-3.

[7] D. Niyazov and D. A. Lara, "Improvement in cardiac function with enzyme replacement therapy in a patient with infantile-onset pompe disease," *Ochsner J.*, 2018, doi: 10.31486/toj.18.0049.

[8] C. J. Hendriksz, P. Harmatz, R. Giugliani, J. Roberts, and G. S. Arul, "Risks of long-term port use in enzyme replacement therapy for lysosomal storage disorders," *Mol. Genet. Metab. Reports*, 2018, doi: 10.1016/j.ymgmr.2018.02.007.

[9] N. Erdem, T. Buran, I. Berber, and I. Aydogdu, "Enzyme Replacement Therapy in a Gaucher Family," *J. Natl. Med. Assoc.*, 2018, doi: 10.1016/j.jnma.2017.06.013.

[10] T. Kaneda *et al.*, "Limited effects of long-term enzyme replacement therapy on the cardiac conduction system in Fabry disease," *J. Cardiol. Cases*, 2018, doi: 10.1016/j.jccase.2018.01.004.

[11] F. Antonini, S. Crippa, M. Falconi, G. Macarri, and R. Pezzilli, "Pancreatic enzyme replacement therapy after gastric resection: An update," *Digestive and Liver Disease*. 2018. doi: 10.1016/j.dld.2017.10.025.

[12] J. C. van der Meijden, M. E. Kruijshaar, L. Harlaar, D. Rizopoulos, N. A. M. E. van der Beek, and A. T. van der Ploeg, "Long-term follow-up of 17 patients with childhood Pompe disease treated with enzyme replacement therapy," *J. Inherit. Metab. Dis.*, 2018, doi: 10.1007/s10545-018-0166-3.

[13] R. Walther, J. Rautio, and A. N. Zelikin, "Prodrugs in medicinal chemistry and enzyme prodrug therapies," *Advanced Drug Delivery Reviews*. 2017. doi: 10.1016/j.addr.2017.06.013.

[14] B. Fejerskov, M. T. Jarlstad Olesen, and A. N. Zelikin, "Substrate mediated enzyme prodrug therapy," *Advanced Drug Delivery Reviews*. 2017. doi: 10.1016/j.addr.2017.04.013.

CHAPTER 3

RECENT DEVELOPMENTSAND OUTLOOKS IN INDUSTRIAL APPLICATIONS OF ENZYMES

Jayashree Balasubramanian, Associate Professor
Department of ISME, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- jayashree.balasubramanian@atlasuniversity.edu.in

ABSTRACT:

When used as industrial biocatalysts, enzymes provide a number of benefits over conventional chemical processes in terms of efficiency and sustainability. Enzyme catalysis has been scaled up for commercial processes in the pharmaceutical, food, and beverage industries, but more stability and biocatalyst functionality improvements are needed for the best biocatalytic processes in the energy sector for the creation of biofuels and the conversion of natural gas. A multidisciplinary approach is required for the creation of immobilized biocatalysts suitable for such industrial-scale operations, according to the technological challenges involved with the use of immobilized enzymes. The next generation of immobilized biocatalysts and the effective scaling up of their induced processes will be specifically defined by the overlap of technological skills in enzyme immobilization, protein, and process engineering. This review focuses on the analysis tools necessary for the multi-scale implementation of enzyme immobilization for increased product yield at maximum market profitability and minimum logistical burden on the environment and user. It also discusses how biocatalysts has been successfully implemented and how enzyme immobilization can improve industrial processes.

KEYWORDS:

Chemical Processes, Enzymes, Industrial Applications, Product Selectivity, Recent Developments.

INTRODUCTION

Because of their numerous unique advantages, including their operation in milder reaction conditions, their exceptional product selectivity, and their lower environmental and physiological toxicity, enzymes are highly effective biocatalysts that are being researched for industrial-scale catalysis. When used as biocatalysts in chemical processes, it has been shown that the benefits indicated above translate into lower operating costs. The pharmaceutical, food, and beverage sectors have therefore partly benefited from their simpler manufacturing processes, reduced waste generation, and lower energy needs as well as their reduced energy requirements. However, more research has to be done to show that biocatalysis is economically viable in other sectors, such as the generation of biofuels and natural gas conversion. The application of enzyme catalysis in chemical processes is also constrained by the lack of enzyme stability at high temperatures or in turbulent flow regimes, as well as in potentially toxic solvents, across the various industries where biocatalysis can be used. The discovery and manufacturing of reliable, stable biocatalysts suited for use in a wider variety of industrial contexts are therefore the focus of focused efforts ranging across several disciplines [1], [2].

This study focuses on the favorable applications of enzyme catalysis in chemical processes and the industries that may best use enzyme catalysis going forward. In-depth discussions of the most recent enzyme immobilization methods are also included in this review, along with

information on how enzyme immobilization can help create fully optimized biocatalysts and the technical know-how needed to scale up these economically viable immobilized-biocatalytic processes for use in industrial applications.

Implementing Enzymes

While other sectors, such natural gas conversion and fine chemical synthesis, are just lately exploring their usage, the pharmaceutical, food and beverage, detergent, and biofuel industries have enjoyed the benefits of enzyme catalysis in commercial-scale applications. Enzymes are appealing catalysts because of their mild reaction conditions, high product selectivity, and minimal environmental impact; as a result, they have been used for both streamlined chemical synthesis routes and improved chemical process economics; the advantages of biocatalysts in industrial-scale chemical production are frequently multifaceted.

Immobilization of Enzymes for a Wider Range of Applications

Studies on the immobilization of enzymes, or the attachment of the biocatalyst to a substance with desired physical, chemical, electrical, or mechanical properties, have demonstrated that immobilizing biocatalysts can improve their activity and stability across a wider range of operating conditions. The additional functionality imparted depends on both the method of immobilization and the inherent properties of the materials used in such immobilization. Further evidence that immobilized biocatalysts are novel was provided by the fact that their use simultaneously permits a reduction in the number of processing steps due to the ease with which the biocatalyst can be easily separated from the reaction mixture, the retention of catalytic activity, and the resulting appreciable degree of reusability [3]–[5].

Immobilization of Carrier-Bound Enzymes through Physical and Chemical Binding

The biocatalyst is attached to a solid substance that has been prepared in carrier-bound enzyme immobilization, and the suitable immobilization techniques are chosen to enable for the catalytic performance to be optimized.

The two most popular techniques for immobilizing carrier-bound enzymes are physical adsorption and chemical adsorption. Physical adsorption has the advantage of being a generally universal and simple immobilization technique because the binding mechanism is not dependent on a chemical reaction between the enzyme and the support, whereas covalent bonding necessitates chemical interactions between the enzyme and the support or the use of a cross-linking agent.

DISCUSSION

Although many organic and inorganic supports, such as ceramics and metal oxides, nanomaterials, and polymers have been studied as supports for enzyme immobilization, the application of such physically adsorbed enzyme-support conjugates is constrained by enzyme leaching as well as a decline in the enzyme's catalytic efficiency. For example, Falus et al. reported immobilizing subtilisin A onto different silica gels to produce racemic N-Boc-phenylalanine ethyl thioester continuously, which is a crucial pharmacological step. For the dynamic kinetic resolution of racemic N-Boc-phenylalanine ethyl thioester in three reactors in succession, subtilisin A was physisorbed to surface-grafted silica gel and employed as packing. The immobilized subtilisin a retained catalytic activity after 120 hours of continuous flow operation, resulting in a 97% conversion of the substrate at an enantiomeric excess of 99.5% in the process under ideal circumstances. The greater thermostability of the enzyme

after physisorption was said to account for the improved activity retention and shelf life, which was estimated to be up to 1 year.

The catalytic activity of lipase, an enzyme that has been extensively researched for the synthesis of biodiesel, was assessed after it was immobilized onto magnetic nanoparticles. Due to the porous silica coating's design, Tran et al. discovered that methyl-grafted Fe3O4-SiO2 nanocomposites showed a high affinity for lipase (29.5 mg lipase g1 nanocomposite being adsorbed). However, a higher Km value and a lower Vmax value were reported for the immobilized enzyme, indicating that the immobilization process decreased the enzyme's efficiency as well as its catalytic activity. This was probably caused by the enzyme active site's non-specific attachment and deformation, as well as its increased mass transfer resistance. When olive oil was transesterified with methanol to create fatty acid methyl esters (FAMEs), the immobilization also increased the reusability and separation of lipase. Due to its improved stability from multi-point hydrophobic contacts with grafted methyl groups, lipase that was physically deposited onto magnetic methyl-grafted Fe3O4-SiO2 nanoparticles was demonstrated to maintain considerable activity for up to 10 reaction cycles.

According to a study by Zhang et al., catalase was immobilized on carbon nanotubes for use in nanoelectronics, biosensing, and high-resolution imaging. Due to their high surface area to volume ratio and biocompatibility, carbon nanotubes have been widely researched as supports for enzymes. For the physisorption of catalase onto oxidized single wall nanotubes (O-SWNT), an ideal enzyme loading (1.88 mg m2) was discovered. As demonstrated by Fourier transformation infrared spectroscopy and circular dichroism (CD) analyses, the Km value for O-SWNT-catalase conjugates, relative to that of the free enzyme, was reported to be 27.0%. This finding suggests that the adsorptive interactions caused conformational changes in the secondary structure of the enzyme.

According to one study, the Vmax of O-SWNT-catalase conjugates was 6.3 times lower than that of free enzyme. The hydrogen bonding between the enzyme and O-SWNT generated an increase in enzyme stiffness, which in turn boosted activity retention, according to an examination of CD spectra for immobilized catalase. Last but not least, Nidetzky's team has shown that even under physiological pH levels, chimeras of target enzymes may be coupled with silica binding modules (SBM) by noncovalent contact and become very securely attached to such underivatized glass. Additionally, the study revealed that the immobilized enzymes exhibited complete biological activity, indicating that their binding to a glass surface may be influenced by the way in which they are positioned at the SBM interface [6], [7].

It has been shown that immobilization by covalent attachment provides a strong chemical connection that hinders considerable enzyme leaching and further reduces the loss of enzyme active sites. Physical adsorption is less labor-intensive than covalent binding approaches, which sometimes demand for activation steps that might cause enzyme denaturation. The choice of an enzyme to be covalently immobilized must also be carefully considered to ensure maximum catalytic efficiency. As a result, the covalent bond between the enzyme and the support, for example, shouldn't affect the amino acids linked to the enzyme active site, or the immobilization process may result in a reduction in catalytic activity. Candida rugosa lipase from Zhu and Sun was effectively immobilized onto poly (vinyl alcohol-co-ethylene) (PVA-co-PE) nanofibrous membranes for the hydrolysis of p-nitrophenyl palmitate. It was discovered that covalent bonding resulted in slower substrate diffusion and reduced enzyme mobility at the interface, which in turn produced an increase in Km and a drop in Vmax. Additionally, it was discovered that immobilized lipase retained about 90% of its activity after 75 minutes at 55 °C in a phosphate buffer environment, compared to just 20% for free

enzymes. After 30 days of storage at 4 °C, much greater activity than free lipase was also preserved, most likely as a result of a decrease in denaturation.

In order to create 2-phenylethyl acetate, the main aromatic ester of rose scent, Kuo et al. immobilized the same enzyme. In this work, lipase was covalently attached to a polyvinylidene fluoride (PVDF) membrane and activated with glutaraldehyde and 1, 4-diaminobutane, resulting in a 1.71 mg enzyme g1 PVDF enzyme loading. Due to the covalent immobilization process' ability to preserve the tertiary structure in the organic medium, the immobilization strategy significantly increased catalytic activity in n-hexane with just a minor decrease in catalytic efficiency. In addition, a research by Mendes et al. showed that covalent attachment to an epoxy-silica-polyvinyl alcohol composite is the best carrier-binding technique for immobilizing lipase from Penicilliumcamembertii. It was discovered that the physical adsorption lipase had a higher, more stable enzyme loading capacity than the covalently attached lipase. The best example for covalently bound lipase also produced a hydrolytic activity that was about twice as high as that of physical adsorption and had a higher activity retention. Compared to free lipase, covalent attachment of lipase to epoxy-silica-polyvinyl alcohol also led to better thermostability.

The use of epoxide hydrolase (EH) in the manufacture of valuable, enantiomerically pure pharmaceutical intermediates and other bioactive compounds has also been researched. For the enantioselective hydrolysis of p-nitrostyrene oxide, Petri et al. suggested the covalent attachment of EH from Aspergillus niger to epoxide-activated silica gel. In comparison to free EH, immobilized EH was shown to maintain over 90% of its activity and to have excellent storage stability over a few months. Immobilization onto the silica gel produced a comparatively high immobilization yield of around 70%. The enantiomeric selectivity of p-nitrostyrene oxide hydrolysis was unaffected by the covalent immobilization of EH, and the stability of EH in 20% DMSO organic solvent was significantly increased [8]–[10].

Due to their large specific surface area for volume-efficient catalysis and negligible mass transport restrictions, nanomaterials have been investigated as supports for biocatalysts. Li et al., for instance, developed a covalent support for catalase from bovine liver in an electrospunpolyacrylonitrile-glycopolymernanofibrous membrane. Although immobilized catalase activity was shown to be stable across a wider range of temperatures and pHs, it was only approximately 50% as active as free catalase. Additionally, it was shown that free catalase lost all of its relative activity when stored at 4 °C for 30 days, but covalently immobilized catalase maintained around 80% of its relative activity. Catalase chemical attachment to Eupergit C, a macroporous derivative of methacrylamide that is said to be chemically and mechanically stable as a catalyst for use in batch and plug flow reactors, was improved by Alptekin et al. Kcat/Km, a ratio used to compare the catalytic effectiveness of free and immobilized catalase, was computed and found to be approximately two orders of magnitude lower in the immobilized enzyme, indicating that it was less effective in converting substrate to product. Immobilization, however, has been shown to increase enzyme stability and shelf life when used as a biocatalyst in batch and plug flow reactors. According to studies, free catalase became inactive after only 11 days of storage but immobilized catalase kept about 78% of its original activity when assessed 28 days following immobilization. In a plug flow reactor, immobilized catalase maintained 50% activity at 82 minutes.

Enzyme Capture

The immobilization of a biocatalyst inside carriers with variable degrees of porosity and permeability is known as enzyme entrapment. Due to tighter control over their

microenvironment, enzymes immobilized by entrapment are more stable and have been shown to be more catalytically active at higher temperatures in organic solvents as well as to be readily detachable from the substrate-product reaction combination. For the use of biocatalysts in the production of organic compounds as well as for innovative biosensing systems, immobilization by entrapment in a range of carriers, including as sol gels, hydrogels, polymers, and nanomaterials, has been investigated. In addition, it has been suggested that lipase immobilization be used to create compounds for taste and scent. For instance, Ferraz et al. examined the possibility of synthesizing geranyl propionate utilizing lipase from Penicilliumcrustosum as the biocatalyst. A crosslinking reaction between calcium chloride and sodium alginate resulted in the encapsulation of lipase in beads that were over 0.5 cm in diameter. Lipase-enhanced calcium-alginate beads were examined for reusability and further refined for the conversion of geraniol and propionate. Results indicate that immobilized lipase's ability to retain activity declined linearly with regard to the number of cycles it was used, indicating that each cycle's enzyme leaching was the cause of activity loss . The immobilization of inulinase from Kluyveromycesmarxianus, a crucial biocatalyst in the creation of high fructose syrups, was examined by Risso et al. using the same entrapment technique. The kinetic characteristics of inulinase, which was trapped in calcium-alginate beads, as well as its thermostability and pH stability in various concentrations of organic solvents, were determined. At ideal mass fractions of organic solvent, immobilized inulinase's Km value was discovered to be much lower than that of free inulinase, although its Vmax value was equivalent to that of free inulinase under the same circumstances. However, in the kinetic study of the immobilized biocatalyst, mass transfer resistances, which would probably be the rate-limiting mechanism, were not taken into account.

For use in reactor system applications, Arica et al. suggested encasing catalase from cow's liver in thermally reversible poly (isopropylacrylamide-co-hydroxyethylmethacrylate) cylinders. The catalytic activity and enzyme-substrate affinity of immobilized catalase decreased, and it maintained less activity at higher temperatures than free catalase. Additionally, it was discovered that a rise in temperature resulted in lower hydrogel swelling and greater mass transfer resistance. The temperature-dependent behavior of the hydrogel carrier itself was primarily blamed for the apparent kinetic parameters of the immobilized catalase. The entrapment method improved storage stability and allowed for the reuse of enzymes. Similarly, immobilized catalase kept 78% of its activity after 20 days of storage at 4 °C, but free catalase lost all of its activity under the same circumstances. Additionally, it was shown that hydrogel-entrapped catalase maintained around 95% of its activity for 6 cycles in the batch reactor system [11], [12].

The kinetic and stabilizing effects of encapsulating bovine liver catalase in hollow silica nanoparticles (HSNPs) were investigated by Singh et al. Catalase and hydrogen peroxide absorption peaks were not seen in the liquid supernatant leftover from the immobilization process, suggesting an immobilization yield of over 100%. It was also discovered that the encapsulation method reduced the enzyme's activity and its affinity for its substrate. However, immobilized catalase shown much better stability throughout a wide pH and temperature range. When evaluated for activity at 70 °C, free catalase was entirely denatured, but encapsulated catalase was shown to exhibit maximum catalytic activity at 80 °C. The thermostability data for the immobilized enzyme showed that catalase was not physically adsorbing onto HSNPs but rather was enclosed inside them. Physically adsorbed enzyme is predicted to exhibit a decline in catalytic activity close to the denaturation temperature of free enzyme.

The industrial application of bovine carbonic anhydrase (BCA), a metalloenzyme studied for applications in carbon capture and biocatalytic enrichment of natural gas, is constrained by the almost complete loss of enzyme catalytic activity at 63 °C due to the irreversible aggregation of BCA. Yan et al. reported the successful nanogel encapsulation of BCA. Acryloylation of BCA and subsequent in-situ polymerization to create single BCA nanogels were done in order to provide the enzyme molecular structural stability while reducing mass transfer restrictions. Similar to free BCA, BCA nanogels demonstrated catalytic activity and considerable activity retention at temperatures over 63 °C. The secondary structure of BCA was found to be retained by nanogel encapsulation, which prevented irreversible aggregation and permitted catalytic activity even at 81 °C.

Another unique method for enhancing enzyme activity as a consequence of biocatalyst-carrier interactions is enzyme entrapment in biocompatible nanoparticles and solid supports. It is feasible to "lock" immobilized enzymes into more catalytically active conformations under the right immobilization circumstances, according to studies of enzyme entrapment in solid carriers. For instance, Prakasham et al. looked at the stability and kinetic characteristics of amylase trapped in matrices made of nickel-impregnated silica paramagnetic particles. For all of the investigated pHs and temperature ranges, it was found that the imprisoned amylase hydrolyzed starch more quickly than the free amylase. The immobilized amylase had a lower Km value, which likely means that the entrapment method produced a more effective, reliable biocatalyst.

In a metal-organic framework, the enzymes glucose oxidase (GOx) and horseradish peroxidase (HRP) were easily co-immobilized, according to Wu et al. The enzyme-embedded zeolitic imidazolate framework (GOx&HRP/ZIF-8) was created by mixing solutions of zinc nitrate, GOx, HRP, and 2-methylimidazole under ambient conditions for 0.5 hours. This conjugate's catalytic activity was compared to a combination of GOx/ZIF-8 and HRP/ZIF-8 in order to identify any efficiency variations brought on by the co-immobilization method. Due to a considerable reduction in mass transfer resistance, analysis revealed that GOx&HRP/ZIF-8 had a 2 times greater activity than the combination of single-immobilized conjugates. Additionally, it was discovered that GOx&HRP/ZIF-8 retained noticeably greater activity than free enzyme in organic solvent and while kept at ambient temperature. However, the mass transfer restrictions of substrate via carrier material, enzyme leaching, and low total catalytic mass of enzyme-carrier conjugate currently preclude the practical scaling up of entrapped enzymes for biocatalysis . Last but not least, Lin et al. reported on the capture of HRP in aqueous solution and inorganic interfaces constructed using cooper phosphate supports. Results revealed that compared to the free enzyme in solution, the hierarchical flower-like spherical structures significantly increased enzyme activity. In addition, even after several cycles of testing the active hydrogen peroxide (H_2O_2) release, the hybrid interfaces still showed outstanding reusability and repeatability [13], [14].

Aggregates of cross-linked enzymes

Since its invention for use in industrial bio-transformations of fine chemicals and pharmaceuticals, the immobilization of enzymes by the production of cross-linked enzyme aggregates (CLEAs), one of the newest classes of immobilization methods, has also received much attention. In order to make CLEAs, soluble enzymes are first aggregated with a precipitating agent like ammonium sulfate, acetone, ethanol, or tert-butanol, and then copolymerized with a cross-linking agent, most frequently glutaraldehyde. The precipitating and cross-linking agents must be carefully chosen to ensure that immobilization does not negatively affect enzyme activity, as analysis revealed that aggregate cross-linking is not a universal immobilization method and should, therefore, be optimized for each target

biocatalyst. The benefits of improved operational stability, reusability, and exceptional resistance to the leaching of immobilized biocatalyst in aqueous media were demonstrated for CLEAs, while they were shown to be free of substrate diffusion restrictions that might have reduced catalytic activity. This phenomenon, known as hyperactivation, was attributed to the aggregation of enzyme in a pre-organized tertiary structure that rendered it permanently insoluble upon cross-linking. In some cases, CLEAs were shown to possess higher catalytic activities than the corresponding free enzymes. Due to their high catalytic productivity and low-cost immobilization techniques, CLEAs therefore shown a significant promise for use in industrial-scale processes. However, CLEA's mechanical characteristics needed to be improved and continuous process separation requirements needed to be clearly defined if applications were to scale up successfully. For the synthesis of microalgal oil and biodiesel from maize, respectively, Lai et al. reported the direct generation (from fermentation broth) and stability study of CLEAs using lipase from Penicilliumexpansum (PEL) in different solvents. This research discovered that PEL-CLEAs were less catalytically active than free PEL, which was probably caused by the big substrate molecules' restricted mass transfer. However, it was shown that the clumping of PEL-CLEAs and loss of enzyme active sites caused a reduction in the production of biodiesel. PEL-CLEAs also demonstrated enhanced stability over free PEL at increasing temperatures and in varied pH settings. In nonaqueous solutions, PEL-CLEAs also displayed significant activity retention, indicating that the immobilization technique may be suitable for biodiesel synthesis in industrial scales.

A cascade chemical reaction was catalyzed by Nguyen and Yang's combined cross-linked enzyme aggregates (combi-CLEAs) of GOx and HRP, which are suitable to glucose monitoring biosystems and pharmaceutical wastewater treatment. To ensure maximum catalytic activity and enzyme stability, the cross-linking density and mass ratio of GOx to HRP were tuned for the combi-CLEAs. After optimization, combi-CLEAs exhibited similar catalytic activity to free enzymes, but lower values of Km were likely caused by two factors: the co-immobilization technique's significant reduction of the hydrogen peroxide intermediate's distance of mass transfer and the cross-linking of GOx, which led to a reduction in the inhibitory effect of H2O2. Similar encouraging results for the application of combi-CLEAs on three commercial enzyme mixes demonstrating feruloyl esterase activity were reported by report. In ternary combinations of n-hexane, 1-butanol, and water, the scientists reported on the immobilization by aggregate cross-linking and evaluated its kinetic activity in comparison to free enzyme. By comparing 10 aggregating agents, Combi-CLEAs were created to maintain the maximum amount of catalytic activity, and the efficiency was then improved by adjusting the cross-linking agent's concentration. When the enzyme was precipitated using ammonium sulfate and cross-linked at a 100 mM glutaraldehyde concentration, a product yield of 97% was obtained. Since ammonium sulfate's solvation is an endothermic reaction, it has been discovered that using it with this precipitating agent is favorable. The fact that the activity of enzyme aggregates was greater than the activity of the free enzyme before cross-linking is significant because it provides proof that the right immobilization procedures may lock enzymes in highly active conformations. Even though the immobilized enzymes had low activity retention and stability, combi-CLEAs could be readily extracted from the reaction mixture that included unreacted methyl ferulic esters, produced 1-butyl ferulate by centrifugation, and then employed again for furuloyl esterase activity.

Rhamnopyranosidase (Rhmnase), a hydrolytic enzyme used in the production of valuable pharmaceutical substances like lipoprotein associated phospholipase A2 inhibitors, which are administered in the treatment of atherosclerosis, was converted by Martins et al. into magnetic cross-linked enzyme aggregates (mCLEAs). With various cross-linking and

precipitating agents, these magnetic aggregates were tested for their catalytic activity before being compared to Rhmnase for reusability in a batch reactor setup. Following five 24-hour reutilization cycles, Rhmnase were shown to preserve about 100% of their activity; however, following seven reutilization cycles, Rhmnase demonstrated a considerable reduction in activity. As a result of the improved physical stability of the magnetic aggregates, Rhmnase, on the other hand, first showed a reduction in activity of around 40% after one reutilization cycle and then exhibited almost constant activity moving forward. The choice of immobilization materials should be carefully considered for assuring high biocatalytic turnover. Due to their increased stability and reusability, magnetic enzyme aggregates were shown to be better biocatalysts for a scaled-up process.

Other authors reported using Pseudomonas sp. CLEAs. For the enantioselective resolution of (S)-N-(2-ethyl-6-methylphenyl) alanine, a chemical precursor utilized in the creation of common herbicides, lipase (CLEA-PSL) was employed as a biocatalyst. The optimal cross-linking and precipitation conditions were established for the production of CLEA-PSL, and the kinetic parameters of free and immobilized lipase, respectively, were established. It was discovered that CLEA-PSL was more active than free lipase, and it was also noticed that free lipase took 48 hours to obtain a 50% substrate conversion, but immobilized lipase only required 12 hours to achieve the same conversion and enantiomeric excess. The time difference was most likely caused by an immobilization-induced transition to a more catalytically active enzyme configuration. The analysis of kinetic parameters revealed that CLEA-PSL also had an enhanced affinity for substrate, most likely as a result of the secondary structural modifications brought about by immobilization. Last but not least, it was discovered that the immobilized lipase was more thermostable than the free lipase, retaining about 80% of its original activity after ten reutilization cycles in a batch reactor with no enantioselectivity loss detected.

CONCLUSION

With current trends for biofuel generation and natural gas conversion, commercial-scale enzyme catalysis has been used in a variety of sectors, including pharmaceutical and food. Enzymatic catalysis was found to control product yield in these industries when used under softer process conditions, with less energy consumption, with reduced waste generation, and with exceptionally high product selectivity, leading to improvements in process economics and environmental sustainability. However, constraints in biocatalyst stability significantly hinder the scaling up of enzymatic activities. In order to achieve these goals, enzyme immobilization was suggested and investigated as a promising strategy for extending the range of enzyme catalysis and enhancing process effectiveness. While comprehensive knowledge of the economic forces that surround process development is still needed to ensure that the implementation of enzyme catalysis in commercial-scale processes is appropriate, critical analysis of immobilization strategies for facilitating the development of optimal enzymes is still necessary.

Critical study of immobilized enzyme processes, which places a premium on the overlapping of skills in protein engineering, enzyme immobilization, process engineering, and life cycle analysis, will ultimately determine the effective industrial implementation of next generation immobilized biocatalysts. Future prospects for implementation will need to take into account the structure-function relationship at the level of the enzyme and the platform used for immobilization, as well as the optimized product yield at low implementation costs and with the combination of experimental and computational approaches for an integrated combinatorial strategy.

REFERENCES:

[1] A. Madhu and J. N. Chakraborty, "Developments in application of enzymes for textile processing," *Journal of Cleaner Production*. 2017. doi: 10.1016/j.jclepro.2017.01.013.

[2] R. Salim *et al.*, "Food processing applications of enzymes," *J. Pharmacogn. Phytochem.*, 2017.

[3] Manisha and S. K. Yadav, "Technological advances and applications of hydrolytic enzymes for valorization of lignocellulosic biomass," *Bioresource Technology*. 2017. doi: 10.1016/j.biortech.2017.05.066.

[4] J. Bzdrenga *et al.*, "Biotechnological applications of quorum quenching enzymes," *Chem. Biol. Interact.*, 2017, doi: 10.1016/j.cbi.2016.05.028.

[5] S. H. Toushik, K. T. Lee, J. S. Lee, and K. S. Kim, "Functional Applications of Lignocellulolytic Enzymes in the Fruit and Vegetable Processing Industries," *J. Food Sci.*, 2017, doi: 10.1111/1750-3841.13636.

[6] "Fermentative Production of Microbial Enzymes and their Applications: Present status and future prospects," *J. Appl. Biol. Biotechnol.*, 2017, doi: 10.7324/jabb.2017.50414.

[7] A. K. Patel, R. R. Singhania, and A. Pandey, "Production, Purification, and Application of Microbial Enzymes," in *Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications*, 2017. doi: 10.1016/B978-0-12-803725-6.00002-9.

[8] M. Loi, F. Fanelli, V. C. Liuzzi, A. F. Logrieco, and G. Mulè, "Mycotoxin biotransformation by native and commercial enzymes: Present and future perspectives," *Toxins*. 2017. doi: 10.3390/toxins9040111.

[9] S. A. de Rose, H. Novak, A. Dowd, S. Singh, D. A. Lang, and J. Littlechild, "Stabilization of a lipolytic enzyme for commercial application," *Catalysts*, 2017, doi: 10.3390/catal7030091.

[10] X. Lian *et al.*, "Enzyme-MOF (metal-organic framework) composites," *Chemical Society Reviews*. 2017. doi: 10.1039/c7cs00058h.

[11] A. Vimal and A. Kumar, "Biotechnological production and practical application of L-asparaginase enzyme," *Biotechnology and Genetic Engineering Reviews*. 2017. doi: 10.1080/02648725.2017.1357294.

[12] S. Parte, V. L. Sirisha, and J. S. D'Souza, "Biotechnological Applications of Marine Enzymes From Algae, Bacteria, Fungi, and Sponges," in *Advances in Food and Nutrition Research*, 2017. doi: 10.1016/bs.afnr.2016.10.005.

[13] R. Tramontina *et al.*, "The Coptotermes gestroi aldo-keto reductase: A multipurpose enzyme for biorefinery applications," *Biotechnol. Biofuels*, 2017, doi: 10.1186/s13068-016-0688-6.

[14] J. I. Reyes-De-Corcuera and J. R. Powers, "Application of Enzymes in Food Analysis," 2017. doi: 10.1007/978-3-319-45776-5_26.

CHAPTER 4

A COMPREHENSIVE REVIEW ON RECENT DEVELOPMENTS IN ULTRASOUND-ASSISTED ENZYME PROCESSING IN AGRI-FOODS

Thiruchitrambalam, Professor
Department of ISME, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- thiru.chitrambalam@atlasuniversity.edu.in

ABSTRACT:

The sustainability of the biorefinery idea depends on the intensification of processes. Because the cavitation effect generated by this technology has been found to increase the effectiveness of the biocatalysts, enzyme catalysis aided by ultrasound may provide attractive potential in the agri-food industry. The most recent developments in this field are covered in this review, with a particular emphasis on three key applications, UAEE, US hydrolysis reactions, and US-assisted synthesis reactions for the production of agri-food products and ingredients, allowing for the upcycling of agro-industrial waste. Also covered are several theoretical and experimental aspects of US that must be taken into account. The primary factor influencing the catalytic activity of enzymes is ultrasonic intensity, yet there is no accepted method for quantifying it, making it difficult to compare findings. UAEE of bioactive substances has seen the largest use of enzyme catalysis supported by US in agri-foods. The production of juices and beverages using US hydrolysis processes comes in second, with some intriguing uses for creating bioactive peptides. Last but not least, some work has been done on synthesis processes, mostly via Trans and esterification to create structured lipids and sugar esters, while early attempts at synthesising oligosaccharides have shown encouraging results. The majority of the time, US has increased the reaction yield, although it is yet unclear how various sonication settings impact the kinetic parameters. Future studies should use a multidisciplinary approach in order to better understand a highly complicated process that happens over very brief intervals of time.

KEYWORDS:

Biocatalysts, Enzyme, Food Processing, Ultrasonic.

INTRODUCTION

In very effective food processes, enzymes are highly active and focused biological catalysts. In contrast to conventional chemical catalysts, they function in benign circumstances and with very high molecular precision, increasing the pace of reaction by lowering activation energy. Enzymes are appealing catalysts for use in a variety of food processes, such as maceration and filtration in brewing, baking, producing glucose and fructose syrups from starch, making wine, tenderizing meat, processing juice, creating low-calorie sweeteners, and a wide range of applications in the dairy industry. Enzymes create a special network of intricate reactions that adapt to cell metabolism under physiological settings, performing astoundingly well and supporting the survival of all species. To change the milieu that normally protects them, however, they are often extracted from their cellular confinement for industrial use. Enzymes are thus impacted by the reaction's operating circumstances, which vary significantly from their natural environment in terms of temperature, pH, substrate concentration, and the presence of inhibitors or activators. Therefore, one of the biggest challenges in biocatalysis is to turn these physiological catalysts into process catalysts that can work in the typically demanding reaction conditions of an industrial process. Enzyme immobilization is emphasized among the many tools available to carry out this

transformation due to the demonstrated benefits of immobilized enzymes over their soluble counterparts in terms of robustness, operational stability, ease of separation from the product stream, and the potential for multiple reuses in a repeated batch operation or prolonged use in a continuous operation [1], [2].

An immobilized enzyme's characteristics and abilities are determined by the enzyme itself, the immobilization technique, and the support material. Adsorption, covalent attachment and physical entrapment to inert supports, aggregation and crosslinking of the enzyme protein, or containment within semipermeable membranes in membrane bioreactors are just a few of the numerous immobilization strategies that have been thoroughly reviewed in the literature. The main obstacles to the widespread use of immobilized enzymes for food processing, despite impressive advancements in the field, are decreased activity per unit of volume, mass transport restrictions, labor-intensive immobilization processes, and the additional costs related to the necessary support material and reagents. Additionally, the immobilization method must be in line with the enzyme's intended application. This may be especially important if the end products of the enzymatic process are meant for human consumption. For the majority of enzyme immobilization techniques, several chemical additions are employed. Surprisingly, there is little knowledge of the possibility of product contamination caused by the leaking of these chemical components into the product and the long-term cumulative impact of those effects.

