REVIEW ARTICLE

Application of Enzymes in Drug Discovery Research: A Review

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ABSTRACT

Enzymes have effectively substituted many conventional chemical catalysts-reliant pharmaceutical drug manufacturing procedures. From this v-prospect, the use of enzymes in drug discovery research is quite encouraging. Numerous enzymes have been demonstrated to perform detoxification at the cellular level, for instance, the antioxidant enzymes, catalase, and superoxide dismutase (SOD). These enzymes work simultaneously to neutralize oxygen-free radical species. The enzyme-mediated pharmaceutical procedures include the synthesis of diverse semi-artificial antibiotics, dynamic enantiomers of medicines through kinetic determination, production of enantiomerically unadulterated types of amino acids, and others. Enzymes are being used to treat cancer and infectious disorders where antibiotics are no longer effective because of antibiotic resistance. Thus, the present article aims to review the diverse use of enzymes in the drug industries and discuss the features of enzymes that make them suitable drug candidates.

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Introduction

Enzymes are biocompounds usually proteins, or nucleic acids (ribozymes), which lower the activation energy required to perform a chemical reaction. Nucleic acids accelerate only protein synthesis, while protein enzymes are for other bioprocesses. These biocatalysts regulate the progression of biochemical responses at rates appropriate for cellular functions, proliferation, and growth. [1-3] Even before their complete elucidation,

the enzyme and its applications were well known, and Wilhelm Friedrich K¨uhne was the first individual to offer a scientific vocabulary for this class of biomolecules.[4] Enzymatic processes have been demonstrated since the prehistoric Egyptian artifacts where microorganisms and their enzymes were utilized to preserve drinks and food. Lazzaro Spallanzani, a renowned Italian catholic priest, first discussed the relevance of this biomolecule in his work on biogenesis in 1783. He claimed that certain types of non-living materials had an inherent ability to start life, which enables live germs to produce them. [5] Gottlieb Sigismund Kirchhoff investigated the conversion of starch to glucose in 1812. He investigated and wrote a paper on the use of these biomolecules as catalysts in this procedure.[6] The first enzyme, diastase, was isolated and characterised by French scientist Anselme Payen in 1833 and [7] in 1960, NOVO started producing protease on an industrial scale using Bacillus lichnformis. After 1980, a lot of scientists began systematically using genetic engineering techniques to improve the efficiency of producing enzymes and change their properties. [8]

Naturally occurring enzymes have been applied extensively since primeval times and in leather, linen, and indigo manufacturing. All the procedures for creating these products rely on either the enzymes created by microbes or the enzymes found in other sources such as the papaya fruit or calves' rumen. [9] Because of their utility in the dairy and detergent industries, proteases continue as the primary type of enzyme under investigation from natural sources. [10] The second major group that underwent specific interventions are the carbohydrases, principally cellulases, and amylases due to their utility as detergents alongwith in the baking and textile industries. [11] The capability of enzymes maintain operational and carry out catalysis activities, even outside of their organism of origin [12], permits their application in several industrial procedures dependent upon chemical alterations of substrates to their identical products.[13]

The utility of enzymes far exceeds just their use within the human body; within the human surrounding can be seen in many of their approaches in several manufacturing applications. About 200 bacterial enzymes from 4000 known enzymes are used commercially. [14] Three companies create almost 75% of the overall enzymes: specifically, United States-based DuPont, Denmark-based Novozymes, and Switzerland-based Roche. [15] The remaining 25% are made by other companies, including Novus International Inc, El du Pont de Nemours and Company, ABF (Associated British Foods), a Dutch multinational corporation known as Koninklijke DSM NV, and AB Enzymes GmbH. Following the report presented by Mordor Intelligence named "Industrial Enzymes Market: Growth, Trends, and Forecast (2019–2024)", the international market of industrial enzymes is anticipated to rise at an approximate yearly development rate of 6.8% during the years 2019–2024. [16,17]

As a result of the relative importance of the enzyme industry and the potential effects of this growing industry on the world at large, an explanation associated with the previous market prediction of the most extensively utilized enzymes such as carbohydrases, proteases, and lipases and their market for the sectors of healthcare and pharmaceutical is presented for the reader's reference. Global Market Insights Industrial's Report [gm insights] predicted that the worldwide market for proteases is expected to increase to approximately 2 billion United States dollars by 2024. On the other hand, industrial wastes are expected to decrease through these enzymes as companies would rather pay to clean the mess than pay a fine. The use of enzymes in modern industry is discussed further in the following section.

