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Medical, Diagnostic and Therapeutic Applications of Enzymes

Ali M. Elshafei*

Microbial Chemistry Department, Genetic Engineering and Biotechnology Research Division, National Research Centre, 33 Elbohouth st., Dokki, 12622 Giza, Egypt.

Abstract

Enzymes from different sources are specific and non-specific biological catalysts facilitating and activating appropriate reactions in cells and tissues and serve in recent years as drugs for many diseases. Errors in their biosynthesis and regulators lead to disturbances and appearance of different diseases. Three possible ways for the application of enzymes in medicine namely: in diagnosis and prognosis of diseases, as therapeutic agents and as diagnostic reagents. In this article, we give a short survey of some representative medical applications of enzymes.

Key words: Medical enzyme applications, Therapeutic enzymes, Diagnostic enzymes, Diagnosis of diseases.

1. Introduction

Enzymes are the biological substances or biological macromolecules that are produced by living organisms and act as catalysts in biological/biochemical reactions where enzymes help to accelerate the reaction. Therefore, these are generally known as "Biocatalyst". Enzymes from different sources are specific and non-specific biological catalysts facilitating and activating appropriate reactions in cells and tissues and serve in recent years as drugs for many diseases. In medical field, based on their role, enzymes are also called as therapeutic enzymes or diagnostic enzymes [1-3].

2. Therapeutic Enzymes

In recent years many diseases have been described due to deficiency of some enzymes, such as lysosomal enzymes, which can be treated by administration of enzymes [4-5]. In this connection different approaches to

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Correspondence to: Elshafei AM, Microbial Chemistry Department, Genetic Engineering and Biotechnology Research Division, National Research Centre, 33 Elbohouth st., Dokki, 12622 Giza, Egypt.
Email: alielshafei@yahoo.com

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enzyme therapy exist which can be categorized into six main groups as follows: a) Replacement enzymes, e.g., digestive enzymes which are mainly used after surgery of the gastrointestinal tract when the organism itself is not sufficient for effective food digestion, b) Antitumor enzymes which have the specific ability to degrade some amino acids required for tumor growth, c) Disinfection enzymes which are active against some bacterial and viral infections, d) Thrombolytic enzymes, which can act by activation of fibrinolytic blood system or lyzing thrombi, e) Enzymes of inherited storage diseases treatment, f) Anti-inflammatory enzymes acting on necrotic tissues. The replacement digestive enzymes are usually administered orally [1, 6] using tablets or capsules to avoid gastric acidity; examples of this category include proteases, lipases and lactase.

The therapeutic action of antitumor enzymes is based on their ability to decrease the amino acids levels required for tumor growth. The formulation of these antitumor enzymes should fulfill the following properties [1]: a) maximal physiological pH values, b) Low K_m value towards the specific substrate, c) Minimal impurities such as endotoxins, d) Stable in biological fluids and blood, e) Long Life-time, f) Resistant against reaction product, g) No requirement for a cofactor for their activity. The antitumor enzyme asparaginase is one of the most frequently used in the treatment of malignant diseases and lymphoblastic leukemia (LL), which is produced by prokaryotic and eukaryotic microorganisms and hydrolyses asparagine into aspartic acid and ammonia (Figure 1) [7]. The therapeutic action of this enzyme is based on the high requirement of tumor cells for asparagine and this achievement was discovered previously by suppressing tumour growth of guinea pig serum lymphoma by administration of asparaginase. L-asparaginase can be used alone or in combination with other antitumor drugs to potentiality their activity. Recently L-glutaminase has attracted attention with respect to its wide applications in pharmaceuticals as an anti-leukemic agent [8-9]. The enzyme catalyzes the hydrolysis of L-glutamine to L-glutamic acid and ammonia (Figure 2) and regulates the cerebral concentrations of glutamine and glutamate, which are very important in processes such as ammonia detoxification [10]. We previously described the production of L-asparaginase and L-glutaminase from different filamentous fungi [11-13].