Contrarily, ultrasonography is a new sustainable technology that increases the speed and efficiency of a number of food processing industrial operations. US uses a transducer that transforms electrical energy into vibrational energy to create sound waves at a frequency over the human hearing threshold. Continuous cycles of compression and rarefaction are created when these waves travel across space, encouraging the growth of cavitation bubbles in the liquid medium, which may interact in various ways with a liquid-solid interface. Acoustic cavitation is a phenomena that occurs when the pressure changes when ultrasonic waves move through a liquid, falling below the liquid's vapor pressure and creating tiny, vapor-filled gaps that result in bubbles within the liquid. A "near" adiabatic heating of the bubble's contents occurs as a result of the bubbles' development and collapse inside the liquid medium, which take place over a relatively brief period of time of around 1 s and result in localized temperatures and pressures of up to 1000 atm . As a consequence, hotspots are created, increasing the rates at which mass and heat are transferred, sonochemical reactions are developed, high turbulence is formed, and liquid jets are created [3], [4].

DISCUSSION

Two approaches have been used to study the cavitation effect that ultrasound has on enzymes during food preparation. The first has to do with enzymes that degrade food quality, such as polyphenoloxidase in vegetables and lipases and lipoxygenases responsible for rancidity in high-fat diets. In these circumstances, it has been shown that high-intensity ultrasonic cavitation may deactivate the undesirable enzymes, extending the shelf-life and improving the quality of numerous food goods without causing the loss of thermolabile bioactive substances, as is the case with traditional heat processing. A second viewpoint discusses using the US to enhance enzyme bioprocesses. A study claim that US has been shown to speed up enzymatic processes by affecting a variety of targets, including the structure of the enzyme and substrate and the rate at which substrate is transferred within the reaction system.

The development of novel food applications, such as the extraction of bioactive substances, nutrient-rich and quality-improved food production, and the removal of food safety concerns, has fueled enzymatic processing's continuous rise in the agri-food industry . The development

of sustainable processes for the biorefinery concept is required given the current consumer and industry demands for production in greener systems. US-supported enzyme catalysis may present intriguing opportunities for improved biocatalytic performances. A few studies from recent years have brought out the dearth of information on the circumstances of ultrasonic processing and how they affect enzyme catalysis, emphasizing the need to provide comprehensive information about the data in order to adequately compare findings. To the best of our knowledge, there are no published reviews that directly address the use of US in enzyme agri-food processing. The goal of this work is to review recent developments in the field with a particular emphasis on three key applications: extraction, hydrolysis, and synthesis of bioactive compounds carried out with enzyme reactions assisted by US for the manufacturing of agri-food produce and ingredients with improved yields, as well as a method for upgrading agro-industrial waste into useful compounds. Included are a theoretical backdrop, some contentious experimental findings, and the processes via which US processing affects the enzymes [5], [6].

Technology using ultrasound

Ultrasound is a hybrid technology distinguished by its robust processing and cost effectiveness. For activities important to the food sector, such as extractions of bioactive substances, emulsification, degassing, membrane filtration, and convective air drying, among others, it enables shorter processing times with reduced use of water, solvents, and energy. The use of ultrasound in food processing can be divided into two categories: low intensity and high frequency ultrasound, which is primarily used for non-invasive and non-destructive food quality monitoring, and high intensity and low frequency ultrasound, which has a significant impact on the physical, biochemical, and mechanical properties of foods. Transducers that transform electrical pulses into acoustic energy are what produce ultrasound. Both transducers based on the piezoelectric principle and those based on the magnetostriction effect exist. The former is the change in length per unit length brought on by applying a magnetic field to a material having ferromagnetism features, which results in magnetization and the subsequent production of vibrations. The majority of transducers used in ultrasonic food preparation, however, are piezoelectric. They are made up of two ceramic components that respond to an electric field by varying in size. As a result, the ceramic components move up and down in a very repeatable way when an alternating field is applied.

Ultrasound Technology

The most affordable setup, ultrasonic baths are mostly employed to extract bioactive compounds, while they may also be utilized for certain enzyme applications. US baths are available in a variety of sizes and ultrasonic frequencies, ranging from 20 kHz to 1 MHz. The majority operate at a single set frequency, however a few commercially available devices provide the option of using two or more frequency levels. The reaction medium container is often placed into the bath with an overhead stirrer, allowing the solution to be continuously mixed. Although this setup is helpful for exploratory research, there are a number of restrictions that should be kept in mind when using it. Because the location, thickness, form, and distance of the vessel from the transducers have a large influence on the acoustic field provided to the reaction media, US baths are notorious for having limited repeatability of findings. The water in the bath and the glassware are also significant attenuators of the supplied intensity. In actuality, this kind of setup cannot be scaled up since the bath's water volume is several times more than the reaction volume. Commercial ultrasound probes, on the other hand, offer the benefit of immediately sending the ultrasonic field to the reaction with little energy loss. The typical operating range for these devices is between 20 and 100 kHz, however some of them allow other frequency ranges by switching out the tip. In reality,

the majority of commercial probes include choices for modifying the shape and size of the tip depending on the response volume since the probe tip also has a significant influence on the amount of ultrasonic intensity that is delivered to the solution [7]–[9].

For instance, tiny volumes of very concentrated intensities are produced using microtips. However, it is typical for the device maker to limit the maximum amplitude utilized in these applications for security concerns. On the other hand, bigger diameter tips may be utilized with the equipment's maximum power levels. Most probes have tips that range in diameter from 1 to 2 cm by default. However, the greatest volume in which these probes can provide cavitation is limited because of how quickly their radial and axial ultrasonic intensities diminish. As a consequence, these devices' findings cannot be directly scaled up. In addition, the reaction vessel has to be jacketed and linked to a heat exchanger to maintain a constant temperature in order to prevent the quick thermal inactivation of the enzyme. This is due to the influence of the thermal rise generated by ultrasonic cavitation. The latter is one of the major problems with US-assisted enzyme processes, which are often carried out in batches. However, continuous operations in the "flow-through" mode enable the processing of greater volumes with enhanced ultrasonic exposure homogeneity, easier temperature control, and results practical for scaling up purposes. This is made possible by the availability of US reactor chambers.

Ultrasound's Impact on the Structure of Enzymes

Enzymes are polypeptide chains organized in certain ways that determine their ability to catalyze reactions. The active site, a tiny region of the molecule made up of a few amino acid residues that are directly engaged in the process of substrate transformation, is where the catalytic activity is located. A small change in the medium conditions and/or composition may result in significant changes in the three-dimensional structure of the enzyme, which is necessary for performing catalysis, even though the information regarding the precise mechanism by which the enzyme is excited by US waves has not been studied in detail. In this sense, enzyme activity is governed by the configurations of the active site. Between 20 and 100 kHz, the majority of these changes in protein structure that are reflected in enzyme activity have been noted. Understanding how the enzyme molecules change under US waves is crucial because structural changes to enzymes lead to changes in their function. In order to understand the changes that enzymes go through in reaction media, methods like X-ray diffraction or scanning electron microscopy are not useful since the crystalline structure of a protein varies dramatically from its structure in solution.

Fluorescence spectroscopy, which investigates the conformational changes in the immediate surroundings of tryptophan units, is one useful approach. Tryptophan's surroundings has an impact on its fluorescence, which enables scientists to learn more about the structure of proteins and the workings of enzymes. An indication of the modification in the secondary structure of the enzyme caused by the exposure of tryptophan residues has been the modulation of the intrinsic fluorescence of an enzyme molecule. Because the active site is exposed, the substrate may interact with the enzyme more readily, speeding up the reaction rate. This has a beneficial impact on enzyme activity. It has been postulated that when an enzyme is in the proper US state, it goes through an unfolding process that exposes the active site, allowing the substrate to get to it more quickly and speeding up the reaction rate. Additionally, it has been suggested that US waves enhance transport phenomena when combined with enzyme unfolding, increasing the contact between the enzyme and substrate and subsequently the rate at which the product is released.

A useful, non-destructive method to investigate the structure of proteins in solution is circular dichroism. This method enables analysis of the conformational changes in molecules with optical activity. One of its traits is CD, which is a simple, reliable, and quick technique for keeping track of conformational changes in protein structures. UV-CD spectra in enzyme molecules allow for the identification of alterations to the secondary structure of enzymes. When the enzyme is subject to any kind of perturbation in solution, as is the case with US, this technique detects variations in the percentage of -helix, -sheet, -turns, and random coil structures. It has been shown in studies by that an enzyme subjected to US treatment experiences changes in its UV spectra at wavelengths below 200 nm, which suggests differences in the ratio of -helix and -sheet structures. Fourier Transform Infrared spectroscopy is an additional popular method for examining changes in enzyme structure. This method is a dependable resource for the investigation of protein 3D structures and molecular processes of reaction, unfolding, and misfolding because it is very sensitive to the chemical composition and distribution of molecules. It has been claimed that the IR absorption bands between 2551 and 724 cm1 may provide important details about the side chains of amino acids in proteins. An increase or decrease in peak intensity in the amino or carboxyl sections of the spectrum is one of the changes in enzyme structure that FTIR has detected [9], [10].

The three methods stated above may be useful in researching how US affects the structure of enzymes. This information, however, does not fully explain whether the ultrasonic waves influence the enzyme and substrate structure alone or if transport events also take place in the reaction fluid, which may lead to enhanced kinetic parameters and/or greater enzyme activity. Despite this gap in information, it is widely acknowledged that UI is the key factor influencing how enzymes behave when catalyzing reactions. By creating stable cavitation bubbles, changes in the enzyme's 3D conformation boost its activity. However, this requires using a low-intensity, low-frequency US irradiation. A transition phase occurs when UI intensity rises and above the ideal level, which causes the polypeptide chain to denaturize and reduce enzymatic activity. Some of these modifications may be brought on by mechanical stress, while others could be brought on by sonochemical processes taking place in the medium. Three zones for sonochemistry have been found, within the cavitation bubble, at the cavitation bubble surface, and inside the bulk liquid around the bubble. Surface-active materials that collect at this interface are more vulnerable to sonochemical assault because the interface between the bubble and the surrounding liquid has the largest concentration of hydroxyl radicals. Enzymes in free solution have the capacity to interact with the cavitation bubble surface and function as surface-active agents. In this situation, enzymes are vulnerable to radical assault brought on by sonochemistry, which may result in the breakage of hydrogen bonds and van der Waals interactions in the polypeptide chains, changing the secondary and tertiary structures of the protein.

The irradiation duty cycle is another crucial factor since it regulates the amount of time the enzyme is exposed to sonication. The enzyme is most active when a low duty cycle is utilized, but if continuous and protracted irradiations are used, more heat is produced, which might possibly lead to partial dissociation of the enzyme structure and subsequent deactivation. Therefore, it follows that the enzyme's inherent qualities as well as ultrasonic processing and the reaction media will affect whether or not enzymatic activity increases or decreases. As a result, the enormous diversity of enzymes prevents the proposal of a general mechanism, opening a research area to be investigated on a case-by-case basis where kinetic modeling may provide a crucial supplementary insight into the mechanisms by which a biocatalyst is affected under US.

Ultrasound-Assisted Enzyme Bioprocessing in Agri-Foods

A biorefinery is a novel idea created to address the issues of the twenty-first century that allows the majority of secondary and primary metabolites from plant matrices to be valued. In this context, the employment of green technologies, such as US, needs particular attention. Additionally, strong enzyme-based biocatalytic systems constitute a useful strategy for enhancing the food production's economic and environmental sustainability. Combining these two ideas might lead to some intriguing chances for the agri-food industry to create value. US applications for enzyme food processing can be carried out both before and during the reaction, typically to increase the reactivity of substrates and improve accessibility, or when treating with complex macromolecules or in systems that present mass transfer limitations, such as in immobilized enzymes. The use of this hybrid technology is discussed in the next section in relation to three distinct agri-food-related scenarios: enzymatic extractions supported by US, enzymatic hydrolysis assisted by US, and US in enzymatic processes of synthesis.

UAEE has had success with colloidal dispersions that are more concentrated. Using a commercial cellulose, Haji and Taghian assessed the suitability of US pretreatment to extract oil from peanut seed powder in n-hexane at a material-solvent ratio of 1:4. P could be represented as UI, which varied from 1.85 W/cm2 to 11.09 W/cm2, since they gave data on the transducer diameter. The results demonstrated a rise in extraction performance with UI up to 7.49 W/cm2, which corresponded to a peak in enzyme activity at this value and a subsequent decline once surpassed. The ideal processing parameters were: 50 g/L of raw material concentration; 4.5 g/L of enzyme dosage; 50 °C for extraction; 70 min for extraction; and 6.65 W/cm2 of UI. The amount of arabinoxylan extracted more than doubled when US was used. Two facts were used by the authors to explain this improvement: the cavitation effect creates a more homogeneous mixture, which improves the movement of the reagents towards the enzyme's active site; and the oscillation of cavitation can induce a radiation force and microstreaming, which can change the enzyme's active stereo-configuration, resulting in a change in the active site's availability and improved activity.

UAEE has also been assessed for its ability to enhance the fruit juices' nutritional value, production, and quality. There is used an enzyme and US concurrently to enhance the mulberry must's quality. The processing parameters were frequency, enzyme concentration, and maceration period while maintaining a constant temperature, power, and duty cycle mode. The authors came to the conclusion that UAEE might be used to improve color by boosting the concentration of phenolic and flavonoid components in the dust and shortening the maceration period. The enzyme structure seemed to be unharmed at the ideal processing conditions. After employing US pretreatment to extract the juice from banana pulp, Bora et al. Employed cellulase and pectinase to extract the enzymes. Cellulase concentration, pectinase concentration, and ultrasonication period were the research variables. US pretreatment by itself did not substantially enhance the yield of juice extraction, but when coupled with both enzymes, a much greater yield was achieved than in the control. UAEE has also been used to study the recovery of valuable compounds from agro-industrial waste in the oil from perilla seeds, lycopene from tomato processing residues, phenolic compounds from Trapa quadripoints residues, and oil from pomegranate seeds. With comparison to traditional extraction techniques, considerable performance gains were realized in every scenario [11]–[13].

It's vital to note that response surface approach was used to optimize UAEE in the majority of the situations stated above. With the least amount of experimental data possible, RSM uses a combination of mathematical and statistical methods to assess the impacts of several elements

and their interactions, identifying the most important variables and their influence swaths. Although this strategy could be helpful, care must be taken in how the data is interpreted. For instance, the many combinations that the experimental design creates lead to reactions that are carried out at various enzyme-substrate ratios, which in turn varies the reaction rate, whether enzyme concentration, the solute-solvent ratio, or both are chosen as variables. Determining whether a future improvement in the UAEE is caused by the US cavitation itself or by another kinetic condition is thus vital. Enzymes are also well known to be more susceptible to inactivation when utilized at low concentrations, a circumstance that also happens when they are exposed to US, therefore this should be taken into account when designing an experiment.

CONCLUSION

Review of recent developments in US-aided enzyme food processing. When coupled in the reaction system, this hybrid technology may improve mass transfer or alter the structure of the enzyme and substrate. However, since several trials lacked crucial information, great care and attention should be used when comparing findings presented in the literature. Furthermore, as it is widely acknowledged to be the primary factor determining the catalytic activity of enzymes, the necessity for consistency in the quantification of UI is of utmost significance. According to the quantity of publications, US-assisted enzyme food processing has mostly been investigated for the extraction of bioactive chemicals, followed by hydrolysis reactions for the manufacture of juice and beverages as well as bioactive peptides. Since transesterification and esterification processes are used to create structured lipids and sugar esters, it is important to note that far less study has been done on enzyme-catalyzed organic synthesis reactions. US application for the production of prebiotic oligosaccharides yields encouraging findings at a relatively early stage of study. The Response Surface Methodology has been used in the majority of studies in this sector, but despite its value, little is known about the precise pathways by which the enzymes are affected either favorably or unfavorably by US. However, since there are so many different enzymes and reaction scenarios, case-by-case research is required. Certain enzymatic structures and molecularly complex substrates have been shown to be affected by US using techniques like CD, FTIR, or SEM. However, this knowledge is not sufficient to fully explain whether the ultrasonic waves are affecting the structure of the enzyme, the structure of the substrate, or the transport phenomena taking place in the reaction media. For a better understanding of the complicated event that happens in fractions of a second, research employing a multidisciplinary approach via food science, enzyme kinetic modeling, computational chemistry, and dynamic bubble modeling would be very beneficial. Finally, in order to determine if this kind of technical innovation can successfully transfer into more intensive and sustainable food processing, with scaling-up potential to be created for the biorefinery idea, it is essential to evaluate energy consumption and environmental indicators.

REFERENCES:

[1] I. Schomburg, L. Jeske, M. Ulbrich, S. Placzek, A. Chang, and D. Schomburg, “The BRENDA enzyme information system–From a database to an expert system,” *Journal of Biotechnology*. 2017. doi: 10.1016/j.jbiotec.2017.04.020.

[2] H. H. Nguyen and M. Kim, “An Overview of Techniques in Enzyme Immobilization,” *Appl. Sci. Converg. Technol.*, 2017, doi: 10.5757/asct.2017.26.6.157.

[3] G. Huang *et al.*, “Effects of ultrasound on microbial growth and enzyme activity,” *Ultrasonics Sonochemistry*. 2017. doi: 10.1016/j.ultsonch.2016.12.018.

[4] C. Glorieux and P. B. Calderon, "Catalase, a remarkable enzyme: Targeting the oldest antioxidant enzyme to find a new cancer treatment approach," *Biological Chemistry*. 2017. doi: 10.1515/hsz-2017-0131.

[5] M. Malakouti, A. Kataria, S. K. Ali, and S. Schenker, "Elevated liver enzymes in asymptomatic patients – what should i do?," *Journal of Clinical and Translational Hepatology*. 2017. doi: 10.14218/JCTH.2017.00027.

[6] J. Åqvist, M. Kazemi, G. V. Isaksen, and B. O. Brandsdal, "Entropy and Enzyme Catalysis," *Acc. Chem. Res.*, 2017, doi: 10.1021/acs.accounts.6b00321.

[7] M. Loi, F. Fanelli, V. C. Liuzzi, A. F. Logrieco, and G. Mulè, "Mycotoxin biotransformation by native and commercial enzymes: Present and future perspectives," *Toxins*. 2017. doi: 10.3390/toxins9040111.

[8] H. B. L. Jones *et al.*, "A complete thermodynamic analysis of enzyme turnover links the free energy landscape to enzyme catalysis," *FEBS J.*, 2017, doi: 10.1111/febs.14152.

[9] M. Chen, G. Zeng, P. Xu, C. Lai, and L. Tang, "How Do Enzymes 'Meet' Nanoparticles and Nanomaterials?," *Trends in Biochemical Sciences*. 2017. doi: 10.1016/j.tibs.2017.08.008.

[10] B. A. Amrein, F. Steffen-Munsberg, I. Szeler, M. Purg, Y. Kulkarni, and S. C. L. Kamerlin, "CADEE: Computer-Aided Directed Evolution of Enzymes," *IUCrJ*, 2017, doi: 10.1107/S2052252516018017.

[11] A. Yarman *et al.*, "Enzymes as tools in MIP-sensors," *Chemosensors*. 2017. doi: 10.3390/chemosensors5020011.

[12] A. I. Martinez-Gonzalez, Á. G. Díaz-Sánchez, L. A. De La Rosa, C. L. Vargas-Requena, I. Bustos-Jaimes, and E. Alvarez-Parrilla, "Polyphenolic compounds and digestive enzymes: In vitro non-covalent interactions," *Molecules*. 2017. doi: 10.3390/molecules22040669.

[13] J. Bzdrenga *et al.*, "Biotechnological applications of quorum quenching enzymes," *Chem. Biol. Interact.*, 2017, doi: 10.1016/j.cbi.2016.05.028.

CHAPTER 5

CURRENT SITUATION AND PROSPECTS FOR PROTOCOLS AND SUPPORTS FOR ENZYME IMMOBILIZATION

Rajesh Kumar Samala, Assistant Professor
Department of ISME, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- rajesh.samala@atlasuniversity.edu.in

ABSTRACT:

The market for industrial enzymes has been expanding steadily, at a rate of 7% annually, and is expected to reach $10.5 billion in 2024. Triglyceride hydrolysis is a natural process carried out by the hydrolase enzymes known as lipases. They are the industrial biocatalysts that are employed the most widely, having widespread usage across a wide variety of industries. However, these biocatalytic processes are often constrained by the poor stability of the enzyme, the half-life, and the complexity and lack of industrial scale applicability of the methods necessary to tackle these issues. Emerging technologies provide more durable, environmentally friendly, and affordable biocatalysts by developing novel materials for enzyme carriers and sophisticated immobilization principles. As a result, this review addresses the popular research and commercial uses of the materials and procedures for lipase immobilization, weighing the benefits and drawbacks of each. It concludes by summarizing the problems lipases are now facing and possible solutions.

KEYWORDS:

Biocatalysis, Enzyme, Immobilization, Lipase.

INTRODUCTION

New research areas in biocatalysis are being opened up by the markets and academias ongoing interest in biotechnological solutions to the waste of byproducts and power associated with traditional industrial processes. However, there are still a lot of untapped potentials in this area. The enzyme market is expanding by around 7% annually, depending on novel strategies for making their usage less expensive and the benefits of specificity, low byproduct production, product separation, biodegradability, and high efficiency that are widely recognized. Triacylglycerol ester hydrolases, or lipases, work on a broad range of substrates, including triacylglycerides, fatty acid esters, and lipids derived from synthetic or natural oils. Lipases are triacylglycerol ester hydrolases. Triglycerides are hydrolyzed by their natural activity into free fatty acids and glycerol. One of the most often employed enzymes in industrial processes is the acyl transfer reaction on the hydrolysis of ester bonds, which may also produce C-C bonds and function in a variety of solvents. The biotransformation of oils and fats in the food, pharmaceutical, cosmetic, and energy production sectors is their primary biotechnological use [1], [2].

Animal, microbial, and plant sources of lipases all have different characteristics. However, only yeasts and filamentous fungi account for over 50% of the commercial amount of lipases. The lipase structure is based on the hydrolase fold, which consists of a core of eight parallel filaments that are most prominent, producing a twisted central sheet, and a variable number of helices around it. Nucleophilic serine, histidine, and glutamate or aspartate make up their catalytic triad. Additionally, they feature an oxyanion opening that serves to stabilize the oxygen ion produced as a byproduct of catalysis. The lid, which is typically a chain of hydrophobic amino acids covering the lipase's active site and serves to limit substrate access,

gives the enzyme an open-closed shape that can be conformationally altered by the presence of a polar-nonpolar interface. Despite the benefits mentioned, lipase synthesis has poor yields and is not reproducible throughout the culture stage. Free-state lipases have drawbacks with respect to their use, such as sensitivity to the reaction medium and limited operational stability, making industrial usage only feasible via their immobilization. The use of immobilization techniques is a suitable substitute because they may increase the activity of the enzyme, make it easier to recover the biocatalyst, modify its selectivity and specificity, and increase its resistance to inhibitors. The interest in using immobilized lipases in industrial processes leads to increased funding for related studies, leading to the discovery and advancement of new support materials and immobilization processes in line with current public policies for green and sustainable development. In this regard, this paper provides the most recent developments in lipase biocatalyst synthesis and industrial applications [3], [4].

Innovative Lipase Immobilization Methods

The connection of the immobilized biocatalyst is defined by physical and chemical interactions between supports and enzymes. Adsorption, encapsulation, covalent bonding, entrapment, and crosslinking are only a few of the fundamental tactics that are still used in the advanced technologies and procedures for this operation. They may thus keep both the positive and negative aspects of their basic technique. The simplest approach, adsorption, needs reactants to work. The enzyme interacts with and is retained by the physical and chemical groups of the matrix. As a result, this technique is more prone to enzyme leakage. The process of encapsulation offers the enzyme high mechanical and storage durability while preserving its initial structure. On the other hand, the substrate could have trouble getting to the enzyme's active site, which would restrict mass transfer and result in lower reaction yields. Although entrapment employs a different kind of matrix, it is easier to apply, less expensive, and allows for greater substrate diffusion. But typically speaking, it has worse operational stability and more enzyme leakage. The most common technique, covalent bonding, depends on chemical reactants to produce functional groups that will link with the enzyme. The biocatalyst may benefit greatly from this approach in terms of operational stability and reusability, but during the immobilization process, the enzymes may undergo conformational changes that lower or even completely shut off their activity. The ideas briefly discussed in this paragraph serve as a foundation for the subsequent subsections, which outline the most recent lipase immobilization techniques.

DISCUSSION

Immobilization by enzymatic aggregates is described as a practical and uncomplicated method that offers benefits like permanent insolubility and exceptional heat stability. By using this technique, the enzyme's structure is preserved, preserving the catalyst's activity. Additionally, compared to previous immobilization techniques, it enables the co-immobilization of two or more enzymes more rapidly. Recently developed non-solid support immobilization techniques, such as crosslinked enzymatic aggregates, have received interest because to their convenience of application, resilience, promotion of high specific activity, and lack of need for highly purified enzymes. This methodology streamlines and accelerates the preparation process by combining many processes, including purification, precipitation, and immobilization. The protein is then combined with precipitating agents in aqueous solutions to create aggregates, which are then prepared by enzymatic precipitation.

Thus, a crosslinking agent, often glutaraldehyde, is used to crosslink these enzyme aggregates. An insoluble biocatalyst with excellent stability and activity is produced by the crosslinking process, which creates covalent connections between the amino acids of the

protein molecules. Different glutaraldehyde concentrations may affect how the immobilized enzyme is being released from its fixation. The process of enzyme fixation is simplified by low glutaraldehyde concentrations. A larger glutaraldehyde concentration, however, makes it possible for more aldehyde groups to bind with the enzyme, improving enzyme immobilization and preventing leaching. In immobilized enzymes, glutaraldehyde acts as a functional binding agent and promotes high stability. Aldehyde and enzyme amino group covalent connections reduce the flexibility of molecules. This data strongly shows that proteins have improved heat stability and enhanced conformational stability [5]–[7].

Because it doesn't employ costly preexisting carriers and often impacts heterogeneous biocatalysts with large volumetric activity, the production method for CLEAs is economical. In order to improve inter-enzyme crosslinking, coaggregation agents such polymers or proteins are recommended. It has been successfully used to immobilize a variety of enzyme types used in the production of chemical products, including hydrolases, oxidoreductases, lyases, transferases, and isomerases. It has been the subject of an increasing number of analyses. In a research, glutaraldehyde and ethylene glycol-bis were used as crosslinking agents to immobilize a lipase from Penicillium notatum via crosslinked enzyme aggregates. In comparison to GLA aggregates, EG-NHS aggregates demonstrated increased hydrolytic activity and esterification. At pH 9.0 and 42 °C, the CLEAs' maximum enzyme activity was attained. Additionally, following immobilization, heat resistance significantly increased. The fact that GLA and EG-NHS crosslinked lipase CLEAs maintained 63.62% and 70.9% of their original activity following ten reuse cycles in aqueous medium, respectively, indicates that this novel CLEA lipase has promise in several industrial applications.

The lipase r27RCL from Rhizopus chinensis was crosslinked in octyl-modified microcellular foams in another investigation by Jin et al. Therefore, compared to their adsorbed state, the crosslinked enzymatic lipase aggregates displayed higher esterification activity. After five reaction cycles, the biocatalyst was still 69% active and shown remarkable thermal and mechanical stability.

A more recent study by Muley et al. in 2017 used fractional precipitation with ammonium sulfate and crosslinking with glutaraldehyde to create crosslinked enzymatic aggregates of lipase from Aspergillus niger. Overall, lipase CLEAs outperformed lipase in free form in terms of thermostability and retained more than 65% of their activity for up to four cycles. They also shown excellent storage stability for 12 days when held at 4 2 °C. They also had success with their application to epoxidize lemongrass oil.

COFs: Covalent Organic Frameworks

When organic binders are polymerized, they form COFs, which are described as porous organic polymeric materials that are typically crystalline. They can withstand challenging circumstances including acidic and basic environments to preserve ordered structures and crystallinity, and they have atomically accurate porous architectures that can have exceptional chemical stability in organic solvents. Additionally, the pure covalent bond and metal-free structures contribute to their great stability. COFs perform exceptionally well in the fields of gas storage and separation, analytical chemistry, catalysis, electrical and storage devices, optoelectronics, and drug detection due to their excellent properties, including high thermal and chemical stability, large surface area, excellent pore properties, traceable physical and chemical properties, and ease of operation . One of their key characteristics that sets them apart from other adsorbents is their sustainability; they are very stable at 250 °C and 450 °C in an inert environment.

In general, the solvothermal method is used to synthesize these COFs under challenging conditions, including high temperature, high pressure, and strict deoxidation. It is difficult to build regular crystalline solids in this fashion because of the intermolecular interactions that might occur when monomer molecules have more groups. Because their organic structure may be altered to change their characteristics, COFs also have the benefit of facilitating synthesis. Due to their exceptional and distinctive qualities, such as their notable stability, porosity and crystalline structure, in addition to a sizable accessible surface area, COFs have recently been used as supports for enzyme immobilization, which has up new frontiers for researchers. There are many ways that enzymes and COFs might interact, including physical adsorption, direct covalent interaction, or the use of a binding molecule [8], [9].

Compared to conventional catalysts, catalysts based on COFs offer certain distinct benefits. A deeper knowledge of the activity and exploration of the catalytic mechanism is provided by their structures and compositions that are projectable by chemical crosslinking. As opposed to this, mass transfer, screening, confinement, and access to catalytic sites are made possible by the very uniform and customizable pore shapes and sizes. Unlike certain MOFs and inorganic zeolites, the strong thermal and chemical stabilities of COFs guarantee that they continue catalytic reactivity in a variety of environments. There isn't much study being done right now on the immobilization of enzymes in COFs. However, research has been done with encouraging outcomes. The accessible synthesis of a core-shell magnetic COF composite immobilizing Rhizomucormiehei lipase for use in biodiesel production was reported by Zhou et al. Because of this, the magnetic structure of COF-OMe performs very effective immobilization and recovery processes and substantially preserves lipase activity. In real-world applications, the novel biocatalyst outperformed the free lipase. Additionally, it effectively created biodiesel from Jatropha curcas oil under ideal circumstances, with a yield of around 70%.

Finally, the porous structure, high stability, modulability, crystalline, flexible surface area, and presence of various functional groups in COF materials promote the process of enzyme immobilization. Due to the many interactions that these functional groups make possible via hydrogen bonds, molecules may adsorb to them more effectively. In this situation, physical adsorption over covalent immobilization is a better method for immobilizing enzymes in COFs. It is feasible to print COFs with immobilized enzyme molecules to create flexible and selective materials as well as the potential for multi-enzyme and porous systems for industrial use.

MOFs: Metal-Organic Frameworks

Inorganic nodes and maybe metal ions or clusters with organic ligands are used to construct metal-organic frameworks, also known as porous coordination polymers. This group of materials appears to be a promising class with several distinctive properties like high porosity, diverse composition, adjustable pore structure, and versatile functionality, bringing merit to various applications ranging from water remediation to storage, separation, and catalysis. In contrast to conventional inorganic materials, MOFs allow for careful control of their composition, morphology, pore properties, and function through the incorporation of intelligent functionalities, thereby enhancing and increasing their efficiency in particular applications. Uncoordinated metal centers or functional groups connected to the structure's ligands are what give MOFs their catalytic activity. Additionally, since the MOF cage affords stability to active catalysts and may serve as a size-selective catalyst support, catalysts like nanoparticles, metal complexes, or biomolecules can be placed within the cage or tethered to its surface.

More enzymes can be loaded onto MOFs with the biggest surface area and tunable porosity than on traditional carrier materials. Additionally, their structure's shielding effect enables the stabilization of the conformational structure of the enzyme, increasing its stability. Encapsulation, surface immobilization, and pore trapping using resynthesized MOFs are the three main methods used to prepare enzymes that are immobilized in MOFs. Enzymes that are enclosed in porous substances, such as reversible micelles or porous nanoparticles, are more resistant to harsh environmental factors including high temperatures, organic solvents, and severe pH levels [10]–[12].

Enzyme trapping is often used in conjunction with 3D-printed support materials, and trapping in this physical substance also enables the retention of the enzyme in the reactor and offers adequate purification. Utilizing various enzymes and environmental factors, the application of 3D-printed carriers has been examined, demonstrating the universal performance of these materials and their methods. As a result, the idea of extending the useful life of enzymes is encouraged by this novel immobilization technique. Network-shaped geopolymers were effectively generated by direct ink writing in the research by Santos et al. to serve as carriers for the immobilization of Candida rugosa lipase. The hydrolysis of leftover cooking oil, a necessary step in the production of biodiesel, was used to assess the biocatalyst. The geopolymer's surface was successfully changed to enable the CRL's covalent bond immobilization method. After the first reuse, the hydrolytic activity remained substantial at 847.7 9.7 U/g. The hydrolysis of WCO yielded a free fatty acid content of 75% by weight, confirming the effectiveness of immobilization and the applicability of network-shaped geopolymers as supports for biocatalysts.

An eco-friendly 3D printing macro-scaffold was created by Zhang et al. using reinforced polylactic acid, phenyl groups with various bond lengths, and two distinct kinds of combining groups as anchors for connecting Burkholderiaambifaria lipase YCJ01. The outcomes assured 137.3% activity recovery, enhanced the payload, boosted enzyme expression, and raised specific activity. Using a binary solvent system with excellent stereoselectivity and the biocatalyst, racemic 1-indanol was successfully resolved. Finally, it demonstrated strong operational stability with repeated usage for nine cycles, benefiting the production of a pure product with high enantiomeric value via feasible separation without demanding operation. This brings up a number of opportunities for the immobilization of lipases and other enzymes with 3D architectures. It is possible to pre-cast these structures, including porous membranes and microstructures with various pore diameters. The material generated is more competitive for industrial applications thanks to these structures with various groups, receptors, sensitivity to perform immobilization, and manufacturing and inexpensive materials to accomplish these aims.