Importance of Enzymes in Various Industries

Due to their many advantages, which range from their functioning in temperate response conditions to their remarkable product selectivity and their little physiological and atmospheric toxicity, enzymes are extremely effective biocatalysts researched for industrial-level catalysis. [18-20] These advantages of enzymes are in addition to cost-effectiveness when used as biocatalysts in chemical procedures. The enzymes have even 2000 times more TON (turnovers) and 1000 times higher lifetime than inorganic and nonbiological catalysts[21,22]. Due to their minimal energy needs, alleviation of waste generation, and primary routes of production, [23-24] enzymes have been partly appreciated in the beverage, pharmaceutical, and food industries [25-31]. The connection of the biocatalyst to a medium with additional electrical, physical, mechanical, or chemical properties has been the focus of research on the immobilisation of enzymes, and it has been shown that immobilising biocatalysts can improve their stability and activity across a wide range of working situations.[32-36]

Applications of Enzymes in Pharmaceutical Research

Recently, drug discovery is essential for the consistent application of different approaches. It enhances the effectiveness, executes novel technologies, and enhances the drug quality. Existing strategies observe detection in four steps: 'hit' selection, selection of lead, optimization of lead, and development selection.[37] Multiple research studies discovered the deprived properties that cause the deliberate destruction of development organizations. The candidate drugs select through experiments to ensure that the compounds with poor properties without progress.[38] In numerous cases, properties are determined to optimize pharmacokinetics, and the properties that influence human absorption and metabolism are applied. [39] If a compound has poor pharmacokinetic characteristics, it may not be therapeutic in vivo even while it is very dynamic in vitro. If a less effective substance's qualities allow for better exposure in vivo, it might produce a better healing effect. [40-41] One of the most critical obstacles

faced by pharmaceutical organizations is assigning their drug discovery reserves toward medicinally appropriate protein targets. Chemical explorations give a process to focus upon the efforts of preliminary novel target recognition towards proteins that are more effortlessly authenticated almost certainly to be efficient targets of a drug, thus generating a higher prospective for achievement. [43]

Principles of early drug discovery

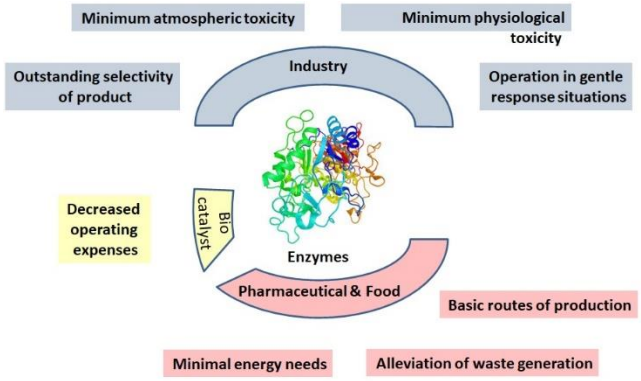

Figure 1. Working of release of new drug

When a disease or clinical condition exists for which there are no suitable pharmaceutical treatments available on the market, a drug development programme is initiated, and this unmet clinical need serves as the project's primary driving force. Drugs that fail in clinical trials typically do so for one of two reasons: either they are hazardous or useless. In order to strengthen confidence in the connection between the target and disease, target selection and validation is one of the key steps in the development of a novel drug.

