



ENZYMES: REVOLUTIONIZING DRUG DISCOVERY, MANUFACTURING AND TREATMENT

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ABSTRACT

Enzymes stand at the forefront of transformative advancements in pharmaceutical and therapeutic realms, reshaping the landscape of drug development, manufacturing, and targeted delivery. This comprehensive review delves into the multifaceted applications of enzymes in these sectors, elucidating their profound influence on drug discovery, formulation, and therapeutic strategies. In drug discovery, enzymes accelerate high-throughput screening, allowing for rapid evaluation of potential drug candidates. Protein engineering, using enzymes like DNA polymerases, facilitates the creation of custom-designed proteins, leading to more precise drug development. Additionally, enzymes contribute to structure-based drug design by providing insights into protein-ligand interactions, enabling the rational design of effective therapeutics. The influence of enzymes extends to pharmaceutical manufacturing. Their catalytic power optimizes chemical reactions, promoting sustainability by reducing reliance on harsh chemicals. The burgeoning field of biopharmaceuticals, encompassing monoclonal antibodies, vaccines, and recombinant proteins, further emphasizes the indispensability of enzymes. Despite these advancements, enzyme sensitivity to environmental conditions and storage stability remain a major challenge and require further attention. Ongoing research in enzyme engineering and synthetic biology aims to create novel enzymes with enhanced properties. As the role of enzymes in the pharmaceutical and therapeutic realms continues to evolve, they promise innovative solutions to tackle current healthcare challenges.

Keywords: Biocatalysis, Biopharmaceuticals, Drug discovery, Enzymes, Protein engineering, Therapeutic enzymes

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INTRODUCTION

Enzymes play an indispensable function in pharmaceutical and therapeutic processes, bringing about significant advancements in drug development, manufacturing, and personalized medicine. These biocatalysts offer remarkable specificity, efficiency, and environmental advantages compared to traditional chemical methods. The demand for enzymes in producing active pharmaceutical ingredients for drugs and health-related supplements within the pharmaceutical industry is substantial [1].

Enzymes, whether in the form of functional proteins or Ribozymes (a ribonucleic acid enzyme), serve as biocatalysts, facilitating the performance of biochemical processes necessary for the functioning and growth of all living systems, whether unicellular or multicellular, including plants and animals [2]. Biocatalysis is the utilization of enzymes for transforming molecular substrates into various natural or synthetic chemicals [3]. These enzymes may exist in various forms, including intact cells, cell lysates, or purified forms, frequently produced as recombinant

proteins expressed within the cells of their native origin or within diverse host cells [4]. Enzymes exhibit a distinctive capacity to maintain their viability and catalytic activity even when removed from their native source, making them valuable for various industrial applications that involve chemical conversions from substrates to end products. Enzyme-catalyzed reactions are highly efficient, occurring under natural environmental factors, including temperature, pressure and pH, which may vary based on the physiology of the native organisms and their environment. This versatility allows enzymes to be utilized in various industrial processes, from pharmaceutical synthesis to beverage production, baking, agrochemicals, flavors, biopolymers, waste treatment, and more [5].

Most of industrial enzymes are microbial-sourced due to their convenience, facilitating rapid production, scalability, recovery, purification, over-expression from strain modification, and enzyme activity and specificity modulation [6]. Selecting the appropriate microbial strains is vital for successful industrial enzyme production, primarily through fermentation technology using microorganisms, classified as Generally Recognized as Safe [6, 7]. The production procedures include solid-state and

submerged fermentation, each with its own merits and constraints. Although the submerged fermentation method is commonly preferred for enzyme production in most industries, recently solid-state fermentation is once again being considered for specific applications [7].

Enzymes as biocatalysts provide several benefits over conventional chemical processes, such as specific catalytic reactions, simplified synthetic routes, high yields with exceptional selectivity, reduced energy requirements, and minimal by-product generation. These advantages make them ideal for sustainable chemical processes [8, 9]. Enzymes are capable of performing bulk operations under benign circumstances at high rates, with exceptional specificity and minimal physiological and environmental toxicity. Despite their immense potential, biocatalysis faces challenges when extreme industrial conditions are considered, requiring compatibility with properties like thermostability, substrate specificity, catalytic efficiency, and operational stability in complex environments [9].

This review delves into the wide-ranging applications of enzymes in the pharmaceutical and therapeutic sectors, emphasizing their significant impact on drug discovery, formulation, and delivery.

Enzymes in drug discovery and design

The discovery and development of pharmaceutical drugs represent intricate and resource-intensive processes necessitating innovative approaches to address the escalating global health challenges. The integration of enzymes into drug discovery and design processes underscores their multifaceted roles in the pharmaceutical industry. From drug targets to drug synthesis and high-throughput screening, enzymes play indispensable roles in the pursuit of novel therapeutic agents, expediting assays, and facilitating the investigation of target interactions [10].

As drug targets, enzymes are pivotal components in various biological pathways and are frequently implicated in the pathogenesis of diseases. They offer promising targets for drug intervention. The inhibition or modulation of specific enzymes can be an extremely effective strategy in the development of therapeutic agents. An increase in AChE is a common feature of AD and not impairment of the breakdown of the neurotransmitter. For instance, in Alzheimer's disease, Acetylcholinesterase (AChE), the key enzyme responsible for the breakdown of acetylcholine becomes a target for most clinically used drugs. These

drugs aim to inhibit AChE activity, thereby increasing acetylcholine levels in the brain and potentially alleviating Alzheimer's symptoms [11, 12, 13]. Kumar and others [14], gave an account of the efficiency of the monoamine oxidase enzyme as a potential drug target for various neurological disorders. Additionally, stearoyl-CoA desaturase (SCD), the rate-limiting enzyme in synthesis of monounsaturated fatty-acid, has been identified as a vital control point in regulating hepatic lipogenesis in type-2 diabetes mellitus [15]. In drug metabolism, enzymes are paramount for predicting a drug's efficacy and safety. The cytochrome P450 (CYP) enzyme family, mainly located in the liver, is essential for drug metabolism [16]. These enzymes break down and transform drugs, influencing their effects in the body. Genetic variations in CYP enzymes can lead to interindividual variability in drug responses, which has significant implications for personalized medicine [17].