Electrospinning

Due to the nature of enzymes and the usefulness of immobilization, immobilization approaches have become a logical project topic. Some typical restrictions might be addressed thanks to advancements and innovations in enzyme immobilization methods. For the purpose of creating ultrafine fibers with diameters in the nanoscale range, electrospinning is a useful, straightforward, and highly effective technical technique. The underlying idea behind this technique is the creation of nanofibers using electrostatic forces. In order to create electric field jets that assist the creation of fibers by solvent evaporation throughout the procedure, high voltage is delivered to a polymer solution and the sample collector. The approach is used to concentrate on the key issues that are inextricably linked to the sector; it is seen as an innovative, effective technique that is affordable and adaptable and can be used in a variety of industrial domains. Because of their many benefits, including their high surface area, multiple

fixation points to the support, high porosity, interconnectivity, high thermal resistance, pH stability, and ability to bind to a variety of solvents, electrified nanofibers have drawn interest from enzyme engineering and biocatalysts. Immobilizing enzymes in electrified fibers often encourages the retention and enhancement of biological catalytic activity and permits the simple separation of the enzyme from the suggested reaction environment.

Be aware that encapsulation refers to the electrospinning of the enzyme and polymer combination, whereas surface fixation refers to the physical adsorption or fixation of enzymes in pure or functionalized nanofibrous supports chemically or physically. Electro fused nanofibers may be employed as ideal supports for the immobilization of a number of enzymes since they have a high potential for overcoming dispersion issues, mass transfer limitations, and limited recyclability. In conclusion, the recognition of electrification as a cutting-edge method is closely related to the viability of nanofibers. They have a low mass transfer barrier in addition to being industrially useful due to factors including the variety of polymers that may be electrophile, high porosity, and chemical interaction with the electrophile support . When choosing this approach, one should pay close attention to the physical properties of the enzyme and the support, constantly checking the congruence and suitability of the size of the enzyme and the pore of the support since each enzyme interacts with the support differently.

Different ideas for immobilizing enzymes in electrophile nanofibers have been published in a number of research. These suggestions mostly center on encapsulating the enzyme and attaching it to the surface of nanofibers. In this regard, Kuang et al. described the creation of *Burkholderiacepacia* -SiO_2 nanofiber membrane bioreactors that were made using a combination of electrospinning and enzyme immobilization techniques. Significantly greater lipase loading capacity and a little improvement in the thermal and solvent stability of the biocatalyst created by electrospinning emphasize the effectiveness of the method for this procedure. After five cycles, activity was still over 80%. In contrast, Isik et al. effectively deployed the lipase into electrospun nanofibers after synthesizing PVA/Zn2+ nanofibers via electrospinning. The results demonstrated the protocol's effectiveness via an increase in the biocatalyst's immobilization characteristics. The catalytic derivative generated exhibited an improvement in the stability features of the enzyme, such as heat stability, pH stability, and reusability, according to the interpretation of the immobilization data. It is also notable that 90% of the catalytic activity was still preserved by the immobilized nanoelectrospun biocatalyst after 14 reuses. This suggests that the recovery of heterogeneous biocatalysts produced by electrospinning methods is preferred for use in the wastewater treatment, pharmaceutical, and cosmetic sectors.

Electrospraying

A promising approach related to electrospinning for creating polymeric nanoparticles or bioactive fiber-based materials utilized in different processes is the electrospraying method, also known as electrohydrodynamic atomization. The electrospraying process is traditionally described as atomizing the liquid using electrical forces. The adjustment of the solution's characteristics, such as solvent content and viscosity, as well as process variables, such as flow rate, distance from the needle tip to the collector, and primarily the voltage utilized, is the key distinction between electrospraying and electrification. Additionally, the Taylor cone jet is stable at high solution concentrations, and elongation happens through the whip instability process. It is interesting that variations in parameters might cause the customized cone jet to split into drops during the electrospraying process, leading in the creation of particles of various sizes and shapes. With a strong loading power and consistent particle size distribution, this method creates micro- and nanoparticles.

Coaxial electrospraying first became popular a few years ago as a cutting-edge technique for creating products with two miscible or immiscible core and wall materials. Coaxial electrospraying is a more practical one-step process that increases the industrial applications of dry nanoparticle production. The ability to construct and vary the form and size of nanoparticles simply by changing the experimental conditions is one of the primary benefits of this method for the immobilization of enzymes. This feature confirms the technique's promise for the stability of enzymes in solid matrices. Electro-pulverized nanoparticles serve as a support structure for the immobilization of enzymes in this way. Excellent residual activity, which is supported by the significant increase in surface area and porosity, is a benefit of enzyme crosslinking for particles subjected to electrospraying. The effectiveness of immobilized enzymes may also be increased by shrinking the carrier substance. As a result, enzyme catalytic activity is often maximized.

Given the inexpensive cost of creating procedures and the strong catalytic response provided by the emulsified catalytic derivative, the field of enzymatic biocatalysis has been particularly interested in this approach. This vehemence is due to the substantial "oil-water" interface area, which enables biphasic reaction systems to optimize hydrogenation, oxidation, and enzymatic reactions with great efficacy. Wang et al. described a straightforward method for immobilizing Candida sp. lipase. Through covalent enzyme coupling with CHO-JNPs for organic/aqueous biphasic catalysis at the oil/water interface of Pickering emulsions. A methodology identical to Wang et al. Sun et al. executed the ultrasound-assisted procedure, however, in addition to having employed the lipase from Candida rugosa as an enzyme, to improve the immobilization process. In this regard, Sun's more potent catalytic derivative had an activity of 177 mg/g whereas Wang's was only 23.3 U/mL. Wang's derivative also maintained this activity farther, keeping the catalytic power at 88.6% after 10 cycles, compared to Sun, who only kept 75% after nine reactional cycles. Despite this, it is clear that ultrasound is a particularly effective optimization technique in these situations as it often enables the enzyme to make greater contact with the water/oil interface.

In order to effectively encapsulate the lipase of Candida a study introduced a novel Pickering interfacial biological catalysis platform. Has a strong catalytic efficiency and binary particle composition. The enzymatic derivative demonstrated outstanding reuse, structural integrity, and stability. This method has made it possible to get around one of Pickering emulsions' key drawbacks, which is simple leaching. The use of reinforcement particles, which provide a larger surface area and greater affinity for the active functional groups of the emulsion with the inactive region of the enzyme, is crucial, according to the authors, in order to improve the interaction of the enzyme with the emulsion interface. Due to its low-cost methods and many other benefits, this innovative approach to enzyme immobilization has drawn growing attention from major enterprises, who prioritize the latent potential of its development on an industrial scale nearly without operational constraints. Pickering emulsion-based enzymatic lipase biocatalyst manufacturing has certain obstacles. Stability, activity, and durability must all be increased in this situation. They will be able to be made in a large-scale, repeatable, and profitable industrial application.

Using Peptides to Immobilize

Different immobilization techniques are now being explored with the goal of enhancing the catalytic properties of these enzymes. Peptides are thus used in these techniques to boost immobilization effectiveness. These peptides' hydrophobic properties enable their usage in polarized or aqueous media as well as non-polarized media like oils or bio-solvents. Peptides may also be altered at the side chain level to more precisely specify their hydrophobic and hydrophilic characteristics. No matter how polarized a peptide is, these alterations to its

chains ensure that it may be applied to additional substrates. It has been shown via simulations and analysis that most peptides have a helical form in nonaqueous environments. Combining proteins with peptides would conduct a selective and suitably orientated binding as another method of using peptides in enzyme immobilization. With this mixture, the enzyme's biological activity and binding to the support may both be preserved. The Escherichia coli biofilm served as support for peptide-guided immobilization, which preserved the enzymes' catalytic characteristics. A difference in this instance would be the ability to sever the link when utilizing low pH solutions, enabling the materials to be used in more applications in the future.

CONCLUSION

Biocatalysis is taking up more and more area thanks to nanotechnology. With the use of this tool, carriers may be designed with certain characteristics that enhance operational stability, immobilization, and reaction yields. Even Nevertheless, the primary obstacle to biocatalyst usage in industry is their high manufacturing costs. The usage of waste materials is a fascinating option since they are affordable and satisfy the market's expanding environmental demands.

Other hot topics in study include co-immobilizing numerous lipases on the same matrix, genetic modification of lipases, and hybrid materials. These add to the complexity and expense of the procedure and may be used further in the commercial use of immobilized lipases. Regarding novel immobilization methods, it is important to stress that the choice of approach, support, and enzyme has a direct impact on the procedure and is influenced by a number of variables.

The many examples mentioned throughout the text illustrate the outcomes attained by several researchers using a variety of settings that not only included these three elements but also a number of others. Knowing the elements that affect a lipase's activity and the qualities that are ideal for a certain application are crucial for the selection of a lipase. Choosing a technique also requires taking into account factors like the expense of the procedure, the toxicity of the chemicals, and the regeneration and deactivation of the enzyme.

REFERENCES:

[1] H. H. Nguyen and M. Kim, “An Overview of Techniques in Enzyme Immobilization,” *Appl. Sci. Converg. Technol.*, 2017, doi: 10.5757/asct.2017.26.6.157.

[2] R. R. De Melo *et al.*, “New heterofunctional supports based on glutaraldehyde-activation: A tool for enzyme immobilization at neutral pH,” *Molecules*, 2017, doi: 10.3390/molecules22071088.

[3] S. Ali, W. Zafar, S. Shafiq, and M. Manzoor, “Enzymes Immobilization: An Overview Of Techniques, Support Materials And Its Applications,” *Int. J. Sci. Technol. Res.*, 2017.

[4] K. J. Stine, “Enzyme Immobilization on Nanoporous Gold: A Review,” *Biochem. Insights*, 2017, doi: 10.1177/1178626417748607.

[5] C. Ding, H. Sun, J. Ren, and X. Qu, “Immobilization of enzyme on chiral polyelectrolyte surface,” *Anal. Chim. Acta*, 2017, doi: 10.1016/j.aca.2016.11.047.

[6] K. Khoshnevisan *et al.*, “Immobilization of cellulase enzyme onto magnetic nanoparticles: Applications and recent advances,” *Molecular Catalysis*. 2017. doi: 10.1016/j.mcat.2017.09.006.

[7] H. H. Je, S. Noh, S. G. Hong, Y. Ju, J. Kim, and D. S. Hwang, "Cellulose nanofibers for magnetically-separable and highly loaded enzyme immobilization," *Chem. Eng. J.*, 2017, doi: 10.1016/j.cej.2017.04.110.

[8] C. Hoffmann, M. Pinelo, J. M. Woodley, and A. E. Daugaard, "Development of a thiol-ene based screening platform for enzyme immobilization demonstrated using horseradish peroxidase," *Biotechnol. Prog.*, 2017, doi: 10.1002/btpr.2526.

[9] D. Valikhani, J. M. Bolivar, M. Viefhues, D. N. McIlroy, E. X. Vrouwe, and B. Nidetzky, "A Spring in Performance: Silica Nanosprings Boost Enzyme Immobilization in Microfluidic Channels," *ACS Appl. Mater. Interfaces*, 2017, doi: 10.1021/acsami.7b09875.

[10] P. J. Allertz *et al.*, "Approaching immobilization of enzymes onto open porous basotect®," *Catalysts*, 2017, doi: 10.3390/catal7120359.

[11] F. Zhao *et al.*, "Enzyme-inorganic nanoflowers/alginate microbeads: An enzyme immobilization system and its potential application," *Process Biochem.*, 2017, doi: 10.1016/j.procbio.2017.03.026.

[12] F. Auriemma *et al.*, "Nano-in-Nano Approach for Enzyme Immobilization Based on Block Copolymers," *ACS Appl. Mater. Interfaces*, 2017, doi: 10.1021/acsami.7b08959.

CHAPTER 6

RECENT ADVANCES IN BIOTECHNOLOGY APPLICATIONS OF LIPASES: FROM STRUCTURE TO CATALYSIS

Ashwini Malviya, Associate Professor
Department of uGDX, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- ashwini.malviya@atlasuniversity.edu.in

ABSTRACT:

Due to their unique properties, such as the capacity to use a variety of substrates, high activity and stability in organic solvents, and region and/or enantioselectivity, microbial lipases are highly valued as biocatalysts. These enzymes are presently used in a wide range of biotechnological processes, such as the synthesis of biodiesel and biopolymers, the manufacturing of food, detergent preparation, cosmetics and paper, food processing, and the biocatalytic resolution of medicinal compounds, esters, and amino acids. However, the expensive cost of lipases still restricts their application in several industrial sectors. Therefore, finding low-cost, highly active, and stable lipases that may be used in a variety of industrial applications is of tremendous interest. The creation of unique enzymes for each kind of process is now a key strategy for overcoming the limits of natural enzymes. These days, it is feasible to "order" an enzyme that has been "customized" to have the characteristics necessary for the creation of the desired bioprocess. This review aims to compile recent developments in the biotechnological application of lipases with a focus on different strategies for enzyme improvement, such as protein engineering and the use of structural data for rational modification of lipases in order to produce more active and selective biocatalysts.

KEYWORDS:

Biocatalysts, Biopolymers, Catalysis, Lipases.

INTRODUCTION

The breakdown of fats and oils into free fatty acids, diglycerides, monoglycerides, and glycerol is catalyzed by lipases. Additionally, these enzymes catalyze a variety of synthetic processes in organic media, including as esterification, acidolysis, alcoholysis, and interesterifications. Lipases often exhibit excellent regio- and/or stereoselectivity in catalysis, are very stable in organic solvents, may function under moderate circumstances, and have a wide substrate specificity. Because of their adaptability, lipases are among the most used types of biocatalysts for biotechnological processes. Lipases have uses in the food industry, detergent formulation, the cosmetic and pharmaceutical industries, the leather, textile, and paper industries, the generation of biodiesel and biopolymers, and the pretreatment of wastewaters that are lipid-rich. Numerous kinds of mammals, plants, microbes, and fungus all have these enzymes. Additionally, microbial lipases are the most desirable due to their practical qualities, such as adaptability and simplicity of mass manufacturing [1], [2].

Detergents, pulp and paste, and leather

Incorporating lipase into detergents, which are primarily used in commercial laundry and residential dishwashers to remove stains containing fat, is the most economically significant use for lipase. Detergent lipases from thermomycesspecies, produced in recombinant Aspergillus oryzae strains, as well as those from Pseudomonas species, are most often utilized. High broad substrate specificity, activity and stability at alkaline pH and

temperatures above 40°C, and compatibility with various detergent components, such as metal ions, surfactants, oxidants, and proteases are possible requirements for lipase application in the detergent industries. These enzymes are also used to remove "pitch" from pulp used for the paper industry. Since the early 1990s, this enzymatic pitch control approach has been widely employed. Additionally, fats and grease may be eliminated from skins and hides using lipases. When compared to leather produced using conventional techniques, this approach results in a product of greater quality.

Pretreatment of Wastewaters Rich in Lipids

If not appropriately handled, the high concentrations of lipids and proteins in the wastewater from dairies, slaughterhouses, and seafood processing facilities may seriously harm the environment. Prior to further biological treatment, an enzymatic hydrolytic phase might decrease the particle diameter, increase their surface area, and encourage the microbial consortium's absorption of organic materials. In order to promote biological breakdown of fatty wastewaters and speed up this process, lipases may be used in effluent treatment. However, if the cost of the commercial enzymatic preparations is considerable, the additional pretreatment method becomes economically unviable. Thus, the creation of affordable enzyme preparations becomes crucial [3], [4].

Processing Food and Increasing Quality

Microbial lipases have been used in the dairy sector to selectively hydrolyze fat triglycerides to produce free fatty acids, which are then utilized to flavor goods including cheese, butter, margarine, milk chocolate, and sweets. Certain microbial lipases are often employed to produce new oils and fats via interesterification processes because they are extremely regio- and fatty acid-specific. Enzymatic transesterification can also be used to change the properties of triacylglycerol mixtures and produce fats with optimal melting characteristics and free of Trans fatty acids, targeting commercial applications. Sellami et al. looked at the enzymatic transesterification of mixtures of palm stearin and palm olein to produce a trans-free fat for margarine formulation. The spreadability of the transesterified fat-based margarine was comparable to that of a commercial product. The main benefits of using lipase are energy savings and a reduction in lipid breakdown brought on by high temperatures.

DISCUSSION

Since only one of the enantiomeric forms of most pharmaceuticals often exhibits bioactivity, stereospecificity is particularly crucial for the production of bioactive compounds. In this setting, lipases are often used to synthesis the chiral building blocks needed to produce high enantiomeric purity insecticides, agrochemicals, and medicines. The practicality, high efficiency, selectivity, ease of separation from the unreacted substrates, and gentle reaction conditions that these biocatalysts provide over conventional chemical methods are only a few of their benefits. It is crucial to emphasize that the cost of lipase does not impede the creation of goods with added value, such as medicines. Machado et al. looked into the kinetic resolution of -1,2-isopropylidene glycerol ester derivatives catalyzed by several lipases. In the production of diglycerides, glyceryl phosphates, tetraoxaspiroundecanes, and several physiologically active substances such glycerolphospholipids, -blockers, prostaglandins, and leucotrienes, solketal is a crucial chiral synthon. Utilizing TBME as the solvent and vinyl acetate as the acetylating agent, excellent conversions and enantiomeric ratios were achieved in a brief reaction period. Over a longer length of time, the lipase remained steady. Numerous pharmaceutical firms all over the globe are presently using lipases to prepare optically active intermediates on a kilogram scale. Despite the abundance of literature in this area, there aren't many commercially viable lipase-based enantioselective methods.

One of the most significant natural scents and tastes is produced by organic esters from short-chain fatty acids. These esters include butyl butyrate, isoamyl acetate, ethyl valerate, and butyl acetate, as well as anthranilic acid alkyl esters. Lipases are used to produce higher-quality goods that are suited for the fragrance and taste industries. Monoacylglycerols are nonionic surfactants that make up the majority of the emulsifiers found in prepared foods, cosmetics, and medicinal preparations. Lipases may be utilized to alleviate the problems of chemical glycerolysis, which is carried out at high temperatures using alkaline catalysts and high pressures. These downsides include dark color, thermal deterioration, and burned flavor. By employing 1,3-regiospecific lipases, glycerolysis of fats or oils, and esterification of fatty esters with glycerol, MAGs may be synthesized enzymatically. Da Silva et al. reported the direct esterification of glycerol with capric acid, which was catalyzed by a commercially available immobilized lipase, to produce monocaprin. The finished product's composition complied with the World Health Organization's guidelines for food emulsifiers. Antioxidants having use in the food, cosmetic, and pharmaceutical sectors include tyrosyl lipophilic derivatives. Using Novozyme 435 as a biocatalyst, Aissa et al. investigated the direct esterification of tyrosol with various fatty acids to produce tyrosyl fatty acid esters. TyC8, TyC10, and TyC12, which are derivatives of tyrosyl esters, showed antibacterial and antileishmanial properties [5], [6].

Biofuel and bio lubricant production

As an alternative and sustainable energy source, biodiesel has garnered a lot of attention recently. The utilization of lipases as biocatalysts for the manufacture of biodiesel has received a lot of interest lately. These enzymes may replace existing alkaline procedures that employ high-quality raw materials, create unfavorable byproducts, make it challenging to separate the catalyst from the glycerol, and produce trash that is very alkaline. Lipases are able to catalyze the esterification of free fatty acids and the transesterification of triacylglycerols to produce monoalkyl-esters.

They need waste oils with a high acidity and water content to execute their catalytic action, as well as oils from other sources. The enzymatic technique also makes it simple to separate from the waste, glycerol. Several research teams have produced enzymatic biodiesel utilizing both extracellular and intracellular lipases as well as raw materials from various sources, including animal fat, vegetable oil, leftover cooking oil, and acid waste from the manufacturing of vegetable oils. With conversion rates exceeding 90%, these papers demonstrate successful outcomes. The expensive cost of the enzymes, however, continues to be a barrier to the enzymatic manufacture of biodiesel. In order to increase conversion yields in less time and recycle the enzyme for as many batches as feasible, efforts have been concentrated on the creation of low-cost enzyme preparations and more stable and active biocatalysts [7], [8].

Another area of intense industrial interest is biolubricants, where lipases may function as biocatalysts. In accordance with the patent EP 2657324 A1, an enzymatic method is used to produce biolubricant by transesterification with trimethylolpropane from methyl ricinoleate and/or from a combination of methyl oleate and linoleate. Castor oil and Jatropha oil biodiesel conversions of 80% to 99% were accomplished, and the end product had excellent viscosity, viscosity index, pour point, and oxidation stability characteristics. Using immobilized commercial lipases, Aguieiras et al. studied the production of biolubricants from oleic acid and methyl ricinoleate. The synthesized product had excellent values for pour point, viscosity, and viscosity index, while Novozym 435 demonstrated the greatest performance.

Biodegradable polymer manufacturing

The creation of practical, biodegradable biopolymers like polyesters from sustainable natural resources is another area where lipases are put to use. Ring-opening polymerization and polycondensation are the two main polymerization modes for lipase-catalyzed polymerization processes. The latter might include polycondensation of oxyacids or their esters as well as dicarboxylic acids or their derivatives with diols. Through a variety of methodologies, this polymerization strategy has been effectively used by various studies. Aliphatic diols and bissebacate were both polymerized using rhizomucormiehei lipase. When 12-hydroxydodecanoic acid was polymerized with Novozym 435, Mahapatro et al. observed a 91% conversion to oligomers with large molecular weights. Bódalo et al. created ricinoleic acid polyesters with a 72% conversion rate utilizing immobilized lipase from Candida rugosa. These findings show that lipases may be used for several paths of polymerization processes. Numerous attempts have been made to comprehend how these enzymes function at the molecular and atomic level in light of the numerous uses and significance of lipases in biotechnology. Numerous three-dimensional structures of lipases from various species have been revealed since the BRIDGE-T lipase project up to the present, providing insight into the mechanism these enzymes employ during catalysis.

Lipases' structural characteristics

Despite having little in common in their basic sequences, lipases have a highly similar fold. Other enzymes with comparable structural characteristics include esterases, proteases, dehalogenases, epoxide hydrolases, and peroxidases. These enzymes together make up the hydrolase family. The core -sheet of the hydrolase fold has eight parallel -strands, with the exception of strand number 2, which is antiparallel to the others. A left-handed superhelical twist on the middle -sheet creates a 90° angle between the first and final strands. Helices A and F pack against the convex side of the central -sheet, whereas helices B, C, D, and E pack against the concave side, connecting strands 3 to 8. One nucleophilic residue, one catalytic acidic residue, and one histidine residue make up the highly conserved catalytic triad that makes up the active site of hydrolases. The nucleophile in lipases has traditionally been identified as a serine residue. The nucleophilic residue is found in the pentapeptide Sm-X-Nu-X-Sm, which is highly conserved. Sm stands for a short residue, often glycine, but it may rarely be replaced by alanine, valine, serine, or threonine. The conformation of this strand-loop-helix motif causes the nucleophilic residue to adopt energetically unfavorable backbone dihedral angles that impose steric constraints on vicinal residues. This pentapeptide forms a very sharp turn between strand 5 and helix C known as the "nucleophilic elbow." The most conserved structural component of the hydrolase fold is the "nucleophilic elbow". A typical hydrolase fold places the catalytic acid in a reverse loop after strand 7, where it forms a hydrogen bond with the catalytic histidine. The catalytic acid, however, may be found after strand 6 in several enzymes. The only hydrolases that have a glutamic acid in the catalytic triad are lipases. The sole residue in the catalytic triad that is completely conserved is histidine. This residue may be found in a loop that follows strand. Various members of this family may have very different shapes and lengths for these loops [9]–[11].

Mechanism of Enzymes

In order to stabilize the negatively charged transition state that happens during hydrolysis, the oxygen atom of the catalytic serine attacks the carbon atom of the ester linkage carbonyl group, resulting in a tetrahedral intermediate that forms hydrogen bonds with backbone nitrogen atoms in the "oxyanion hole." The acyl-lipase complex is left behind when an alcohol is produced, and it is finally hydrolyzed to liberate free fatty acids and regenerate the

enzyme. In order to demonstrate that the active site of these enzymes is negatively charged in their preferred pH range, mapped the electrostatic surface of a number of lipases and esterases. Thus, in the so-called "electrostatic catapult" process, the ionized carboxylic acid is promptly ejected from the active site following the ester cleavage because of electrostatic repulsion between its negatively charged carboxyl group and the negative electrostatic potential of the active site.

A movable subdomain known as lid is present in many lipases, and it regulates how substrate molecules may contact the catalytic core. This domain is capable of adopting two different conformations, the closed and the opened states, as shown by crystallographic structures. The lipase is rendered inactive in the closed condition because the solvent cannot reach the active site and the enzyme's surface is mostly hydrophilic. The enzyme is functional in the opened form because the active site is accessible and reveals a sizable hydrophobic surface. It is still unclear how the activation mechanism that causes the lid to move works. Several transition methods have been suggested, depending on the structural design of the lid. It has been proposed that the transition in lipases with a lid made up of only one propeller involves a rapid rigid body motion. The secondary structure of the lid changes during the opening process of lipases with simpler lids, such as Candida rugosa lipase, and as a result, a partial refolding is anticipated. This may cause a bottleneck in the kinetics of the enzyme. The interfacial activation phenomena is connected to the conformational rearrangement for lid opening. When the substrate concentration surpasses the solubility limit, or when the substrate is a hydrophobic interface, several lipases show a large increase in their enzymatic activity.

It is still difficult to characterize significant conformational shifts at the atomic level in real time using biophysical experimental techniques. X-ray methods give a poor dynamic perspective but vital information regarding the static conformational states. Due to the difficulty of using NMR spectroscopy on large molecular weight proteins, as many lipases are, only a small number of lipases, including cutinase and Pseudomonas mendocina lipase, have been structurally described by solution NMR. Therefore, additional technologies like molecular dynamics modeling have drawn a lot of interest in an effort to anticipate and comprehend the conformational changes of macromolecular systems. In order to learn more about the conformational changes of the lid domain, the impact of the environment, and the dynamic characteristics of different lipases, a number of molecular dynamics experiments have been conducted. The molecular specifics of this crucial component of lipase activity are still unclear since only a small number of studies have looked at all aspects of the transitions between open and closed conformations [12], [13].

Numerous investigations on the three-dimensional structure of lipases have been carried out throughout the last several decades. The most popular method for comprehensively describing an enzyme's high selectivity and catalytic activity as well as for illuminating the structural underpinnings of interfacial activation is X-ray crystallography. In 1990 and 1991, respectively, the first crystal structures of lipases from the fungus Rhizomucormiehei and Geotrichumcandidum were determined. Pseudomonas glumae's lipase, the first crystal structure of a bacterial lipase, was discovered in 1993.

The identification of the catalytic triad and the existence of a lid that regulates substrate access to the active site were both initially disclosed by the structure of this bacterial lipase from the I.2 family. Another aspartate found at position 241 was shown to be crucial for enzyme activity, despite the fact that its acidic group was not required for lipase action. This residue is capable of binding a calcium ion that is critical for maintaining the protein's

stability. Additional research has shown that bacterial lipases from the I.5 family, which also have a single calcium binding site, have comparable properties.

Another fungus lipase structure was figured out in 1993. The Candida rugosa lipase structure has made it possible to compare it to its homolog from Geotrichumcandidum and has revealed fresh information on interfacial activation. CRL has three loops close to the active site, unlike the crystal structure of Geotrichumcandidum lipase, which has an inaccessible active site and is covered by loops; the interfacial activation of CRL is linked to conformational changes involving those loops. One solitary loop that blocked the active site in the inactive state was discovered to be involved in structural rearrangement when this enzyme's closed conformation was crystallized in 1994. A significant amount of the enzyme's total hydrophobic surface is exposed surrounding the active site in the open state, increasing by 9%.

Candida antarctica's lipase B, the most common fungal lipase employed in biotechnological operations, had its first crystallographic investigation in 1994. CalB departs from the conventional /-hydrolase fold by having only seven -strands. In contrast to most lipases, CalB's conserved pentapeptideGxSxG, which surrounds the catalytic serine, has a threonine residue in lieu of the first glycine. The crystal structure revealed a somewhat deep, thin channel that leads to an open active site with an oxyanion hole. The high degree of stereospecificity of the enzyme is presumably a result of the structure of this channel. The mobility of -helix 5 allowed for the identification of a potential lid. Only fourteen years later, CalA, a lipase from Candida antarctica, had its structure solved, showing a significantly bigger lid made up of 92 residues. In specified organic solvents, CalB molecular dynamics simulations revealed a 10% reduction in the hydrophilic surface area and a 1% increase in the hydrophobic surface area of the protein. The dielectric constant of the solvent turned shown to be a major determinant of the dynamics of CalB, exhibiting considerable flexibility in water and poor flexibility in organic solvents. The amount of water molecules adhering to the enzyme's surface increased significantly, which reduced the dielectric constant and spread the water network.

Molecular dynamics simulations on free and dialkyl phosphate-bound Rhizomucormiehei lipase were not carried out until 1997, seven years after the establishment of the first crystal structure of Rml. This research showed that during the activation process, which is brought about by a shift of the active site helix, hydrophobic residues are exposed while polar residues are concealed. Motions were mostly seen in loop areas, particularly in loops Gly35-Lys50 and Thr57-Asn63, and helices shown to be very stiff. The active site lid opens wider in the presence of a lipid patch made up of substrate molecules than in an aqueous environment, according to another research employing Rml complexed with a substrate or a product in the presence of a lipid aggregate [14], [15].

A bacterial lipase from the I.1 family has its first crystal structure determined in the early 2000s. The PAO1 lipase from Pseudomonas aeruginosa had a unique hydrolase fold san the first two -strands and one -helix. Because helix E is absent, a stabilizing intramolecular disulfide bridge forms between Cys183 and Cys235. The coordination of a calcium ion stabilizes the active site loop that contains the catalytic histidine. There were three pockets that could fit the sn-1, sn-2, and sn-3 fatty acid chains. The primary determinants of the enzyme's regio- and enantio-preference are its acyl pocket size and interactions with the substrate, particularly with the sn-2 fatty acid chain.

A rare lipase that does not display interfacial activation at oil-water interfaces was crystallized in 2001. In the I.4 family of bacterial lipases, LipA from Bacillus subtilis

displayed a compact minimum /-hydrolase fold with a six-stranded parallel -sheet encircled by five -helices. The catalytic serine was solvent exposed, and no lid domain was seen. The first crystallographic structure of a lipase from a thermophilic organism was determined by Tyndall et al. Less than 20% of the amino acids in any other lipase that has previously crystallized were same in Bacillus stearothermophilus P1 lipase. It has a zinc binding site that may be crucial for heat stability and considerable insertions into the canonical / hydrolase fold. In comparison to other previously examined lipases, BSP lipase contains a lot more salt bridges, -helical content, proline, and aromatic residues.

Several structural analyses of I.3 lipase family members have been done during the last ten years. Three calcium binding sites are found in this particular bacterial lipase family, which has two lids covering the active site. By anchoring lid1, the most frequent lipase lid, the Ca1 site is necessary for the complete opening of the active site. The enzyme structure is stabilized by the Ca2 and Ca3 sites, and Ca2 is necessary for complete enzyme activation. To define the function of lid2, Pseudomonas sp.

A lid2 deletion mutant was created using lipase MIS38 as a model. Since both mutant and natural proteins needed calcium for lipase activity, the crystal structures suggested that the enzyme only works when lid1 is completely open. The hydrophobic surface area given by lid1 and lid2 in an open conformation is significantly reduced by lid2 deletion, according to the comparison of DL2-PML models in a closed and open conformation with the crystal structures of PML. This work made the claim that lid2 is crucial for the interfacial activation of PML because the hydrophobic surface area is required to firmly keep the micellar substrates in the active region.

The significance of lid2 for the complete activation of PML has been shown in earlier investigations. In the absence of micelles, lid2 shuts first while lid1 remains open, according to simulations of PML in the open conformation. Similar to this, molecular dynamics simulations of PML in the closed conformation revealed that lid1 opens while lid2 stays closed in the absence of Ca2+ and in the presence of octane or trilaurin micelles. These findings imply that Ca1 is required for both the completely opened conformation of lid 1 and the beginning of the following opening of lid 2.

The crystal structure of the closed conformation of the thermoalkalophilic lipase from Geobacilluszalihae was recently determined. It was suggested that the lid domain consists of helices 6 and 7, which are joined by a loop, based on the structural study of lipases from the I.5 family. Since the large structural rearrangement of the lid domain was only seen in the water-octane interface due to the interaction between the hydrophobic residues of the lid and the octane solvent, the activation process is controlled by interfacial activation coupled with a temperature-switch activation.

In another recent research, the expected structure and function of the psychrophilic lipase AMS8 were examined using computer-aided software. At various temperatures, MD simulations were run to analyze the flexibility and stability of the structure. According to the findings, the enzyme is best stable between 0°C and 5°C. Even though the noncatalytic domain exhibits more flexibility, the N-terminal catalytic domain is more stable than the C-terminal noncatalytic domain. Amazing biocatalysts called lipases are employed in many different bioprocesses. However, in several industries, lipases' industrial use is still limited by their high price. This inspires scientists to design new methods for producing enzyme preparations with a more cost-effective profile. When creating lipases for particular uses, structural data is crucial. Recent advancements in structure-based rational design and protein engineering have enabled the customisation of lipases for various bioprocesses.

CONCLUSION

In conclusion, the topic of enzyme engineering is a vibrant one that offers a wide range of opportunities and many fascinating studies. By combining directed evolution with structure-based rational design, lipase catalysis looks to move toward the "ideal biocatalyst paradigm," allowing for the creation of more active, selective, and stable catalysts. The article then focuses on the many biotechnological uses for lipases, including their critical function in the manufacturing of biodiesel as well as their engagement in the food, pharmaceutical, and detergent sectors. It highlights contemporary advancements in enzyme engineering, immobilization methods, and bioprocess optimization, all of which improve catalytic performance and commercial feasibility. Finally, "From Structure to Catalysis" emphasizes the crucial part lipases play in biotechnology. This research establishes the groundwork for future innovation in the sector, providing bright possibilities for sustainable, environmentally acceptable, and commercially viable industrial processes by bridging the structural knowledge and catalytic applications divide. It advances the biotechnological use of lipases and is a useful resource for both academics and businesses.

REFERENCES:

[1] J. H. Kim *et al.*, "Alginate/bacterial cellulose nanocomposite beads prepared using Gluconacetobacter xylinus and their application in lipase immobilization," *Carbohydr. Polym.*, 2017, doi: 10.1016/j.carbpol.2016.09.074.

[2] F. Xiao, Z. Li, and L. Pan, "Application of microbial lipase and its research progress," *Prog. Appl. Microbiol.*, 2017.

[3] D. Guerrand, "Lipases industrial applications: Focus on food and agroindustries," in *OCL - Oilseeds and fats, Crops and Lipids*, 2017. doi: 10.1051/ocl/2017031.