Additional ways of identification include measuring mRNA/protein levels to ascertain the disease's expression and whether they are related to the disease's development or exacerbation. Another useful tactic is to look for genetic associations, such as whether a genetic variant is merely functional or is associated with the risk or progression of a disease. Phenotypic screening is an alternate method to find disease-relevant targets. Target identification and mechanism of action should be clarified very early in the drug discovery process due to the practical benefits that this knowledge provides.[44,45]

As more software systems for the prioritisation of pharmaceutical targets are created, they heavily rely on efficacy estimates based on ratings for the target-disease associations. Here, novel computational approaches are introduced that can be used to more precisely evaluate the efficacy and safety of potential treatment targets. The suggested efficacy scores enhance the inference of target-disease connections by utilizing tissue/disease-specific networks and existing gene expression data. [46]

Target proteins may be recognized via chemical explorations that can be potential drug targets.

Chemical probes can also be applied to validate research in cellular models or animals at both early periods of ailment model selection and throughout experiments of early target confirmation. [48] Additionally, specific chemical probe components and reactive clusters may serve as the cornerstone of a strategy for molecule inhibitors. Chemical investigations have already been used to identify

cysteine proteases involved in processes like apoptosis, [50] cataract formation, [47] and malaria infection. [48] Additionally, the number of reported chemical probes is increasing, and various mechanism-dependent affinity labelling inhibitors or reagents have been identified that could be used to create new categories of chemical probes. The protein phosphatases and protein kinases are two crucial families of enzymes that have been the focus of medical discovery efforts and are particularly amenable to chemical proteomics applications. These enzymes are important regulators of metabolism and cell signalling, and they are frequently thought of as potential therapeutic targets.[48, 49] A chemical proteomic method may be useful for the family of protein kinase enzymes. More than 500 members of this family provide various substrate and cellular functions.[50,51] Due to their involvement in numerous cell-signaling processes, protein phosphatases are becoming increasingly prominent as therapeutic targets.[52] Over 600 industrial goods are produced by enzymes, and there is a growing need for them in businesses looking for long-lasting solutions. [53]

Enzyme therapy

At the industrial level, catalysis by enzymes has been successfully used to produce compounds with pharmacological functionality. [54] The strong chemo-, regio-, and stereo-selectivity with which enzymes alter substrates to a resulting product is one of the most significant advantages of catalysis using enzymes over classical catalysis.[23,55] Due to the efficient product production processes and consistent improvement in the industry's economics, the high degree of product specificity is vastly preferred in these types of pharmaceutical operations.[56,57] On an industrial scale, the multidisciplinary strategy is appropriate for biocatalysts. It is highlighted by Merck's development of the enzyme-catalyzed sitagliptin, a medication used to treat type 2 diabetes mellitus. [58] Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) DPP-4 inhibitor that lowers diabetes-induced tight connection disassembly and avoids an increase in the blood-retinal barrier (BRB).[59] To produce sitagliptin traditionally, enamine is elevated-pressure hydrogenated using a catalyst supported by rhodium, followed by carbon management to remove trace amounts of rhodium and express sitagliptin in excess of 97% enantiomers.[60] A bicyclic proline intermediate, a significant precursor in the production of boceprevir, an NS3 protease inhibitor used for the management of persistent hepatitis C infections, has been desymmetrized using an MAO (monoamine oxidase)-catalyzed procedure that has been expanded in research by Merck and Codexis. [23, 61]

Use of enzymes as oral and inhalable drugs

An advanced marketable-level production technique for (S, S)-reboxetine succinate—a nor-epinephrine anti-depressant for the treatment of fibromyalgia—was reviewed by Hayes et al. in their study. Pfizer was in the last stages of developing this drug for the treatment of fibromyalgia. [62,63]

Pregabalin, a neuroactive drug with antiepileptic, analgesic, and anti-apprehension activity that is used to treat anxiety, epilepsy, and irrational social fear, was synthesised at an industrial level by Martinez et al. using a novel generation. [64, 65] Since de Duve proposed a therapeutic enzyme as part of alternative therapy for inherited inadequacies in the 1960s, the concept of the remedial enzyme has been around for at least forty years. [66] The FDA approved Alteplase (Activase; recombinant plasminogen activator for human tissue), the first recombinant enzyme preparation, in 1987. This 'coagulate-buster' enzyme, which was the second recombinant protein therapy to be promoted on the market (the first hereditarily created preparation was insulin in 1982), is used in the management of cardiac crises, which are caused by the occlusion of a coronary artery by a thick clot.[67,68] Numerous additional enzymes that are used as coagulant or anti-clotting medicines have received FDA approval. Peg Damage Bovine (Adagen), a type of bovine ADA treated with PEG, received approval in 1990 to treat individuals suffering from a form of SCID (severe combined immunodeficiency disease), which is caused by a persistent insufficiency of adenosine deaminase. It is significant to remember that the first healing enzyme recognised by the Food and Drug Administration under the Orphan Drug Act, or ODA, was pegadamase bovine (Adagen). The Orphan Drug Act was created in the USA in 1983 to encourage the pharmaceutical industry to create treatments for diseases affecting only a tiny population (not more than 200,000).