Enzymes are not only important drug targets but also invaluable tools in drug synthesis. The utilization of natural or engineered enzymes to perform chemical reactions offers an environmentally friendly and cost-effective approach to drug manufacturing [18, 19]. Protein engineering, using enzymes like DNA polymerases, allows the creation of tailor-made proteins for drug development [20, 21]. Their role in structure-based drug design provides insights into protein-ligand interactions, thereby enhancing the rational design of therapeutic agents [21]. The synthesis of antibiotics such as amoxicillin attests to the efficiency and selectivity of enzymes in chemical transformations [19].

Synthesis of 7-aminocephalosporanic acid (β -lactam antibiotic)

Cephalosporin-derived antibiotics have proven highly effective in treating bacterial infections and are derived from the heterocyclic structure found in cephalosporins. Notable examples include cefdinir, cefotaxime, cefuroxime, and ceftriaxone, which have demonstrated efficacy against severe MRSA (methicillin-resistant *Staphylococcus aureus*) caused infections [7]. The synthesis of 7-ACA (7-aminocephalosporanic acid) a vital precursor for these antibiotics (Figure 1), is typically initiated from cephalosporin C, which is readily available and cost-effective through microbial fermentation. Furthermore, cephalosporin C is a corresponding intermediate to 7-ACA through splitting of the amide bond [22].

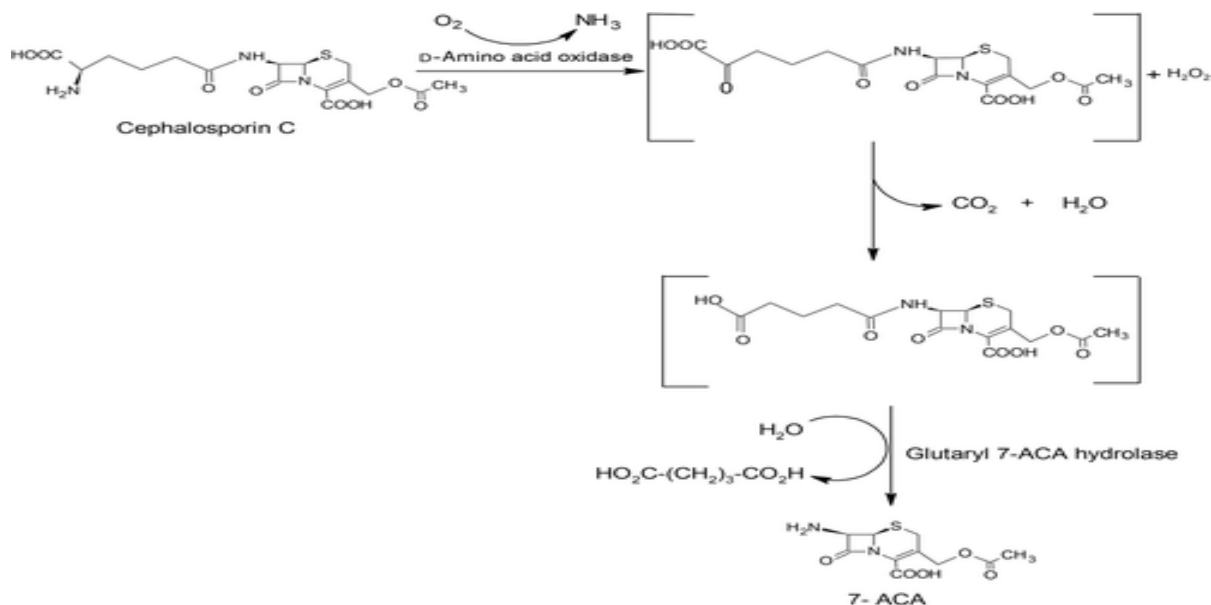


Figure 1: The synthesis of 7-ACA from cephalosporin (7-aminocephalosporanic acid) [7]

Enzymatic production of 6-amino penicillanic acid

Penicillin acylases are special enzymes that break apart penicillin compounds by cutting off a specific side chain resulting in the production of 6-APA (6-amino penicillanic acid) along with the respective organic acid (Figure 2). These enzymes can be found in various microbes, including bacteria, actinomycetes, fungi, and yeasts. They are categorized into ampicillin acylases, penicillin V acylases, and penicillin G acylases based

on specificity of their substrate. The use of penicillin acylases for synthesizing 6-APA has substituted traditional processes and offers economic advantages, particularly when the enzymes are immobilized. Immobilization facilitates enzyme separation, reuse, and stability. Penicillin acylases obtained from organisms like *Alcaligenes faecalis*, *Bacillus megaterium*, and *Escherichia coli* have been capacitated to enhance their efficiency [23].

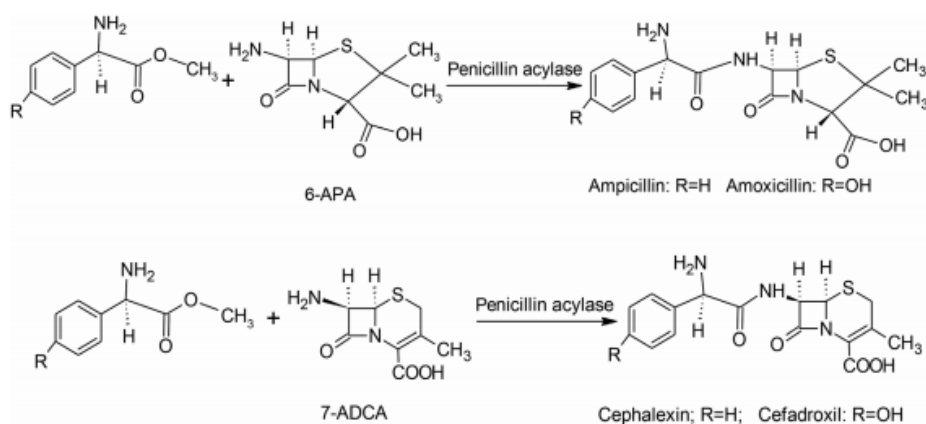


Figure 2: Enzymatic production of semi-synthetic β -lactam antibiotics [7].