[4] A. Mehta, U. Bodh, and R. Gupta, "Fungal lipases: A review," *Journal of Biotech Research*. 2017.

[5] N. Matuoog and Y. Yunjun, "Review: Immobilization and application of lipase," *IOSR J. Pharm. Biol. Sci. (IOSR-JPBS*, 2017.

[6] X. Chen *et al.*, "Synthesis of dihydropyrano[4,3-b]pyranes via a multi-component reaction catalyzed by lipase," *Green Chem. Lett. Rev.*, 2017, doi: 10.1080/17518253.2017.1285442.

[7] Y. Fan, F. Su, K. Li, C. Ke, and Y. Yan, "Carbon nanotube filled with magnetic iron oxide and modified with polyamidoamine dendrimers for immobilizing lipase toward application in biodiesel production," *Sci. Rep.*, 2017, doi: 10.1038/srep45643.

[8] Z. Wang *et al.*, "A mild and efficient dakin reaction mediated by lipase," *Green Chem. Lett. Rev.*, 2017, doi: 10.1080/17518253.2017.1375159.

[9] P. Esakkiraj *et al.*, "Molecular characterization and application of lipase from Bacillus sp. PU1 and investigation of structural changes based on pH and temperature using MD simulation," *Int. J. Biol. Macromol.*, 2017, doi: 10.1016/j.ijbiomac.2017.04.111.

[10] M. Zarinviarsagh, G. Ebrahimipour, and H. Sadeghi, "Lipase and biosurfactant from Ochrobactrum intermedium strain MZV101 isolated by washing powder for detergent application," *Lipids Health Dis.*, 2017, doi: 10.1186/s12944-017-0565-8.

[11] M. Zhao, H. Cai, Y. Li, J. Lai, H. Zhang, and F. Feng, "Yeast Cell Surface Display for Lipase and Its Applications," *J. Chinese Inst. Food Sci. Technol.*, 2017, doi: 10.16429/j.1009-7848.2017.05.020.

[12] K. Zhao *et al.*, "Synthesis, characterization and optimization of a two-step immobilized lipase," *Renew. Energy*, 2017, doi: 10.1016/j.renene.2016.11.035.

[13] S. A. de Rose, H. Novak, A. Dowd, S. Singh, D. A. Lang, and J. Littlechild, "Stabilization of a lipolytic enzyme for commercial application," *Catalysts*, 2017, doi: 10.3390/catal7030091.

[14] M. G. Pereira *et al.*, "Different covalent immobilizations modulate lipase activities of hypocrea pseudokoningii," *Molecules*, 2017, doi: 10.3390/molecules22091448.

[15] S. Cui *et al.*, "Immobilization of lipase onto N-succinyl-chitosan beads and its application in the enrichment of polyunsaturated fatty acids in fish oil," *J. Food Biochem.*, 2017, doi: 10.1111/jfbc.12395.

CHAPTER 7

LINES MEMBRANE INTEGRITY IN RESPONSE TO CAUSAL ENZYMOLOGY AND PHYSIOLOGICAL ASPECTS: A REVIEW

Suresh Kawitkar, Professor
Department of ISME, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- suresh.kawitkar@atlasuniversity.edu.in

ABSTRACT:

The plant cell membranes are important for appropriate development of the many surface structures developed on aerial plant organs in addition to their importance in stress protection. We also point out that certain physiological and enzymatic factors, including MDA, ion leakage, and the observation of certain phytozymes engaged in lipid and cellulose metabolisms, are responsible for membrane remodeling and integrity. These latter ones were evaluated in the context of durum wheat dehydrin transgenic plants, proline metabolic mutants, and the wild-type plant, and are connected to the membrane structure. We also present docking data supporting the hypothesis that dehydrin-phytozyme interactions and the inhibition of lipase activity by the durum wheat LTP4 are responsible for the observed causal relationships between enzymological behaviors and membrane integrity.

KEYWORDS:

Cells, Enzymology, Membrane, Plant Cells, Water.

INTRODUCTION

It is important to emphasize from the outset that up to 50% of the world's irrigated lands suffer from excessive salinity. Additionally, the majority of plants that thrive in salty environments acquire more low-molecular-weight water-soluble solutes in their cells than other plants. Osmotic correction is the last step. Osmotic adjustment and salt tolerance have been suggested to be related, albeit this has never been demonstrated. The buildup of organic osmolytes like proline is the most well-studied biochemical reaction of plant cells to osmotic stress. It was originally noted that this unusual amino acid accumulated in leaves, and since then, research has shown its crucial significance as an osmoprotectant effector under a variety of stress situations, particularly salt stress. Salt content, exposure time, and plant genotype all have an impact on how salt stress affects plants. Because of the reduced external water potential caused by salt in the environment, plants experience a water shortage, and ion toxicity and nutritional changes disrupt ion transport systems. Salt stress also damages membranes, changes the amounts of growth regulators, inhibits certain enzymes, and interferes with photosynthesis, all of which may result in plant mortality. It's vital to remember that proline is said to mimic the aldolation process by acting as a catalytic component. In fact, there has been a lot of discussion over the proline-catalyzed aldol reaction mechanism, or at least many theories have been brought forth. The Hajos-Parrish-Eder-Sauer-Wiechert reaction is the first highly enantioselective organocatalytic transformation, as well as the first asymmetric aldol reaction ever devised by chemists [1], [2].

On the other hand, the formation of ROS is one of the recognized plant responses to salt stress. Because too much ROS may damage lipids, proteins, and nucleic acids and cause cell damage and death, plant cells must control how much ROS they produce. It has been shown that phospholipid signaling is a key mediator of the ROS generated by NADPH oxidase

activity. Phospholipid phosphatidic acid, the second messenger, targets certain proteins to affect cellular and physiological adjustments that enable plants to respond to abiotic stressors. When phospholipase D hydrolyzes structural phospholipids at the terminal phosphoesteric link with release of the hydrophilic head group, phosphatidic acid is produced. The most common phospholipase family in plants is phospholipase D. When exposed to diverse environmental conditions including cold, drought, salt, and damaging ones, their activity quickly increases. It's also quite intriguing to note that in Arabidopsis thaliana, phospholipase D activity was shown to be a negative regulator of proline accumulation.

On the other hand, we see that lipid transfer proteins are several tiny members of the PR-14 protein family that are thought to be involved in plant defensive responses. Numerous biological functions, including antimicrobial defense, signaling, cell wall thinning, and participation in the development of salt tolerance, have been hypothesized and shown. Significantly, LTPs may play a key role in abiotic stress conditions such salinity as well as direct protection against fungi. Here, the current study focuses on the identification of some physiological parameters as well as causal enzymatic behaviors that may explain the integrity of the membrane in the case of the response of Arabidopsis thaliana lines, transgenic, and metabolic mutant to salt stress treatment. Therefore, we discuss the probable role of phytozymes such cellulases and lipases, which are engaged in membrane remodeling and integrity, as well as the effect of the tolerance level on MDA and ion leakage. Through the analysis of the molecular dynamics of the interaction between phytozymes and dehydrin or one of these active sites, in silico studies further support the function performed by transgenic environment. Finally, it is crucial to recognize the LTP4's potential involvement as an inhibitor during the in vitro lipase evaluation [3], [4].

Plant Constituentsand Salinity Control

The wild type utilized in this investigation is Arabidopsis thaliana ecotype Columbia. Hence, Wt, the transgenic Arabidopsis lines overexpressing durum wheat dehydrin Dhn-5 gene, and the truncated forms have been previously described by and also the proline metabolic mutant lines were grown on MS agar medium for one week under light/dark cycle condition of 16-hour light/8-hour dark cycle at 22°C and then transferred to MS medium supplemented or not with NaCl at the concentration of 100 mM. Following the application of salt stress for eight days, the impact of adding NaCl to the medium was evaluated by evaluating several catalytic activities as well as various physiological parameters as the quantity of H2O2, MDA, and ion leakage.

DISCUSSION

A cold solution with 100 mM Tris-HCl buffer, 10 mM EDTA, 50 mMKCl, 20 mM MgCl2, 0.5 mM PMSF, and 2% PVP was used to homogenize aliquots of frozen fresh shoot material. The supernatant from the centrifugation of the homogenate at 14.000 g for 30 min at 4°C was used to calculate the enzyme activity. The Bradford technique was used to assess protein concentration [5], [6].

Potentiometric Lipase Activity Assay Using pH-stat

According to, lipase activities were quantified potentiometrically at 40 °C and pH 8, which automatically titrated the free fatty acids produced from pure egg L-phosphatidylcholine as substrate, as previously reported. Triglyceride emulsions that had been physically agitated were used to test lipase activity. LTP4 isolated from durum wheat is added to the reaction mixture at a concentration of 40 g/mL1 to measure its inhibitory impact on lipase activity.

A-Glucosidase Activity Assessment

According to Saibi and Gargouri's materials and methods section, -glucosidase activity is measured by incubating the enzyme preparation at the proper dilution with 0.2 mL of p-nitrophenyl--D-glucopyranoside as substrate for 15 min at 50°C. By adding 0.6 mL of 0.4 M glycine-NaOH buffer pH 10.8, the process was halted. At 400 nm, the released p-nitrophenol was detected. P-nitrophenol has a molecular extinction coefficient of 18,000 M1.cm1. The quantity of enzyme needed to release 1 mol of p-nitrophenol per minute under the test conditions was measured as one unit of enzymatic activity.

Assays for Membrane Lipid Peroxidation

Malondialdehyde levels in tissue were measured to determine the extent of lipid peroxidation. We homogenized fresh leaf and root samples in 10% TCA. Centrifuging the homogenate at 15,000 g for 20 min. at 4°C. The supernatant was collected, then 0.5% thiobarbituric acid in 20% TCA was added. Samples were heated in a water bath for 25 minutes at 95°C before cooling on ice. The samples were centrifuged at 10,000 g for 10 min to measure the solutions' absorbance at 532 and 600 nm. The extinction coefficient for MDA expressed in nmol MDA g1 DW was used to compute the MDA level.

Test for Electrolyte Leakage

From leaves from the examined Arabidopsis thaliana lines, five leaf discs were cut and put into glass tubes with 5 ml of deionized water. After that, tubes were left at room temperature for 24 hours while being gently agitated. The electrical conductivity of the glass tube solution was measured using a conductivity meter to assess the amount of electrolyte leakage, and the results were represented as mS.cm1 as per [7]–[9].Modeling server phyre 2 used the crystal structure of the 1-acyl-sn-glycerophosphate 2 acyltransferase, plsc, from Thermotogamaritima to model the phospholipid/glycerol acyltransferase. The lipase class 3 family was created using the fungal lipase as a template. The thioesterase's crystal structure was modeled after the murine soluble epoxide hydrolase 2 crystal structure. Using collagen I alpha 1 as a template, the dehydrin ORF was modeled. Using the ModRefiner tool, the resultant models were then refined via two-step atomic-level energy minimization. Then, to assess and verify models, the web program RAMPAGE generated Ramachandran plots. It was done by using the PyMol Molecular Graphics System to create graphical representations of the built-in model structure. The GRAMM-X server, which is accessible at, was used to conduct protein-protein docking of dehydrin and the three partners' interacting proteins. GRAMM-X automatically selects the optimum course of action after analyzing the input structures.

Analytical Statistics

One-way analysis of variance was used to evaluate the data, and Duncan's multiple range tests were used to determine treatment mean separations at the 5% level of significance. It is essential to state from the outset that membranes are one of the most significant cellular structural elements. As a result, they serve as the cell's protective barrier, enclosing the protoplasm and acting as an interface for interaction between the inner and exterior components. They perform a number of crucial tasks include receiving messages and helping to transfer substances both inside and outside. The membrane in this situation also comprises lipids, phospholipids, and galactolipids in addition to cellulose and hemicellulose. Particularly, those latter substances were thought to play crucial roles in membrane functioning and integrity in the face of diverse biotic and abiotic stressors [10]–[12].

However, it is crucial to note that durum wheat dehydrin is a member of the LEA-group 2 family that was isolated and described in order to be researched and understood for its physiological functions. Our team also discovered DHN-5 overexpression in Arabidopsis thaliana. Indeed, we demonstrated that DHN-5 confers salinity tolerance to the dehydrin transgenic Arabidopsis lines by modulating a number of metabolic pathways, including the proline metabolism one and the ROS scavenging system, as well as some phytozymes like the cysteine and aspartyl proteases.

Process for Persuasive Salt Tolerance

Additionally, and in accordance with our earlier transcriptional level research, our team demonstrated the deniability of 77 genes that were either up- or down-regulated in DH4 line subjected to salt stress treatment. In fact, some characteristics of phospholipid/glycerol acyltransferase , which was upregulated as shown in the list of upregulated genes in transgenic Arabidopsis seedlings overexpressing DHN-5 , as well as lipase class 3 family protein , and furthermore esterase/lipase/thioesterase family protein , which was downregulated as shown It is crucial to note that these three proteinsphospholipid/glycerol acyltransferase, lipase class 3 family proteins, and esterase/lipase/thioesterase family proteinsare involved in lipid metabolism, which is closely linked to membrane integrity and structure. This fact is made possible by the fact that LTP4, an essential and upregulated member, is present. The three target proteins, lipase class 3 family protein, and esterase/lipase/thioesterase family protein were studied for protein interactions with the Arabidopsis thaliana proteome. The results respectively. We may list more than one partner of phospholipid/glycerol acyltransferase engaged in metabolism associated to tenacity and excellent membrane structure and integrity. We hypothesize that this enzyme's beneficial effects on the dehydrin transgenic Arabidopsis lines tolerance acquisition process are caused by their contribution to the maintenance of membrane integrity via dynamic stability in lipid and phospholipid metabolism in plants. We employed the research of protein-protein interaction to better comprehend the role played by the transgenic environment in the establishment of the salt tolerance process through the interaction of DHN-5 with these potential phytozymes. We have used molecular modeling inside this framework to produce the most amount of data used to support our recommendations [13], [14].

As a result, the models for phospholipid/glycerol acyltransferase, lipase class 3 family, thioesterase, and dehydrin were developed and refined using ModRefiner. The related Ramachandran plots were then used to verify the results. More than 98% of residues were in the preferred and permitted locations, according to the model analysis. As a result, these models were verified and kept. Dehydrin in particular has a disorganized structure. Furthermore, the K-segment domain is present in two copies as the Lys-Ileu-Lys-Glu-Lys-Leu-Pro-Gly sequence in the dehydrin structural model. The consensus sequence Asp-Glu-Tyr-Gly-Asn-Pro and the sequence known as the S- segment are both conserved in the N-terminal region. According to the docking study between the dehydrin and those proteins, the dehydrin was superior to acyltransferase and thioesterase for lipase class 3 in terms of the number of contact hydrogen bonds. Dehydrin-protein complexes had atomic contact energies of -297 kcal/mol and -148 kcal/mol, respectively, while lipase, the acyltransferase, and the thioesterase had atomic contact energies of -69.96 kcal/mol. According to the estimated dehydrin interface areas for lipase, acyltransferase, and thioesterase were 1316 2, 1277 2, and 1136 2, respectively. Ultimately, the in silico discoveries support the information gathered from the transcriptional study carried out by our team and further support the notion that causal enzymology plays a significant role in the maintenance of membrane integrity [15]–[17].

The Potential Role of LTP4 in the Development of Salt Tolerance

Salt stress, abscisic acid stimulation, and other biotic and abiotic challenges all include the lipid transfer protein 4. Additionally, it was mentioned as an inhibitor of several glycosyl hydrolase enzymes and some lipid metabolism enzymes. LTP4 is also a protein that is increased, as seen in. It is significant to note that LTP4 was reported as an inhibitor of several enzymes involved in the metabolism of carbohydrates and other metabolisms connected to the integrity of the membrane. By modifying some of the implicated phytozymes in the outlined process, it seems that LTP4 may play a protective function during the development of salt tolerance. Testing the impact of the durum wheat LTP4 on the lipase activity is essential in this situation. The development of salt tolerance under the salt-treated condition may finally be explained by the inhibitory impact of the durum wheat LTP4 detected on the lipase activity. In fact, the decrease in lipase activity under stressful circumstances helps safeguard the integrity of the membrane as well as the membrane structure from potential harm from lipolytic activity.

CONCLUSION

Finally, and in light of the data presented here, it is crucial to suggest that the acquisition of salt tolerance in Arabidopsis thaliana lines may be accompanied by certain causal enzymatic as well as physiological factors that explain the membrane integrity. Given that DHN-5 is a multifunctional protein with important roles as a thermoactivator and thermostabilizer of biocatalysts and the capacity to chelate metals, it appears to be an important protein acting as a heat-protective protein that also interacts with numerous partners involved in various pathways and other metabolites. Dehydrin may also be a strong candidate to interact with catalysts involved in proline metabolism like P5CS one due to its multifunctionality. All of these discoveries highlight how crucial this protein is to plants' ability to respond to stress. We may dare to raise this last question in order to open up opportunities for new study and inquiry methods.

REFERENCES:

[1] P. K. Robinson, “Enzymes: principles and biotechnological applications,” *Essays Biochem.*, 2015, doi: 10.1042/BSE0590001.

[2] M. K. Go, J. Wongsantichon, V. W. N. Cheung, J. Y. Chow, R. C. Robinson, and W. S. Yew, “Synthetic Polyketide Enzymology: Platform for Biosynthesis of Antimicrobial Polyketides,” *ACS Catal.*, 2015, doi: 10.1021/acscatal.5b00477.

[3] C. Khosla, “Quo vadis, enzymology?,” *Nature Chemical Biology*. 2015. doi: 10.1038/nchembio.1844.

[4] H. Luhavaya, M. V. B. Dias, S. R. Williams, H. Hong, L. G. De Oliveira, and P. F. Leadlay, “Enzymology of Pyran Ring A Formation in Salinomycin Biosynthesis,” *Angew. Chemie - Int. Ed.*, 2015, doi: 10.1002/anie.201507090.

[5] P. F. Fox, T. Uniacke-Lowe, P. L. H. McSweeney, and J. A. O’Mahony, “Enzymology of Milk and Milk Products,” in *Dairy Chemistry and Biochemistry*, 2015. doi: 10.1007/978-3-319-14892-2_10.

[6] B. Stauch, S. J. Fisher, and M. Cianci, “Open and closed states of Candida Antarctica lipase B: Protonation and the mechanism of interfacial activation,” *J. Lipid Res.*, 2015, doi: 10.1194/jlr.M063388.

[7] M. J. Rust, “Computational Recipes in Enzymology,” *Cell Systems*. 2015. doi: 10.1016/j.cels.2015.09.006.

[8] J. C. Cochran, "Kinesin Motor Enzymology: Chemistry, Structure, and Physics of Nanoscale Molecular Machines," *Biophys. Rev.*, 2015, doi: 10.1007/s12551-014-0150-6.

[9] R. P. Dick, *Methods of soil enzymology*. 2015. doi: 10.1002/9780891188582.

[10] Y. Fichman, S. Y. Gerdes, H. Kovács, L. Szabados, A. Zilberstein, and L. N. Csonka, "Evolution of proline biosynthesis: Enzymology, bioinformatics, genetics, and transcriptional regulation," *Biol. Rev.*, 2015, doi: 10.1111/brv.12146.

[11] T. A. Kunkel and D. A. Erie, "Eukaryotic Mismatch Repair in Relation to DNA Replication," *Annu. Rev. Genet.*, 2015, doi: 10.1146/annurev-genet-112414-054722.

[12] Y. Liu *et al.*, "Digestion of nucleic acids starts in the stomach," *Sci. Rep.*, 2015, doi: 10.1038/srep11936.

[13] R. P. Dick and R. G. Burns, "A brief history of soil enzymology research," in *Methods of Soil Enzymology*, 2015. doi: 10.2136/sssabookser9.c1.

[14] L. E. Tailford *et al.*, "Discovery of intramolecular trans-sialidases in human gut microbiota suggests novel mechanisms of mucosal adaptation," *Nat. Commun.*, 2015, doi: 10.1038/ncomms8624.

[15] H. Luhavaya, M. V. B. Dias, S. R. Williams, H. Hong, L. G. de Oliveira, and P. F. Leadlay, "Enzymology of Pyran Ring A Formation in Salinomycin Biosynthesis," *Angew. Chemie*, 2015, doi: 10.1002/ange.201507090.

[16] R. J. Kutta *et al.*, "The photochemical mechanism of a B 12-dependent photoreceptor protein," *Nat. Commun.*, 2015, doi: 10.1038/ncomms8907.

[17] M. Kovermann, J. Ådén, C. Grundström, A. Elisabeth Sauer-Eriksson, U. H. Sauer, and M. Wolf-Watz, "Structural basis for catalytically restrictive dynamics of a high-energy enzyme state," *Nat. Commun.*, 2015, doi: 10.1038/ncomms8644.

CHAPTER 8

METABOLIC ENZYMES' FUNCTION IN CONTROL OF INFLAMMATION

Somayya Madakam, Associate Professor
Department of uGDX, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- somayya.madakam@atlasuniversity.edu.in

ABSTRACT:

Responses to environmental stimuli result in substantial metabolic reprogramming in immune cells. These metabolic pathways, which for a long time were thought of as being routine maintenance chores, are now known to play a crucial role in the immune response by controlling the activation, differentiation, and subsequent effector roles of both lymphoid and myeloid cells. Several enzymes play important roles in controlling inflammation within the intricate metabolic networks linked to immune activation and may be used as therapeutic targets in human illness. In certain instances, these enzymes regulate the flow across the various metabolic or energy pathways necessary to satisfy the demands of the immune response. In other instances, important enzymes regulate the levels of immunoactive metabolites that have a direct signaling function. Finally, and probably most intriguingly, a number of metabolic enzymes have developed side jobs that play roles in the immune response completely unrelated to their normal enzymatic functions. Here, we discuss important metabolic enzymes that control inflammation, emphasizing mechanistic insights and therapeutic intervention potential.

KEYWORDS:

Enzymes' Function, Control of Inflammation, Direct Signaling Function, Metabolic.

INTRODUCTION

Immunologic reactions are sophisticated and complicated. To create responses that are correctly focused and efficient, immune cells of various kinds must integrate a variety of environmental signals and collaborate with one another. This results in substantial but precisely timed alterations in proliferation and function. In the last two decades, it has become clear that cellular metabolism, which was formerly thought to just play a supporting role in immunity, is really a crucial immune function regulator. Immunological signals result in a significant reprogramming of metabolic pathways, which affects the activation, differentiation, and effector activities of immune cells. In order to find pathways that may be targeted in human disorders, such as autoimmune diseases defined by dysregulated inflammation, this control of immune responses by metabolic pathways referred to as "immunometabolism" has become a prominent area of study [1], [2].

Even while our mechanistic knowledge of metabolism's function in immunology is constantly expanding, a few universal concepts have come into sharper clarity. In order to supply precursors and satisfy the energy requirements particular to certain immunologic states, metabolic reprogramming seems to be required. Additionally, metabolites themselves have the potential to function as signaling molecules that control inflammatory reactions directly. Within this concept, metabolic enzymes have been shown to govern flux into important pathways and/or the fluctuating amounts of immunoactive metabolites to control inflammation. Many of these enzymes have evolved into "moonlighting" proteins that function in immunologic signaling pathways completely unrelated to their normal enzymatic

functions, demonstrating the elegance and effectiveness of evolution. These enzymes are prospective therapeutic targets for inflammatory diseases in humans because they are important regulators of inflammation. The feasibility of pharmacologically targeting metabolic enzymes to modify immunity is supported by animal and, in some circumstances, human investigations. Here, we will go through the expanding list of metabolic enzymes that are essential for controlling immunological responses, emphasizing how amenable both people and animals are to pharmacological treatment. Given the variety of metabolic processes that have been shown to affect immune function, we have organized our study using a few important metabolic pathways [3], [4].

Master regulators of metabolism

A serine/threonine kinase known as the "mechanistic target of rapamycin" has significant control over the metabolism, growth, and survival of cells. In order to produce a wide range of effects, mTOR, which can exist within two different complexes, combines knowledge about the availability of cellular energy and nutrients with external stimuli. These effects include the induction of nutrient transporter expression, encouragement of glycolytic enzyme activity, increase in lipid synthesis, induction of ribosome synthesis, and encouragement of the transcription and translation of various proteins. It is true that there are many ways in which mTOR triggers metabolic reprogramming, both within and outside of immune cells, and it is beyond the purview of this review to list them all. It is important to remember that mTOR functions as a master regulator of immune responses, preparing myeloid and lymphoid cells for the pro-inflammatory state by causing extensive metabolic alterations. When the T cell receptor and CD28 are simultaneously stimulated in T cells, mTOR is activated downstream of AKT/PI3K signaling. Toll-like receptors and cytokine receptors both contribute to the activation of mTOR in myeloid cells. mTOR signaling, which primarily functions through the transcription factors HIF-1 and Myc, is essential for the upregulation of metabolic pathways necessary for inflammatory activation and effector functions in both myeloid and lymphoid cells. These pathways include glycolysis, the pentose phosphate pathway, and glutaminolysis. The well-known mTOR inhibitor rapamycin prevents organ transplant rejection by suppressing the immune system.

DISCUSSION

Differentially regulated by mTORC1 and mTORC2 signaling is CD4+ T cell differentiation. Th1, Th2, and Th17 cell development is hampered by a lack of mTOR, which affects both complexes, although regulatory T cell differentiation is aided. Increased Foxp3+ Treg production requires a deficit in both mTORC1 and mTORC2 because both complexes prevent Treg differentiation. Th1 and Th17 differentiation was shown to be prevented by the selective impairment of mTORC1 signaling caused by a defect in its upstream regulator Rheb whereas Th2 differentiation was found to be promoted. A reciprocal impact on differentiation was seen with mTORC2 deficiency. A separate research, however, discovered that CD4+ cells lacking mTORC2 were unable to develop into either Th1 or Th2 cells. AMP-activated protein kinase, which reciprocally suppresses mTOR signaling and activates opposing metabolic pathways, serves as the counterweight to mTOR in the control of metabolism. By turning on the TSC1/TSC2 complex and phosphorylating Raptor, the mTOR binding partner, AMPK inhibits mTOR. While AMPK is triggered by an increase in the intracellular AMP/ATP ratio and switches on catabolic processes to restore energy balance, mTOR reacts to nutrient excess and supports anabolic activities. To replenish the cell's ATP supply, it inhibits gluconeogenesis, lipid synthesis, and protein synthesis while activating fatty acid oxidation. Most significantly, AMPK inhibits acetyl-CoA carboxylase by phosphorylating it, which results in a switch from lipid synthesis to fatty acid oxidation [5], [6].

The exact function of AMPK in inflammation is complex, and research is ongoing in this area. Although AMPK is not necessary for the growth or homeostasis of T cells, AMPK activity is momentarily elevated after TCR activation. AMPK controls the formation of memory CD8+ T cells, the recall response, and T cell effector responses. Activating AMPK simultaneously may stop pathological inflammation. AICAR, an AMPK activator, reduced ulcerative colitis and multiple sclerosis models as well as avoided sepsis in mouse models. Metformin, which partly activates AMPK, has been found to reduce inflammation in lupus and allograft rejection models. In contrast, compound C's inhibition of AMPK made sepsis worse in mouse models.

Glycolysis

One of the initial findings in immunometabolism, that glycolysis is upregulated in response to immunological challenge of naive T cells and is essential for T cell effector activities, was the significance of glycolytic reprogramming in immune activation. This increase in glycolytic flow, which is similar to the Warburg effect, which was originally identified in cancer, and which preferentially converts pyruvate to lactate rather than oxidation in mitochondria, largely describes the inflammatory response in both adaptive and innate immune cells. As mentioned previously, mTOR activation and the downstream transcription factors HIF-1 and Myc are essential for glycolytic reprogramming. Glycolytic reprogramming also affects the activity of regulatory cell types, even though this transition to aerobic glycolysis has repeatedly been connected with the differentiation and effector activities of inflammatory cells. For instance, it has been shown that Treg cell proliferation is increased but that the suppressive capabilities of Treg cells are compromised when glycolysis is upregulated in response to TLR activation or GLUT1 overexpression. More recently, it was shown that TNF receptor 2 activation increased glycolysis in thymus-derived Treg cells, improving both proliferation and suppressive activity. However, these cells oxidized pyruvate rather than secreting lactate in a Warburg-like manner. Similarly, recent studies using the glycolysis inhibitor 2-deoxyglucose suggested that glycolysis is also necessary in these cells to support OXPHOS and fatty acid synthesis, despite the fact that upregulating OXPHOS is crucial for the alternative activation of anti-inflammatory M2 macrophages. A more recent research, however, asserted that glycolysis is not necessary for M2 differentiation and that 2-DG's inhibitory effects are based on off-target effects unrelated to glycolysis.

Regarding the need of glycolytic upregulation after immune activation, many theories are commonly accepted. One reason, for instance, is that enhanced glycolysis generates vital biomass by delivering the building blocks for the production of nucleotides, lipids, and proteins. Another argument is that, while having lower efficiency than oxidative phosphorylation, glycolysis's quick kinetics may sustain higher bioenergetic demands by supplying more ATP per second. Despite the biological plausibility of these concepts, it is now known that other pathways play a role in the need of increased glycolysis in inflammatory reactions. As a result, whereas certain glycolytic enzymes seem to play a crucial role in the regulation of inflammation merely by regulating metabolic flow, others have surprising and unusual secondary functions or regulate the availability of metabolites that serve as both signaling molecules and precursors [7], [8].

Hexokinase

The first stage of glycolysis, the conversion of glucose to glucose-6-phosphate, is catalyzed by hexokinase. Hexokinase 2, in particular, has a crucial role in regulating glycolytic flow and is increased as a result of TCR and IL-2 receptor activation. The transcription factors HIF-1 and Myc as well as the activation of mTOR all contribute to this. By assisting HIV-

infected cells in surviving, hexokinase is also crucial for macrophage HIV infection. The hexokinase enzyme activity is pharmacologically inhibited, which reduces the inflammatory response. The glycolytic inhibitor 2-DG reduces T cell proliferation and effector functions, shifts the balance between effector, regulatory, and memory T cells, prevents the pro-inflammatory activation of dendritic cells, and macrophages, and is beneficial in animal models of autoimmunity like lupus and rheumatoid arthritis. In addition, in RA and MS mouse models, the direct hexokinase inhibitor 3-bromopyruvate inhibits immune activation and lessens illness.

The function of the hexokinase enzyme changes somewhat during viral infection. Retinoic acid-inducible gene 1-like receptor family proteins recognize viral RNA in cells that have been infected by a virus. These proteins then interact with the mitochondrial antiviral signaling protein to cause the type 1 interferon response. Hexokinase activity has recently been demonstrated to be increased by MAVS's interaction with the enzyme at the mitochondrial outer membrane. The MAVS-hexokinase association was broken down by binding to RLR, resulting in a reduction in glycolytic flow, which was necessary for the synthesis of type 1 interferon. Hexokinase causes inflammation in addition to its typical enzymatic action by double-acting as a pattern recognition receptor. Hexokinase is inhibited by N-acetylglucosamine, which is a part of the peptidoglycan present in bacterial cell walls, according to research. Hexokinase dissociates from the mitochondria as a result of this inhibition, activating the NLRP3 inflammasome and triggering the release of pro-inflammatory cytokines. This result persisted when different hexokinase inhibitors were used [9]–[11].

GAPDH, or glyceraldehyde-3-phosphate dehydrogenase

Another glycolytic enzyme that is important in inflammation is glyceraldehyde-3-phosphate dehydrogenase, or GADPH. In addition to its primary function as a glycolytic enzyme, GAPDH also plays a distinct and tightly controlled secondary role as an mRNA-binding protein that inhibits the translation of inflammatory cytokines. This function is covered in more detail below. In the Warburg circumstances, which characterize activated immune cells, GAPDH also seems to be essential for controlling glycolytic flow. GADPH inhibits the translation of pro-inflammatory cytokines IFN and GM-CSF by binding to AU-rich regions in their 3′ untranslated region. These mRNAs bind GAPDH in a competitive manner with NAD+/NADH, and following immunological activation, the enhanced glycolytic engagement of GAPDH results in release and elevated translation. Similar to this, GADPH inhibits the translation of TNF mRNA in monocytes and macrophages, a process that is reversed after exposure to LPS. Therefore, the increase of glycolysis and the generation of inflammatory cytokines are linked through GAPDH's reduced inhibition of cytokine mRNA translation. Post-translational modification controls GAPDH's side job as an mRNA-binding protein. Malonyl-CoA causes malonylation, a lysine modification. Following LPS stimulation, GADPH is malonylated in monocytes and macrophages. This increases the activity of the GAPDH enzyme while diminishing its ability to bind to mRNA, enabling inflammatory cytokines including IFN-, IL-6, and TNF- to be translated into protein.

The fact that GADPH becomes rate-limiting under Warburg circumstances while not being a rate-limiting enzyme under baseline conditions may be directly related to its function in controlling immunological responses. The immunomodulatory medication dimethyl fumarate, FDA-approved for the treatment of MS, post-translates GAPDH at its active location and renders it inactive. The anti-inflammatory actions of DMF are mediated through the enzymatic inhibition of GAPDH by the medication, which prevents glycolysis in activated immune cells but not in resting immune cells. It's interesting to note that DMF does

not influence GAPDH-mRNA binding. Itaconate, an anti-inflammatory metabolite generated from the TCA cycle, was later shown to similarly inactivate GAPDH enzyme function. These results indicate that GAPDH enzyme activity, either by restricting glycolytic flow generally or by controlling the amounts of immunoactive metabolites, is necessary for pro-inflammatory reactions independent of mRNA binding. For instance, GAPDH inhibition raises methylglyoxal concentrations, which has been shown to reduce inflammation by influencing the KEAP1-NRF2 axis.

Enolase

The ninth stage of glycolysis, which changes 2-phosphoglycerate into phosphoenolpyruvate, is carried out by the enzyme enolase. Enolase, a protein found in large quantities on bacterial cell surfaces, binds to plasminogen and promotes bacterial invasion of the host organism. According to data showing that blocking the interaction between enolase and plasminogen or immunizing against enolase before being challenged with pathogenic bacteria significantly changed the course of the infection in mouse models, enolase has been shown to be essential for the virulence of several strains of bacteria. It has been shown that the recruitment of macrophages in inflammatory lung disease depends on -enolase's binding to plasminogen. Enolase also contributes to the production of Treg by acting as a transcriptional regulator in the background. Enolase localizes to T cell nuclei to produce Tregs in the periphery, according to one research. Enolase directly impacts the expression of the splicing variant Foxp3-E2 in the nucleus by binding to regulatory areas of FOXP3. This relationship was confirmed in peripheral blood samples from people with type 2 diabetes and relapsing-remitting multiple sclerosis [12], [13].

