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Innovations in Enzyme Technology: Applications in Industry and Medicine

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Annotation: Enzymes are biological catalysts that perform specific functions, such as food digestion and synthesis of biomolecules. They have diverse applications in industrial and medical arenas. Many biomolecules can now be synthesized from simple precursors using properly engineered biocatalysts. Since the commercialization of the protease, trypsin, over a hundred years ago, advances in biocatalyst development have accelerated, allowing previously expensive and infeasible biomolecule syntheses to be carried out on increasingly larger scales. The most active research area in enzyme technology is that of enzyme immobilization. The immobilization of enzymes on solid supports, nanoparticles, or within ceramics, fibers, and polymers can improve enzyme stability, target enzymes to specific sites in the body, and reduce enzyme concentrations. This essay attempts to survey the many areas of enzyme technology that have progressed in an incomplete and piecemeal fashion over the past three decades.

The rapid expansion of enzyme applications arose from our increased understanding of enzyme structure and function. New technologies introduced in this mid-20th century age of molecular biology made this possible. The exponential increase in our understanding of enzyme structure and function can be shown by the fact that the number of protein-related entries in the public protein databases doubles in number about every 10 years. In 1976, there were only two protein structures solved; in 1986, 89; in 2000, several thousand; in 2007, about 20,000 were known; and in 2012, the PDB had approximately 83,000 protein structures, including those of over 7,500 unique protein sequences. The origin of such an increased number of protein structures is directly linked with technological developments in X-ray crystallography, multidimensional nuclear magnetic resonance spectroscopy, infrared absorption, and imaging techniques, together with early quantum mechanics and quantum chemistry.

1. Introduction to Enzyme Technology

Enzymes are molecules that serve as biological catalysts in a multitude of biochemical processes. They were once considered harmless curiosity—mere side products of scientific research in cell physiology. Little did anyone know that over the following century or so, they were to become one of the most powerful instruments of scientific research and one of the driving forces of technological advancement. More than that, these tools have found effective applications in nearly every branch of industry and also in medicine. They are used to increase productivity and lower the costs of many chemical transformations. They are also extensively employed to streamline the recovery and isolation of desired products in biotechnology.

Enzymes perform a number of crucial functions in living organisms. They can accelerate the rates of biochemical reactions as much as a million times or more; this property forms the basis for a number of their practical applications. The rapid rate, choice of substrates, and specificity of enzymes have directed their development for both industrial and medical purposes. In industries, enzymes are used to improve food quality, in biofuel production, as ingredients in detergents and cleaning agents to enhance the breakdown of protein, starch, and fats in waste, or in technical applications like recovering valuable metals. Curative therapies process applications of enzymes in medicine. Innovations in terms of enzyme technology are critical considering that medical and industrial advances are equally reliant on biotechnological applications. [1][2][3]

1.1. Definition and Importance of Enzymes

Enzymes are proteins that catalyze (i.e., accelerate) chemical reactions. Enzymes are found in all living systems: plants, animals, fungi, and microbes. They carry out vital roles in several functions, including metabolic pathways for energy and ATP production; synthesis of cellular building blocks, including nucleic acids, proteins, and lipids; and degradation and breakdown of waste materials. Enzymes also contribute to the general maintenance of cellular functions and regulation, working in protein-protein and protein-nucleic acid complexes. Each enzyme is specific for a particular reaction, or set of reactions, and in many cases is able to react with only one of the two enantiomers of a chiral molecule. This property is called stereospecificity. Enzymes are also able to carry out reactions under much milder conditions of temperature and pressure than those required to carry out the equivalent process by chemical synthesis.

Enzymes function with both efficiency and specificity. Efficiency is gained through the ability of enzymes to accelerate the velocity of reaction by as much as hundreds of millions of times faster than the same reaction without the enzyme. This is recorded by the second-order rate constant, which gives an indication of the diffusion-limited rate of the enzyme. Enzymes can also be specific in their choice of substrates. Typically, enzymes will catalyze one or a few very closely related chemical reactions and will interact selectively with these particular substrates and no others. Enzymes are grouped into families based on their related amino acid sequences; all enzymes within a family are thought to have descended from a common ancestor by gene duplication and evolution. There are multiple thousands of different enzymes now recognized. The versatility and specificity of enzyme action have desirable applications in medicine, diagnostics, and industry, as well as being of fundamental importance in our understanding of the activity of important biological systems. Enzymes are responsible for maintaining the flow of biological systems and thus sustaining life. The benefits of incorporating enzymes into technology include sustainability, both economically and environmentally. Additionally, enzymes have established popularity in applications of health with ever-increasing developments, notably in diagnostics and therapeutics. With the endless list of established and possible uses for enzymes, there is little requirement for environmental microorganisms to have new enzymes if they are to outcompete their rivals. [4][5]