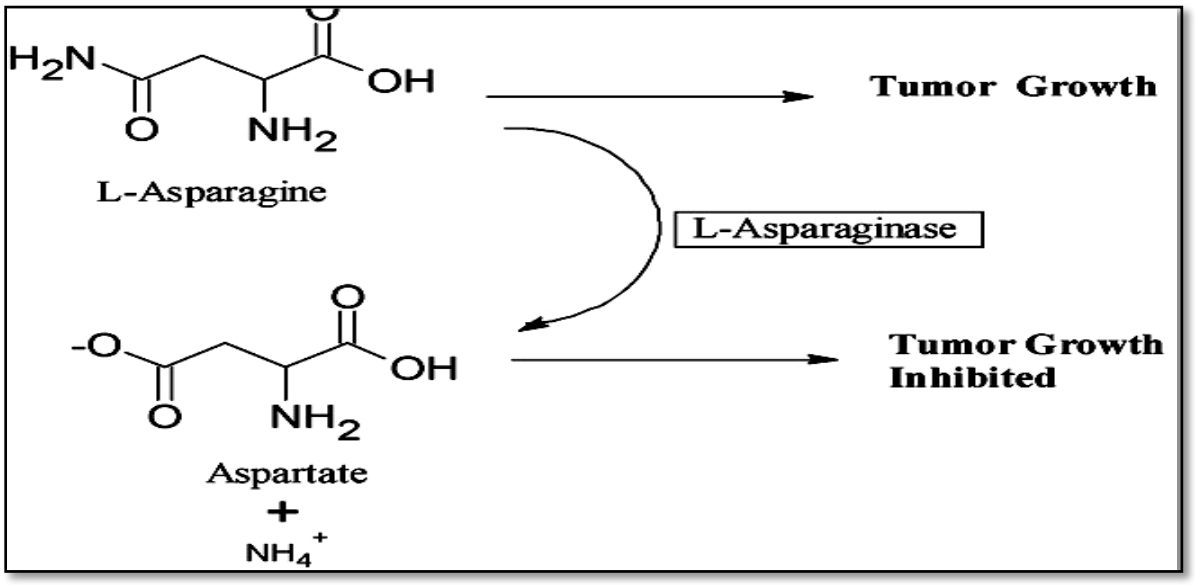

Figure 1. Schematic presentation of the reaction mechanism of L-asparaginase.

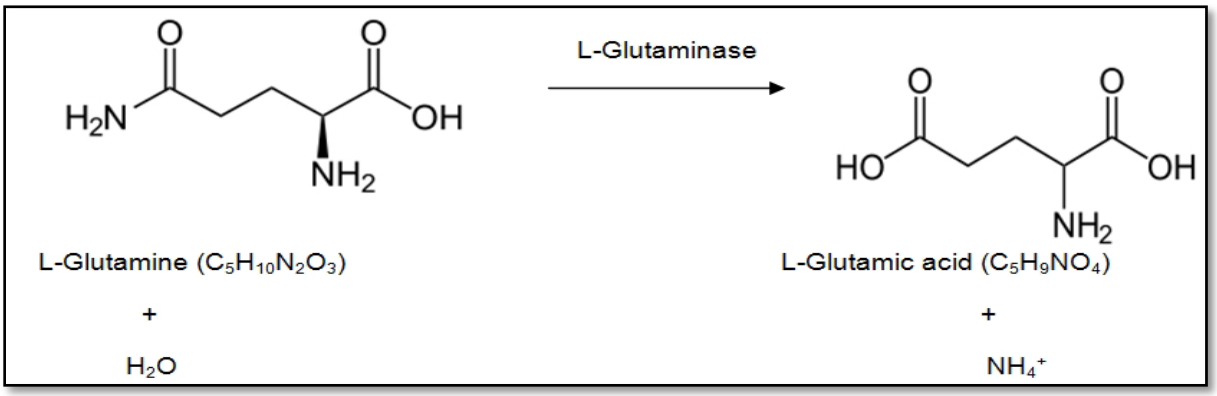

Figure 2. Schematic presentation of the reaction mechanism of L-glutaminase.