The rate-limiting stage and last reaction in glycolysis is the conversion of phosphoenolpyruvate into pyruvate by the enzyme pyruvate kinase. Different PK isoforms exist. The M1 isoform occurs as a tetramer with strong glycolytic activity and is constitutively expressed in the majority of differentiated tissues under baseline circumstances. The pyruvate kinase M2 isoform preferentially expresses under Warburg circumstances, such as in cancer cells and activated immune cells, and it supports the inflammatory response through a variety of pathways. PKM2 differs from PKM1 in that it may either be a dimer with low glycolytic activity or a tetramer with strong glycolytic activity. Perhaps counterintuitively, it is the low-activity PKM2 dimer that stimulates aerobic glycolysis and inflammation, mostly by way of side functions that aren't glycolytic.

PKM2 is more expressed in response to pro-inflammatory stimuli including TCR ligation in T cells and LPS activation of macrophages. By phosphorylating the mTOR inhibitor AKT1 substrate 1 and boosting serine production from the glycolytic metabolite 3-phosphoglycerate, PKM2 in turn activates mTORC1. PKM2, while having a reduced intrinsic enzyme activity, upregulates glycolysis by moonlighting as a transcriptional co-activator and supporting the HIF-1 transcriptional program. Increased expression of PKM2 is caused by HIF-1. PKM2 also attaches to HIF-1, moves to the nucleus, and then promotes the transcription of genes that are HIF-1 target genes.

The inflammatory activation of macrophages and T lymphocytes depends on PKM2's non-canonical second job as a transcriptional co-activator of HIF-1. Dimerized nuclear PKM2 activates a pro-inflammatory transcriptional pathway that induces IL-1 after LPS stimulates macrophages. Small molecules like TEPP-46, which cause PKM2 to tetramerize and thereby increase its canonical enzyme activity while inhibiting its nuclear functions, block the effects of LPS on inflammation and glycolytic reprogramming while increasing the expression of anti-inflammatory cytokines like IL-10 . Similar to this, TEPP-46 treatment of CD4+ T cells

suppresses glycolytic reprogramming, inhibits PKM2 nuclear translocation, and lowers T cell activation, proliferation, and cytokine production. In the experimental autoimmune encephalomyelitis animal model of autoimmune neuroinflammation, administration of TEPP-46 inhibits the differentiation of pro-inflammatory Th1 and Th17 cells and ameliorates illness, designating PKM2 as a possible therapeutic target. A genetic PKM2 defect protects mice against EAE, according to research from two other groups. One of them showed that PKM2 was knocked down by shRNA in isolated CD4+ cells, which in turn lowered glycolysis and Th1/Th17 differentiation while also reducing their pathogenicity in an adoptive transfer model of EAE. Although they noted that PKM2 was necessary for Th17 differentiation through actions on STAT3 rather than through actions on HIF-1 and metabolic reprogramming, the other group discovered that the CD4+ T cell-specific knockout of PKM2 impaired Th17 differentiation and attenuated the course of active-immunization EAE. A different study discovered that PKM2 increases the production of IL-17 in CD4+ cells in response to lactate absorption via activating STAT3.

PKM2 has been shown to directly activate the NLRP3 inflammasome in macrophages in addition to its nuclear activities. The genetic deletion of PKM2 and a pharmaceutical inhibitor of PKM2 enzyme activity both blocked AIM2 and NLRP3 inflammasome activation, indicating that PKM2's canonical enzyme activity may be significant. PKM2 controls the flow of upstream glycolytic metabolites into the pentose phosphate pathway for the production of NADPH in NK cells, where it modulates the inflammatory response independently of HIF-1 and its nuclear activities. Therefore, canonical PKM2 enzyme activity may be significant in certain cell types and/or environmental circumstances. Pyruvate, the result of glycolysis, may either enter the pyruvate dehydrogenase complex to form acetyl-CoA and join the TCA cycle, or it can enter the lactate dehydrogenase complex to be converted to lactate. NAD+ is replenished when pyruvate is converted to lactate, which permits the continuation of glycolysis and the Warburg phenotype. Pyruvate dehydrogenase kinase 1 , a kinase that inhibits PDH and directs pyruvate away from the TCA cycle and toward lactate synthesis, is expressed and active at higher levels in both CD4+ and CD8+ T cells after TCR stimulation . Though Treg cells have minimal PDHK1 activity, pro-inflammatory Th17 cells do. Dichloroacetate, a tiny chemical that inhibits PDHK1, reduces aerobic glycolysis, encourages pyruvate entrance into the TCA cycle, and reduces Th17 while enhancing Treg formation from naive CD4+ cells and decreasing the production of pro-inflammatory cytokines in CD8+ cells. Animal models of a variety of autoimmune illnesses, such as inflammatory bowel disease, RA, MS, and asthma, respond well to treatment with DCA in vivo. These results point to PDHK1 as a possible therapeutic target for autoimmune disease as well as a crucial regulator of aerobic glycolysis and inflammation.

LDH, or lactate dehydrogenase

As shown above, under Warburg conditions, the conversion of pyruvate to lactate by LDH sustains high glycolytic flux and regenerates NAD+. LDH controls cellular inflammatory responses through a number of methods. LDH-A, an LDH isoform with high enzymatic activity, is expressed more often as a result of CD4+ cells' pro-inflammatory activation. By maintaining high levels of acetyl-CoA, which in turn stimulates histone acetylation and IFN transcription, LDH-A activity aids in the inflammatory response. Mice lacking LDH-A were shielded from autoimmune assaults. It has been shown that suppressing LDH using the small chemical FX11 -4-propylnaphthalene-1-carboxylic acid) prevents macrophages from secreting pro-inflammatory cytokines. LDH has been discovered to bind and suppress the translation of mRNAs encoding inflammatory cytokines, releasing them upon activation of its enzymatic activity. This is similar to how GAPDH does. Finally, it has been shown that

lactate itself has a direct signaling function in inflammation. It has been shown that lactate absorption in CD4+ cells through the transporter SLC5A12 promotes Th17 development and inhibits migratory egress from areas of inflammation, and that lactate uptake blockage ameliorates illness in an inflammatory arthritis model.

But in several additional research, it was shown that lactate has anti-inflammatory effects on lymphocytes and macrophages. Previous research has shown that lactate generated in the tumor microenvironment suppresses the activity of cytotoxic and effector T lymphocytes. Contrarily, it has recently been shown that Foxp3 alters the metabolism of Treg cells, enabling them to live and work in a low-glucose, high-lactate TME. A different investigation discovered that lactate particularly encourages Foxp3 expression. Extracellular lactate similarly generates regulatory phenotypes in tumor-associated macrophages via a number of pathways, aiding tumor evasion. More recently, it was shown that lactate causes histones to undergo a post-translational alteration called lactylation that controls transcription and causes cells to take on the anti-inflammatory M2 phenotype after being stimulated with LPS. It's interesting to note that lactate inhibits type 1 interferon production by infected cells during viral infection through a direct interaction with MAVS. As a result, mice lacking in LDH-A showed increased type 1 interferon response and increased resistance to vesicular stomatitis virus infection.

Metabolism of One Carbon

One-carbon metabolism, which includes the movement of one-carbon units inside the cell, is essential for several biosynthetic activities, such as the production of nucleotides, amino acids, and fatty acids, as well as for the methylation-based epigenetic control. This route may get one-carbon units from the metabolism of folate or from amino acids like serine, glycine, or methionine. One-carbon metabolism is more important for immune cells that are activated. While LPS stimulation in macrophages activates both the serine synthesis route and one-carbon metabolism to generate the epigenetic modifications necessary for the production of pro-inflammatory cytokines like IL-1, TCR stimulation upregulates one-carbon metabolism. One-carbon metabolism generally promotes immune activation, proliferation, and survival via a variety of mechanisms, including the provision of biosynthetic precursors for anabolic processes , the regulation of redox status by regulating glutathione levels , and the provision of the substrate for histone methylation.

It has been shown that a number of crucial enzymes involved in one-carbon metabolism control inflammation. Methionine adenosyl transferase suppression via genetic or pharmaceutical means, as well as therapy with the SAH hydroxylase inhibitor 3-deazaadenosine, both reduced the amount of IL-1 that macrophages produced. Mice were protected from LPS-induced sepsis by inhibiting serine hydroxymethyl transferase 1 and 2, which feed one-carbon metabolism via serine. This reduced the generation of inflammatory cytokines.

Similar effects on T cell proliferation were seen with the genetic and pharmacologic suppression of SHMT isoforms.One of the most popular and well-established anti-inflammatory medications available is methotrexate, and no discussion of one-carbon metabolism would be complete without addressing it. As a dihydrofolate reductase inhibitor, methotrexate prevents the metabolism of folate, one-carbon metabolism, and nucleic acids. However, its exact mode of action in the therapy of autoimmune illnesses is debatable. According to some data, it may function by releasing extracellular adenosine and activating AMPK. Other cutting-edge medications that target this route, however, have shown promise in pre-clinical cancer or autoimmune disease models.

Protein Metabolism

The availability of amino acids and the metabolism of proteins are important immune response regulators. Amino acid transporters are increased during T cell activation in order to allow them to transport amino acids into the cell. After T cell activation, leucine and glutamine transporters are both increased, and genetic ablation of both transporters prevents the T cell response. Additionally, the growth of T cells depends on the availability of amino acids in the extracellular environment. For instance, cell culture conditions include excessive amounts of glutamine, therefore limiting glutamine will limit in vitro T cell proliferation. Cysteine and cysteine are depleted in the tumor microenvironment by myeloid-derived suppressor cells in cancer, which prevents T cell activation. The activation of T cells and macrophages is significantly regulated by serine availability, which comes from both external pools and de novo synthesis, as was previously mentioned.

A number of enzymes are crucial in controlling amino acid availability to manage the immune response. Tryptophan is depleted by the enzyme indoleamine 2,3-dioxygenase, which inhibits T cell responses. For the purpose of fostering feto-maternal tolerance and preventing T cell activation, IDO is produced at high levels in the placenta. Cancer researchers are attempting to therapeutically modify this route since kynurenine, the IDO's downstream metabolite, also suppresses the immune system. By regulating de novo NAD+ production, tryptophan metabolism through the kynurenine route also controls macrophage activity. The pro-inflammatory response to LPS is increased when quinolatephosphoribosyltransferase , which produces the NAD+ precursor nicotinic acid mononucleotide , is genetically or pharmaceutically inhibited. The ectopic expression of QPRT, on the other hand, reduces inflammation and encourages a homeostatic macrophage phenotype. Arginase-1, an enzyme that selectively depletes arginine and is only expressed in M2 macrophages, prevents the generation of the pro-inflammatory nitric oxide produced by M1 macrophages. To manage the inflammatory response during Mycobacterium TB infection, macrophages in the lung granulomas pathognomonic for the illness release high quantities of Arg1.

The process by which the cell breaks down glutamine and transforms it into intermediates of the TCA cycle and other metabolites is known as glutaminolysis. The increase of glutamine catabolism in activated T cells occurs in a Myc-dependent manner, fueling polyamine synthesis, refueling the TCA cycle, and coordinating with glucose catabolism to enable amino acid, nucleotide, and lipid biosynthesis. Additionally, the glutamine metabolism enzyme glutamate oxaloacetate transaminase 1 promotes inflammation by generating 2-hydroxyglutarate, which suppresses FOXP3 expression and prevents the development of Tregs. The glutamine analog 6-diazo-5-oxo-L-norleucine, which was used to limit glutamine metabolism, reduced inflammation in mice models of acute lung damage and avoided allograft rejection. Although reducing glutamine metabolism in autoimmunity models reduces the autoimmune phenotype, doing so in cancer models results in a stronger anti-tumor immune response. Researchers discovered that blocking glutamine metabolism with DON or a similar prodrug produced effector CD8+ T cells capable of a potent anti-tumor response in a number of cancer model systems. By increasing the activity of acyl-coenzyme A synthetase short-chain family member 1, glutamine antagonism specifically boosted OXPHOS in tumor-infiltrating CD8+ cells, enabling the TCA cycle to be fueled by acetate.

The metabolic process through which cells break down and reuse biological components is known as autophagy. In vitro activation of murine immune cells was reduced by the deletion of the autophagy-essential gene Atg3, which has been demonstrated to be required for immune cell activation. This research also shown that when hematopoietic cells experience

metabolic stress, autophagy is used to provide lipids to the cell. Immune cells increase autophagy after TCR or TLR activation to provide the nutrients required for the cell's metabolic needs. While the HIV glycoprotein gp120 binds to CXCR4 on T cells and triggers apoptosis, autophagy also contributes to the induction of cell death during HIV infection. However, this impact was reversed by the ablation of autophagy-critical enzymes such Beclin-1 or Atg7. In addition, autophagy is crucial for immune cell development. The cell can control the destiny of Treg and memory T cells by employing autophagy to produce the lipids required for the FAO-dependent metabolic phenotype of these cells. Autophagy is turned off by mTOR and turned on by AMPK. In myeloid cells, autophagy also regulates inflammatory responses; loss of autophagy-critical genes resulted in an M1 inflammatory phenotype and a reduction in M2 polarization. Due to the upregulation of cell-intrinsic survival pathways during autophagy, it is also important for the neutrophil response to infection.

CONCLUSION

With several intricate and interrelated metabolic pathways now known to affect immune activation and inflammation, immunometabolism is a fast developing topic. We have tried to draw attention to important metabolic enzymes that play important roles in controlling inflammation within these intricate networks, as well as their potential for pharmacological modification.

Modulating inflammation by targeting metabolic enzymes has the potential to advance the field of immunometabolism from the bench to the bedside in the treatment of inflammatory diseases, with a number of drugs targeting these enzymes currently in pre-clinical or clinical trials, and in some cases already in clinical use.

REFERENCES:

[1] A. Castello, M. W. Hentze, and T. Preiss, “Metabolic Enzymes Enjoying New Partnerships as RNA-Binding Proteins,” *Trends in Endocrinology and Metabolism*. 2015. doi: 10.1016/j.tem.2015.09.012.

[2] K. P. Kim *et al.*, “Vitamin C stimulates epidermal ceramide production by regulating its metabolic enzymes,” *Biomol. Ther.*, 2015, doi: 10.4062/biomolther.2015.044.

[3] J. Tian, G. He, K. Mai, and C. Liu, “Effects of postprandial starvation on mRNA expression of endocrine-, amino acid and peptide transporter-, and metabolic enzyme-related genes in zebrafish (Danio rerio),” *Fish Physiol. Biochem.*, 2015, doi: 10.1007/s10695-015-0045-x.

[4] G. Pacioni *et al.*, “Truffles contain endocannabinoid metabolic enzymes and anandamide,” *Phytochemistry*, 2015, doi: 10.1016/j.phytochem.2014.11.012.

[5] P. S. Shyne Anand *et al.*, “Effect of dietary supplementation of periphyton on growth, immune response and metabolic enzyme activities in Penaeus monodon,” *Aquac. Res.*, 2015, doi: 10.1111/are.12385.

[6] K. Nakatsukasa, F. Okumura, and T. Kamura, “Proteolytic regulation of metabolic enzymes by E3 ubiquitin ligase complexes: Lessons from yeast,” *Crit. Rev. Biochem. Mol. Biol.*, 2015, doi: 10.3109/10409238.2015.1081869.

[7] G. Zhao *et al.*, “Study of the serum levels of polyunsaturated fatty acids and the expression of related liver metabolic enzymes in a rat valproate-induced autism model,” *Int. J. Dev. Neurosci.*, 2015, doi: 10.1016/j.ijdevneu.2015.04.350.

[8] A. de Mesquita Barbosa, S. dos Santos Costa, J. R. da Rocha, C. A. Montanari, and S. Giorgio, "Evaluation of the leishmanicidal and cytotoxic effects of inhibitors for microorganism metabolic pathway enzymes," *Biomed. Pharmacother.*, 2015, doi: 10.1016/j.biopha.2015.07.040.

[9] A. Castello, M. W. Hentze, and T. Preiss, "Special Issue: Systems Approach to Metabolic Disease Metabolic Enzymes Enjoying New Partnerships as RNA-Binding Proteins," *Trends Endocrinol. Metab.*, 2015.

[10] M. Pröschel, R. Detsch, A. R. Boccaccini, and U. Sonnewald, "Engineering of metabolic pathways by artificial enzyme channels," *Frontiers in Bioengineering and Biotechnology*. 2015. doi: 10.3389/fbioe.2015.00168.

[11] N. S. Madhukar, M. O. Warmoes, and J. W. Locasale, "Organization of enzyme concentration across the metabolic network in cancer cells," *PLoS One*, 2015, doi: 10.1371/journal.pone.0117131.

[12] G. D. Moghe and R. L. Last, "Something old, something new: Conserved enzymes and the evolution of novelty in plant specialized metabolism," *Plant Physiol.*, 2015, doi: 10.1104/pp.15.00994.

[13] H. Kwon, S. Oh, X. Jin, Y. J. An, and S. Park, "Cancer metabolomics in basic science perspective," *Archives of Pharmacal Research*. 2015. doi: 10.1007/s12272-015-0552-4.

CHAPTER 9

ROLE OF HUMAN ENZYMES IN VARIOUS METABOLIC PATHWAYS AND DISEASES

Suresh Kawitkar, Professor
Department of ISME, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- suresh.kawitkar@atlasuniversity.edu.in

ABSTRACT:

The essential proteins known as enzymes carry out the fundamental cellular functions. Enzymes may also cause illnesses in people, and research is being done to understand the molecular processes behind the genotype-to-phenotype link for the sake of diagnosis and medical treatment. Here, we emphasize enzymes that participate in several metabolic processes and function as significant nodes in protein interaction networks. Our current understanding of human metabolic pathways is derived through statistical analysis using the Kyoto Encyclopaedia of Genes and Genomes database. We discovered that the enzymes activity aldehyde dehydrogenase, designated by the Enzyme Commission number EC 1.2.1.3, and activity acetyl-CoA C-acetyltransferase are the ones that are most frequently involved. We identified the proteins that are often engaged in metabolic pathways by assigning functional activities to enzyme proteins. Our study revealed that these proteins had the largest numbers of anticipated interaction sites as well as the highest numbers of interaction partners when compared to all the enzymes in the pathways. We concentrate on the cytosolic and mitochondrial forms of acetyl-CoA acetyltransferase and alpha-aminoadipic semialdehyde dehydrogenase. We demonstrate using computational methods that it is feasible to highlight hints of enzymes' numerous functions in various pathways and of suspected mechanisms favoring the correlation of genes with illness by beginning with the structure of the enzyme.

KEYWORDS:

Diseases, Enzymes, Metabolic, Protein.

INTRODUCTION

It is well known that enzymes are proteins with specialized molecular properties that, when coordinated, produce the diversity of biological activities that are the foundation of the physiology of the cell complex. The question of whether various enzyme molecules prefer to momentarily congregate in the cell environment in order to produce the necessary coordinated activity and references therein is still up for dispute. Any coordinated biological activity involving enzymes is modeled by a metabolic network or pathway that details the biochemical sequential interactions and/or cycles that form the cornerstone of cellular metabolism. Through curated databases like the Kyoto Encyclopaedia of Genes and Genomes and REACTOME, information on which models of metabolic pathways and reactions are known for a given organism is also accessible. Each enzyme is a protein molecule that has a unique four-digit EC number that completely identifies the biological process that it catalyzes and may also have an atomic solved structure that is often accessible in the Protein Data Bank. This makes it easy to comprehend how sequence, structure, and function relate to the catalytic processes at the active site and the potential function of effectors at the binding site [1], [2].

The reference databases for sequences and three-dimensional structures, respectively, are PDB and UniProt/SwissProt. Numerous enzymes have been linked to somatic disorders

including cancer as well as genetic diseases, as described in OMIM. When non-synonymous mutations have been linked to certain illnesses, it is now feasible to infer information about specific molecular pathways. We are also aware of the participants in interactions in the cell milieu thanks to extensive proteome research, which are kept in databases like IntAct and BioGRID. For thorough functional annotation of enzymes, a number of databases are now accessible, including BRENDA, Enzyme Portal, and M-CSA. GeneCards also contains information, including available data on the degree of enzyme expression in various human organs. In order to generate general laws of molecular functioning, which reconcile molecular processes to physiological models connected to particular phenotypes, we increasingly need to interconnect various databases as more data accumulates. Enzymatic activities are organized into a hierarchical structure of subnetworks and mesonetworks matching KEGG categorization and containing structural data in the most current version of Manet.

Here, we specifically consider the number of human enzymes shared by several metabolic pathways. The goal is to draw attention to the intricate networks of networks where certain proteins are engaged in several biological processes at once and to provide evidence of potential connections to information on protein-protein interactions and molecular hints. In light of the human KEGG metabolic maps, we include these enzymes together with, where known, their associations with diseases. We discover an intriguing correlation between the most prevalent enzymes in the KEGG metabolic maps, the quantity of cell environment interactors, and the quantity of anticipated interaction sites [3], [4].

We next look at one of these enzymes, ALDH7A1, which belongs to subfamily 7 of the aldehyde dehydrogenase gene family, on a molecular level. The detoxification of aldehydes produced by lipid peroxidation and alcohol metabolism is said to be greatly aided by the enzymes. The protein, which has at least three distinct isoforms, is found in the cytosol, mitochondrion, and nucleus and is connected to a variety of biological processes. We study the structural characteristics of the enzyme that may be suggestive of its significant function using computational techniques, and we highlight potential pathways for its failure, which is mostly related to pyridoxine-dependent epilepsy. Similar to this, we discuss the molecular experimental and anticipated features of ACAT1 and ACAT2, which function as acetyl-CoA C-acetyltransferases in humans and do so in the cytosol and mitochondria, respectively.

DISCUSSION

We obtained data for our study from SwissProt/UniProt. The current list of human proteins on SwissProt contains 20,365 proteins, of which 3428 are enzymes, each of which is given a full enzyme commission number. Enzyme proteins will be referred to as EC proteins moving forward. Through our database eDGAR, which uses OMIM, HUMSAVAR, CLINVAR, and curated DisGeNet as key sources of data, we correlate 7316 proteins with genetic illnesses. We used the April 2018 KEGG version for KEGG pathway annotation, making a difference between KEGG pathways and KEGG metabolic pathways and referring to human genes. The sources for protein-protein interactions include IntAct and BioGRID. ISPRED4, a machine-learning-based predictor operating at the state of the art, calculates the chance of a protein's lateral side chain interacting with other proteins. With an accuracy rate of up to 85% and a very low percentage of false positive predictions, it can accurately predict the interaction sites of proteins from their structural data. When a structure is unavailable, an internal version of ISPRED4 that just takes sequence data into account is used. We used INPS to calculate the impact of missense variants on protein stability. A non-linear regression based on machine learning techniques is carried out by INPS using data retrieved from protein structure or sequence, and it achieves a Pearson's correlation coefficient of up to 0.76. The related standard error for the calculated G values is about 1 kcal/mol [5]–[7].

The KEGG Metabolic Pathways and EC Proteins

We concentrated on analyzing all potential connections between biological processes as indicated by EC numbers and KEGG pathways in order to deal with the complexity of the network of human biochemical reactions. There are 320 biological pathways included in the Kyoto Encyclopaedia of Genes and Genomes, 90 of which are referred to as metabolic pathways. We included KEGG keywords for pathways to annotate EC human proteins. We discover that 6904 proteins are linked to 320 KEGG pathways using the human protein portion of SwissProt as a reference. 1642 EC proteins engage in 90 metabolic pathways when concentrating on proteins linked to metabolic KEGG pathways. We discovered 770 EC proteins linked to 90 metabolic pathways by limiting our search to proteins that are enzymes and disease-related. 930 EC numbers are linked to the 770 proteins.

Interactions between EC Proteins

All the possible interactions that each protein might have can be modeled by a network of networks. In order to take use of this option in light of the data, we concentrated on the EC proteins that are linked to 10 or more KEGG metabolic pathways in order to emphasize the number of potential interactors. From IntAct and BioGRID, interactions that are experimental and physical are collected. IntAct reports 337,389 interactions among 36,815 proteins when limiting to interactions involving human proteins, while BioGRID has 471,774 interactions involving 25,420 proteins. Therefore, the average number of interactors per protein in IntAct and BioGRID is equivalent to 18 and 37, respectively. The characteristics of the distribution of the number of reported interactors in IntAct and BioGRID are compared among the following classes: proteins involved in only one metabolic KEGG pathway; proteins involved in at least ten metabolic KEGG pathways; and all EC proteins. When compared to those that are less commonly engaged, EC proteins that are involved in a large number of KEGG metabolic pathways also have a large number of interactors.

Alpha-Aminoadipic Semialdehyde Dehydrogenase Case Study

The human protein alpha-aminoadipic semialdehyde dehydrogenase, also known as antiquitin , is a multifunctional enzyme that mediates significant protective effects. It is encoded by the gene ALDH7A1. The protein participates in lysine catabolism and protects cells from oxidative stress by metabolizing lipid peroxidation-derived aldehydes. A vital physiological osmolyte and methyl donor, betaine is produced as a byproduct of the metabolism of betaine aldehyde. There are three distinct isoforms of it, one of which is exclusively mitochondrial. The gene is linked to 59 human phenotypes in the human phenotypic ontology, according to GeneCards, as well as eight distinct REACTOME and 13 KEGG metabolic pathways. The expression data in Gene Cards indicate that the protein is present in several tissues. As confident expression organs, GeneORGANizer mentions the brain, cranial nerve, eye, head, liver, lung, peripheral nervous system, and peripheral nerve. ALDH7A1 is linked to 34 processes in 17 distinct subsystems, including the cytosol, endoplasmic reticulum, lysosome, mitochondria, and peroxisome, according to the Human Protein Atlas. More than 100 papers have been written on it because of its importance to the biology of the cell. The protein interacts with other proteins, and it is found in the cytoplasm, the mitochondrion, and the nucleus. It has undergone 15 crystallizations. Here, we concentrate on a full version of the biological unit, a homotetramer solved at 0.170 nm resolution with the highest coverage of the sequence P49419 and no mitochondrial target peptide. Recently, significant protein variations that are linked to PDE and inhibit its action have also been resolved with atomic precision. Finally, the protein lacks intrinsically disordered regions, which is a key characteristic, according the MobiDB database. We are interested in emphasizing some of the

protein's molecular characteristics that are connected to its function in various metabolic pathways and illnesses [8], [9]. There are 232 variants in the protein sequence P49419, which consists of 539 residues. 195 of these variations are linked to illness in 160 distinct places, and 117 of them are linked to PDE. In order to be thorough, we calculated the probability that each protein variation would have an impact on protein stability and discovered, as predicted, that variants that aren't necessarily tied to illness are causing problems with protein folding. We discovered that the PDE-related variants V278L, Q281H, M285V, and K375R correspond to expected interaction locations at the solvent-accessible protein surface without impacting protein stability. The features of every protein variant found in various databases and linked to certain disorders. The positions of each variation in the protein reference sequence P49419, the location of the variation in the protein three-dimensional structure, and the projected impact on protein stability, calculated by INPS for each variation. Additionally, it shows if the disease-related residue appears in the target peptide, in the tetrameric interface, in the active sites, or in any of the locations annotated in the associated UniProt file. When available, the ISPRED projections are shown. The transit peptide, a unique N-terminal peptide in the protein sequence regulating mitochondrial import, is 26 residues long and has a wide range of variants. This shows that a protein's unpaired translocation to the mitochondrial compartment may potentially contribute to illness. For the purpose of comparison, we have highlighted some PDE disease-related variations that are known to occur in the aldehyde substrate binding site and that have recently been examined at the atomic level for their impact on protein structure and function. P197S, G202V, and W203G are predicted by INPS to affect protein stability.

Acetyl-CoA C-Acetyltransferase Case Study

Acetyl-CoA C-acetyltransferases catalyze the condensation of an acetyl-CoA and an acyl-CoA, which results in the synthesis of an acyl-CoA with a longer fatty acid chain. These enzyme proteins are listed for the activity EC 2.3. The residue chains of the two enzymes, which are encoded by two distinct genes, have 39% sequence similarity. ACAT2 and ACAT1 encode the cytosolic and mitochondrial, respectively, of the two enzymes. Both proteins' 3D structures have been determined at the atomic level. Two sample structures, or a lack of acetyl-CoA acetyltransferase, is an inborn mistake of isoleucine catabolism, and is shared by ACAT1 and ACAT2. ACAT1 is linked to 118 human phenotypes in the human phenotypic ontology, according to GeneCards, whereas ACAT2 is linked to 23 human phenotypes. According to GeneORGANizer, both ACAT1 and ACAT2 are confidently expressed in the brain and cranium. The liver, oesophagus, and stomach all express ACAT1. ACAT1 has been linked to two processes in the cytosol, mitochondria, and peroxisome in the Human Protein Atlas. The two proteins are the focus of several articles that may be accessed through GeneCards due to their importance for cell biology. IntAct lists 32 interactors for the ACAT1 protein while BioGRID lists 108 interactors for the ACAT2 protein. These statistics are much higher than the average number of interactions per protein in the whole sample, especially for ACAT1. Finally, none of the proteins had intrinsically disordered regions, according to the MobiDB database.

First, we concentrate on a homotetramer solved with a resolution of 0.185 nm that represents the entire shape of the ACAT1 biological unit. The target peptide-depleted mature form of P24752 is completely covered by the monomeric chain. We are interested in emphasizing some of the protein's molecular characteristics that are connected to its function in various metabolic pathways and illnesses. We discovered that changes in potential interaction sites often lead to the impairment of protein function, just as they did in the case of ALDH71. This is the situation with eight variants caused by acetyl-CoA acetyltransferase deficiency. The 33

residue-long mitochondrial target peptides have an interesting five variants, which suggests that the illness may possibly be caused by an unpaired translocation of the protein to the mitochondrial compartment [10]–[12].

We used the PDB entry 1WL4 for the ACAT2 protein to represent the interaction areas and map the changes. The entry includes a homotetrameric form that was solved with a 0.155 nm precision. All of the chains span the whole sequence. The ACAT2 subunit chain A and depict anticipated interaction residues, tetrameric interaction areas, and locations containing disease-related mutations using the same approach. The single documented variant of ACAT2 related with the deficiency of acetyl-CoA acetyltransferase occurs at the solvent exposed protein surface and is predicted with ISPRED4 as an interaction site, supporting the earlier studies on the significance of interaction areas. Additionally, this alteration has a negligible impact on protein stability, supporting the idea that changes to interaction sites might impede protein-protein interactions and cause illness without impacting protein stability.

Diseases may be linked to protein variations. By determining if the variation is present at a prospective interaction site and/or whether it affects the protein stability, it is possible to study potential indicators of the impact of disease-related changes. We picked the ALDH7A1 gene as a test example since it is one of the genes found most often in KEGG metabolic pathways, according our data. In several databases, the protein is linked to 232 variants. It turns out that 27% of the disease-related alterations in protein structure impair the protein stability, regardless of whether they are located in active sites, biological assembly interfaces, or areas of the protein that are exposed to solvents. According to Intact and BioGrid, the protein physically interacts with 23–62 distinct interactors. In the solvent-exposed protein surface, 21 residues are predicted to function as interaction sites. Seven of them are disease-related, and four of them are connected to PDE. This shows that any disease-related alteration in the protein's external surface, which affects how well it interacts with all of its diverse partners without impacting protein stability, may impair the protein's effectiveness in each of the several metabolic pathways where it is active. The investigation of ACAT1 and ACAT2, which are typical of the second EC number, came to similar findings. Another example of the importance of changes at the protein solvent accessible interface as favorable to illnesses may be found by delving more into the molecular characteristics. Our analysis shows that it is possible to highlight enzyme proteins that are essential to biochemical pathways and to identify potential molecular mechanisms underlying their association with particular diseases using the data currently available and computational tools [13], [14].

CONCLUSION

The creation of a three-dimensional model of cell metabolism is one of the objectives of system biology. As a first stage, we now deal with the issue of creating connections between various databases that break down cell complexity into meaningful and significant pieces of data, addressing cell components from various angles and using various methodologies. By limiting our analysis to human enzymes and their relationships to KEGG metabolic pathways and PPI interaction maps, we are able to examine the issue of connecting the network of protein-protein interactions to the KEGG metabolic pathways. We discovered that when enzymes are the focal points of metabolic pathways, they often interact with a large number of proteins, as shown by various experimental techniques, and they also have a large number of predicted interaction sites. Our findings imply that protein-protein interaction networks include centres for enzymatic metabolic activity. When compared to the other EC proteins in the networks, hubs are consistently provided with the largest average number of projected interaction sites.

REFERENCES:

[1] C. Dölle, R. Skoge, M. VanLinden, and M. Ziegler, "NAD Biosynthesis in Humans - Enzymes, Metabolites and Therapeutic Aspects," *Curr. Top. Med. Chem.*, 2015, doi: 10.2174/1568026613136660206.

[2] T. Bhattacharya, T. S. Ghosh, and S. S. Mande, "Global profiling of carbohydrate active enzymes in human gut microbiome," *PLoS One*, 2015, doi: 10.1371/journal.pone.0142038.

[3] A. Varghese, J. Savai, N. Pandita, and R. Gaud, "In vitro modulatory effects of Terminalia arjuna, arjunic acid, arjunetin and arjungenin on CYP3A4, CYP2D6 and CYP2C9 enzyme activity in human liver microsomes," *Toxicol. Reports*, 2015, doi: 10.1016/j.toxrep.2015.02.008.

[4] F. X. Ruiz *et al.*, "Structural Determinants of the Selectivity of 3-Benzyluracil-1-acetic Acids toward Human Enzymes Aldose Reductase and AKR1B10," *ChemMedChem*, 2015, doi: 10.1002/cmdc.201500393.

[5] S. Masaki *et al.*, "Identification of the specific interactors of the human lariat RNA debranching enzyme 1 protein," *Int. J. Mol. Sci.*, 2015, doi: 10.3390/ijms16023705.

[6] Y. Liu and T. J. Flynn, "CYP3A4 inhibition by Psoralea corylifolia and its major components in human recombinant enzyme, differentiated human hepatoma HuH-7 and HepaRG cells," *Toxicol. Reports*, 2015, doi: 10.1016/j.toxrep.2015.03.006.