2. Fundamentals of Enzyme Structure and Function

Enzymes are biological catalysts that determine the rate of almost all metabolic reactions in living organisms. Their biological activity is a direct result of their detailed three-dimensional structure, with the activity and specificity of each enzyme being linked to its shape and the microenvironment provided by the intricately ordered assembly of its atoms. Hence, there is a very specific relationship between the structure of an enzyme and its function and biological role. An understanding of the molecular basis of the properties of enzymes is critical to those who need to design and/or modify their properties for use in the biotechnological, pharmaceutical, and medical fields. The structure of enzymes can be considered at several levels, including primary, secondary, tertiary, and quaternary structures, and these structural levels are detailed further below. In industrial and medical applications, numerous factors influence the use of an enzyme. One of the most important of these is the effect of temperature, since this parameter exerts a fundamental influence on enzymatic activity. Typically, the activities of most enzymes are highest at temperatures in the range of about 35-65°C. In addition, all enzymes have an optimum pH, with the pH optimum for most human enzymes being in the neutral range. An understanding of the relevant principles that govern the structure and function of enzymes is an essential beginning for considering how the properties of enzymes might be modified in innovative ways. [6][7][8]

2.1. Primary, Secondary, Tertiary, and Quaternary Structures

Enzymes are proteins that catalyze metabolic and synthetic reactions in living organisms. In this section, we will elaborate on the structure of enzymes. The structure of enzymes is crucial to their function in this series. The structure of enzymes forms a basis for enzyme applications. Enzymes have several structures, and we discuss several aspects. There are four enzyme structural levels, namely primary, secondary, tertiary, and quaternary structures. The primary structure arises from the linear arrangement of amino acid residues in enzyme polypeptide chains, which are connected through peptide bonds to form the protein backbone. The order of amino acids specifies this one-dimensional covalent backbone. The one-dimensional nature of an enzyme's structure often makes it tempting to reduce the network of interactions and phenomena involved from the analysis and design.

The secondary structure arises from the arrangement of the polypeptide chains in a repeating pattern to produce a specific three-dimensional shape. The common secondary structural elements are the alpha-helix, beta-sheet, beta-turn, and random coil. The repetitive patterns in this two-dimensional enzyme spatial organization lead to a three-dimensional enzyme structure organization. The alpha-helix and beta-sheet provide rigid and stable local enzyme structures, which are important for positioning enzyme catalytic groups in enzyme active sites. The arrangement of enzyme alpha helices and beta sheets also determines some enzyme functions. The tertiary structure is the arrangement of the enzyme secondary structures in a specific way to give rise to a complex three-dimensional enzyme shape. This is important because the three-dimensional shape of enzyme active sites determines the enzyme substrate specificity. The enzyme active sites must have a very complementary shape for the enzyme substrate to bind and react. The most important forces in the enzyme tertiary structure are disulfide, hydrophobic, electrostatic, and hydrogen bonds. Disulfide bonds are covalent bonds that occur between oxidized cysteine amino acids, hydrophobic interactions occur between the nonpolar amino acids on the enzyme interior, electrostatic interactions occur between the acidic and basic residues, and the hydrogen bond is the weakest bond that occurs between polar residues.

The quaternary structure arises from the assembly of protein subunits, each of which forms its own tertiary structure and then comes together with its neighbors in the same way to form enzyme oligomers. An important feature of this multimeric structure is that the combined activities of the protein subunits can provide properties that individual large proteins cannot. The majority of enzymes are globular proteins; many are multisubunit complexes. The relationship between primary structure and function refers to the sequence of amino acids in the enzyme protein polypeptide chain that determines secondary and tertiary structural organization. Therefore, the primary structure in part determines enzyme substrate specificity. Understanding the makeup of enzymes in the context of exploring enzyme applications can give us a clear picture and lead to the rational and improved design of enzymes. Understanding the enzyme structure in the four structural levels (the primary, secondary, tertiary, and quaternary structures), including the specific role of active sites in the enzyme structure and the correlation of structure with function, is essential for applications in industrial biocatalysts. [9][10][11]

3. Recent Innovations in Enzyme Technology

The rapid development of the fields of enzyme technology and biochemical engineering becomes evident when one reviews the proceedings of the First International Enzyme Engineering Conference organized at the Massachusetts Institute of Technology in 1970. The proceedings featured around twenty oral presentations and less than fifty shorter reports. Today, the number of publications referencing enzymes in a given year is counted in the millions across various scientific platforms. One factor facilitating the recent explosion of enzymes in reportable data is the development of screening conditions, coupled with the rise of genetic engineering tools, that enable the identification of novel biocatalysts that function under a variety of chemical reactions, temperatures, and pH environments.