Enzymes in amino acid synthesis

The amino acid market has experienced significant growth since the 1980s, primarily due to cost-effective production and isolation methods [24]. Fermentation and enzymatic catalysis have emerged as predominant techniques for amino acid production, renowned for

their cost-efficiency and environmental friendliness [25]. Amino acids are of fundamental importance for human and animal health and nutrition, serving as essential building blocks for life. Their significance is amplified by their chirality, making them crucial in chemical synthesis [26]. Notably, amino acids such as

l-leucine, l-valine, l-isoleucine, l-lysine, l-methionine, l-histidine, l-phenylalanine, l-tryptophan, and l-threonine, cannot be produced by humans as well as animals, necessitating their dietary intake.

Enzymes and whole-cell biocatalysts offer a powerful approach for the production of diverse amino acids. This includes proteinogenic amino acids used to build proteins, as well as nonproteinogenic amino acids with various functionalities. Notably, these biocatalysts enable the selective production of both L- and D-enantiomers of amino acids, and even the generation of highly purified amino acid products. These amino acids serve as essential components for the manufacturing of medications, cosmetics, and agrochemicals [7]. For instance, the production of L-methionine, a dietary essential, has been achieved through enzymatic resolution employing *Aspergillus oryzae* acylase [27]. Innovative enzyme membrane reactor technology has enabled the annual production of hundreds of tons of l-valine and l-methionine, significantly reducing enzyme loss.

Furthermore, enzymes have facilitated the efficient production of various amino acids. L-aspartic acid is obtained through the action of aspartase, catalyzing the transfer of an amine group from

ammonia to fumaric acid, leading to the direct production of l-aspartate, widely utilized in the industrial production of L-aspartame [7]. The production of L-cysteine has evolved towards industrial processes incorporating enzymatic hydrolysis and racemization of substrates, with notable progress attained using fermentation processes involving an *E. coli* strain with an interrupted l-cysteine regulatory system [28].

Enzymes play a critical role in the stereoselective production of non-proteinogenic amino acids, enabling the generation of both L- and D-isomers, offering an environmentally friendly approach through biocatalytic resolution of racemic mixtures or direct production using d-specific acylases [27]. The production of building blocks for semisynthetic antibiotics, such as p-hydroxy-d-phenylglycine and d-phenylglycine, relies on hydantoinase/carbamoylase enzymes (Figure 3). Advanced molecular biology techniques, including directed evolution, have enabled the transformation of hydantoinases from d-specificity to l-specificity. Additionally, coexpressing racemases with L-selective or D-selective carbamoylases and hydantoinases in recombinant whole-cell systems can produce various d- and l-amino acids [7].

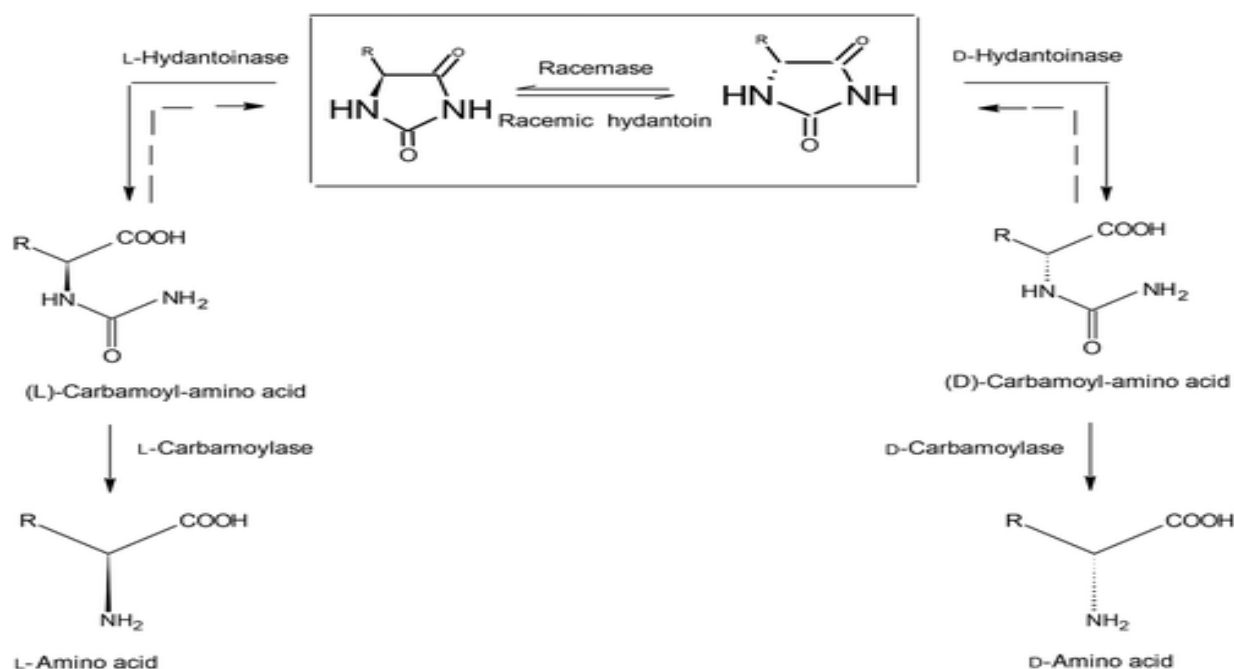


Figure 3: L- and D- amino acid production via carbamylases/hydantoinases [7]

In the context of high-throughput screening (HTS), it is a crucial step in drug discovery involving the testing of vast compound libraries for potential drug candidates. Enzymes are a significant part of the druggable genome and offer valuable drug targets. Enzyme assays

are the cornerstone of HTS methods, allowing for rapid and reliable identification of compounds that interact with specific targets. A survey of published papers between 1990 and 2018 revealed that kinase enzymes were the most frequently investigated in HTS. Assays

based on fluorescence were the most often used readout technique, and the therapeutic areas of focus included cancer and bacterial infections [29]. For instance, the development of kinase inhibitors for cancer therapy heavily relies on enzyme-based HTS assays [30]. High-throughput experimental pipelines, often incorporating microfluidics, are essential for functional screening and biochemical characterization of target enzymes to facilitate the development of effective therapeutic enzymes [10].