[7] Á. J. Espejo-Mojica *et al.*, "Human recombinant lysosomal enzymes produced in microorganisms," *Molecular Genetics and Metabolism*. 2015. doi: 10.1016/j.ymgme.2015.06.001.

[8] W. Sumsakul, W. Chaijaroenkul, and K. Na-Bangchang, "In vitro inhibitory effects of plumbagin, the promising antimalarial candidate, on human cytochrome P450 enzymes," *Asian Pac. J. Trop. Med.*, 2015, doi: 10.1016/j.apjtm.2015.10.016.

[9] F. P. Guengerich, "Human cytochrome P450 enzymes," in *Cytochrome P450: Structure, Mechanism, and Biochemistry, Fourth Edition*, 2015. doi: 10.1007/978-3-319-12108-6_9.

[10] S. Rendic and F. P. Guengerich, "Survey of human oxidoreductases and cytochrome P450 enzymes involved in the metabolism of xenobiotic and natural chemicals," *Chemical Research in Toxicology*. 2015. doi: 10.1021/tx500444e.

[11] A. Duda-Chodak, T. Tarko, P. Satora, and P. Sroka, "Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review," *European Journal of Nutrition*. 2015. doi: 10.1007/s00394-015-0852-y.

[12] W. He *et al.*, "Inhibition of human cytochrome P450 enzymes by licochalcone A, a naturally occurring constituent of licorice," *Toxicol. Vitr.*, 2015, doi: 10.1016/j.tiv.2015.06.014.

[13] M. Coughtrie, "Ontogeny of Human Conjugating Enzymes," *Drug Metab. Lett.*, 2015, doi: 10.2174/1872312809666150602151213.

[14] D. Nokinsee, L. Shank, V. S. Lee, and P. Nimmanpipug, "Estimation of inhibitory effect against tyrosinase activity through homology modeling and molecular docking," *Enzyme Res.*, 2015, doi: 10.1155/2015/262364.

CHAPTER 10

ENHANCING THERAPEUTIC ENZYME PERFORMANCE USING LIPOSOMES

Swarna Kolaventi, Assistant Professor
Department of uGDX, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- swarna.kolaventi@atlasuniversity.edu.in

ABSTRACT:

Over the last several decades, the idea of pharmaceuticals has evolved, leading to the acceptance of both macromolecules and low-molecular-weight entities as pharmaceutics' bioagent components. A separate class of molecules, particularly proteins in general and enzymes in particular, have entered a new age as a result of this. Delivery issues and the need for suitable carrier systems have been made more difficult by the use of enzymes as medicines. We shall concentrate on therapeutic enzymes and their uses in this overview, mentioning a few that have made it to the pharmaceutical market. It will include issues with their clinical use and nanotechnological solutions to some of their shortcomings. The topic of drug delivery systems will be covered, with a focus on liposomes as they are the most well-researched and practical nanosystem for in vivo enzyme administration. The favorable pre-clinical outcomes of two enzymes, L-asparaginase and superoxide dismutase, after their association with liposomes will be fully explored. Examples of liposomal enzymatic formulations in development will be given.

KEYWORDS:

Drug delivery, Enzymes, Liposomes, pharmaceutical.

INTRODUCTION

Research and biotechnological developments are to blame for the so-called "change in paradigm" of molecules as medications, which saw a shift from virtually entirely low molecular weight compounds to bioactive agents. The use of biological molecules in research and medicine is already a reality, with interest in this field growing throughout time. Various proteins and genetic material are a few examples of those substances utilized as bioactive agents. The use of enzymes as therapeutic agents and the interest in developing nanoformulations as an alternative to standard formulations with the goal of enhancing enzyme activity will be covered in this study. In all animal and vegetable cells, enzymes are extensively distributed and play a significant role in all cellular metabolisms by taking part in important processes required for the continuation of life. Diseases that are often difficult to treat and manage with traditional medicines might result from the lack or dysfunction of a single enzyme. In fact, via enzymatic substitution or replacement, biopharmaceutics, including enzymes, constitute the sole therapy option for several uncommon disorders. Since the 19th century, reports of the use of digestive aids such amylases and proteases as medicinal agents have been made. Later, in the 1960s, it was realized that enzymes, because of their almost unique features that result in high activity and selectivity, may form a promising class of bioagents for the treatment of many illnesses. The natural conformation must be maintained, and they must be properly formed, stabilized, and preserved [1], [2].

The development and advancement of genetic engineering technology allowed for the manufacture of enzymes with the necessary properties of purity, selectivity, and quantities suitable for clinical usage, making the enzymatic treatment approach more feasible. Enzymes

have a very high therapeutic potential, which may be used to treat anything from cancer to cardiac issues to metabolic and inflammatory illnesses. Since 1987, when the Food and Drug Administration first approved the recombinant tissue plasminogen activator, Alteplase, for the treatment of acute ischemic stroke, the market has grown rapidly due to the availability of enzymes with pharmaceutical properties and a wide range of applications. Although enzymes with high specificity and selectivity to the substrate, high activity, and production capabilities have many benefits, their use for therapeutic purposes has been constrained by a number of disadvantages, including immunogenic reactions, short residence times in living things, rapid metabolization and/or degradation, and loss of activity upon storage [3], [4].

Strategies including the production of enzyme derivatives, association with polymers, immobilization in inorganic supports, or in drug delivery systems have been developed throughout time in an effort to get around such restrictions and enhance therapeutic enzyme characteristics. In order to cure lysosomal storage disorder, Gregory Gregoriadis and Brenda Ryman first added enzymes to lipid vesicles, or liposomes, in 1972. Since this groundbreaking study, a broad range of biomolecules have been included using liposomes and other DDS, giving rise to a new idea of nanoformulations and nanopharmaceutics and enhancing the field of nanomedicines . The rationale behind the molecules' association with DDS is to enhance the performance of the loaded material through a number of concurrent mechanisms, including defense against biological degradation, modification of the pharmacokinetic profile, and site-specific targeting, which will improve the therapeutic result. After 50 years of research in this area, there are several examples of success, both from an academic and an industrial perspective. The industry has already commercialized a sizable number of nanoformulations.

Therapeutic Properties of Enzymes

The unique binding and activity on their targets with high specificity and affinities identify enzymes as a subclass of proteins. They may be derived from plant, animal, or antimicrobial sources. Enzymes may now be generated in vast quantities without having to be removed from their natural sources, with the benefits of better purity and reduced costs, thanks to advancements in biotechnological techniques. Enzymes have a variety of uses for various diseases because of their exceptional and one-of-a-kind characteristics, as well as their wide range of targets and potential actions in living things. From minor digestive issues to significant conditions including cystic fibrosis, metabolic and cardiac abnormalities, inflammatory illnesses, and malignancies, among others, they span the gamut. More than 100 enzymes with therapeutic potential and functioning via various pathways have been discovered, according to Drug Bank online. Since they may be discovered in recent revision articles, the current study does not aim to be thorough in its selection of therapeutic enzymes.

DISCUSSION

Early attempts to provide therapeutic enzymes intravenously based on passive absorption by target cells and tissues had ineffective therapeutic effectiveness. With enzyme-nanocarrier association, the subject of considerable and rewarding scientific effort, improved activity, decreased immunogenicity, and clearance were accomplished. Typically, nanocarriers are characterized as particles in the nanosize range; however, for therapeutic applications, the mean diameter might range between 10-1000 nm. Several nanocarrier systems were created for the delivery of therapeutic enzymes and drugs more generally. The benefits of including enzymes in such carriers are well acknowledged. In essence, the important factors are maximising encapsulation effectiveness while preserving enzyme catalytic activity by keeping their quaternary structure.

Additionally, the increased stability of enzymes in bodily fluids makes it possible for enzyme availability to have a therapeutic impact. In order to optimize transport and targeting of loaded enzymes, this represents a technical advancement based on the careful selection of carrier composition and assembly procedures. Different compositions, topographies, and forms are possible for nanocarriers. The primary established categories among the many nanostructures that have been created to be coupled with macromolecules for therapeutic or diagnostic reasons include liposomes, nanoparticles, virosomes, extracellular vesicles, and erythrocytes. Liposomes are the most frequently used methods for enzyme inclusion. They consist of biocompatible phospholipid nanovesicles with customizable trapping properties that may be used for active or passive delivery of low- and high-molecular-weight drugs [5]–[7].

Liposomes as Flexible Enzyme Delivery Vehicles

Liposomes, one of the most researched DDS of bioactive compounds, have led to a number of commercially available formulations and a large number of others that are in advanced phases of clinical testing. They have a number of benefits over other nanocarriers, including their adaptable structural design, biocompatibility, biodegradability, non-toxic nature, and lack of immunogenicity. They are spherical structures that are predominantly composed of amphiphilic molecules and arranged in bilayers. Bangham and Horne initially described lipid vesicles in 1964. On the outside of the vesicles or within the compartments, the hydrophilic parts of the lipids are water orientated. The hydrophobic areas are contained inside the bilayer and are not in direct contact with water. Van der Wall forces that hold the tails of the phospholipid's nonpolar chains together and hydrogen bonds between the water molecules and the phospholipid's polar heads maintain the liposome construction and stability. As seen in Figure 1, several vesicle generations and various liposome types have been identified.

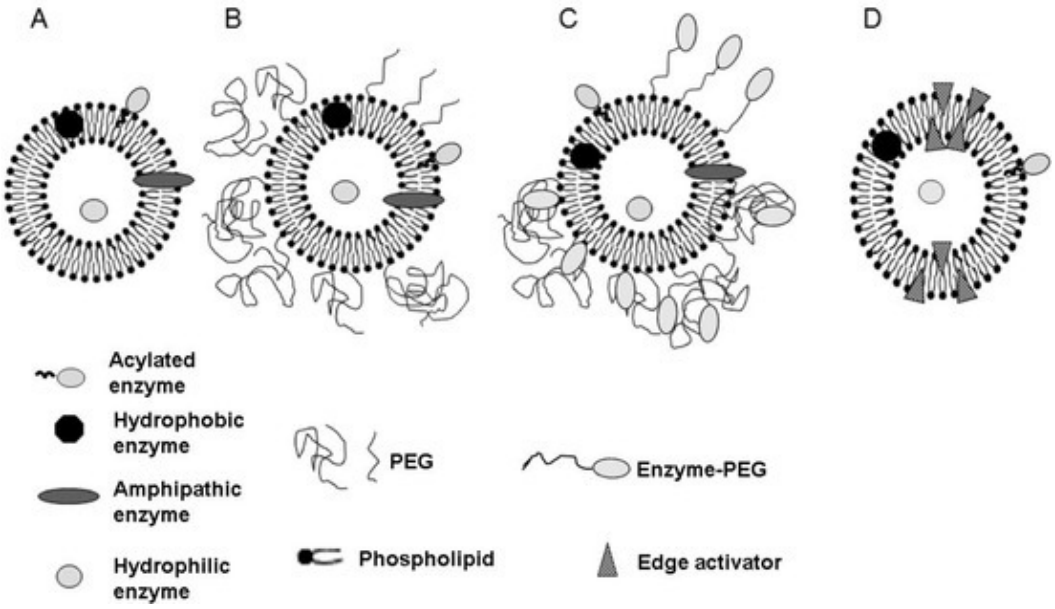


Figure 1: Schematic representation of different types of liposomes

The lipid content and manufacturing process of liposomes have a direct impact on their physicochemical characteristics. The primary elements of the vesicular carriers are lipid molecules like cholesterol and phospholipids. Through the selection and blending of their elements, lipids and others, their composition may be greatly altered. As a result, they can have a variety of physicochemical characteristics depending on the following factors: the charge of the phospholipid polar head groups, the saturation and length of the phospholipid

acyl chains, the presence of cholesterol or other non-lipid-charged molecules, and the relative amounts of the different constituents. Drug-vesicle interactions are also influenced by the pH of the environment. The preparation technique used may affect the encapsulation of hydrophilic molecules, which depends on the vesicle's interior aqueous volume. Additionally, it has been suggested several times to boost the effectiveness of encapsulating hydrophilic pharmaceuticals while maintaining the loaded material within the vesicles by include cholesterol in the composition of fluid phospholipid vesicles, which limits the permeability of lipid bilayers . As a result, almost infinite variations of liposomes may be produced under control, and there in vivo behavior can be altered depending on various factors.

After parenteral administration, conventional liposomes are unable to avoid being engulfed by the mononuclear phagocytic system. Delivery methods have been thought of to avoid their quick elimination from the circulation. The creation of so-called long-circulating liposomes, also known as "stealth liposomes", one of the most researched methods, was made possible by covering the liposome surface with polyethylene glycol. Other tactics included attaching various ligands, including as immunoglobulins, glycoproteins, transferrin, peptides, folate, etc., to the liposome surface in order to target them more specifically to overexpressed receptors at afflicted areas. To functionalize the exterior of liposomes, other molecules, such as enzymes, may be covalently joined to PEG chains. In addition, Cevc and Blume originally described an exclusive kind of liposome known as ultradeformable vesicles in 1992 and gave it the name Transfersomes , consisting of unilamellar vesicles containing a surfactant responsible for their deformability . These nanocarriers were created especially for the cutaneous and transdermal delivery of drugs. The lipid composition used for liposome production determines their properties. As a result, they may exhibit neutral, positive, or negative surface charges. Higher aggregation is anticipated for non-charged, non-coated liposomes since they are often less stable. The intermembrane repulsion is increased and the tendency of liposomes to assemble is decreased by charged liposomal formulations, on the other hand [8]–[10].

In disorders affecting the liver and spleen, negatively charged vesicles were employed because they interact more favorably with the MPS. Since negatively charged nucleic acids electrostatically interact with positively charged vesicles to enable cell transfection, cationic liposomes were first designed for gene transport. Other uses include delivering chemotherapeutic medications to established tumor blood vessels or allowing pharmaceuticals to effectively cross the blood-brain barrier and enter the central nervous system. The creation of stimulus-responsive liposomes, which may be tailored to release the medications they contain in the presence of certain stimuli like an in vivo pathogenic trigger, is another targeting technique that has been created. The stimuli may be produced by the tissue itself or by outside forces. Phosphatidyl choline from egg yolk or soybean, hydrogenated phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidyl serine, and phosphatidic acid are the phospholipids often utilized to form liposomes. More stable phospholipids are produced by alteration of the nonpolar and polar areas than in the corresponding native forms. PEG may be replaced with other substances such poly, heparin, dextran, and chitosan. Antioxidants and complexing agents can also be added to liposomes to boost their chemical stability. Additionally, cyclodextrins may be effectively used to include hydrophobic molecules into the aqueous core of vesicles.

The most popular liposome categories, while there are others, are based on structural characteristics, production techniques, or in vivo behavior. The number of lipid bilayers, which may be uni, multi, or plurilamellar, is commonly used to categorize liposomes. Drug

loading or drug incorporated per amount of lipid in liposomal form, captured volume, captured volume, and incorporation/encapsulation efficiency, expressed as a percentage and corresponding to the quotient between the final and initial ratios of incorporated subunits are all characteristics of vesicles. The traditional method of making liposomes is incredibly straightforward and involves dissolving a mixture of the lipid constituents including any bioactive agents that are hydrophobic in a compatible organic solvent, followed by drying the mixture in a flask with a flat bottom while applying reduced pressure or a stream of nitrogen to create a lipid film. In order to create liposomes in suspension, an aqueous solution must be added while being stirred and must include the bioactive substance if it is hydrophilic. Multilamellar vesicles, also known as multilamellar liposomes or multilamellar liposomes containing the integrated chemical, are produced using this technique. This technique of production, although being straightforward, has some limitations since the liposomes it produces have a wide range of mean sizes and poor integration rates.

By subjecting big liposomes, such as MLV, to the action of ultrasound or extrusion, small unilamellar vesicles, also known as SUV, may be produced. Following the solubilization of the lipid mixture and the bioactive ingredient in detergent and its gradual removal by dialysis, large unilamellar vesicles may be produced. All of these liposome types MLV, LUV, and SUV are referred to as conventional or from the first generation, and they were gradually superseded by more advanced ones that permitted the integration of larger solute concentrations. One of these preparation methods is lyophilizing liposomes that have the integrated material in them, followed by controlled rehydration. The integration efficiency of this sort of liposomes, known as dehydration-rehydration vesicles, may rise from around 10% in conventional liposomes to 90%. They are one of the first significant milestones in liposome technology. A totally distinct technique known as "active loading" enables the inclusion of bioactive substances into liposomes that have already been created while maintaining all of their structural integrity. This only applies to bioactive substances that are weak bases or acids, however. To homogenize the vesicle size of liposomes, liposome solutions prepared using any technique are extruded [11], [12].

Encapsulating enzymes in liposomes is a twofold technique designed to both target enzymes in vivo and prevent them from degrading. In order to ensure that both an enzyme-loaded liposome and a free enzyme displayed a conventional Michaelis-Menten profile, enzyme entrapment techniques have recently been predicated on the kinetic parameters of the enzyme as well as the optimal pH and temperature. Additionally, liposomes loaded with enzymes have been prepared using microfluidics technology. Superoxide dismutase-loaded liposomes with great encapsulation efficiency and preservation of the enzyme's biological activity have recently been produced using microfluidics. This preparation process has benefits over traditional preparation methods, including a minimal time commitment and an easy method for microfluidic assembly that can be scaled up.

An oxyreductase enzyme called catalase has antioxidant properties and works by reducing the amount of hydrogen peroxide that is created during inflammatory processes. The nanoformulation was able to cause tumor hypoxia after being co-loaded in liposomes with a cytotoxic agent, cisplatin, producing a synergistic therapeutic effect. In a different work conducted by Hei and colleagues, catalase was also included in liposomes to combat tumor hypoxia while displaying at their surface a monoclonal antibody against programmed death ligand 1 to improve immunotherapeutic effects against melanoma. This combined treatment improved liposome accumulation at tumor locations, inhibited tumor growth, and increased induced animal survival rates. A class of metallocenzymes known as superoxide dismutases works as an antioxidant for the treatment of illnesses caused by reactive oxygen species.

Superoxide dismutase has been investigated as a potential therapeutic agent for more than 30 years in several animal models and a few successful human studies. Although superoxide dismutase may have therapeutic benefits, its quick removal from the bloodstream with a plasma half-life of 6 and 20 minutes in rats and humans, respectively restrains it's in vivo application. Superoxide dismutase has been chemically modified or associated with polymeric or lipid-based systems, such as liposomes, in order to increase circulation time and, as a result, enhance treatment outcomes. In many animal models, including the rat model of adjuvant arthritis, the ear oedema model, and the ischemia reperfusion injury, the incorporation of superoxide dismutase in liposomes with various properties has led to higher therapeutic activity in comparison to the respective free form.

A summary of many therapeutic experiments using standard liposomes to deliver superoxide dismutase may be found elsewhere. After being exposed to hyperoxia in a rat model, the i.v. Superoxide dismutase-containing traditional liposome injections provided effective protection against oxygen poisoning. In a mouse model of rheumatoid arthritis, the addition of SOD to conventional liposomes improved therapeutic efficacy. The kind and average size of the liposomes that were potentiated for formulations with enhanced blood circulation qualities were responsible for this in vivo result. The therapeutic efficacy of the superoxide dismutase long circulation liposomes was further boosted in comparison to the standard formulation by the improvement in pharmacokinetic characteristics and the improved passive targeting ability to inflamed areas. Superoxide dismutase liposomes' mean size and method of delivery revealed that size is a crucial factor in allowing the vesicles to be drained from the s.c. administration location. In actuality, superoxide dismutase liposomes s.c. had a less therapeutic effect. Compared to the identical superoxide dismutase formulation given intravenously, the mean size of the supplied dose was 450 nm. Attained was injected. When using tiny liposomes, these changes were not seen. These various in vivo outcomes were linked to the various bloodstream superoxide dismutase concentrations [13], [14].

The combination of enzyme chemical alteration followed by inclusion in liposomal lipid bilayer was researched in order to enhance the therapeutic impact of superoxide dismutase. When compared to the unmodified form of superoxide dismutase liposomal formulation, these enzymatic nanoformulations, called enzymosomes, displayed a faster therapeutic effect in the rat model of adjuvant arthritis. This is because the chemically modified enzyme, which is partially exposed at the vesicles surface, is able to exert its enzymatic activity without the need for prior release from the nanosystem. Superoxide dismutase is covalently linked to the lipid component's terminal in another combination strategy to achieve long-circulating liposome characteristics and demonstrate superoxide dismutase activity at the nanosystem's exterior. The extended circulation period of the nanosystem was not hampered by the enzyme's presence at the liposomal surface. Due to its uniqueness, superoxide dismutase encapsulated in liposomes had a later therapeutic action in the rat model of adjuvant arthritis. The therapeutic action is required during the first 24 h, which is not possible when superoxide dismutase is encapsulated in the internal aqueous compartment of liposomes inhibiting its release at such an early time-point. More recently, superoxide dismutase-enzymosomes were more effective in reducing IRI in comparison to superoxide dismutase encapsulated in liposomes.

Non-invasive ways of delivery of superoxide dismutase nanoformulations have also been researched in addition to parenteral administration. On the back of rats with adjuvant-induced arthritis, a superoxide dismutase lipid-based nanoformulation was applied epicutaneously in one of these cases. In this instance, enzyme-loaded deformable lipid vesicles called Transfersomes that were created specifically for transdermal distribution were able to reduce

animal illness symptoms including paw edema and inflammatory hematological and biochemical markers in contrast to provoked and untreated animals. Additionally, it was shown that superoxide dismutase-loaded Transfersomes were able to collect in inflamed paws after being applied epicutaneously and enter blood circulation in a therapeutic dosage. A recombinant version of superoxide dismutase was packaged in negatively charged liposomes and demonstrated a decrease in colon inflammation in an experimental colitis model. In addition, Vorauer-Uhl and colleagues found that rh-CuZn-superoxide dismutase liposomal formulation reduced skin swelling and prevented a fully necrotic zone of stasis when compared to direct rh-CuZn-superoxide dismutase injection into the lesion or by spreading an rh-CuZn-superoxide dismutase gel.

After topical treatment to the arachidonic acid-induced mouse ear oedema model, the anti-inflammatory activity of superoxide dismutase-loaded, catalase-loaded, and co-encapsulated superoxide dismutase/catalase in ultradeformable vesicles was assessed. The biological activity was contrasted with enzyme solutions and traditional vesicles that had been filled with enzymes. As a consequence of their co-association with deformable vesicles, both enzymes were better able to penetrate the intact epidermal barrier and exert their therapeutic impact. Urokinase is a serine protease, whereas streptokinase is a protein that breaks down fibrin. They are enzymes that are often used to treat various thrombosis conditions, including myocardial infarction, acute cerebral infarction, and pulmonary embolism. Urokinase works by catalyzing the creation of plasmin, which results in the breakdown of the fibrin mesh structure in blood clots. Streptokinase promotes bond cleavage to create plasmin. Their shortened biological half-lives are significant limitations that restrict therapeutic application. Streptokinase loaded in long circulating liposomes was used in pharmacokinetic studies that led to an increase in the enzyme's half-life in comparison to the corresponding free form, and higher thrombolytic activity than the free enzyme was attained in a human clot inoculated rat model. When compared to the free enzyme, the lipid nanoformulation was much more effective in binding to activated platelets and releasing the payload. In a research by Zhang and colleagues, urokinase was added to a cyclic RGD and functionalized long circulating liposomes, which increased the enzyme's half-life. In a mouse mesenteric thrombosis model, this nanoformulation demonstrated a 4-fold greater thrombolytic activity when compared to urokinase in the free form.

The enzyme uricase catalyzes the breakdown of uric acid into byproducts that may be quickly excreted by the kidneys. The clinical use of uricase has been constrained, as has been shown with other therapeutic enzymes, by inappropriate biological characteristics, including early degradation and inactivation by endogenous proteases, among others. Uricase inclusion in liposomes led to an extended half-life, improved uric acid reducing effectiveness, and reduced immunogenicity compared to free enzymes, following a common technique previously observed for other enzymes.

CONCLUSION

Although enzymes have been used as therapeutic agents for over a century, their utilization as biopharmaceutics is still far from what would be anticipated for such a powerful family of chemicals. This fact has been linked to negative side effects and a brief residence period in living things, both of which are problems that must be resolved. On the plus side, advances in DNA technology now make it possible to produce enzymes with the right amounts and properties for therapeutic usage. However, by combining DDS, or liposomes, with pharmaceutical nanotechnologies, it is now feasible to alter how enzymes are presented to human cells, enhancing the therapeutic efficacy of these cells. In spite of all the advantages of including therapeutic enzymes in liposomes, there are always significant obstacles to

overcome throughout the transition from the lab to the clinic. In order to retain the biological activity of enzymes as well as to prevent immunogenic reactions, one of the primary challenges is connected to the preservation of the protein 3D structures. Each enzyme's lipid composition must be carefully chosen throughout the construction of the nanoformulation in order to optimize loading and preserve the integrity of the enzyme.

Additionally, the compositions of liposomal membranes must be developed to deliver the enzyme with the proper biodistribution profile for the particular organ or tissue that it is meant to target. The liposomal membrane's chemical and physical stability presents another difficulty. In reality, problems with liposome stability that were seen for traditional liposomes, which were mostly employed in the 1980s and 1990s, have been resolved as a consequence of vigorous academic efforts, leading to the development of many approaches to produce stable liposomes.

Furthermore, the mass manufacture of liposomal formulations is a tried-and-true method that has previously resulted in a number of commercially successful products. There are more than a dozen liposomal nanoformulations that have been successful in clinical usage. This clinical offer is anticipated to grow as a result of the liposome technology's ongoing development and complexity, as well as its expanded range of applications. These DDS will continue to play a key role in introducing novel enzymatic nanomedicines to the market since liposome technology is now established. We firmly think that enzymatic treatment will take its proper position in the batteries of the next generation of biomedicines after being properly formulated in DDS, such as liposomes.

REFERENCES:

[1] D. B. Oh, “Glyco-engineering strategies for the development of therapeutic enzymes with improved efficacy for the treatment of lysosomal storage diseases,” *BMB Reports*. 2015. doi: 10.5483/BMBRep.2015.48.8.101.

[2] H. Xiong *et al.*, “Nanosomal microassemblies for highly efficient and safe delivery of therapeutic enzymes,” *ACS Appl. Mater. Interfaces*, 2015, doi: 10.1021/acsami.5b05758.

[3] C. E. Callmann, C. V. Barback, M. P. Thompson, D. J. Hall, R. F. Mattrey, and N. C. Gianneschi, “Therapeutic Enzyme-Responsive Nanoparticles for Targeted Delivery and Accumulation in Tumors,” *Adv. Mater.*, 2015, doi: 10.1002/adma.201501803.

[4] F. Dabbagh, M. B. Ghoshoon, S. Hemmati, M. Zamani, M. Mohkam, and Y. Ghasemi, “Engineering Human Urate Oxidase: Towards Reactivating It as an Important Therapeutic Enzyme,” *Curr. Pharm. Biotechnol.*, 2015, doi: 10.2174/1389201016666150907113055.

[5] F. Cheng *et al.*, “A Competitive Flow Cytometry Screening System for Directed Evolution of Therapeutic Enzyme,” *ACS Synth. Biol.*, 2015, doi: 10.1021/sb500343g.

[6] H. Xie, J. K. Chung, M. A. Mascelli, and T. G. McCauley, “Pharmacokinetics and bioavailability of a therapeutic enzyme (idursulfase) in cynomolgus monkeys after intrathecal and intravenous administration,” *PLoS One*, 2015, doi: 10.1371/journal.pone.0122453.

[7] M. Sanzey *et al.*, “Comprehensive analysis of glycolytic enzymes as therapeutic targets in the treatment of glioblastoma,” *PLoS One*, 2015, doi: 10.1371/journal.pone.0123544.

[8] L. P. Cousens, L. Moise, and A. S. de Groot, "Novel methods for addressing immunogenicity of therapeutic enzymes," *AAPS Adv. Pharm. Sci. Ser.*, 2015, doi: 10.1007/978-1-4939-2543-8_5.

[9] B. W. Bigger, M. Saif, and G. E. Linthorst, "The role of antibodies in enzyme treatments and therapeutic strategies," *Best Practice and Research: Clinical Endocrinology and Metabolism*. 2015. doi: 10.1016/j.beem.2015.01.006.

[10] Y. Liu, J. Li, and Y. Lu, "Enzyme therapeutics for systemic detoxification," *Advanced Drug Delivery Reviews*. 2015. doi: 10.1016/j.addr.2015.05.005.

[11] B. Sun, E. Brooks, and D. Koeberl, "Preclinical Development of New Therapy for Glycogen Storage Diseases," *Curr. Gene Ther.*, 2015, doi: 10.2174/1566523215666150630132253.

[12] M. L. Corvo *et al.*, "Superoxide dismutase enzymosomes: Carrier capacity optimization, in vivo behaviour and therapeutic activity," *Pharm. Res.*, 2015, doi: 10.1007/s11095-014-1447-7.

[13] J. Lopez-Pastrana, Y. Shao, V. Chernaya, H. Wang, and X. F. Yang, "Epigenetic enzymes are the therapeutic targets for CD4+CD25+/highFoxp3+ regulatory T cells," *Translational Research*. 2015. doi: 10.1016/j.trsl.2014.08.001.

[14] K. M. Lorentz, S. Kontos, G. Diaceri, H. Henry, and J. A. Hubbell, "Engineered binding to erythrocytes induces immunological tolerance to E. coli asparaginase," *Sci. Adv.*, 2015, doi: 10.1126/sciadv.1500112.

CHAPTER 11

RECENT ADVANCES IN ENZYME-MEDIATED WASTEWATER REMEDIATION

Mohamed Jaffar A, Professor
Department of ISME, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- mohamed.jaffar@atlasuniversity.edu.in

ABSTRACT:

Diverse bodies of water, including lakes, rivers, and oceans, have been shown to contain several kinds of manufactured pollutants, often known as emerging pollutants. Numerous studies have shown the catastrophic consequences that these new contaminants may have on aquatic and terrestrial life. The primary cause of these newly emerging contaminants in the aquatic environment is their insufficient removal in the wastewater treatment facilities that are now in operation. Several additional treatments, including a variety of physicochemical and biological techniques, may be added to current WWTPs to help remove these contaminants. The usage of enzymes, particularly oxidoreductases, is being investigated more and more for its potential to degrade many kinds of organic molecules. To encourage their implementation as a practical and recyclable remediation technique, these enzymes have been immobilized on various substrates. Sadly, several of these methods have been demonstrated to have detrimental effects on the enzyme, including denaturation and loss of catalytic function. This review focuses on current developments in this field as well as the main obstacles that researchers working on peroxidase immobilization face. It focuses on four key areas: the stability of enzymes following immobilization, enzyme engineering, and enzyme evolution; recyclability and reusability, including immobilization on membranes and solid supports; the cost of enzyme-based remediation; and scaling-up and bioreactors.

KEYWORDS:

Enzymes, Immobilization, Pollutants, Water.

INTRODUCTION

Recent years have seen a dramatic rise in pollution due to the ongoing growth of the global population. The environment is continually being impacted by new difficulties and issues. A significant issue is emerging pollutants or contaminants of emerging concerns. Diverse types of synthetic organic chemicals that are being found in diverse water bodies across the globe are considered emerging contaminants. Current water quality laws do not cover these contaminants. Pesticides, medications, personal care items, hormones, and dietary additives are some of them. Numerous studies have shown how these contaminants may have an impact on aquatic and terrestrial life. According to some assessments, women's reproductive capacities are declining and their chance of breast cancer is rising. For instance, the insect repellant N, N-diethyl-meta-toluamide may cause the central nervous system enzyme acetylcholinesterase to be inhibited in both mammals and insects. High levels of emerging contaminants have been found in lakes, rivers, and oceans by several investigations. From ng/L to a few hundred g/L, developing contaminants are present in several water bodies. Various pharmaceuticals, such as metoprolol, ibuprofen, bezafibrate, and naproxen, have been detected in ground water at various amounts [1], [2].

Alphamethrin, Fluometuron, Lambda-cyhalothrin, and Lindane have all been identified as pesticides in Lake Vistonis, with values of 0.161 g/L, 0.088 g/L, 0.041 g/L, and 0.030 g/L,

respectively. It should come as no surprise that the scientific community and scholars have given the existence of developing contaminants in various water bodies throughout the globe a lot of attention. An overview of the literature on certain new contaminants identified in water at high amounts. The environment is severely harmed and damaged by emerging contaminants. The constant influx of EPs into the ecosystem, which might reduce the pace of removal and transformation, is another problem. The poor removal of EPs from wastewater treatment facilities is the main source of EPs in the environment, including ground and drinking water. As a result, a significant amount of EPs and their metabolites elude removal in WWTPs and enter aquatic ecosystems. This is because WWTPs are not intended to get rid of these chemical compounds. How these substances get into aquatic bodies is summarized. Emerging contaminants provide a difficulty since there is a lack of ecotoxicological and risk assessment data. It is also challenging to predict the effects of new contaminants on aquatic ecosystems because we lack analytical capabilities to identify trace quantities of these pollutants. With further toxicological and analytical study on these chemicals, these difficulties are being solved. For instance, sensitive methods for identifying EPs at trace quantities in environmental matrices include liquid chromatography with mass spectrometry and gas chromatography with mass spectrometry.

Removal of Emerging Pollutants Using Different Techniques

To remove developing contaminants from various water sources, including drinking water, groundwater, and wastewater, and therefore lessen their danger to humans and marine life, several techniques and strategies have been devised. Physical, chemical, and biological treatments are the three basic methods used. Additionally, there is a hybrid system that uses two or more different types of treatment to get rid of developing contaminants. Filtration, coagulation, and adsorption are physical processes. Advanced oxidation processes, electrolysis, and ozonation are examples of chemical procedures. Enzymes and microorganisms are used in biological processes [3], [4].

Advanced oxidation processes are the most well-known chemical techniques. In this procedure, an oxidizing chemical is used, and both ultrasonic and ultraviolet radiation are used to activate it throughout the process. The principal oxidizing agent used in AOPs is hydrogen peroxide. The first step in treatments is the production of hydrogen peroxide radicals, which start oxidation processes against emerging pollutants and cause pollutants to degrade into less dangerous and safer chemicals. According to research, the breakdown of the antibiotic sulfamethoxazole using UV radiation and H2O2 resulted in two intermediates that were much less harmful to L. than sulfamethoxazole, stavia seeds. Chemical approaches have a number of drawbacks, including the formation of a lot of sludge, the potential for toxic byproduct development, and high operating costs. Ozone, for instance, may be expensive to utilize in the chemical procedure of ozonation since a constant supply is required, and O3 only has a 20-minute half-life.