Recently, a wide range of state-of-the-art enzymatic assays have been described that improve the throughput of high-throughput screens, providing the user with a rapid phenotypic assessment of catalytic activity, ligand binding, or protein stability. In parallel with the development of new assays, a suite of innovative approaches has been employed by researchers to evolve novel enzymes aimed at solving problems ranging from bioremediation and resource recovery to commodity chemical and pharmaceutical production. Thus, most of the innovation in the field of enzyme technology is dedicated to endowing enzymes with innovative properties, not the least, enhanced activity or stability in physicochemical changeable processes that characterize industrial applications. [12][13][5]

3.1. Directed Evolution and Protein Engineering

3.1. Directed Evolution and Protein Engineering - New Approaches in Enzyme Technology Directed evolution mimics natural selection. Candidate enzymes are produced either through error-prone PCR or gene shuffling to produce a library containing between 1,000 and 1,000,000 variants. This library is then used to screen the proteins for the preferred trait(s). The best enzymes are isolated, characterized, and the process is repeated, if necessary, with mutated individual clones to improve traits even further. This is one of the most effective ways to obtain enzyme variants with improved properties. As this is an accelerated evolution of the protein, all potential catalytic diversity of the natural protein is available to 'select' from to produce the desired change in specificity, selectivity, stability, or one of the other modulating effects. Protein engineering, as described above, is a delightful complement to directed evolution. It uses the principles of chemistry, biochemistry, and molecular biology to design enzymes that outperform wild-type proteins. Engineered enzymes from the chemical and pharmaceutical industry are just beginning to have an impact on the types of reactions that can be performed with enzymes and the amount of energy used in the process, and are thus increasing the potential economic and environmental implications of using nature's catalysts. There are an increasing number of case studies where enzyme variants have been used to develop pharmaceutical targets to act as antibiotics or as drug targets. The potential for this as an alternative to the search for entirely synthetic targets is immense, opening up new disease sectors through this approach. With health lines becoming blurred between medicine, pharmaceuticals, and biopharma, the possibilities of this approach are only going to increase. Further, the ability to debottleneck enzyme-based technologies is immense, particularly in human and animal health treatments where resistance against synthetic small molecule medicines is increasing. These are indeed exciting times for this technology. Case Studies Further Work Future Developments in Directed Evolution and Protein Engineering Sustained success in meeting the challenges of carrying out a broad diversity of evolved engineered proteins in order to perform or modify compounds required for complex biosynthetic pathways. Develop the tools available for directed evolution to be more efficient, widespread, and accessible. Where are we now, what do we need to get there, and how can we best get there? Develop the automated systems to screen a population of 1 in a billion. Develop cheaper, faster, and more reliable techniques/screening strategies. Improve our understanding and ability to recognize the mutational changes required to evolve enzyme function-structure relationships. The evolution of allostery and novel metal binding sites, mimicking the power of nature's evolution to provide us with new tools. Protein X-ray crystallography and NMR studies are useful to vastly help in evolving new functionalities in existing proteins; however, only a tiny fraction of proteins have been crystallized. Develop computational approaches to help us in visualizing this uncrystallizable space and therefore design or evolve new functions. The biggest challenge in the accelerated creation of new biocatalysis of the 21st century will be to be able to decide the exact inputs required to evolve a target, and apart from the correct type of screening to carry out, we will need to find ways to know qualitatively and quantitatively what makes one enzyme function better than another. The field is evolving rapidly to this end. [14][15]

4. Applications of Enzyme Technology in Industry

Enzyme technology can be used to catalyze a large number of valuable chemical reactions. It plays an important role in many growing areas of modern industry. The technology enables chemical reactions to become more environmentally friendly, as it can directly decrease the formation of waste, undesirable by-products, and the demand for energy. Additionally, its use is biocompatible and highly efficient, as it operates under mild conditions, uses mild reagents, and has high selectivity for synthesis. The uses of enzymes within industrial processes span across multiple industrial sectors, including applications in food, fuel, biopolymer synthesis, textile processing, fine chemicals, biofuels, detergents, and pharmaceuticals, to name a few.