Catalysts for greener drug manufacturing and targeted delivery

Enzymes have emerged as indispensable tools in the realm of drug manufacturing, offering remarkable catalytic properties, selectivity, and eco-friendly attributes. Their adoption in the synthesis of Active Pharmaceutical Ingredients (APIs) has significantly increased [31]. Biocatalysis, the application of natural or engineered enzymes for chemical transformations, presents several advantages in drug manufacturing processes ([19, 31]. One of the critical applications of enzymes in drug manufacturing is the resolution of chiral compounds, a crucial step in the synthesis of enantiopure drugs [9]. Enzymes such as lipases, esterases, proteases, and oxidoreductases are employed to catalyze these enantioselective reactions. This approach not only enhances the purity and efficacy of the final drug product but also reduces the reliance on costly and environmentally harmful chemical processes [18, 9].

The pharmaceutical industry is under increasing pressure to adopt greener and more sustainable practices. Biocatalysis is recognized as a key technology within the realm of green chemistry, embraced by the chemical and pharmaceutical sectors to produce chemicals with enhanced yields [32]. Enzymes align with the principles of green chemistry, offering a way to mitigate the environmental impact of drug manufacturing processes. Proteases and amylases, for instance, are utilized to replace harsh chemicals in processes such as cleaning and purification, thereby reducing the generation of hazardous waste and contributing to a more sustainable pharmaceutical industry [33]. Dunn [34] highlighted the utilization of water as an eco-friendly solvent in the production of synthetic pharmaceutical compounds. Examples include the production of Pregabalin through a lipase-based resolution, the utilization of a transaminase in the final step for Sitagliptin synthesis, and the creation of the intermediate for Rosuvastatin using a deoxyribose-aldolase (DERA) enzyme, a process that simultaneously forms two C-C bonds and chiral centers.

Enzymes, with their remarkable specificity and catalytic prowess, have emerged as key players in the pharmaceutical landscape, making significant contributions to drug formulation and delivery. Enzyme-based nanoparticles, liposomes, and micelles have proven invaluable in enhancing drug solubility and bioavailability, ensuring precise dosing and targeted delivery to specific tissues or cells [35]. Furthermore, enzymes exhibit a vital role in the design of enzymatic prodrugs, which are biologically inactive compounds strategically engineered to be converted into their active form by enzymes at the target site. A notable example is the prodrug capecitabine, which is transformed into the anti-cancer drug 5-fluorouracil by the enzyme thymidine phosphorylase [36]. While enzymes are commonly harnessed for drug activation, they can also be targeted for inhibition to modulate pathological processes. Enzyme inhibitors are essential in the formulation of drugs aimed at diseases where enzyme overactivity is a key factor [37].

Enzymes are instrumental in the development of targeted drug delivery systems, where they are strategically employed to trigger drug release at specific sites within the body. For instance, glucuronidases can be exploited to release drugs selectively in tumor tissues [35]. The use of therapeutic enzymes like asparaginase in cancer treatment exemplifies the immense potential of enzymes in drug delivery systems [38]. The gastrointestinal tract poses formidable challenges for drug delivery, and encapsulating enzymes such as amylases, proteases, and lipases with drugs improves their stability in the harsh digestive environment, thus enhancing oral drug delivery and bioavailability [39].

Biopharmaceuticals and enzyme therapies

Biopharmaceuticals, or biologics, are medicinal products derived from living organisms through biotechnology. This diverse category includes monoclonal antibodies, therapeutic proteins, vaccines, gene therapies, and nucleic acid-based drugs, offering new avenues for treating various diseases, including cancer, autoimmune disorders, and genetic diseases [40]. Enzymes, such as restriction endonucleases, play a crucial role in genetic engineering techniques used for the production of biopharmaceuticals [41]. Monoclonal antibodies, widely embraced in cancer therapy, have revolutionized treatment approaches. For instance, trastuzumab, an antibody-based therapy, has significantly enhanced results for patients with HER2-positive breast cancer [42]. Enzyme replacement therapy (ERT), a prominent example of the therapeutic potential of enzymes, has been instrumental in treating lysosomal storage disorders. The administration of recombinant glucocerebrosidase has shown improved clinical outcomes for Gaucher disease patients [43].

Additionally, enzymes play a fundamental role in gene therapies, as exemplified by the recent success of Luxturna, a gene therapy for inherited retinal disorders that utilizes an adeno-associated virus to deliver a functional RPE65 gene [44].

Enzyme therapies, a subset of biopharmaceuticals, involve the administration of enzymes to address enzyme-related disorders and correct deficiencies in various health conditions. Enzymes contribute to critical physiological processes, including food digestion, detoxification, immune system support, muscle function, and reducing strain on vital organs like the pancreas. Enzyme therapies have potential applications in treating metabolic disorders, pancreatic insufficiency, lactose intolerance, cystic fibrosis, and addressing conditions like cancers or tumors [7].

Enzyme replacement therapy (ERT) represents a milestone in treating enzyme deficiencies. In Pompe disease, for instance, recombinant human acid alpha-glucosidase (rhGAA) is administered to substitute the deficient enzyme [45]. The use of Miglustat in Substrate Reduction Therapy (SRT) is another strategy used to mitigate the accumulation of substrates due to enzyme deficiencies. In Gaucher disease for instance, lack of the enzyme, glucocerebrosidase which degrade glycosphingolipids leads to the accumulation of the substrate (glycosphingolipids) resulting to various symptoms associated with Gaucher disease [46]. Miglustat as an enzyme inhibitor, is utilized to inhibit glucosylceramide synthase, an essential enzyme for the synthesis of most glycosphingolipids thereby preventing its accumulation and aiding in the management of Gaucher disease.

Recently discovered or engineered enzymes have showcased rapid advancements in enzyme technology and their potential impact on the development of novel therapeutics, diagnostics, and biotechnological applications in the pharmaceutical and healthcare industries. Here are some of the enzymes recently discovered or gaining prominence in the industry.

CRISPR-Cas gene editing enzymes: The CRISPR-Cas9 system, particularly the Cas9 endonuclease, has revolutionized genome editing technology, enabling precise and efficient gene editing with numerous applications in drug discovery, gene therapy, and personalized medicine [47, 48]. CRISPR-Cas9 operates by utilizing a guide RNA and the Cas9 nuclease to introduce targeted modifications to the DNA [47].