Proteolytic and glycolytic enzymes

The elevated levels of adenosine's toxicity to the immune system are lessened by the enzyme adenosine deaminase, which is present in the circulation of these patients. The change of adenosine deaminase with polyethylene glycol is crucial to the management's success.[69] Because the medication is derived from cattle, polyethylene glycol reduces the possibility of immunological reactions while increasing the enzyme's half-life (which is actually less than thirty minutes). Another enzyme used to treat CSID is sacrosidase, as patients with congenital sucrase-isomaltase deficiency are unable to absorb the disaccharide sucrose. [70] The amount of enzymes is used to manage the digestive issues that are triggered by a lot of sweets. The enzyme -galactosidase is taken as a digestive aid by people who have. The enzyme -galactosidase is taken as a digestive aid by people who have abdominal gas, bloating, and diarrhoea after consuming meals like beans, Brussels sprouts, broccoli, cabbage, and other members of the Brassica family of vegetables. [71] Lactase and -galactosidase are currently available as dietary supplements in some sets. When eating foods containing lactose, such as milk and milk products, those who have lactose intolerance experience gastrointestinal distress because they are unable to digest the sugar. [72] These folks have access to lactase supplements like lactase powder and lactase-enhanced milk. These supplements lessen lactose intolerance symptoms like gas, abdominal bloating, and diarrhoea by facilitating the dissociation of

lactose into its monomers, galactose and glucose. [73, 74] Following genetic modification, glycolytic enzymes should increase hydrogen production to an industrial-scale level, enabling them to replace current techniques for producing the element.[75]

Enzymes as anti-inflammatory drugs

When phenylalanine hydroxylase levels are low or nonexistent, phenylketonuria develops. This enzyme helps to maintain the body's normal levels of phenylalanine by converting it into tyrosine. [76] A plant's PAL (phenylalanine ammonia-lyase), which was obtained from recombinant yeast, was used to construct an oral treatment for the condition. This treatment is now on the market under the brand name Phenylase TM. Additionally, it has been demonstrated that the GIT's phenylalanine is hydrolyzed by phenylalanine ammonia-lyase. [77]

Based on the current knowledge of pancreatic secretions, a mixture of pancreatic enzymes, comprising lipases, proteases, and amylases, was developed as an all-purpose digestive support for the benefit of immunosuppressed patients. It has been demonstrated that the pancreatic enzyme mixture effectively lowers lipid malabsorption in HIV patients. [78,79] The management of pancreatic insufficiency, which occurs frequently in CF and chronic pancreatitis, is also aided by this cocktail of enzymes.[76] A mixture of pancreatic enzymes with the market name “TheraCLECTotalTM” is accessible commercially.

Conclusion

Enzymes find great applicability in drug development, and they are proven to be excellent drugs mainly owing to two of their properties. The first of these properties is that enzymes can bind specifically to their target site, and the second is that enzymes are catalytic, which aids the conversion of various target substrates to desired products. With these outstanding features, enzymes excel as a significant therapeutic component for a wide range of disorders that many other drug candidates can not achieve. Advent in biotechnology has had the pharmaceutical industries develop safe, cost-effective, high-specificity enzymes in the last decades. In line with scientific progress, drug laws have evolved, and drug administration bodies have taken initiatives to support the development of enzyme drugs. In the future, further interventions are expected to exploit more potential of the enzymes in the treatment of severe and rare diseases.

Conflict of Interest

The author started there is no conflict of interest.

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