DISCUSSION

Adsorption is a physical technique for clearing water of developing contaminants. It is possible to utilize a variety of sorbents, including activated carbon, wood chips, peat, zeolites, and silica gel. Adsorption methods have been shown to be very effective in getting rid of and removing organic pollutants. Adsorption has a few advantages over other methods, one of which is its low operating cost, which makes it economically feasible for industrial scale-up. Activated carbon is the sorbent that is most often used to remove organic pollutants from water. The kind and quantity of carbon employed determine this method's efficacy.

Membrane separation is another physical technique for removing contaminants from water. Numerous benefits come with this approach, including minimal maintenance requirements, straightforward functioning, and simple installation. However, this approach has significant drawbacks, such as a costly initial investment and the potential for membrane blockage. Today, if the water being treated contains a little quantity of color, membrane separation may be employed for water treatment in textile facilities. Another form of physical technique is ion exchange. The capacity to recover the solvent and the adsorbent is one benefit of ion exchange. Its downsides include its expensive price and poor efficacy. Physical and chemical processes still face significant difficulties and have some restrictions, although now operating on a big scale. These technologies' high energy and operating costs, as well as the substantial amounts of sludge created during the reactions, are their main obstacles.

The employment of biological techniques, which make use of enzymes and microorganisms like bacteria, is a more environmentally friendly option. Compared to physiochemical approaches, bioremediation is seen as being safer, more ecologically friendly, less disruptive, and less expensive. However, there are several drawbacks to using biological techniques for water treatment. The effectiveness of biological approaches may be decreased by harsh environmental conditions since they may disrupt the organisms utilized.

Mutagen-Based Bioremediation

The adaptability and activity of the microorganisms have a role in the effectiveness of microbial bioremediation. The capability of microorganisms to digest new contaminants has been shown in several research. These investigations have been made on various bacterial species to learn more about their functions in the breakdown of dyes, especially azo dyes. Low cost, environmental friendliness, and less sludge and mineralization are benefits of utilizing bacteria in color degradation. For instance, it was discovered that a Bacillus cereus isolate from petroleum waste was able to break down a variety of aromatic dyes, including reactive black 5, toluidine blue, ponceau BS, and Congo red. The capacity of Bacillus cereus to break down numerous developing contaminants, including Fluometuron, Sulfamethoxazole, and Prometryn, was also shown in the same research [5], [6].

Typically, the azo linkage must be broken by enzymes like laccase and azoreductase that are created at the start of the stationary phase in microbial development in order for bacteria to degrade azo dyes. After 120 hours of incubation with an isolated strain of Pseudomonas entomophila BS1, Khan and Malik were able to breakdown 93% of reactive black 5. Another research looked at how Pseudomonas rettgeri strain HSL1 and Pseudomonas species SUK1 degraded four distinct azo dyes. According to Nnenna et al, bacteria may break down the medicines ciprofloxacin and erythromycin. While erythromycin works against Gram-positive bacteria, ciprofloxacin only works against Gram-negative bacteria. Wastewater treatment facilities have been discovered to contain these antibiotics. Some isolated bacteria, including Shigella sp., Micrococcus sp., and Pseudomonas sp. These antibiotics were successfully biodegraded by and Bacillus sp. The breakdown of these antibiotics by the bacterial strains was shown to be unaffected by pH variations. Oxygen, wetness, and the absence of additional sources of nitrogen and carbon have all been shown to have significant effects on therapy. Oxygen, pH, the concentration of the pollutant, as well as its structure, redox mediator, electron donor, temperature, and other sources of nitrogen and carbon, may all have an impact on the bacterial breakdown of pollutants. For instance, oxygen may have an influence on microbial breakdown since it is essential for cell development, which affects the physiological properties of the cell. Excess oxygen may obstruct the breakdown of pollutants by acting as an electron acceptor. Oxygen is regarded as lethal for anaerobic bacteria and might directly block azoreductase enzymes. The optimal pH for dye degradation by bacteria

is a neutral pH, and when pH values are excessively acidic or alkaline, the rate of degradation substantially decreases. Other biological variables may have an impact on the bioremediation process in addition to the physiochemical ones. A plasmid's genes, which encode particular enzymes and give substrate specificity, may be responsible for several fundamental characteristics of bacteria that impact their capacity to break down various substrates. Bacterial chemotaxis, which is seen as a benefit to improve bacteria's capacity to break down resistant organic molecules, is another element. Additionally, intricate interacting networks of distinct microbial communities may be necessary to effectively breakdown different chemicals and improve the bioremediation process in general. The efficacy of microbial bioremediation has been improved by the use of two frequently used techniques. One method is known as "bioaugmentation," which entails the inclusion of pre-grown microbial cultures to speed up the breakdown of pollutants. Another method, known as biostimualtion, involves introducing nutrients and other additional components into the local microbial community in order to encourage growth and reproduction. By accelerating the degradation process, these techniques may increase the effectiveness of bioremediation [7]–[9].

Even though bioremediation is a great technology for cleaning up contaminated environments, there are still some issues that need to be taken into account, such as how these microorganisms grow, how they act, how difficult it is to cultivate them, and how their regulatory systems work. Recent developments in omics technologies, as well as in-silico analyses, have addressed these issues by making it possible for researchers to gather biological data about the microbial communities that live in contaminated environments, their physiological and cellular mechanisms, and the enzymes involved in bioremediation. The chemistry and routes must thus be understood in order to improve environmental monitoring and bioremediation effectiveness.

The use of peroxidase and laccase enzymes in enzymatic bioremediation

A technique for cleaning up water is enzymatic bioremediation. Oxidoreductases are the most popular enzymes used in enzymatic bioremediation. Laccase and peroxidase enzymes are members of this family of enzymes. Multicopper enzymes known as laccases are abundantly found in fungi and plants throughout nature. Due to their capacity to oxidize a variety of phenolic and nonphenolic chemicals, laccase enzymes are useful in a variety of biotechnology applications. Application areas for laccase enzymes in water treatment are many. The endocrine disruptor bisphenol A was effectively digested by Asadgol et al. utilizing pure laccase enzyme from Paraconiothyriumvariabile. Approximately 60% of the pollutant was destroyed by the enzyme after 30 minutes of treatment. The Trametes versicolor laccase enzyme was used by Hongyan et al. to breakdown bisphenol A. Using laccase in synthetic water and municipal wastewater effluent, Auriol et al. showed the oxidation of three natural estrogens, estrone , 17-estradiol , and estriol , as well as a synthetic estrogen, 17-ethinylestradiol. This investigation concentrated on the impact of the wastewater matrix on laccase-mediated treatment, even though the ideal pH for the elimination of these hormones in synthetic water was 6.0. The matrix of the effluent had no discernible impact on the treatment at pH 7 and 25 1 °C. Additionally, both synthetic water and municipal wastewater completely lost these hormones when 20 U/mL laccase was present. Antimicrobial triclosan has been discovered in a variety of sediments and water surfaces. The laccase enzyme was able to degrade 56.5% of this pollutant in 24 hours without the use of a redox mediator. The breakdown of triclosan improved to around 90% when tested with the two separate redox mediator’s 1-hydroxybenzotriazole and syringaldehyde, which led to the production of low-molecular-weight transformation products. When compared to the original pollution, the toxicity of those transformation products was either much reduced or harmless.

Additionally, several research have shown how laccase enzymes may be used to break down and remove different kinds of aromatic dyes [10], [11].

Peroxidases are the other oxidoreductase enzymes used in water purification. These enzymes are widely spread throughout nature and are mostly found in bacteria and plants. The great heat stability and high redox potential of peroxidase enzymes are two of its distinguishing characteristics. Heme peroxidases and nonheme peroxidases are the two primary classes into which these enzymes are divided. When hydrogen peroxide or other peroxides are present, heme peroxidase enzymes have a wide spectrum of contaminants they may oxidize. This reaction's process begins with a native peroxidase interacting with hydrogen peroxide to create "Compound I," which is a peroxidase in its cation radical form. This radical form of the enzyme, Compound I, may react with a variety of contaminants to create radicals, which can subsequently be converted to Compound II, the second form of the enzyme. Compound II, the enzyme in this form, may undergo further reactions with toxins before reverting to its original state.

For use in water cleanup, peroxidase enzymes have undergone extensive research. A substantial body of research demonstrates peroxidases' capacity to convert hazardous developing contaminants into safer intermediaries. An analysis of the degradation of a thiazole pollutant by chloroperoxidase and the UV light + H_2O_2 technique. The results showed that the intermediates created by the CPO enzyme were much less hazardous than those created by the UV light + H_2O_2 approach. This finding supports the superior and safer choice of using enzymes for water cleanup. CPO, lactoperoxidase , and lignin peroxidase are a few peroxidases that have been employed in water remediation . However, using enzymes may have several disadvantages, including enzyme reusability and enzyme stability during the course of water cleanup. Enzyme immobilization may help with some of these problems.

Immobilization of Enzymes

An enzyme is said to be immobilized when it is attached to an inert, insoluble support material, which results in a partial or complete loss of the enzyme's mobility. It offers a variety of benefits, such as stability, reusability, the capacity to recover enzymes, and improved pH and temperature tolerance. When reducing the acid orange 7 dye, It was found that immobilized horseradish peroxidases on calcium alginate cell beads were more durable in terms of pH and temperature than free HRPs. On the other hand, due to confirmational changes in the enzyme, steric hindrance, and mass transfer limits, enzyme immobilization might result in problems, such as a reduction in enzymatic performance. These and other factors make it necessary to create new techniques and materials that improve upon the drawbacks of various immobilization techniques. There are two main methods of immobilization. The physical attachment of the enzyme to the support materials is one method, while chemical binding is another. There are four distinct strategies that fall under these two categories: trapping, adsorption, covalent attachment, and cross-linking.

There are two types of physical immobilization: adsorption and entrapment. These two techniques rely on weak contacts between the support and the enzyme, such as van der Waals forces and ionic binding. Physical immobilization may aid in maintaining enzyme function since it has no impact on the natural enzyme structure. Through hydrogen bonds, van der Waals forces, and ionic binding, the enzyme is physically attached to the support surface during adsorption. This technique for immobilization is the most straightforward and is also reversible. Additionally, it is the most often utilized method for scaling-up in industrial applications and is inexpensive and simple to make. In order to show the immobilization of the lipase enzyme on sol-gel dried silica, Lee et al. used carbon nanotubes. Immobilized

lipase exhibited superior activity and a longer lifespan, according to the findings. Both inorganic and organic support materials may be used in the adsorption process. Silica is one of the inorganic support materials. Mesoporous silica SBA-15 with hexagonal arrays of holes and pore sizes ranging from 5 to 30 nanometers in diameter is the kind of silica that is often employed on a wide scale. Carriers that have high mechanical strength and thermal stability are silica gels. The typical silica gel particle size ranges from 70 to 150 m. Chitosan, calcium alginate, cellulose, and agarose gel are utilized as organic carriers.

Specific functional groups on both the enzyme and the carrier must exist for the adsorption of the enzyme on the support material to be effective. If these groups are absent, effective immobilization may be achieved by chemical modification. A modifying agent having at least two reactive groups is used to alter chemicals. One of these groups is required to physically engage with the enzyme in order to immobilize it, while the other group is required to chemically link the modifying group to the support. Having two aldehyde groups, glutaraldehyde is one of the most often used modifying agents. Entrapment is yet another physical method of immobilizing enzymes. Despite being physical, this process cannot be reversed. Since the enzyme is contained inside a porous matrix support, other products may flow through it but not the enzyme. Metal-organic frameworks, gel/fiber entrapment, and microencapsulation are examples of entrapment. Due to the enzyme's sheltering from severe circumstances and protection from denaturation, entrapment significantly increases both thermal and storage stability. The entrapment approach was used by Cui et al. to demonstrate that CPO immobilized on Fe3O3 magnetic nanoparticles successfully degraded more than 90% of the aniline blue dye.

Chemical immobilization methods include cross-linking and covalent adhesion. These methods employ substances like glutaraldehyde to form a covalent link between the support material and the enzyme. This may cause the enzyme's conformation to alter and block the active site, which would reduce the enzyme's activity. However, stiffness and strong chemical bonding make up for this. The covalent attachment approach is characterized by the creation of a covalent link between the support material and the functional groups of the enzyme, such as amino, carboxylic, and hydroxyl groups. Covalent attachment was employed by Bilal et al. to immobilize HRP on a calcium alginate substrate. The findings demonstrated that fixed HRP was more enzymatically stable and efficient than free HRP. One of the reasons this method outperforms other forms of immobilization is because covalent connection may prevent enzyme leaching, keeping the enzyme intact. Cross-linking, a different technique for chemical immobilization, creates intermolecular cross-links between the enzymes utilized by substances like glutaraldehyde and diazonium salt rather of using support materials or matrices. Sun et al. used the cross-linking agent diethylene glycol diglycidyl ether to immobilize HRP on nanocomposites. Improved durability, enhanced activity, reusability, and resistance to microbial assault were all shown by immobilized HRP. Enzyme denaturation during the immobilization process, which results in the enzyme losing its catalytic activity, is one disadvantage of this method. This method also has large operating expenses and makes it difficult to manage the response.

Major Obstacles and Recent Developments in Enzyme-Based Methods

It is well acknowledged that the greatest obstacle to employing free enzymes on both a laboratory and commercial scale is their poor stability when stored or exposed to severe environments, such as high and low pH, high temperature, organic solvents, ionic liquids, and oxidizing agents like H2O2. However, enzyme immobilization, enzyme engineering, and enzyme evolution are developing into effective techniques for enhancing enzyme stability in many conditions. To address the limited stability of free enzymes, many research groups have

been drawn to immobilization techniques. In terms of pH, immobilization has often led to the immobilized enzyme being active at various pH values, a change in the optimal pH, or a wider profile with no improvement. However, stability under other circumstances might be significantly enhanced. In order to immobilize HRP, Rong et al. created a multiarmed magnetic graphene oxide composite. The stability of immobilized HRP in storage was examined in the research. The immobilized HRP maintained 85.5% of its original activity after 30 days and 72.5% after 60 days of cold storage at 4 °C. However, free HRP maintained just 10.2% of its activity after 60 days while maintaining 42.3% of its activity after 30. Additionally, immobilization significantly increased the enzyme's thermostability.

Melamine, an amine, and triformylphloroglucinol, an aldehyde, were used in the Schiff base reaction to create a covalent organic framework that effectively immobilized -amylase. The enzyme was immobilized using a simple adsorption technique, which improved its thermal stability at a higher temperature of 90 °C compared to the free form of the enzyme. Laccase enzyme was immobilized by Wu et al. on a Fe3O4-NH2@MIL-101 magnetic metal-organic framework. By combining adsorption and covalent bonding techniques, laccase was immobilized. This led to high laccase activity recovery, improved acidic pH tolerance, increased temperature, great thermostability, and excellent storage stability. The laccase enzyme retained 98% of its original activity after 28 days of storage. After six hours at 85 °C, 49.1% of its initial activity was still present. Additionally, the enzyme's stability in several organic solvents, including methanol, ethanol, acetonitrile, acetone, and dimethyl sulfoxide, was significantly enhanced. For instance, in such chemical solvents, free laccase lost more than 70% of its activity in only 12 hours.

When immobilized laccase was kept in methanol, ethanol, and acetonitrile for storage, there was around 80% relative activity left after immobilization. Impressively, when it was incubated in acetone and dimethyl sulfoxide, it retained more than 90% of its activity. In order to remove the contaminant 2, 4-dichlorophenol from water, the laccase-MOF was used. After 12 hours, it was 87% effective in removing pollutants from water, with the MOF helping to increase the rate of adsorption during the first hour. After the reaction was finished, a magnet could be used to extract the immobilized enzyme from the reaction fluid. Furthermore, it was examined if the laccase-MOF could maintain its adsorption and degradation capacities in the presence of various salt concentrations. The MOF's adsorption was impacted by the salt content, but laccase's propensity to degrade was largely unaffected since immobilization increased its structural stability.

Ionic liquids have recently gained popularity as a safe substitute for poisonous, dangerous, extremely flammable, and volatile organic solvents, especially for enzyme-based applications. This is because ILs have a number of appealing benefits, such as improved catalytic activity, solubility, and stability. These benefits heavily rely on ILs being biocompatible, since some of them may be harmful to enzymes. However, by adjusting the cation and anion combinations and/or the attached substituents, their toxicity may be reduced. Immobilization of enzymes has a tremendous potential to improve the stability of enzymes in ionic liquids, in addition to the creation of microemulsions by mixing enzymes with a suitable surfactant to overcome IL restrictions. For instance, laccase Y20 was coated with poly -block-polylactide using water in an oil emulsion. Studying PEG-PLA-laccase's activity and stability in an ionic liquid—specifically, 1-ethyl-3-methylimidazolium hexafluorophosphate—resulted in the discovery that free laccase had somewhat greater enzymatic activity than PEG-PLA-laccase. This could be because employing a polymer has a mass transfer constraint; however, this limitation can be removed by using substrates that are IL soluble. However, the synthesized polymer-laccase demonstrated a much greater level of

storage stability in ILs. For instance, after 12 hours of storage in an IL at 40 °C, about 70% of the initial activity of PEG-PLA-laccase was still present, but only around 20% of the original activity of free laccase was still present. The authors suggested that the increased structural stiffness of the glycoprotein was responsible for the decrease in enzymatic denaturation after immobilization. Ionic liquids increased the productivity of immobilized laccase overall despite the fact that enzymatic activity was lowered.

CONCLUSION

Although promising, enzyme-based remediation techniques still face a number of significant obstacles. Recent developments in oxidoreductases for enzymatic wastewater treatment processes were described, and their applications were compiled. Effective enzyme immobilization can get around some of these difficulties by making many enzymes more stable and expanding the pH and temperature ranges where they can function. However, further research is still required to create novel and/or hybrid materials that may solve some of the issues with the supports that are now on the market. Additionally, there is still a significant gap between the scaling-up and bioreactor applications for these enzymes as well as the lab-level research conducted in the field. Future studies should concentrate more on the practical use of enzymes in already operating wastewater treatment facilities.

REFERENCES:

[1] Y. Wang *et al.*, "Deubiquitinating enzymes regulate PARK2-mediated mitophagy," *Autophagy*, 2015, doi: 10.1080/15548627.2015.1034408.

[2] S. Alonso *et al.*, "Human bone marrow niche chemoprotection mediated by cytochrome p450 enzymes," *Oncotarget*, 2015, doi: 10.18632/oncotarget.3614.

[3] S. I. Tanaka, T. Takahashi, A. Koide, S. Ishihara, S. Koikeda, and S. Koide, "Monobody-mediated alteration of enzyme specificity," *Nat. Chem. Biol.*, 2015, doi: 10.1038/nchembio.1896.

[4] D. Neupane, H. Yasuhara, N. Kinoshita, and Y. Ando, "Distribution of mineralized carbonate and its quantification method in enzyme mediated calcite precipitation technique," *Soils Found.*, 2015, doi: 10.1016/j.sandf.2015.02.018.

[5] N. Van Baren and B. J. Van Den Eynde, "Tumoral immune resistance mediated by enzymes that degrade tryptophan," *Cancer Immunol. Res.*, 2015, doi: 10.1158/2326-6066.CIR-15-0095.

[6] G. Zhou, S. Bergeron, and D. Juncker, "High-performance low-cost antibody microarrays using enzyme-mediated silver amplification," *J. Proteome Res.*, 2015, doi: 10.1021/pr501259e.

[7] R. R. Beerli, T. Hell, A. S. Merkel, and U. Grawunder, "Sortase enzyme-mediated generation of site-specifically conjugated antibody drug conjugates with high In Vitro and In Vivo potency," *PLoS One*, 2015, doi: 10.1371/journal.pone.0131177.

[8] J. G. Sandana Mala, D. Sujatha, and C. Rose, "Inducible chromate reductase exhibiting extracellular activity in Bacillus methylotrophicus for chromium bioremediation," *Microbiol. Res.*, 2015, doi: 10.1016/j.micres.2014.06.001.

[9] H. M. Beyer, P. Gonschorek, S. L. Samodelov, M. Meier, W. Weber, and M. D. Zurbriggen, "AQUA cloning: A versatile and simple enzyme-free cloning approach," *PLoS One*, 2015, doi: 10.1371/journal.pone.0137652.

[10] F. Xue *et al.*, "Biological control of Ascosphaera apis in honey bees using restricted enzyme mediated integration (REMI) transformed Trichoderma atroviride mutants," *Biol. Control*, 2015, doi: 10.1016/j.biocontrol.2014.12.015.

[11] S. Sriramula, H. Xia, P. Xu, and E. Lazartigues, "Brain-targeted angiotensin-converting enzyme 2 overexpression attenuates neurogenic hypertension by inhibiting cyclooxygenase-mediated inflammation," *Hypertension*, 2015, doi: 10.1161/HYPERTENSIONAHA.114.04691.

CHAPTER 12

IMPROVING THE CYTOTOXIC ENZYME DELIVERY AS ANTICANCER AGENTS

Shweta Loonkar, Assistant Professor
Department of ISME, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- shweta.loonkar@atlasuniversity.edu.in

ABSTRACT:

Being one of the most deadly human illnesses that yearly results in millions of deaths globally, cancer research is one of the most popular fields of study. Researching the impact of various naturally occurring enzymes of plant and microbial origin on tumor cells is a necessary step in creating effective anticancer tools. Over the last two decades, several intelligent delivery methods based on enzyme medications have been developed. A handful of these delivery systems have even found use in certain cancer therapies. Some of these delivery systems have been developed to the point where they are in the clinical testing phase. For better distribution and targeting, many biological, pharmacological, and physical techniques have been used to increase their efficacy. In this article, we examine cutting-edge enzyme drug delivery methods for application in cancer treatment. The primary findings from in vivo and clinical trials of these cutting-edge delivery systems are presented. These include their structure-based activities, modes of action, and fused forms with other peptides in terms of targeting and penetration, and other important findings.

KEYWORDS:

Cancer Therapy, Enzyme, Glucose, Peroxidase.

INTRODUCTION

Enzymes that cause cell harm or death have drawn a lot of interest since they are crucial for cell metabolism. It seems that cytotoxic enzymes are effective cancer-fighting agents. A protein poison should ideally only need one molecule to cause the death of a single cell. The use of cytotoxic enzymes is hampered by a variety of factors, however. They have reached this point in their evolution as a result of contemporary techniques to medication delivery and genetic engineering. Studies on suspected harmful enzymes' underlying mechanisms have produced important findings after being subjected to intense scrutiny. Sadly, only few of these enzymes have progressed to the clinical stage. Here, we present a handful of the enzyme medicines' methods of action that are currently being employed in cancer treatments [1], [2].

Many of the most effective hazardous enzymes contribute significantly to important procedures like protein synthesis; they transduce harm to cell metabolism and magnify it from a single location. These essential proteins of plant origin that work to limit ribosomal activity include ribosome-inactivating proteins. Popular RIPs like Trichosanthin, Gelonin, and others depurinate several ribosomal sites and harm cells as a result. In comparison to lesser molecular weight anticancer medicines, these proteins' well-documented anticancer and antiviral properties provide benefits. They may even trigger apoptosis and are known to impede tumor cell growth and cell survival. TCS and Gel have also shown effective in their capacity as cytotoxic agents, which harm certain cancer cells. To increase the effectiveness of RIPs, several smart drug delivery methods have been created and extensively researched, particularly TCS and Gel, which are type I RIPs with molecular weights of under 30 kD.

Oxidoreductases

Another crucial element for healthy metabolism and cell development is the oxidation-reduction state. Thus, oxidoreductases the enzymes that control this have emerged as important enzymes of great interest. One of the most effective protein poisons, peroxidases have been extensively studied for their potential anticancer properties. These enzymes have direct pH-changing effects by consuming H_2O_2, one of the primary reactive oxygen species. A disturbance in cell metabolism may result from oxidative stress and cytotoxicity brought on by changes in the pH of the culture media or in the quality and amount of substrates produced by their activities. Studies have looked at the cytotoxic effects of glutathione peroxidase and horseradish peroxidase as anticancer agents. The function of these enzymes in the treatment of cancer has also been previously examined. Another potentially hazardous protein that has drawn a lot of interest in the treatment of cancer is glucose oxidase. Gox catalyzes the conversion of glucose into gluconic acid and hydrogen peroxide, both of which have anticancer potential. H_2O_2 has a well-established involvement in the therapy of cancer and has been demonstrated to interact with a number of cellular components. The inhibition of citrate absorption, which slows tumor development, explains why gluconic acid has anticancer properties. Another possible method for treating cancer is to use less glucose, which is necessary in large amounts for tumor development [3], [4].

Asparaginase

Asparaginase, an enzyme that transforms L-asparagine into L-aspartic acid and ammonia, is regarded as one of the most potentially significant enzymes to have ever become commercially available. Acute lymphoblastic leukemia cells are deprived of asparagine by this substance, which inhibits the proliferation of these cells in the blood by reducing asparagine levels. Other forms of blood cancer have successfully been treated using bacterial asparaginases, which are accessible as anticancer medicines. The most widely utilized technique for extending asparaginase's effectiveness and boosting this medication's effectiveness is PEGylation.

DISCUSSION

Bacterial toxins are a different category of toxic proteins, the effects of which have been extensively researched in the treatment of cancer. Adenine dinucleotide phosphate is ribosylated by a number of bacterial exotoxins, perhaps as a mechanism of action. The use of this activity in cancer treatment has been extensively studied. One of these possible cytotoxic enzyme medicines that has successfully completed clinical trials is Pseudomonas exotoxin, an ADP-ribosyltransferase. Moreover, conjugating PE with various antibodies or their fragments increased its therapeutic activity. The capacity of PEs to penetrate cancer cells was also improved by the fusion of different peptides or protein fragments. Another possible helpful ribosyltransferase inhibitor is diphtheria toxin, which inhibits elongation factor activity. It has been shown that DT can target and penetrate cancer cells more effectively when combined with immunoconjugates and other substances. Several experiments on PE gene delivery have also been conducted utilizing biomimetic systems and synthetic polymers, which express the toxin gene in specifically targeted cancer cells, indicating its usefulness in cancer treatment. In this study, we examine a number of recent developments in TCS, Gel, HRP, peroxidases, asparaginase, PE, and DT drug delivery systems [5], [6].

Trichosanthin

An extensively studied ribosome-inactivating protein is TCS. Numerous studies have shown its antiviral action against the Hepatitis B virus as well as its suppressive impact on HBsAg,

the surface antigen, HBeAg, the viral protein that causes hepatitis, and HSV-1. Additionally, TCS-enrichment was observed to reduce HIV infectivity. Zhao et al. provided more evidence of TCS's capacity to enter HIV type 1 particles. The activation of chemokine receptors produced in HEK293 cells was discovered to be significantly enhanced by TCS' anti-HIV function. The outcomes presented above show TCS's potential. TCS has also been shown to be much more important in the treatment of cancer. Previous research shown that TCS might cause human stomach cancer cells MCG803 to undergo apoptosis. TCS had an effect on cell viability levels that depended on both concentration and duration. In these investigations, p21, a cyclin-dependent kinase inhibitor, was simultaneously elevated with the protein expression levels of p53, which initiates apoptosis. The induction of apoptosis and partial reduction of telomerase activity by TCS were credited with suppressing CNE1 carcinoma epithelioid cells and CNE2 poorly differentiated nasopharyngeal cancer cells.

TCS was more effective in slowing the development of the CNE1 tumor. TCS was shown to cause tumor cells to die. Caspase-3 and -8 were shown to be induced in both the intrinsic and extrinsic apoptosis pathways in studies on the effects of TCS on HL60 leukemia cells. It was also shown that TCS causes apoptosis by inducing the caspase-9-dependent and caspase-4-dependent pathways. In CaSki cervical cancer cells treated with TCS, Smac, an activator of caspases, was discovered to be increased. Smac has been hypothesized to modify chemoresistance to TCS and reduce resistance by reversible action. In Hela and CaSki cervical cancer cells, DNA methyltransferase 1 genes and proteins were shown to be reduced by TCS, and the gene expression of methylation-silenced tumor suppressors was also shown to be restored. In PC3 cells, the synergistic effects of interleukin-2 on TCS were also investigated. In mice carrying PC3 cells, TCS and TCS/IL-2 treatments resulted in significant variations in tumor volume and weight. In addition, TCS dramatically elevated levels of caspase-8, an apoptotic marker in cancer, and markedly enhanced procaspase-8. The apoptotic pathways might vary depending on the kind of tumor cell. Changes in ROS concentrations may also result in the demise of tumor cells.

Even within cancer cells, there are considerable differences in the quantities of TCS absorption by distinct types. According to reports, TCS enters JAR cells through particular receptors in a fast accumulative process. Direct diffusion over the H35 cells' membrane has been hypothesized to cause slow and non-specific penetration. TCS binding has been attributed to low-density lipoprotein receptor-related protein 1. When compared to controls, TCS at a dose of 20–100 g/mL suppressed the viability of JAR and Bewo human choriocarcinoma cells by around 20%. The effects were, however, at least two times less pronounced in HeLa, which was accounted for by the low amount of LRP1 surface expression. Leucine-rich G protein-coupled receptor 5 was shown to be the target of TCS in U87 glioblastoma cells. As a consequence of TCS therapy, LGR5 levels were suppressed, and c-myc, pGSK-3Ser9, and cyclin D1 protein levels were also decreased. These proteins are all essential for the development of cancer [7]–[9].

The participation of host immune cells in tumor elimination was discovered by studies on the effects of TCS on survival in immunocompetent and naked mice containing Lewis lung cancer cells. TCS was discovered to increase interferon and effector T cell numbers. It has been shown that TCS therapy dramatically increases CD4+CD25+ T cells, a subtype of T lymphocytes, and that these TCS-enhanced cells have stronger suppressive activity as effector T cells in vitro. However, cytokine release and cell-cell interactions were necessary for this impact to occur. In HeLa cells, it was discovered that TCS increased cytosolic Ca+2, which further reduced protein kinase C as opposed to protein kinase A. It was discovered that the decrease in cAMP levels brought on by TCS was time- and concentration-dependent.

Previous research found that the PKC/MAPK pathway controlled how well TCS suppressed HeLa cell growth. The activation of the caspase-8 and -9 pathways was connected to the cell death brought on by TCS impact in MCF-7 and MDA-MB-231 human breast cells. The elevation of caspase-3 was followed by further PARP cleavage, and then their activation was dose-dependent.

The NO and caspase-4 pathways have reportedly been used in other TCS processes that result in tumor inhibition and cell death. Over 20 distinct cell types have so far shown extremely effective effects. More research on TCS is needed to use it effectively. These studies have mostly focused on delivering TCS to tumor cells and maintaining its integrity as it circulates in the blood. Too far, a number of strategies have been developed to improve the delivery and targeting of TCS to tumor cells. In this context, substances of greater and lower molecular weights, both synthetic and natural, have been employed. We examine various drug delivery techniques in this section, along with their results.

To get around delivery and targeting problems, sophisticated drug delivery systems are being developed. Cell-penetrating peptides have significantly aided in the development of intracellular delivery methods in this regard. Recombinant proteins, covalent chemical bonding, noncovalent interactions, or surface modifications have all been used to establish a durable relationship between protein medicines and CPPs. As a result, several tactics based on enzyme cleavage, pH triggering, or light-dependent modifications have been devised. Lower molecular weight protamines, which are mostly composed of arginine, matrix metalloproteinase-2 substrate peptide, and PEG, have been used as one clever DDS for the delivery of TCS into tumor cells. It is the function of MMP-2 substrate to enable targeted cleavage at tumor locations. Thus, integrated TCS would no longer have a PEG cover. Further evidence of increased efficiency came from the remaining TCS and LMWP fusion. The maximum tumor growth inhibition was obtained with this approach, which was at least two times more effective than TCS or TCS-LMWP that did not have a PEG coating. The differences in tumor mass were significantly bigger, demonstrating the effectiveness of LMPW and PEG. The effects of PEGylated TCS or TCS-LMWP-MSP in combination with Paclitaxel were investigated in further development on PTX-resistant A549/T NSCLC cells [10], [11].

Gelonin

21 lysine residues make up 21 of the 258 amino acids in the mature plant-derived Gel protein. It shares 33% of its sequence with TCS and the ricin a chain. To target various tumor types, new recombinant versions of Gel have been created and produced. Geloniummultiflorum served as the source of the first study data on the extraction and purification of Gel. On the basis of antibody-associated cancer cell targeting, the antibody targeted, triggered, electrically modified prodrug-type approach system is anticipated to deliver protein medicines extremely well. The interactions between CPPs and heparin are stable throughout blood circulation but are susceptible to disaggregation when protamine is added. T84.66 murine anti-CEA monoclonal antibody was successfully used in earlier experiments to deliver CPP-conjugated Gel. Heparin was conjugated to T84.66 through a thioester linkage to create electrostatic contact between antibodies and toxins. In comparison to free CPP-Gel, the antibody-targeted CPP-Gel system accumulated toxin at levels 43-fold greater in tumor tissues.

When compared to toxin-CPP conjugation, the tumor growth was dramatically reduced by electrostatic interactions between CPP, conjugated with Gel, and Heparin, conjugated with T84.86. Protamine was added to the TAT-Gel/T84.86-Hep system, which enhanced targeting

and delivery effectiveness. In vivo tests on mice treated with this ATTEMPTS system showed a 3-fold reduction of tumor growth. It was discovered that protamine was crucial for TAT-Gel fusions that were electrostatically drawn to T84.66-Hep. These ATTEMPT systems with heparin might be effectively used for cancer treatment, however the anti-coagulation feature may result with unanticipated issues at greater dosages. Another strategy to increase Gel's effectiveness was genetically manipulating the TAT peptide's fusion with it. With the addition of a protease inhibitor, a 6xHis, and a thioredoxin motif, a TAT-Gel fusion gene was created that has an affinity for nickel-charged nitrilotriacetic acid resin. This protein fusion, TRX-6xHis-TEVp-TAT-Gel, when converted into E. coli, was expressed effectively. Initially, the fusion was purified using the TRX-6xHis tagged site by elution with imidazole. TEV protease was used for further purification. The established electrostatic contact gave high stability that could be dissolved by protamine addition, and the resulting TAT-Gel fusion was covered by heparin. The LS174T cells were not affected by the TAT-Gel/Hep system on its own, but when Protamine was added, it lowered cell viability to TAT-Gel levels. In mice carrying LS174T cells, the newly developed TAT-Gel/Hep + Pro system decreased the tumor volume to a third of the original amount. According to reports, the cytotoxicity of the TAT-Gel fusion was at least 53 times more than Gel alone in a variety of cancer cells.