Beyond CRISPR-Cas9, other gene-editing enzymes have been developed to expand the capabilities of genome editing. Base editors, such as cytidine and adenine base editors, enable precise modification of individual DNA bases without

introducing double-strand breaks, thus enhancing the safety and efficiency of gene editing [47, 48]. Prime editors, which combine the properties of CRISPR-Cas9 and reverse transcriptase, allow for targeted insertions, deletions, and base substitutions with high precision [48]. RNA-guided nucleases like Cpf1 (Cas12a) and Cas13 (C2c2) offer distinct properties and advantages over Cas9, further expanding the gene-editing toolbox [49].

RNA-Targeting Enzymes: Enzymes involved in RNA interference (RNAi), such as Dicer and Argonaute, have emerged as promising therapeutic targets for silencing disease-causing genes at the RNA level [50]. Furthermore, RNA-modifying enzymes like RNA methyltransferases are being explored for their role in regulating gene expression and potential therapeutic applications [51]. Ribozymes and deoxyribozymes, catalytic RNA and DNA molecules, respectively, also hold potential for RNAi therapeutics, biosensors, and studying cellular processes [52].

Antimicrobial enzymes: CRISPR-based antimicrobials, exploiting the RNA-guided nuclease activity of CRISPR-Cas systems, offer a novel approach to selectively target and eliminate antibiotic-resistant bacteria, addressing the global challenge of antimicrobial resistance [53]. Additionally, enzymes like aminoglycoside phosphotransferases are being investigated for modifying aminoglycoside antibiotics to reduce toxicity and improve therapeutic properties [54].

Microbiome-modulating enzymes: Enzymes involved in modulating the gut microbiome, such as microbial-derived bile salt hydrolases and polysaccharide-degrading enzymes, have gained interest for their potential therapeutic applications in various diseases, including gastrointestinal disorders, metabolic disorders, and immune-related conditions [55]. These enzymes can influence gut microbial composition and function, with implications for human health.

Viral disease-targeting enzymes: Gene-editing enzymes, including CRISPR-based systems and engineered nucleases like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), are being investigated for their potential in treating viral diseases such as HIV and hepatitis B virus (HBV) [56, 57]. These enzymes offer the possibility of disrupting viral replication or eliminating viral reservoirs, paving the way for new antiviral therapies.

Protein engineering enzymes: Engineered enzymes have emerged as valuable tools in the pharmaceutical industry. Transglutaminases, which catalyze crosslinking between proteins, have applications in biomaterial development, tissue engineering, and drug delivery systems [58].

Engineered proteases, like sortases and transpeptidases, enable site-specific protein modification and conjugation for the production of protein-based therapeutics and diagnostic tools [59]. Additionally, deimmunized enzymes with reduced immunogenicity have been developed to improve the safety and efficacy of enzyme replacement therapies [60]. Engineered lipases, esterases, and glycosidases with improved catalytic properties and specificities have also found applications in the synthesis of chiral building blocks, prodrugs, lipid-based drug delivery systems, and the modification of glycoconjugates for therapeutics and vaccines. Many other enzymes known for their therapeutic use have been documented and these include;

Urokinase: Urokinase, or urokinase-type plasminogen activator (uPA), is a serine protease that plays a crucial role in fibrinolysis, the process of breaking down blood clots. It was first discovered in 1947 by McFarlane and Pilling [61] and is found in human urine, blood, and various tissues. Urokinase is produced using cell culture techniques, including kidney cells from animals like mice and hamsters, with innovations like the addition of phenylalanine to the culture medium to increase yields [62]. The enzyme consists of a serine protease domain, a kringle domain, and an EGF-like domain [63]. It is clinically used as a thrombolytic agent for various thrombotic conditions, such as acute myocardial infarction, pulmonary embolism, and ischemic stroke [61]. Elevated urokinase levels have been associated with tumor malignancy, making it a potential target for cancer therapy [64].

L-Asparaginase: L-Asparaginase is an enzyme used in cancer therapy, particularly for the treatment of acute lymphoblastic leukemia (ALL). It works by depleting asparagine, an essential amino acid for the survival and proliferation of certain cancer cells [65, 66]. L-Asparaginase is produced from various microbial sources, including bacteria and actinomycetes, through submerged fermentation, with extensive optimization of production conditions to maximize yield [67]. Beyond its therapeutic use, L-Asparaginase is also utilized in food processing to reduce the formation of acrylamide, a carcinogenic substance, in fried foods [68].

Collagenase: Collagenase is an enzyme that breaks down collagen, a major component of connective tissue [65]. In the pharmaceutical industry, collagenase is utilized in wound care products to promote the debridement of necrotic tissue and facilitate wound healing [65]. It is commonly used in the treatment of burns, ulcers, and in enzymatic debridement of various types of wounds.

Streptokinase: Streptokinase is an enzyme produced by certain strains of streptococci that

converts plasminogen to plasmin, a key enzyme in the fibrinolytic pathway [69]. It is used therapeutically as a thrombolytic agent to dissolve blood clots in conditions such as acute myocardial infarction, deep vein thrombosis, and pulmonary embolism [65]. Streptokinase functions by activating the body's own plasminogen to plasmin, promoting clot dissolution.

Uricase: Uricase, also known as urate oxidase, is an enzyme involved in the metabolism of uric acid [65]. Recombinant uricase has been developed for the treatment of hyperuricemia and gout by catalyzing the conversion of uric acid into a more soluble compound, allantoin, which can be easily excreted by the kidneys [70]. While uricase is not naturally present in humans, it is used in drug formulations to help reduce elevated uric acid levels associated with these conditions.

Challenges and future directions

Enzymes hold great promise in drug manufacturing, formulation, and delivery but there are several obstacles that need to be addressed to be able to achieve these promises. These obstacles include; the demand for more robust enzymes, scalability issues, concerns about the stability of enzymes, potential immunogenicity, and the need for sophisticated delivery systems. Addressing these challenges will necessitate interdisciplinary research and collaboration between industrial experts in the biotechnology and pharmaceutical sectors and researchers and scientists in the fields of pharmacology, chemistry, and biotechnology. Similarly, the biopharmaceuticals and enzyme therapies are not without their challenges too despite offering immense potentials. These entail high costs, immunogenicity concerns, and the requirement for personalized treatment approaches. Future advancements in gene editing and delivery systems may further broaden the applications of these therapies.

