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**Review Article**

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# Some Important Therapeutic Enzymes and their Uses

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**Abstract** Enzymes are protein molecules that are responsible for many vital reactions including digesting food, building bones, purifying blood and detoxification. Enzymes also, have several clinical uses in treatments including leukemia, metabolic disorders, inflammation, cardiovascular disease and lysosomal storage diseases etc. Specificity, stability, and substrate conversion makes therapeutic enzymes advisable agents more than non-enzymatic drugs. As foreign proteins to the body, enzymes are representing antigenicity that can induce immune response which lead to immunogenicity. PEG coatings have also been utilized for overcoming various biological barriers accompany other modes of administration for efficient enzyme and gene delivery, to increase the systemic circulation time, serum half-life and decrease the immunogenicity of the proteins without affecting activity in anti-cancer chemotherapy. Besides others, exosomes are used as natural carriers for enzymes. The sources of different types of clinical enzymes affect its production costs.

**Keywords** Fibrinolytic enzymes, pancreatic insufficiency, hypophosphatasia, SARS-CoV-2.

## Introduction

Enzymes are designated in replacement therapy whether in enzyme deficient genetic disorders, antineoplastic agents [1], enzymes for fibrinolysis, specific detoxification enzymes in case of acute chemical poisoning to limit tissues damage in reperfusion injuries and enzymes that are used for various digestive and metabolic treatments [2].

Therapeutic enzymes are required in fine quantities with high purity and specificity. Engineered enzymes that maintain point mutation, designing fusion protein, glycoengineering were done to ease production and purification, to enhance delivery to target sites and to reduce side-effects specially immunogenicity upon continuous drug administration. Low Km and high Vmax are the recommended kinetics for maximal efficient of low concentration of the therapeutic enzymes and its substrate [3]. The enzymes sources are chosen carefully to avoid any possibility of undesired contamination by opposite material and facilitate purification [4].

## Production of therapeutic enzymes

Therapeutic enzymes were usually sourced from human or animal tissue, microorganisms and plants. Many of such enzymes were secreted in small insufficient quantities from human tissues to meet clinical demand. Lentiviral vectors (LVVs) are powerful genetic tools that are being used with greater frequency in biomedical laboratories and recombinant protein production trials. LVVs gene therapy is a method by which genes can be inserted, modified or deleted in organism using lentivirus [5,6]. Lentivirus are a family of viruses that are responsible for notable diseases like human immunity virus, which infect by inserting DNA into their host cells genome. Production of microbial enzymes on bulk industrial scale reported downstream processing and its production by fermentation. Manufacture of a batch starts with the fermentation or cell culture stage. After expression of the therapeutic enzyme in a cell

culture bioreactor, protein purification, or downstream processing of the product, it is critical to ensuring safe delivery of these medicines. The use of modern disposable equipment in processing has been greatly improved. Production and testing of therapeutic enzymes are strictly governed by regulatory systems in each country around the world. Once purified, the drug substance undergoes careful quality control testing to enable release to patients [2,7,8].

**Stability and purity of therapeutic enzymes**

Enzymes as fragile macromolecules are particularly sensitive because both proteolysis and unfolding can lead to their inactivation in gastrointestinal tract. In order to overcome this action gastro-resistant delivery systems and the modification of enzyme whose substrate is localized in the gastrointestinal tract structures in the oral delivery of therapeutic enzymes by polymer conjugation or biohybrid were tried. [9]. Polymer or nano- conjugated resistant delivery methods, as well as mutagenesis increase enzyme stability against thermal denaturation, acidic gut environment, proteolysis and immunogenicity. Modification with polymers provide both steric shielding and selective interaction in different digestive compartments specially focus on enzymes that are directly active in the gastrointestinal lumen [10-12].

**Physicochemical properties of therapeutic enzymes**

Fluorescent biosensors represent a class of imaging agents which declare the function and regulation of enzymes in their cellular context. Four important popular fluorescence techniques are used in the study of therapeutic enzymes which are fluorescence tracing, fluorescence resonance energy transfer, fluorescence quenching and fluorescence polarization [13]. X-ray crystallography is another tool for function- structure help understanding of therapeutic enzymes [14-16].