A lower MW human vascular epithelial growth factor was fused with Gel as part of a genetic strategy to enhance Gel's anticancer capabilities. The flexible GGGGS sequence that connected this genetically engineered Gel fusion to VEGF was used. In contrast to rGel, this VEGF121/Gel fusion was shown to drastically diminish tumor volume in mice carrying A375M cells. To further boost its therapeutic effectiveness, VEGF121/Gel was coupled with photochemical internalization and its impact on VEGFR1 and VEGFR2 receptors was identified. This work provided evidence that this chemical may directly target tumor cells without the need for receptors by interacting with VEGFR receptors. When CT26, CT25, and 4T1 cells were exposed to light for 80 or 120 min, respectively, treatment with VEGF-Gel-PCI decreased the cell viability degree by 4- and 10-fold at 48 h, according to MTT test results [12], [13].

The gel fusion containing epidermal growth factor, whose receptor is overexpressed on the surface of tumor cells, was likewise created using flexible short peptides made of GGGGS. Cell viability was affected differently by the Gel-EGF fusion and Gel-EGF mixed with PCI, with PCI increasing the prodrug efficiency. Internalization resulted from cross-phosphorylation and the dimerization of rGel/EGF and EGFR. Additionally, the rGel/EGF-EGFR complex was followed by the TPCS2a photosensitizer, which created ROS and led to the release of rGel-EGF into the cytosol. Gel-EGF-PCI therapy dramatically reduced the levels of cellular viability in comparison to Gel-PCI in the A-431 human epidermoid carcinoma and WiDr colon adenocarcinoma cell lines. However, negative effects were seen in MDA-MB-435 melanoma cells and MES-SA human sarcoma cells, which might be attributed to the EGFR expression levels that were hardly detectable. Under the impact of Gel-EGF-PCI, a substantial decrease in tumor volume as compared to untreated controls was seen. The tumor volume did not significantly alter as a consequence of gel monotherapy or gel-PCI. Thus, when paired with PCI, the fusion with EGF was shown to be a successful therapeutic method. Additionally, it has been shown that PCI increases Gel's or its fusions' effectiveness in different cancer cells. Cancers of the uterine, the breast, the colon, the ovary, the sarcoma, the bladder, the glioma, the skin, and the lung all showed improvements.

The anti-insulin-like growth factor-1 receptor, a 58 amino acid long peptide that can precisely bind to receptors, was fused with Gel in a different genetic recombinant method. No

discernible changes were made to Gel's N-glycosidase activity as a result of this fusion. The cell survival of the glioma cancer cells U87 MG and U251 MG was significantly reduced by this genetically fused protein. In this work, the conjugation of Gel with IGF-1R reduced the viability of U87 MG and U251 cells by 5 and 6 fold, respectively. However, in neither instance did lymph node cancer of the prostate cells show any appreciable alterations. Gel efficiency was also shown to be greatly increased by the fusion of Gel with single, double, and triple F3 peptides. It was discovered that 2F3-Gel and 3F3-Gel fusions were more effective than F3-Gel in suppressing the cell viability levels of LNCaP, PC-3, and DU-145 prostate cancer cells by at least 6-fold. It is clear that F3-combination has a substantial role since U87 MG and 9L glioma cells absorbed F3-Gel at levels that were 2.7 and 3.2 times higher than those of Gel, respectively. F3-Gel drastically reduced the viability levels of U87, 9L, LNCaP, and Hela cells when used at the recommended dose. No appreciable differences were seen in the absorption rates of Gel and F3-Gel by non-cancer HEK 293 and SVGp12 cells, respectively. F3-Gel also significantly reduced the tumor sizes of U87 MG xenografts, down to up to 40% of the mean value of the tumor volumes after Gel therapy.

In U-87 MG glioma cells, it was discovered that the combination of the brain cancer homing peptide chlorotoxin with Gel was preferentially internalized. This chlorotoxin-Gel fusion reduced the cell survival levels of U87 MG and 9L glioma cells by 10- and 8-fold, respectively, as compared to Gel and Gel + chlorotoxin samples. In comparison to unfused Gel, the fused Gel considerably reduced tumor development, resulting in a 4-fold smaller volume compared to that obtained after treatment with PBS. The fused Gel also had equipotent N-glycosidase activity. Gel was combined with B lymphocyte stimulator to target lymphocytes in chronic lymphocytic leukemia. This genetically created Gel-BLyS was able to precisely attach to receptors on cell surfaces. Only 5000 nM Gel could internalize in leukemic cells with an IC50 as low as 3 nM for the fused Gel. Additionally, it has been shown that Gel-BLyS causes interleukin-6 receptor to be downregulated, which has an effect on the STAT3 targets c-Myc, p21, Mcl-1, and Bcl-xL and is highly expressed in diffuse large B cell lymphoma. Moreover, Gel-BLyS therapy was reported to reduce STAT3 phosphorylation levels and STAT3-DNA binding activities. Gel-BLyS was used at a dose of 106 pM to completely suppress the development of the B cell-like ABC-DLBCL cell lines OCI-Ly3 and OCI-Ly10, while leaving SUDHL-4 and SUDHL-6 cells unaltered. Gel-BLyS was thus recommended as a successful choice for treating ABC-subtypes of DLBCL. Nuclear factor kB targets bcl-xL and MCL-1 were similarly shown to be downregulated by gel-BLyS. It has also been proposed that Gel-BLyS's cytotoxic effects are mediated by BLyS receptors, which cause apoptosis by cleaving poly polymerase and activating caspase-3.

Exotoxin

Proteins that are toxic as well as secondary metabolites that are immune to antibiotics or chemical agents are considered to be toxins. Prokaryotes and eukaryotes both manufacture these substances. Since these organisms and toxins may be genetically altered, microbial poisons should be simple to deal with. The microbial toxins that are of bacterial origin have undergone the most thorough research, particularly in regards to their effectiveness against cancer. It has been discovered that exotoxin A, which was isolated from Pseudomonas aeruginosa, is very intriguing as a possible anticancer drug. As a result of this toxin's inhibition of ADP-ribosyltransferase activity, protein synthesis is also hindered. It was shown that a 38-kD protein fragment from P. aeruginosa, known as PE 38, had strong activity against cancer cells, and this served as a valuable starting point for additional research. Anti-Tac-PE38, a recombinant version of PE 38, has been shown to be an effective therapeutic strategy against B cell lymphoma that overexpresses CD25. Early research found three

domains in Pseudomonas exotoxin A, including receptor recognition, cytosol translocation, and elongation factor ribosylation. Exotoxin's PEIII or PE3 catalytic domains prevent ADP-ribosylation, which impairs protein synthesis. Its inactivation was brought on by extensions in catalytic subunits, however there was no discernible impact on protein synthesis. It was determined that the relevance of exotoxin or its derivatives is that they deplete oncogenic signaling molecules and growth factors released by cancer cells.

PE was discovered to function as a carrier that may enhance cytotoxic T-lymphocytes' protective response to antigens in vivo. An ovalbumin protein fragment was introduced into a 64-kD nontoxic mutant PE to illustrate this idea. In order to immunize mice, ovalbumin was fused to nontoxic PE. This protein fusion produced ovalbumin epitope-expressing murine cells-lysing CD8+ lymphocytes. In a different study, tripolyphosphates were used to cross-link chitosan microparticles loaded with the catalytic domain of PE. The cross-linking agent concentration and the release of the 28.7-kD protein PEIII from the created microparticles were both associated. The sensitivity of Drosophila melanogaster S2 cells to PE at picomolar doses was established by experiments. The influence of the insect terminal caspase, diphtamide, and elongation factor modification on the toxin-mediated death of S2 cells was further highlighted by earlier research that demonstrated an increase in caspase activity.

This toxin has been delivered to cells that overexpress the folate receptor using a PE combination with folate. In comparison to a momordin-folate tandem toxin, this exotoxin-folate combination demonstrated a 10-fold greater efficacy against folate-deficient HeLa cells. It has also been shown that FDKB tumor cells take up toxin-folate conjugates at significant quantities. The toxin's translocation domain was implicated in the conjugate's heightened toxicity. Targeting the granulocyte-macrophage colony-stimulating factor receptor, which is overexpressed in certain forms of leukemia and solid tumors, was another method for delivering PE to cancer cells. To do this, a peptide sequence with a high affinity for binding to GM-CSFR was genetically fused to PE. Recombinant PE exotoxin shown very high effectiveness against LS174T, SW403, and N87 gastric cancer cells. In LS174T, SW403, N87, and HTB103 cells, the IC50 values for this toxin were 2.2, 0.9, 0.45, and 9.5 ng/mL, respectively. On promyelocytic, erythroleukemia, and monocytic cells, recombinant versions of DT were shown to have better efficacy, with IC50 values of 0.4, 0.02, and 0.04 ng/mL, respectively. The investigations carried out to find these IC50 values did not reveal any appreciable variations in the GM-CSFR expression levels in the examined cells. As a result, it was discovered that the potency of several toxins on solid and hematopoietic tumor cells varied depending on the amount of receptor expression.

One of the most effective methods for drug delivery is to connect anticancer medications to antibodies. A few studies on PE have been conducted in this field. Trastuzumab, a kind of monoclonal antibody specifically designed to treat stomach and breast malignancies, was conjugated to PE and DT in a study. When compared to PE, this conjugation drastically reduced the viability of SK-BR-3 breast cancer cells. DT produced outcomes that were comparable. It has been suggested that obtaining toxin conjugates is an effective way to lower the therapeutic dosage of an antibody needed. The development of tumor cells that produce an antigen may be inhibited by combining noncovalently linked human or mouse antibodies with an immunoconjugate of a truncated PE with alpha crystallizable fragment antibodies.

CONCLUSION

It has been shown that protein toxins are effective anticancer therapeutic agents. Ideally, it only takes a few molecules to kill cancer cells, according to research. Protein toxins have an

advantage over medications with smaller molecular weight due to this characteristic. However, a number of challenges prevent their widespread use, including immune cell assault, huge weights and large volume, aggregation during storage, and limited productivity. For this reason, various methods have been used to increase their efficacy, such as fusion with peptides that provide targeting and penetrating functions, encapsulation in liposomes and nanoparticles, different releasing options that activate at lower pH, linking with transport proteins like albumin or lactoferrin, providing their expression in targeted cells by delivering appropriate genes, and utilizing physical-stimuli responsive mechanisms. Some of these poisons have now entered the market as a consequence of these various changes.

Ongoing research should concentrate on combining these protein toxins with other pharmacological types that might have additive effects.

REFERENCES:

[1] M. Maggi, L. R. Chiarelli, G. Valentini, and C. Scotti, "Engineering of helicobacter pylori lasparaginase: Characterization of two functionally distinct groups of mutants e0117025," *PLoS One*, 2015, doi: 10.1371/journal.pone.0117025.

[2] J. F. Campos *et al.*, "Antimicrobial, Antioxidant, Anti-Inflammatory, and Cytotoxic Activities of Propolis from the Stingless Bee Tetragonisca fiebrigi (Jataí)," *Evidence-based Complement. Altern. Med.*, 2015, doi: 10.1155/2015/296186.

[3] Y. Y. Chia, M. S. Kanthimathi, K. S. Khoo, J. Rajarajeswaran, H. M. Cheng, and W. S. Yap, "Antioxidant and cytotoxic activities of three species of tropical seaweeds," *BMC Complement. Altern. Med.*, 2015, doi: 10.1186/s12906-015-0867-1.

[4] A. de Mesquita Barbosa, S. dos Santos Costa, J. R. da Rocha, C. A. Montanari, and S. Giorgio, "Evaluation of the leishmanicidal and cytotoxic effects of inhibitors for microorganism metabolic pathway enzymes," *Biomed. Pharmacother.*, 2015, doi: 10.1016/j.biopha.2015.07.040.

[5] M. Khan, A. H. Naqvi, and M. Ahmad, "Comparative study of the cytotoxic and genotoxic potentials of zinc oxide and titanium dioxide nanoparticles," *Toxicol. Reports*, 2015, doi: 10.1016/j.toxrep.2015.02.004.

[6] E. Agostinelli, F. Vianello, G. Magliulo, T. Thomas, and T. J. Thomas, "Nanoparticle strategies for cancer therapeutics: Nucleic acids, polyamines, bovine serum amine oxidase and iron oxide nanoparticles (Review)," *Int. J. Oncol.*, 2015, doi: 10.3892/ijo.2014.2706.

[7] M. S. M. Al-Nimer, H. G. Hameed, and M. M. Mahmood, "Antiproliferative effects of aspirin and diclofenac against the growth of cancer and fibroblast cells: In vitro comparative study," *Saudi Pharm. J.*, 2015, doi: 10.1016/j.jsps.2015.01.002.

[8] E. T. Oh and H. J. Park, "Implications of NQO1 in cancer therapy," *BMB Rep.*, 2015, doi: 10.5483/BMBRep.2015.48.11.190.

[9] B. Liu, L. Qu, and S. Yan, "Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity," *Cancer Cell International*. 2015. doi: 10.1186/s12935-015-0260-7.

[10] S. Y. Fung, M. L. Lee, and N. H. Tan, "Molecular mechanism of cell death induced by king cobra (Ophiophagus hannah) venom l-amino acid oxidase," *Toxicon*, 2015, doi: 10.1016/j.toxicon.2015.01.012.

[11] T. V. Serebryanskaya, A. A. Zolotarev, and I. Ott, "A novel aminotriazole-based NHC complex for the design of gold(i) anti-cancer agents: synthesis and biological evaluation," *Medchemcomm*, 2015, doi: 10.1039/c5md00185d.

[12] T. Otomo *et al.*, "Mannose 6 phosphorylation of lysosomal enzymes controls B cell functions," *J. Cell Biol.*, 2015, doi: 10.1083/jcb.201407077.

[13] H. Aloysius and L. Hu, "Targeted prodrug approaches for hormone refractory prostate cancer," *Med. Res. Rev.*, 2015, doi: 10.1002/med.21333.

CHAPTER 13

IMPACT OF VARIOUS AGRICULTURAL FARMING PRACTICES ON THE MICROBIAL BIOMASS AND ENZYME ACTIVITIES

Somayya Madakam, Associate Professor
Department of uGDX, ATLAS SkillTech University, Mumbai, Maharashtra, India
Email Id- somayya.madakam@atlasuniversity.edu.in

ABSTRACT:

Changes in the microbiological, biological, biochemical, physical, and chemical properties of soil have a direct impact on its quality. Because the cycling of the nutrients, enzymes, and hormones required by plants for healthy growth and development may be sped up, the microbiological activities of soil can have an impact on soil fertility and plant growth. By modifying the soil microclimate, the habitat of the soil microorganisms, and the nutrient cycling, various agricultural management strategies may have an impact on microbial biomass and enzyme activity. Based on this, the current study intended to assess the effects of conventional, low-input, and organic farming methods on microbial biomass and various soil enzyme activities in a vegetable field producing celery. In this research, two sampling times one month after colonization and one month before harvesting were used to compare the impact of various techniques on biological soil quality markers. It was shown that compared to conventional and low-input farming methods, the soil microbial biomass in the organic farming system was substantially greater.

KEYWORDS:

Agriculture, Catalase, Farming, Organic farming, Soil Enzyme.

INTRODUCTION

The soil microbial biomass in December was substantially greater than that in October under an organic agricultural system. For all three of the agricultural management approaches that were utilized in the research, the variance in the soil microbial biomass carbon in the 0–20 cm soil layer was greater than that in the 20–40 cm layer. Additionally, it was noted that the soil total carbon and total organic carbon in the December samples were greater than they were in the October samples. The soil catalase activity was greater in the October samples than it was in the December soil samples, which were obtained from the 20–40 cm layer of soil as opposed to the 0–20 cm layer, under all three of the management strategies that were used. The application of organic fertilizer led to increases in the activity of the soil's protease and urease enzymes. While the samples collected in December from both soil layers showed higher protease activity when organic methods had been used, the soil samples that were extracted from the 0-20 cm and 20-40 cm soil layers in October showed higher protease activity in the samples that were taken from farms using conventional practices as opposed to the samples that were taken from farms using organic and low-input practices. The urease activity of the soil did not significantly differ between the two soil layer samples [1], [2].

The adoption of organic management techniques, followed by low-input and traditional modes, resulted in the maximum urease activity. The soil urease activity for the traditional and low-input techniques showed a clear pattern of change that was connected to the time of sampling, i.e., activity in December was much greater than activity in October. This research was unique in that it used three different management techniques low-input, conventional, and organic systems to measure the microbial biomass carbon and enzymatic activity in a six-

field crop rotation of tomato, cucumber, celery, fennel, cauliflower, and eggplant. The current research shown that outstanding microbial and enzyme activity, which lead to enhanced soil quality, is greatly influenced by the long-term use of organic fertilizers.

A significant portion of the global economy is devoted to agriculture. Sustainable land management may boost agricultural output while reducing biological diversity losses. Conserving the soil quality and the related ecosystem services while improving agricultural yields is one of the key issues in land management. The ability of a soil to function effectively across different ecosystem limits in order to retain biological productivity, maintain environmental quality, and foster plant growth and development is referred to as soil quality. Following the loss of organic carbon, land management practices like conventional, low-input, etc. may cause differences in soil organic matter, which in turn can have an impact on the microbial biomass of a specific soil ecosystem. Generally speaking, conventional farming has caused a decrease in soil structure and soil aggregation, which reduces water penetration, increases soil bulk density, and causes salt and nitrogen leaching, all of which contribute to ground water pollution. Due to its biological nutrient supply and pest control measures, organic farming produces food that is safer and of higher quality than traditional land management approaches [3], [4].

Due to improved soil biodiversity, improved soil structure formation, and increased enzyme activity, microbial biomass, and soil organic carbon, organic farming may produce soil with greater quality than conventional farming under various crop management techniques. The most significant and comprehensive production management approach is organic farming, which enhances biological cycles, biodiversity, soil biological activity, and other aspects of agro-ecosystem health. The use of synthetic substances, plant growth regulators, genetically modified organisms, fertilizers, insecticides, animal food additives, etc. is minimized or eliminated in this form of crop management technique. To maintain the overall soil productivity, organic farming promotes the use of crop residues, animal manures, plant nutrients, legumes, green manure, off-farm organic waste, biofertilizers, etc. The best use of natural resources is achieved through organic farming, which also improves soil physical properties like good tilth, granulation, good aeration, and high water holding capacity, as well as reduces soil erosion.

DISCUSSION

It also helps to maintain the health of the environment and reduces risks to humans and animals. The production of organic food and organic farming have increased globally since 1985. Globally, organic farming occupied 69.8 million hectares of land as of 2017, an increase of 20% from the previous year. Australia is the nation with the largest area dedicated to organic farming, followed by Argentina, China, Spain, the USA, Italy, and other nations. India has the highest concentration of organic producers worldwide, followed by Uganda, Mexico, Ethiopia, the Philippines, and other nations. Around 72.3 million hectares of agricultural land are used for organic farming, which is done in 187 countries worldwide. The Farm to Fork strategy was recently introduced by the European Union as part of the "European Green Deal," which consists of a number of various policies chosen by the European Commission with the intention of introducing new regulations on organic farming and food security. The F2F strategy of the European Commission launched a number of projects with the goal of managing at least 25% of the agricultural land in the European Union using organic farming practices by 2030. The F2F future goals also called for a 50% decrease in pesticide use, a 20% decrease in fertilizer use, a 50% decrease in nutrient loss, a 50% decrease in the use of antimicrobials in agriculture and aquaculture, a 50% decrease in food waste, and the labeling of sustainable foods by 2030.

As indicators of soil quality, many chemical and microbiological measures may be utilized. The physical, chemical, and biological indicators of soil quality may show quick changes in soil conditions as a consequence of various soil management techniques, according to Bell and Raczkowski, Microbial biomass nitrogen, MBC, and the activities of several enzymes were determined to be the most useful markers for tracking soil quality and productivity, according to Qin et al. While soil enzymes serve as crucial components that catalyze the processes necessary for organic matter breakdown and for the cycling of nutrients, MBC stands for the microbial population and serves as an indicator of organic carbon turnover in soil. While phosphatase enzyme activity is necessary for phosphorus cycling, urease enzyme activity is crucial for nitrogen cycling. No-till farming promotes MBC and other enzyme activities while decreasing soil disturbances and erosion and increasing the quantity of organic matter that may enter the soil. Numerous writers have shown that organically managed agricultural soils often have greater microbial populations and enzyme activity than conventional and alternative farming approaches. The productivity level of agricultural soil may be assessed by classifying soils according to soil enzyme activity. The pace of soil material transformation and circulation may be directly influenced by the degree of soil enzyme activity, and it can react fast to changes brought on by environmental parameters like pH, water content, and soil temperature [5]–[7].

The adoption of alternative agricultural techniques that may enhance the biological qualities of soil and, as a result, ensure excellent soil health and high crop output is now an increasing issue among farmers and researchers. Few studies comparing the abundance of microbial communities and enzyme activity in soil under various management regimes have been published over the course of the past few years. Less research, on the other hand, has shed light on how organic farming practices over time affect the often reported microbiological parameters. In light of all of this, it was decided that the current study would compare the soils from three greenhouses that were using three different farming techniques and that were likely to exhibit differences in terms of the microbial biomass carbon and soil enzyme activity at the Quzhou Experimental Station of the China Agricultural University, China. The goal of the current research was to determine the long-term impacts of various agricultural methods on the carbon derived from microbial biomass and the activity of soil enzymes in soil that was being managed by various agricultural techniques.

A celery-growing field served as the research location for the current experiment, and soil samples were collected from the region twice, one month after colonization and one month prior to harvest or maturity for the year 2016. The three greenhouses were split into four samples, each of which was collected using the 5-point sampling approach and in accordance with the model S. Four duplicates of each system's soil samples were collected between the depths of 0 cm and 20 cm. The soil samples were individually put into aseptic bags, tagged, and stored in the laboratory refrigerator at 4 °C until they could be processed as soon as possible after the soil had been properly dried by the sun.

Evaluation of Soil Microbiomass and Soil Enzyme Activities

The chloroform fumigation extraction technique was used to measure the soil's microbial biomass carbon. The soil samples were put in vacuum dryers with a layer of moist filter paper and fumigated with chloroform using a vacuum pump. About 20 gof of the fresh soil samples were used. A soil sample was also moved from a beaker to a concussion bottle that contained 60 mL of 0.5 M potassium sulfate. For 15 minutes, the soil sample was centrifuged at 3000 rpm/min. A liquid storage container was used to store 5 mL of the filtrate, which was then used as the starting point for a TOC analyzer [8], [9].

1. Activity of Soil Enzymes

Additionally, the activity of soil-found enzymes such asurease, catalase, and protease was measured. While catalase and protease activity were determined using the volumetric technique and the ninhydrin colorimetric method, respectively, urea was used as the substrate for the spectrophotometric measurement of urease activity.

2. Activity of Urease

20 g of dry soil samples were put into a 100 mL volumetric flask and exposed to 2 mL of toluene for 15 minutes to measure the urease activity. The volumetric flask spent 24 hours at 37 °C in an incubator. The samples were then transferred to capacity bottles, where they were shaken thoroughly before being mixed to with 20 mL of citric acid buffer and 10 mL of 10% urea solution. Following mixing, the bottles were kept at a temperature of 37 °C for 24 hours in a temperature box. Following centrifugation, the samples were divided, and 3 mL of the supernatant was absorbed into a 50 mL volumetric flask along with 10 mL of distilled water. The control sample was likewise made at the same time, but it did not include any soil samples. A spectrophotometer operating at a 578 nm wavelength was used to measure the urease activity of the final soil solution. The standard curve for ammonium sulfate that has been dissolved in water and diluted to 1000 mL with 0.1 mg of nitrogen was used to determine the urease activity of the soil samples. The amount of milligrams of ammonia released by the enzyme deuresis urea per gram of soil is represented by the enzyme activity in NH3-N. Different agronomic farming methods, such as organic or conventional farming, may have an effect on the soil's enzymatic activity; organic farming has been found to have a considerable impact [10], [11].

3. Catalase Activity in Soil

There are several soil organisms that have the catalase enzyme. The catalase enzyme inhibits the hazardous impact that hydrogen peroxide has on soil enzymes by promoting the breakdown of hydrogen peroxide, a free radical that is damaging to terrestrial plants when it may enter water and oxygen. The oxido-reductase system it functions as is crucial for the synthesis of humus in soil. Catalase is a key component of the soil ecosystem and may be utilized as a biological activity measure to assess a soil's quality. Therefore, research on the differences that might arise in soil catalase under various agricultural techniques is crucial if we are to comprehend the wise use of fertilizers, soil resources, and the creation of long-lasting soil ecosystems. Catalase activity in soils is influenced by dehydrogenase, glucosidase, and esterase activity, as well as microbial biomass, organic oxygen content, changes in CO2, and variations in CO2. High soil fertility and the presence of aerobic microorganisms are predicted by its high activity.

Under all three procedures, the 20–40 cm soil layer had greater catalase activity than the 0–20 cm soil layer. In the same soil layer and under the same conditions, the catalase activity in October was greater than it was in December. Catalase activity in October and December responded in the same order to all management strategies, however conventional farming had higher catalase activity than low-input and organic farming strategies. In contrast to soil that had been grown using traditional farming methods, Kobeirski et al. found that organically grown soil had much higher catalase activity. Furthermore, Filipek-Mazur et al. discovered significant catalase activity in the stagnicluvisol soil type under organic farming techniques and came to the conclusion that soil's enzymatic activity relies on the type of soil and the range of crops that are produced in that soil. In contrast to the current findings, Purev et al. found that catalase activity was highest between 0 and 15 cm deep in hilly, dry steppe, and humidified soils, and that activity quickly declined as depth rose.

4. Active Soil Protease

Protease is a broad class of enzyme that is found in significant quantities in soil and has the ability to hydrolyze a wide range of proteins, peptides, and other substances into amino acids. The transformation of nitrogen nutrition in soil is significantly influenced by the protease activity in the soil, and this index may be used to measure the fertility of the soil. Crop rotation soil often has more soil protease activity than soil from monocultures. Protease activity reveals the potential of the soil's microbial populations to mineralize nitrogen. However, in response to the various methods, the soil protease activity in October and December was inconsistent. In comparison to the organic and low-input practice samples, the conventional practice soil samples had greater levels of protease activity in the 0–20 cm and 20–40 cm soil layers in October. However, in December, the soil samples collected from the greenhouses utilizing organic methods had greater levels of protease activity in both of the soil layers than the soil samples taken from the greenhouses using low-input and traditional forms of cultivation. The soil proteinase activity in the typical practice samples was greater in October than it was in December from the same soil layer, and in the low-input mode, it was higher in December from the same soil layer than it was in October [12], [13].

Purev et al. reported the maximum protease activity in the layer that was 0–15 cm below the soil surface and noted that the activity quickly decreased as the soil depth rose, which is consistent with the findings that were obtained. When compared to soil that had been cultivated using traditional farming methods, the protease activity in the organically grown soil significantly increased. Additionally, it has been shown that the activity of the protease enzyme may rise in organic farming systems with a favorable soil pH and a larger concentration of total nitrogen, organic carbon, etc , a feature that was also seen in our investigation. Similar to this, Niewiadomska et al. reported that increased protease enzyme activity in maize-cultivated soil from an experimental farm at the Department of Soil and Plant Cultivation, Swadzim depended on the kind and quantity of organic fertilizer used to amend the soil.

5. Urease Activity in Soil

The major source of nitrogen for plants is ammonia, which is produced by the enzyme urease, which is extensively distributed in the soil ecosystem. Urease closely correlates with soil nitrogen capacity and serves as a proxy for the level of soil nitrogen supply. High soil urease activity has the potential to quickly hydrolyze externally provided urea into ammonia, which adds to soil nitrogen losses and masks plant deficits. Increased urease activity in the soil promotes the stability of high levels of organic nitrogen and efficient nitrogen transformation. The increase in urease activity indicates that utilizing organic fertilizer to enhance soil nitrogen transformation has a higher impact on improving soil nitrogen supply [14], [15].

The variations in the soil urease activity in the same soil layer over both sampling times exhibited clear consistency, and there was no discernible difference in the soil urease activity between the two soil layers. The maximum urease activity was seen in organic conditions, followed by low-input and conventional modes. The soil urease activity for the traditional and low-input techniques demonstrated a clear pattern of change over time, as the activity in December was much greater than that in October. Under organic farming, the soil urease activity didn't change significantly over time, but there were slight variations based on the month the sample was taken: December and October.Urease activity is highly sensitive to heavy metals in the soil and can be affected by the presence of both organic and inorganic matter . Similar to the current research, Kwiatkowski et al. also found that the organically farmed soil had the maximum urease activity because there was more of it there than in the

organic matter of the soil. They discovered a strong positive link between the activity of the urease enzyme and the organic farming of crops such sugar beet, red clover, oats, and winter wheat. Additionally, Chen et al. reported that sustainable agriculture management practices significantly increased the urease activity of tea plantation soil across a temporal scale , though no differences between various management practices were found to be statistically significant.

CONCLUSION

Based on a comparison of the impacts of various management techniques on soil microbial biomass and soil enzyme activity during two sampling periods, namely one month after colonization and one month before harvesting, the current research was conducted. The current research demonstrated that, when compared to samples taken from soil that had been farmed using conventional and low-input techniques, the soil microbial biomass in the organic farming system was much greater in the samples that were gathered during December. Under all three agricultural management approaches, the soil's microbial biomass carbon at the 0–20 cm soil layer varied the most from the 20–40 cm layer. In the soil samples that had been taken from the 20-40 cm soil layer as opposed to the 0-20 cm layer, the soil catalase activity was greater in October than it was in December. Urease, catalase, and protease activity were the highest under organic farming management practices, followed by low-input and conventional modes. In contrast, samples taken from the 20–40 cm soil layer and collected during December showed higher soil enzymatic activity than samples collected in October. The results of the current study showed that regular use of organic fertilizers may enhance soil quality and have a significant beneficial impact on the quantity of soil microbial communities as well as enzyme activity.

This research also shown that by enhancing the chemical and biological characteristics of the soil, organic farming may increase crop production stability and sustainability. We may draw the conclusion that promoting organic farming will help to create a country that is soon to be environmentally, nutritionally, and economically healthy.

The employment of sustainable agricultural techniques, such as the planting of perennials and legumes, expanding crop rotation systems, and using more organic fertilizers, should be encouraged by the government. Growing organic production should be supported since it not only improves consumer health but also the ecological state of any country and its ability to thrive economically. In order to determine the best agricultural management strategy, more in-depth study should be done in the future utilizing standardized procedures. This will help to not only improve crop output but also to sustain resilient soil health.

REFERENCES:

[1] R. Singh, M. Kumar, A. Mittal, and P. K. Mehta, “Microbial enzymes: industrial progress in 21st century,” *3 Biotech*. 2016. doi: 10.1007/s13205-016-0485-8.

[2] M. D. Stewart, T. Ritterhoff, R. E. Klevit, and P. S. Brzovic, “E2 enzymes: More than just middle men,” *Cell Research*. 2016. doi: 10.1038/cr.2016.35.

[3] F. B. H. Rehm, S. Chen, and B. H. A. Rehm, “Enzyme engineering for in situ immobilization,” *Molecules*. 2016. doi: 10.3390/molecules21101370.

[4] G. de Gonzalo, D. I. Colpa, M. H. M. Habib, and M. W. Fraaije, “Bacterial enzymes involved in lignin degradation,” *Journal of Biotechnology*. 2016. doi: 10.1016/j.jbiotec.2016.08.011.

[5] E. Kuah, S. Toh, J. Yee, Q. Ma, and Z. Gao, "Enzyme Mimics: Advances and Applications," *Chemistry - A European Journal*. 2016. doi: 10.1002/chem.201504394.

[6] V. L. Arcus *et al.*, "On the Temperature Dependence of Enzyme-Catalyzed Rates," *Biochemistry*. 2016. doi: 10.1021/acs.biochem.5b01094.

[7] H. Vaghari *et al.*, "Application of magnetic nanoparticles in smart enzyme immobilization," *Biotechnology Letters*. 2016. doi: 10.1007/s10529-015-1977-z.

[8] G. O. Ribeiro, R. J. Gruninger, A. Badhan, and T. A. McAllister, "Mining the rumen for fibrolytic feed enzymes," *Anim. Front.*, 2016, doi: 10.2527/af.2016-0019.

[9] C. R. M. Araújo, V. L. A. Santos, and A. A. Gonsalves, "Acetylcholinesterase - AChE: A pharmacological interesting enzyme," *Rev. Virtual Quim.*, 2016, doi: 10.21577/1984-6835.20160122.

[10] R. Mohan, V. Sivakumar, T. Rangasamy, and C. Muralidharan, "Optimisation of bromelain enzyme extraction from pineapple (Ananas comosus) and application in process industry," *Am. J. Biochem. Biotechnol.*, 2016, doi: 10.3844/ajbbsp.2016.188.195.

[11] S. Wang, X. Meng, H. Zhou, Y. Liu, F. Secundo, and Y. Liu, "Enzyme stability and activity in non-aqueous reaction systems: A mini review," *Catalysts*. 2016. doi: 10.3390/catal6020032.

[12] V. E. Bosio, G. A. Islan, Y. N. Martínez, N. Durán, and G. R. Castro, "Nanodevices for the immobilization of therapeutic enzymes," *Critical Reviews in Biotechnology*. 2016. doi: 10.3109/07388551.2014.990414.

[13] D. Krah, A. K. Ghattas, A. Wick, K. Bröder, and T. A. Ternes, "Micropollutant degradation via extracted native enzymes from activated sludge," *Water Res.*, 2016, doi: 10.1016/j.watres.2016.03.037.

[14] T. Jesionowski, J. Zdarta, and B. Krajewska, "Enzyme immobilization by adsorption: A review," *Adsorption*. 2014. doi: 10.1007/s10450-014-9623-y.

[15] U. Guzik, K. Hupert-Kocurek, and D. Wojcieszynska, "Immobilization as a strategy for improving enzyme properties- Application to oxidoreductases," *Molecules*. 2014. doi: 10.3390/molecules19078995.