In clinical practice, microbial asparaginases are used successfully when no other antitumor agents can be used. In some cases, the enzyme is not effective and sometimes is accompanied with different moderate to serious side-effects. Promising results were also obtained using some other enzymes having the ability to degrade essential amino acids such as alanine, arginine, cystein, glutamine, glycine, ornithine, glutamic acid, proline, tyrosine, aspartic acid and serine as substrates [14]. In this respect microbial glutaminase and glutaminase/asparaginase can be used to convert the above mentioned amino acids successfully, however unfortunately some of these enzymes demonstrate toxicity towards kidney and central nervous system in clinical trials. Cysteine oxidase and cystein aminotransferase can be used for therapeutic purposes. Many investigators reported the pronounced effect of ribonucleases [15], exonucleases [16] and folate-degrading enzymes in cancer treatment [17]. Treatment of inherited diseases by therapeutic enzymes is of importance due to the accumulation of particular toxic metabolites and insufficient endogenous intracellular enzymes activity [4-5] (Table 1). Gaucher's disease, the condition characterized by the insufficiency of lysosomal gluco-cerebrosidase leads to the increase in liver and spleen size, neurological disorders and bone deformation [4]. Another serious Lysosomal disease (Pompe's disease) is conditioned by the deficiency in alpha-1,4 glucosidase that causes muscle dystrophy and child death after one or two months after birth due to the accumulation of glycogen in lysosomes. The treatment of phenylketonuria (an inherited disease) is also limited and connected with dietotherapy in order to limit the quantity of phenylalanine in the food and the reasonable alternative seems to be the treatment with phenylalanine ammonia lyase. Hemostatic disturbance-hemophilia "A", is an inherited disease mainly caused by the deficiency of blood clotting factor VIII leading to frequent and prolonged bleeding [16]. In this case the transfusion of normal blood containing blood clotting cascade enzymes is used for the treatment of hemophilia [18-19].

Table 1. Examples of some inherited enzyme diseases.

Disease	Enzyme Deficiency	Accumulated Substance	Clinical Manifestation
Pompe's Disease	α-1,4 Glucosidase	Glycogen	Heart failure & Myopathic infection
Gaucher's Disease	β-Glucosidase (Glucocerobrosidase)	Glucocerobroside	Neurologic disorders, Bone deformation
Phenylketonuria	Phenylalanine hydroxylase	Phenylalanine	Imbecility & Neurologic disorders
Hemophilia	Factor VIII (antihemophilic factor)	Bleeding episodes	Bleeding &Secondary anemia

The treatment of blood clotting system abnormalities was highly developed by enzyme therapy [20-23]. Nowadays the market of therapeutic thrombolytic enzymes is growing rapidly and many of these enzymes have been used such as streptokinase, urokinase and tissue plasminogen activator [24, 25]. Many modifications and attempts to increase the efficiency of these enzymes are being developed such as the simultaneous action of urokinase and hialuronidase on myocardial infarction [26]. Investigators also reported the use of free radical detoxifying enzymes such as catalase and superoxide dismutase in combination with other drugs for thrombolytic therapy [27]. Other therapeutic enzymes were reported, such as pronase and collagenase for the treatment of spleen and liver diseases, and elastase for the treatment of arthritis [28, 29].

In this concern, many difficulties faced the application of therapeutic enzymes in medicine, one of them is the high cost of pure enzyme and the second being the complex nature of enzymes as protein macromolecules which are foreign to the recipient patient and lead to low stability at the physiological pH and temperature. The third being the allergic complications exerted by many enzymes that cause pronounced irritation. Therapeutic enzyme immobilization is expected to solve this difficulty through the capability to reduce the enzyme contact with cell receptors [30-31].