Many industrial biocatalysts are produced by fermentation, and the inclusion of recombinant enzymes within food and medical products is approved. The importance and scale of the enzyme technology sector as a whole is underlined by its inclusion within international trade events. In the U.S. alone, enzyme sales for use in biotechnology applications were an estimated $1.8 billion in 2014, and total enzyme sales within the U.S. were expected to grow to more than $5 billion by 2024. In 2010, the global market for industrial enzyme sales totaled approximately $3.76 billion, which is an increase from $3.1 billion in 2009, and the global biofuels market is expected to reach $185.3 billion by 2021.

Enzymes offer a more sustainable approach as they can avoid many of the problems and risks associated with traditional chemical processes of producing specialty and fine chemicals. The addition of enzymes to solid waste treatment systems can be considered an environmentally friendly alternative to incineration. One estimate suggests that the removal of food waste from landfills would add 36 TWh/year of capacity to the U.S. electricity system. These process benefits result in more sustainable industrial processes that require less water and energy and can be more cost-effective. The commercial implementation of biological processes through the use of enzymes is logically seen as highly effective and efficient, and therefore companies are now developing biologically based processes for a variety of applications within biocatalysis. Some successful case studies have underlined the financial benefits of these cleaner, more sustainable, and safer industrial processes. In conclusion, despite the challenges that biocatalysis faces, there are significant business opportunities in the application of this green technology. [16][17]

4.1. Biocatalysis in Chemical Synthesis

Enzymes are biological catalysts that increase the rates of chemical reactions without being consumed during the reaction itself. In a rather simplified way, biocatalysis typically refers to the application of enzymes as catalysts in chemical synthesis. Compared to traditional chemical methods that are inherently more energy-consuming and often display catalytic cycles with the formation of undesirable chemical by-products, one can argue that the turnover numbers and reaction speed and the specific and selective nature of enzymes make biocatalysis the most efficient way for so-called green chemical synthesis to date. From a practical perspective, numerous examples have been published detailing the benefits of using enzymatic catalysis in the production of pharmaceuticals, biofuels, and chemicals such as polymers, insecticides, flavors, and aromas as specialty chemicals.

For pharmaceutical production, enzymes have attracted the attention of industry due to their ability to catalyze the selective functionalization of complex molecules and enantioselective transformations, being considered indispensable in the synthesis of over half of the best-selling drugs worldwide. Thus, the application of biocatalysis in the industrial production of fine chemicals, pharmaceuticals, and bulk chemicals is an area that has been receiving more attention compared to even a decade ago. By using the in vivo produced metabolic intermediates/precursors, genetic methods for improved and increased synthesis yield. An example of this can be found in studies where enzymes are used in the pyruvate–pyruvate route to 2-ketos I-enantiomer meso-2,3-pentanediol catalyzed by the enzyme YerE.

5. Applications of Enzyme Technology in Medicine

There are two main spheres of application of enzyme technology: in diagnostics, usually in the form of simple tests that can be performed by patients themselves, or quickly by medical professionals; and therapeutics, specifically in the form of enzyme replacement therapy. Enzymes in blood are an important diagnostic tool, and a large number of tests are based on the serum level of enzymes. For example, high levels of liver or pancreatic enzymes in the blood, amylase and/or lipase in particular, are used as a diagnostic marker for liver or pancreatic disease. Lactate dehydrogenase is an enzyme found in almost all tissues of the body and is easily released into the blood when cells are damaged. LDH tests are used to indirectly identify diseases that cause increased LDH levels. The rapid rise in lactate dehydrogenase is used as a prognostic indicator that determines whether an individual needs prolonged intubation and/or chemotherapy following a lymphoma diagnosis. The level of carbonic anhydrase 9 in plasma is a useful biomarker for modern oncology.