**Enzybiotics:**

Antibiotic misuse is responsible for the increase in the resistance of pathogens to traditional antibiotics. Enzybiotics and antimicrobial peptides have attracted much attention as potential substitutes for conventional antibiotics. As Peptidoglycan is the major structural component of bacterial cell walls, enzybiotics are a class of antibiotics which are derived from endolysins, (peptidoglycan hydrolase) bacteriophage-encoded enzymes that degrade the bacterial cell wall of the infected cell at the end of the lytic replication cycle. Bacteriophage enzyme that degrade bacterial cell wall help to fight bacterial or fungal diseases by using viruses or viral derived lysins and antimicrobial peptides. Phage encoded endolysins attack and lyse bacteria when added. The term enzybiotic means "enzyme" and "antibiotic" and refers to phages. Enzybiotic are characterized by a rapid and unique mode of action, a high specificity to kill pathogens, a low probability for bacterial resistance development and a proteinaceous nature. Engineered endolysins have been demonstrated to be effective in a variety of animal models to combat both gram-positive and gram-negative bacteria and in addition, mycobacteriophage-encoded endolysins have been successfully used to inhibit mycobacteria in vitro [17].

**Recombinant Enzymes**

Enzyme replacement therapy (ERT), based on the periodic intravenous administration of specific enzymes produced with recombinant DNA technology, is at present the most appropriate available therapy for several lysosomal storage disorders. Other treatment for patients with enzyme or protein deficiencies include substrate reduction therapy, gene therapy, and transplantation. The use of enzymes is still limited because of low yields, low reproducibility, and the not-always optimal performance of these biocatalysts in their native form. Recombinant DNA technology and protein engineering represent suitable ways to overcome these drawbacks, thus allowing the high-level production of redesigned enzymes with improved properties that meet the specific industrial requirements. Enzymes in recombinant forms, means the genes responsible for enzyme production are moved from organisms difficult to grow or handle genetically, e.g., plants, animals, extremophilic microbes, to microbes easily

used in industrial production [18,19]. Systems used for production include non-filamentous and filamentous bacteria, yeasts, fungi and transgenic plants. Recombinants production allows to circumvent the risk of transmission of diseases via infected source material therefore benefiting both production levels and product safety. Most therapeutic enzymes currently marketed are thus recombinant, [20].

**The use of natural nanocarriers in enzyme delivery**

Cells communicate with each other through the use of chemical messengers as intercellular communication tools that transfer cargo to recipient cells, these are predominantly in the form of extracellular vehicles (EVs) which are lipid bound vesicles secreted by cells into the extracellular space. The three main subtypes of EVs are micro vesicles (MVs), exosomes, and apoptotic bodies, which are differentiated based upon their biogenesis, release pathways, size, content, and function [21-24]. The content of EVs consists of lipids, nucleic acids, and proteins associated with the plasma membrane, cytosol, and are involved in lipid metabolism. EVs offers important advantages compared to other nanoparticulate drug delivery systems such as liposomes and polymeric nanoparticles of being non-immunogenic in nature due to similar composition as body′s own cells besides modification to contain specific proteins, genetic lipids, and genetic materials including messenger RNA, microRNA, and other small non-coding RNAs, and genomic DNA from their progenitor cell . EVs may have other multiple advantages over available drug delivery vehicles, such as their ability to overcome natural barriers, their intrinsic cell targeting properties, and stability in the circulation. However, applications of EVs as drug delivery systems is limited due to a lack of methods for scalable EV isolation and efficient drug loading. However, due to their nanoscale measurement, exosomes are considered to be the most favorable tool for drug delivery between neighboring cells by carrying proteins and RNAs even to distant organs. Exosomes bind to cell membranes through receptor–ligand interaction and mediate antigen presentation, cancer progression etc. Exosomes deliver their surface protein and cytoplasm to the recipient cell by fusing with the target-cell membrane at which point they open up, exosomes enter cells, release their cargo and mediate many physiological and pathological processes.

**Some types of therapeutic enzymes**

In contrast to the industrial use of enzymes, therapeutically useful enzymes are required in relatively tiny amounts but at a very high degree of purity and specificity. However, being generally foreign proteins to the body, they are antigenic and can elicit an immune response and their effective lifetime within the circulation may be only a matter of minutes.

**Anticancer enzymes**

Asparaginase which targets amino acid L-asparagine that is necessary for the growth of some tumor cells and metabolize it to L-aspartic acid and ammonia [25], so, it is the major potential enzymes is in the treatment of cancer particularly promising for the treatment of acute lymphocytic leukemia. Its action depends upon the fact that tumor cells are deficient in aspartate-ammonia ligase activity, which restricts their ability to synthesize the normally non-essential amino acid L-asparagine. Therefore, they are forced to extract it from body fluids. The action of the asparaginase does not affect the functioning of normal cells which are able to synthesize enough for their own requirements, but reduce the free exogenous concentration and so induces a state of fatal starvation in the susceptible tumor cells. The enzyme is administered intravenously. Its half-life may be increased 20-fold by use of polyethylene glycol in the modified asparaginase.