In addition to these challenges, the integration of artificial metabolic pathways, nanotechnology approaches, and cell factory design will play a vital role in enhancing biocatalytic processes. This will not only guarantee the productivity and effectiveness of the drugs produced but also extend the reach of biocatalysis into sectors like bioenergy and waste treatment.

CONCLUSION

In conclusion, biocatalysts are poised to make significant strides in pharmaceutical manufacturing and various other challenging sectors. However, addressing the challenges and exploring future directions will be essential to fully realize the potential of enzyme-based therapies and biopharmaceuticals.

REFERENCE

- GADIPELLY, C., PÉREZ-GONZÁLEZ, A., YADAV, G.D., ORTIZ, I., IBÁÑEZ, R., RATHOD, V.K. & MARATHE, K.V. (2014). Pharmaceutical industry wastewater: review of the technologies for water treatment and reuse. *Industrial & Engineering Chemistry Research*, **53**(29): 11571-11592.
- MITCHELL, J.B. (2017). Enzyme function and its evolution. *Current Opinion in Structural Biology*, **47**: 151-156.
- HUGHES, G. & LEWIS, J.C. (2018). Introduction: Biocatalysis in industry. *Chemical Reviews*, **118**: 1-3.
- TRUPPO, M.D. (2017). Biocatalysis in the pharmaceutical industry: The need for speed. *ACS Medicinal Chemistry Letters*, **8**: 476-480.
- NEWTON, M.S., ARCUS, V.L., GERTH, M.L. & PATRICK, W.M. (2018). Enzyme evolution: innovation is easy, optimization is complicated. *Current Opinion in Structural Biology*, **48**: 110-116.
- YANG, H., LI, J., DU, G. & LIU, L. (2017). *Biotechnology of Microbial Enzymes: Production, Biocatalysis and Industrial Applications*. Academic Press Books, Elsevier. pp. 151-165.
- MEGHWANSHI, G.K., KAUR, N., VERMA, S., DABI, N.K., VASHISHTHA, A., CHARAN, P.D., PUROHIT, P., BHANDARI, H.S., BHOJAK, N. & KUMAR, R. (2020). Enzymes for pharmaceutical and therapeutic applications. *Biotechnology and Applied Biochemistry*, **67**(4): 586-601.
- SUN, H. ZHANG, H., ANG, E.L. & ZHAO, H. (2017) Biocatalysis for the synthesis of pharmaceuticals and pharmaceutical intermediates. *Bioorganic & Medicinal Chemistry*, **26**(7): 1275-1284.
- SOY, S., PRABHA, R. & KUMAR N.V. (2021). Potential of Biocatalysis in Pharmaceuticals. *IntechOpen*. doi: 10.5772/intechopen.90459
- VASINA, M., VELECKÝ, J., PLANAS-IGLESIAS, J., MARQUES, S.M., SKARUPOVA, J., DAMBORSKY, J., BEDNAR, D., MAZURENKO, S. & PROKOP, Z. (2022). Tools for computational design and high-throughput screening of therapeutic enzymes. *Advanced Drug Delivery Reviews*, **183**: 114143.
- BIRKS, J. (2006). Cholinesterase inhibitors for Alzheimer's disease. *The Cochrane Database of Systematic Reviews*, **2006**(1): CD005593.
- SINGH, M., KAUR, M., KUKREJA, H., CHUGH, R., SILAKARI, O. & SINGH, D. (2013). Acetylcholinesterase inhibitors as Alzheimer therapy: from nerve toxins to neuroprotection, *European Journal of Medicinal Chemistry*, **70**: 165-88.
- SAXENA, M. & DUBEY, R. (2019). Target Enzyme in Alzheimer's Disease: Acetylcholinesterase Inhibitors, *Current Topics in Medicinal Chemistry*, **19**(4): 264-275.
- KUMAR, B., GUPTA, V. P. & KUMAR, V. (2017). A Perspective on Monoamine Oxidase Enzyme as Drug Target: Challenges and Opportunities. *Current Drug Targets*, **18**(1): 87-97.
- DOBRZYŃ, A. & NTAMBI, J.M. (2005). Stearoyl-CoA desaturase as a new drug target for obesity treatment. *Obesity Reviews*, **6**(2): 169-174.
- ZHAO, M., MA, J., LI, M., ZHANG, Y., JIANG, B., ZHAO, X. & HUAI, C. (2021). Cytochrome P450 Enzymes and Drug Metabolism in Humans. *International Journal of Molecular Sciences*, **22**(23): 12808.
- ZHOU, S.F. (2009). Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clinical Pharmacokinetics*, **48**(11): 689-723.
- ATALAH, J., CÁCERES-MORENO, P., ESPINA, G. & BLAMEY, J.M. (2019). Thermophiles and the applications of their enzymes as new biocatalysts. *Bioresource Technology*, **280**: 478-488.
- WILTSCHI, B., CERNAVA, T., DENNIG, A., GALINDO C.M., GEIER, M., GRUBER, S., HABERBAUER, M., HEIDINGER, P., HERRERO A.E., KRATZER, R., LULEY-GOEDL, C., MÜLLER, C. A., PITZER, J., RIBITSCH, D., SAUER, M., SCHMÖLZER, K., SCHNITZHOFFER, W., SENSEN, C.W., SOH, J. & WRIESSNEGGER, T. (2020). Enzymes revolutionize the bioproduction of value-added compounds: From enzyme discovery to special applications. *Biotechnology Advances*, **40**: 107520.
- TOBIN, P.H., RICHARDS, D.H., CALLENDER, R.A. & WILSON, C.J. (2014). Protein Engineering: A New Frontier for Biological Therapeutics. *Current Drug Metabolism*, **15**(7): 743.
- RIGOLDI, F., DONINI, S., REDAELLI, A., PARISINI, E. & GAUTIERI, A. (2018). Review: Engineering of thermostable enzymes for industrial applications. *APL Bioengineering*, **2**(1): 10-16
- GROEGER, H., PIEPER, M., KOENIG, B., BAYER, T. & SCHLEICH, H. (2017).