Therapeutic enzymes have usually been used in replacing missing or faulty enzymes, often in the field of rare diseases, reducing many of the adverse effects and hence increasing patient lifestyle. Of particular relevance are enzymes for rare diseases such as all types of mucopolysaccharidoses, Pompe disease, Gaucher disease, Hurler syndrome, Fabry disease, chronic gout, and cystic fibrosis. However, while rarely life-saving, enzyme replacement therapy often changes the lives of those receiving it, sometimes dramatically. Engineered enzymes can be designed to target specific tissues in the body, irrespective of blood distribution. The need for oral enzyme administration and enzyme pharmacokinetics can be significantly reduced. Enzymes have also been used in the form of enzyme-potentiated desensitization. An additional but less successful approach has been the use of enzymes to degrade extracellular macromolecules that inhibit drug action or have tumor-promoting features. Some of the problems with developing therapies are long and expensive development times due to safety concerns; although none of the above examples have led to direct patient deaths, recent issues may make regulators more wary when approving new products. There are also concerns based around worldwide availability. While some drugs have been developed, they are not reimbursed by health systems. Nonetheless, use of this system, in particular, is one reason to persist with the development of new technologies in drug therapy. Additionally, researchers are developing new and better therapeutics that use existing technologies, such as new formulations of enzymes or enzyme encapsulation strategies. Ongoing areas of research in enzyme technology include enzyme immobilization and enzyme drug targets. Other research areas in this field tend to move away from the use of enzymes as they are commonly understood towards the use of 'missing' proteins or using protein 'metabolons', which exploit the proximity of enzymes to make processes more efficient. [18][19]

5.1. Enzyme Replacement Therapy

The concept of 'inborn errors of metabolism' was established in 1908. Since then, enzyme technology has become one of the fastest-growing fields of biotechnology. It has many potential applications in the fields of medicine, food technology, fermentation, and environmental biotechnology. It mainly deals with storage diseases, so enzyme technology has an impact on the treatment of genetic diseases. Enzyme replacement therapy (ERT) is a medical treatment that involves the use of recombinant enzymes to supplement the action of the defective enzyme. It is available in the form of an infused medication.

Enzyme replacement therapy (ERT) is an effective treatment for several disorders caused by an inherited deficiency of lysosomal enzymes. Specifically, lysosomal enzymes are membrane-bound intracellular organelles that contain over 60 enzymes responsible for degrading waste products from the patient's body. These waste products, or substrates, cannot be degraded due to receptor-mediated mechanisms or manifestations. Therefore, ERT replaces these missing enzymes so that the substrates can be effectively metabolized. The success of such therapy has brought positive and dramatic changes to these patients, markedly improving and extending their lives, who otherwise may suffer from fatal neurodegenerative illnesses. Such patients have led many normal lives and have shown regular improvement in vigilance, intellectual ability, motor skills, and physical fitness. Despite its high cost and dependence on other similar techniques, ERT has a prominent effect in only some severe and unfortunate metabolic disorders in humans. The drawback of this replacement therapy is mainly the high expenses of acquiring and sustaining regular intravenous high-dose medications. The increasing needs of patients have forced the manufacturing and preparation of huge quantities of enzymes. Such activities could be particularly problematic and costly as very often the recombinant enzymes are processed and purified to remove pyrogens and ensure sterility. Additionally, such enzymes require a lot of time and money for restructuring and regulatory trials. Given this, successful ERT for inherited metabolic disorders is already a huge boost for rare untreated patients who have reached adulthood and should double their lives. But experts are not guaranteed over the period of increased treatment and significantly improved and sustained outcomes. Such a provision can be anticipated by one of the important aspects of ERT, which mainly includes advancements in enzyme engineering, gene therapy, and breakthrough inventions. [20][21][22]

6. Conclusion

The 21st century has seen a significant increase in the commercial, medical and industrial applications of proteins. Although they have been used industrially since 6000 BC, enzymes have this century been recognised and utilised as powerful tools, with numerous applications in both industry and medicine. In the 1980s, site-directed mutagenesis was developed and adapted to proteins and enzyme systems such as restriction endonucleases, LYases and the Mxe Gyr A intein, with the hope that they could be used to create a broader array of site-specific mutants of proteins. More than 30 years later, the manual methods of the 1980s and 1990s have been partially automated, and replaced in many instances by directed evolution and other protein and enzyme engineering and design techniques.

The full extent of the potential applications of the recent revolution in enzyme capability is yet to be realised, but it is already clear that they add a further dimension to their attraction. They have the potential, in combination with suitable biobased feedstocks to produce products more efficiently, with lower waste, and reduced toxicity and hazards, i.e. in a sustainable way. This makes a positive input into many of the sustainable development goals, such as reducing food scarcity, cleaning water supplies, removing greenhouse gases, and using waste as a resource. The science of protein medicine, enzyme technology, and the remaining issues that emerge from it, may be necessary to realise these potential applications. These issues mainly concern the relatively low and/or variable stability of many enzyme formulations and the relatively slow responses of bulky solid enzyme preparations. There is much for enzyme engineers and researchers from other disciplines to do to realise the full potential of the power of enzymes.

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