Some cancer cell lines also display addiction to glutamine in spite of glutamine is a nonessential amino acid that can be synthesized from glucose. Cell metabolism that restarted during tumorigenesis and metastasis as a consequence of oncogene activation and onco-suppressors inactivation, leading to a new cellular homeostasis typically directed towards anabolism. i,e, several oncogenes and tumor suppressors have been linked to the regulation of metabolic processes .Because of these modifications, cells can become dependent on specific substrates like sugars, lipids or amino acids. Cancer addictions or the acquired dependence of a cancer cell on the activity of a single oncogenic gene product have altered metabolic requirements that create addictions to specific nutrients such as glucose and

glutamine is a step for therapy, as removal of an essential substrate can lead to their selective cell-cycle arrest or even to cell death leaving normal cells untouched [26,27].

**Multiple trauma treating enzyme**

L-amino acid -ligase obtained from Empedobacter brevis catalyzes the ligation of two amino acids L-alanine and L-glutamine. This dipeptide i.e. Ala-Gln is easily digested in human body and hence is used in nutritional therapy. In multiple trauma patients, parenteral supplementation of alanyl-glutamine dipeptide was associated with better insulin sensitivity. It also prevents muscle wasting and increases synthesis of protein in muscle. In Bacillus subtilis, a novel enzyme coded by a gene YwfE was identified, which catalyzed this dipeptide formation from unprotected amino acids in an ATP-dependent manner. This novel enzyme was classified into the category of L-amino acid α-ligase (LAL) [28,29].

**Fibrinolytic enzymes**

Thrombosis is formation of blood clots inside the vessel may be due to factors including age, obesity, hypertension, raised blood lipids, and diabetes. Fibrinolytic enzymes have become more substantial for treating cardiovascular diseases disorder since they could lyse the fibrin clot within the blood vessel. Cardiovascular diseases are a group of disorders consisting of coronary heart disease, peripheral arterial disease, cerebrovascular disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism. Severe cardiovascular disease conditions lead to acute myocardial infarction and stroke. Anticoagulants and antiplatelet drugs are used for managing cardiovascular diseases for a long time but they were unable to dissolve existing thrombus. Inability of plasma fibrinolytic system demands better thrombolytic drugs. Major thrombolytic enzymes belonging to plasminogen activators and plasmin like enzymes [30]. Thrombolytic drugs are used to lyse clot to recover the normal flow of blocked blood vessels. Their action involves the activation of the natural fibrinolytic system and thus the activation of plasminogen. Some plasminogen activators are streptokinase, urokinase, etc. Streptokinase is inactive but when it combines with the circulating plasminogen, it forms an activator complex which then results in limited proteolysis of other plasminogen molecules to plasmin. It is antigenic, has ability to cause hypersensitivity reactions and anaphylaxis. Urokinase directly activates plasminogen and it is non-antigenic. Dissolution of clot by converting intrinsic plasminogen present in the clot to active plasmin [29,31].

Lipase is used as digestive aim and also used in the treatment of malignant tumors as it has the ability to activate tumor necrosis factor, while lipase from Candida rugosa synthesizes lovastatin, a drug that has the ability to lower serum level of cholesterol. A new lipase which enantio-selectively hydrolyzes (±)-trans-3-(4-methoxyphenyl) glycidic acid methyl ester, a key intermediate in the synthesis of diltiazem hydrochloride, a coronary vasodilator, was purified from the culture supernatant of Serratia marcescens Sr41 8000 [29, 32].

Natto kinase is a serine proteinase obtained from Bacillus subtilis. It can reduce some factors of blood clotting and lipids that are associated with an increased risk for cardiovascular disease. Oral administration of natto kinase could be considered as a CVD nutraceutical [33]. It decreases the plasma levels of fibrinogen, factor VII, and factor VIII. Natto kinase shows prolonged action of preventing coagulation of blood and dissolving existing thrombus [29, 34].

**Serrati peptidase**

It is useful in the treatment of pain and inflammation via three mechanisms including breaking down fibrin, the insoluble protein byproducts of blood coagulation, thinning the fluids formed from inflammation and injury, and facilitates their drainage which increases the speed of the tissue repair process. It also alleviates pain as it inhibits the release of bradykinin, a specific pain inducing peptide [29,32].