- Industrial landmarks in the development of sustainable production processes for the β -lactam antibiotic key intermediate 7-aminocephalosporanic acid (7-ACA). *Sustainable Chemistry and Pharmacy*, **5**: 72-79.
23. ZHANG, B., WANG, J., CHEN, J., ZHANG, H., YIN, D. & ZHANG, Q. (2017). Magnetic mesoporous microspheres modified with hyperbranched amine for the immobilization of penicillin G acylase. *Biochemical Engineering Journal*, **127**: 43-52.
 24. FAN, Y., EVANS, C.R. & LING, J. (2017). Rewiring protein synthesis: From natural to synthetic amino acids. *Biochimica et Biophysica Acta (BBA)-General Subjects*, **1861**(11): 3024-3029.
 25. CHEONG, J.E., PFEIFFER, C.T., NORTHRUP, J.D., PARKER, M.F. & SCHAFMEISTER, C.E. (2016). An improved, scalable synthesis of bis-amino acids. *Tetrahedron Letters*, **57**(44): 4882-4884.
 26. SIEBERT, A., WYSOCKA, M., KRAWCZYK, B., CHOLEWIŃSKI, G. & RACHOŃ, J. (2018). Synthesis and antimicrobial activity of amino acid and peptide derivatives of mycophenolic acid. *European Journal of Medicinal Chemistry*, **143**: 646-655.
 27. LEUCHTENBERGER, W., HUTHMACHER, K. & DRAUZ, K. (2005). Biotechnological production of amino acids and derivatives: current status and prospects. *Applied Microbiology and Biotechnology*, **69**: 1-8.
 28. KAWANO, Y., OHTSU, I., TAKUMI, K., TAMAKOSHI, A., NONAKA, G., FUNAHASHI, E. & TAKAGI, H. (2015). Enhancement of L-cysteine production by disruption of yciW in *Escherichia coli*. *Journal of Bioscience and Bioengineering*, **119**(2): 176-179.
 29. LLOYD, M.D. (2020). High-Throughput Screening for the Discovery of Enzyme Inhibitors. *Journal of Medicinal Chemistry*, **63**(19): 10742-10772.
 30. FABIAN, M.A., BIGGS, W.H. & TREIBER, D.K. (2005). A small molecule-kinase interaction map for clinical kinase inhibitors. *Nature Biotechnology*, **23**(3): 329-336.
 31. KINNER, A., NERKE, P., SIEDENTOP, R., STEINMETZ, T., CLASSEN, T., ROSENTHAL, K., NETT, M., PIETRUSZKA, J. & LÜTZ, S. (2022). *Recent Advances in Biocatalysis for Drug Synthesis and Biomedicines*, **10**(5): 73-78
 32. CIRIMINNA, R. & PAGLIARO, M. (2013). Green Chemistry in the Fine Chemicals and Pharmaceutical Industries. *Organic Process Research & Development*, **17**(12): 1479-1484.
 33. SHELDON, R.A. (2016). Green and sustainable manufacture of chemicals from biomass: state of the art. *Green Chemistry*, **18**(13): 4369-4380
 34. DUNN, P. (2011). The importance of Green Chemistry in Process Research and Development. *Chemical Society Reviews*, **41**: 1452-1461.
 35. CHEN, L., KONG, Q., TIAN, M., ZHANG, Q., XIA, C. & DENG, C. (2023). Zn 0.4 Mg 0.6 Fe₂O₄ nanoenzyme: a novel chemo-sensitizer for the chemotherapy treatment of oral squamous cell carcinoma. *Nanoscale Advances*, **5**(3): 851-860.
 36. DESMOULIN, F., GILARD, V., MALET-MARTINO, M. & MARTINO, R. (2002). Metabolism of capecitabine, an oral fluorouracil prodrug: (19)F NMR studies in animal models and human urine. *Drug Metabolism and Disposition*, **30**(11): 1221-1229.
 37. LU, C. & LI, A.P. (2010). Enzyme Inhibition in Drug Discovery and Development: *The Good and the Bad*, **10**.1002/9780470538951.
 38. WANG, R., ZHAO, C. & JIANG, S. (2022). Advanced nano-particles that can target therapy and reverse drug resistance may be the dawn of leukemia treatment: A bibliometrics study. *Frontiers in Bioengineering and Biotechnology*, **10**: 1027868.
 39. REYES-REYES, A.L., BARRANCO, F.V. & SANDOVAL, G. (2022). Recent Advances in Lipases and Their Applications in the Food and Nutraceutical Industry. *Catalysts*, **12**(9): 960.
 40. WALSH, G. (2018). Biopharmaceutical benchmarks 2018. *Nature Biotechnology*, **36**(12): 1136-1145.
 41. JONES, D.T., TAYLOR, W.R. & THORNTON, J.M. (1992). The rapid generation of mutation data matrices from protein sequences. Computer applications in the biosciences: *CABIOS*, **8**(3): 275-282.
 42. SLIWKOWSKI, M.X. & MELLMAN, I. (2013). Antibody therapeutics in cancer. *Science*, **341**(6151): 1192-1198.
 43. SAWKAR, A.R., CHENG, W.C., BEUTLER, E., WONG, C.H., BALCH, W.E. & KELLY, J.W. (2002). Chemical chaperones increase the cellular activity of N370S beta -glucosidase: a therapeutic strategy for Gaucher disease. *Proceedings of the National Academy of*

- Sciences of the United States of America*, **99**(24): 15428–15433.
44. RUSSELL, S., BENNETT, J. & WELLMAN, J.A. (2017). Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet*, **390**.10097: 849–860.
 45. KISHNANI, P.S., CORZO, D., NICOLINO, M., BYRNE, B., MANDEL, H., HWU, W. L., LESLIE, N., LEVINE, J., SPENCER, C., MCDONALD, M., LI, J., DUMONTIER, J., HALBERTHAL, M., CHIEN, Y.H., HOPKIN, R. & VIJAYARAGHAVAN, B. (2007). Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology*, **68**(2): 99-109.
 46. COX, T.M. & CACHÓN-GONZÁLEZ, M.B. (2012). The cellular pathology of lysosomal diseases. *The Journal of Pathology*, **226**(2): 241–254.
 47. QIAN, Y., WANG, D., NIU, W., ZHAO, D., LI, J., LIU, Z., GAO, X., HAN, Y., LAI, L. & LI, Z. (2023). A new compact adenine base editor generated through deletion of HNH and REC2 domain of SpCas9. *BMC Biology*, **21**(1): 155.
 48. ANZALONE, A.V., RANDOLPH, P.B., DAVIS, J.R., SOUSA, A.A., KOBLAN, L.W., LEVY, J.M., CHEN, P.J., WILSON, C., NEWBY, G.A., RAGURAM, A. & LIU, D.R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, **576**(7785): 149-157.
 49. GHORBANI, A., HADIFAR, S., SALARI, R., IZADPANAH, K., BURMISTRZ, M., AFSHARIFAR, A., ESKANDARI, M.H., NIAZI, A., DENES, C.E. & NEELY, G.G. (2021). A short overview of CRISPR-Cas technology and its application in viral disease control. *Transgenic Research*, **30**(3): 221-238.
 50. IWAKAWA, H.O. & TOMARI, Y. (2022). Life of RISC: Formation, action, and degradation of RNA-induced silencing complex. *Molecular Cell*, **82**(1): 30-43.
 51. ROUNDTREE, I.A., EVANS, M.E., PAN, T. & HE, C. (2017). Dynamic RNA modifications in gene expression regulation. *Cell*, **169**(7): 1187.
 52. CHI-WONG, B., SHAHID, U. & SIEW TAN, H. (2023). Ribozymes as Therapeutic Agents against Infectious Diseases. *IntechOpen*, doi: 10.5772/intechopen.107141
 53. LI, X., BAO, N., YAN, Z., YUAN, X., WANG, S. & XIA, P. (2023). Degradation of Antibiotic Resistance Genes by VADER with CRISPR-Cas Immunity. *Applied and Environmental Microbiology*, **89**(4): e00053-23
 54. RAMIREZ, M.S. & TOLMASKY, M.E. (2017). Amikacin: Uses, Resistance, and Prospects for Inhibition. *Molecules*, **22**(12): 2267
 55. SHANAHAN, F., GHOSH, T.S. & O'TOOLE, P.W. (2021). The Healthy Microbiome-What Is the Definition of a Healthy Gut Microbiome? *Gastroenterology*, **160**(2): 483–494.
 56. ZHANG, Z., HOU, W. & CHEN, S. (2022). Updates on CRISPR-based gene editing in HIV-1/AIDS therapy. *Virologica Sinica*, **37**(1): 1-10.
 57. HUSSEIN, M., MOLINA, M.A., BERKHOUT, B. & HERRERA-CARRILLO, E. (2022). A CRISPR-Cas Cure for HIV/AIDS. *International Journal of Molecular Sciences*, **24**(2): 1563
 58. VASIĆ, K., KNEZ, Ž. & LEITGEB, M. (2022). Transglutaminase in Foods and Biotechnology. *International Journal of Molecular Sciences*, **24**(15): 12402.
 59. ZHANG, Y., PARK, Y., SUAZO, K.F. & DISTEFANO, M.D. (2018). Recent progress in enzymatic protein labelling techniques and their applications. *Chemical Society Reviews*, **47**(24): 9106.
 60. ZINSLI, L.V., STIERLIN, N., LOESSNER, M.J. & SCHMELCHER, M. (2021). De-immunization of protein therapeutics – Recent advances in experimental and computational epitope prediction and deletion. *Computational and Structural Biotechnology Journal*, **19**: 315-329.
 61. ALTAF, F., WU, S. & KASIM, V. (2021). Role of fibrinolytic enzymes in anti-thrombosis therapy. *Frontiers in Molecular Biosciences*, **8**: 680397.
 62. MUKHERJEE, S., MALIK, P. & MUKHERJEE, T.K. (2023). Mammalian Cells, Tissues and Organ Culture: Applications. In *Practical Approach to Mammalian Cell and Organ Culture*. Springer Nature Singapore. pp. 837-915.
 63. LETH, J.M. & PLOUG, M. (2021). Targeting the Urokinase-Type Plasminogen Activator Receptor (uPAR) in Human Diseases with a view to Non-invasive Imaging and Therapeutic Intervention. *Frontiers in Cell and Developmental Biology*, **9**: 732015.
 64. LV, T., ZHAO, Y., JIANG, X., YUAN, H., WANG, H., CUI, X., XU, J., ZHAO, J. & WANG, J. (2021). UPAR: An Essential Factor for Tumor Development. *Journal of Cancer*, **12**(23): 7026-7040.

65. TANDON, S., SHARMA, A., SINGH, S., SHARMA, S. & SARMA, S.J. (2021). Therapeutic enzymes: Discoveries, production and applications. *Journal of Drug Delivery Science and Technology*, **63**: 102455.
66. JULURI, K.R., SIU, C. & CASSADAY, R.D. (2022). Asparaginase in the Treatment of Acute Lymphoblastic Leukemia in Adults: Current Evidence and Place in Therapy. Blood and Lymphatic Cancer. *Targets and Therapy*, **12**: 55-79.
67. BATOOL, T., MAKKY, A.E., JALAL, M. & YUSOFF, M.M. (2016). A Comprehensive Review on L-Asparaginase and Its Applications. *Applied Biochemistry and Biotechnology*, **178**: 900-923.
68. HENDRIKSEN, H.V., KORNBRUST, B.A., ØSTERGAARD, P.R. & STRINGER, M.A. (2009). Evaluating the potential for enzymatic acrylamide mitigation in a range of food products using an asparaginase from *Aspergillus oryzae*. *Journal of Agricultural and Food Chemistry*, **57**(10): 4168–4176.
69. URANO, T., SUZUKI, Y., IWAKI, T., SANO, H., HONKURA, N. & CASTELLINO, F.J. (2019). Recognition of Plasminogen Activator Inhibitor Type 1 as the Primary Regulator of Fibrinolysis. *Current Drug Targets*, **20**(16): 1695.
70. AZEVEDO, V.F., KOS, I.A. & VARGAS-SANTOS, A.B. (2019). Benzbromarone in the treatment of gout. *Advances in Rheumatology*, **59**: 37.