**Pancreatic enzymes**

These categories were used in deficiencies related with secretion of pancreatic enzymes in chronic pancreatitis, pancreatic carcinomas, and cystic fibrosis. i.e. the use of enteric coated and unprotected replacement pancreatic

enzymes for treatment of malabsorption and pain due to pancreatic insufficiency [35]. Pancreatic enzyme; chymotrypsin, lipase and amylase were highly sensitive to pH conditions. Enteric coated formulations mix poorly with food allowing separation of enzymes and nutrients when emptying from the stomach. The site of dissolution of the enteric coating in the intestine is also unpredictable and enzymes may not be released until the distal intestine causing pain that may be controllable to therapy with pancreatic enzymes of imperfect absorption. Inhibition of endogenous pancreatic secretion can prevent pain associated with pancreatic secretion but requires use of non-enteric coated formulation [36]. Pancreatic enzyme extracts, and particularly trypsin, were suggested as useful agents in the prevention of cancer. This led to the emergence of commercially available trypsin-based formulation.
Lipase-protease-amylase capsule medication contains digestive enzymes to help break down and digest fats, starch, and proteins in food. It is used in conditions where the pancreas cannot make or does not release enough digestive enzymes into the small intestines to digest the food as in post-pancreatectomy, post-gastrointestinal bypass surgery [37-39].

**Serum alkaline phosphatase**

Mutations in the ALPL gene lead to Low activity of the enzyme tissue-nonspecific alkaline phosphatase (TNSALP) resulting in hypophosphatasia (HPP), [40]. a rare inherited disorder form rickets or osteomalacia, tooth mineralization, bone pain, fracture result in multi-systemic complications with mortality in severe cases. Dental manifestations in affected individuals feature most prominently premature loss of deciduous teeth featuring low serum alkaline phosphatase (ALP) activity [41]. A targeted enzyme replacement therapy ERT designed to restore the regulation of metabolic processes in the bones and teeth, and to reduce complications of dysregulated bone mineral metabolism. Asfotase alfa-ERT has proven to be transformative, improving survival in affected infants, children and adults with HPP. The tissue-nonspecific alkaline phosphatase (TNSALP) is expressed by mineralizing cells of the skeleton and dentition and is associated with the mineralization process [42]. Deficient of activity of the TNSALP leads to accumulation of inorganic pyrophosphate that inhibits physiological mineralization. Management of HPP has been limited to supportive care until the introduction of a recently approved enzyme replacement therapy employing bone-targeted recombinant human TNSALP [43-48].

**Hyaluronidases**

Hyaluronidases are a group of enzymes widely distributed in nature that degrade hyaluronic acid which constitutes an essential part of the extracellular matrix. They are initially discovered in bacteria, and are found in many classes including insects, snakes, fish and mammals. Hyaluronidases from animals are limited compared to microbial hyaluronidases which are obtained from many microorganisms as Clostridium, Micrococcus, Streptococcus, and Streptomyces. A Novel Hyaluronidase A50 is produced by Bacillus sp. In the human, six different hyaluronidases, HYAL1-4, HYAL-P1 and PH-20, have been identified [49]. PH-20 exerts the strongest biologic activity, is found in high concentrations in the testicles and can be localized on the head and the acrosome of human spermatozoa. Today, animal-derived bovine or ovine testicular hyaluronidases as well as synthetic hyaluronidases are clinically applied as adjuncts to increase the bioavailability of drugs, for the therapy of extravasations, or for the management of complications associated with the aesthetic injection of hyaluronic acid-based fillers [50].

**SARS coronavirus enzymes**

Severe acute respiratory syndrome (SARS) is a type of pneumonia transmitted by aerosols, or via oral route causing high death rate as it is caused by a previously unknown coronavirus termed SARS coronavirus (SARS-CoV). The main protease of Sars-CoV-2 has been cleared for the first time by the high-intensity X-ray light. The structural details lead to a trial therapy for Covid-19 by creating inhibitors for this enzyme that would reduce viral replication. Sars-CoV-2 protease is a large polyprotein enzyme that is critical for the virus. Scientists developed broad-spectrum inhibitors against this protease, known as α-ketoamide complexes that take place between the protease and α-ketoamide inhibitors, offer a starting point for the development of new inhibitors or drugs. α -ketoamide 13b,

inhibited the main protease of the virus, as well as the related Mers virus in the undergoing trials to develop drugs were recorded [51-53].

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