3. Diagnostic Enzymes

Enzymes are not only employed for the treatment of diseases but also being as an important tool for medical diagnosis of diseases as the latter lead to different cases of tissue damage associated with the release (or decrease) of specific enzymes into circulation and body fluids related to the disease tissue or organ [32]. In this case, the measurement of enzymatic activity in serum and plasma is of importance for the diagnosis of diseases. The increase of serum alkaline phosphatase (ALP) indicates an increased osteoblastic activity, Paget's disease, rickets, osteomalacia, hyperthyroidism, hyperparathyroidism and rheumatoid arthritis [33]. Low levels of ALP were recorded for many diseases such as Hypophosphatasia which results in varying degrees of skeletal abnormality [34]. The enzyme that regulates matrix composition namely Matrix metalloproteinase-9 (MMP-9) or Gelatinase B secreted by different malignant cells, monocytes and tissue macrophages and many investigators reported that MMP-9 mainly used as a marker mostly for autoimmune diseases [35-37]. Elevated level of Lysozyme in rheumatoid arthritis patients, an enzyme that hydrolyses glycosidic bonds in the peptidoglycans of cell wall, serves as an indicator of monocyte-macrophage activity [38]. Many types of acid phosphatases (ACPs) are found in humans namely prostatic, osteoblastic, lysosomal, erythrocytic and macrophage. The level this enzyme in male prostate gland reach one hundred times more than any other body tissue and the assay of ACP is carried out by the prostate specific antigen PSA to diagnose the presence of prostate cancer cells [39]. The presence of high level of alanine transaminase (ALT) indicates liver diseases usually viral hepatitis and liver necrosis. Investigators supported the idea that the presence of minor elevation of ALT is a good proof about the presence of serious liver disease [40]. Glucose-6-phosphate dehydrogenase (G6PD) was found to be a vital enzyme responsible for membrane integrity. The over-expression of G6PD can be regarded as an independent indicator of prognosis of gastric cancer [41]. Leucocyte G6PD could serve as a prognostic and diagnostic tool in chronic myeloid leukemia (CML) and acute non-lymphocytic leukemia (ANLL) [42]. Lactate dehydrogenase (LDH) is a valuable prognostic marker in colon cancer, breast cancer, leukemia and breast cancer. LDH in combination

with other factors and markers may be also employed for identification and management of early cancer patients [43-44] (Table 2).

Table 2. Examples of some diagnostic enzymes and their applications.

Diagnostic Enzyme	Substrate	Disease or Disorder	Reference
Glutamate oxidase	Glutamate	Neuropathologic conditions	[45]
Oxalate oxidase	Oxalate	Kidney stones	[46]
Urease	Urea	Renal disorders	[47]
Lactate oxidase	Lactate	Ischemic myocardium	[48]
Glucose oxidase / Glucose dehydrogenase	Glucose	Diabetes mellitus	[49]
Creatine amidinohydrolase	Creatine	Thyroid, renal and muscle function	[50]
Creatinine amidohydrolase	Creatinine	Thyroid, renal and muscle function	[51]
Carnitine dehydrogenase	Carnitine	Renal insufficiency, carnitine deficiency	[52]

4. Microbial Therapeutic Enzymes

Many advantages were recorded for microbial enzymes (MEs). The following are some of these advantages: a) Variety of MEs available for supplementation, b) Selected according to each microbial enzyme's characteristics, c) MEs exhibit broad ranges of temperature, pH and substrate specificities, d) MEs are selected on their ability to work within the gastro-intestinal system of mammals and their compatibility with body's temperature, e) Supplemented MEs exhibit activity throughout the entire digestive process and consequently improving food nutrient utilization [53], (Table 3).

Table 3. Therapeutic microbial enzymes and their applications.

Therapeutic Enzyme	Application	Source	Reference
α-Galactosidase	Fabry's disease, Blood group transformation	*Streptomyces griseoloalbus*, *Aspergillus sp.*	[54-56]
β-Aminopeptidase	Anti-oxidant	*Ochrobactrum anthropi*	[57]
β –Lactamase	Antibiotic resistance	*Klebsiella pneumonia*	[58]
β -Galactosidase	Removal of lactose from milk	*Aspergillus sp.*	[59]
Urokinase	Blood clots	*Bacillus subtilis*	[60]
Tyrosinase	Treatment of Parkinson s disease, Antitumor	*Streptomyces glausescens*, and *Erwinia herbicola*	[61]
Superoxide dismutase	Antiinflammatory, Anti-oxidant	*Mycobacterium* sp. and *Nocardia* sp.	[61]
Streptokinase	Anticoagulant	*Streptococci* sp	[62]
Ribonuclease	Antiviral	*Saccharomyces* sp.	[63]
Rhodanase	Cyanide poisoning	*Sulfobacillus sibiricus*	[61]
Lipase	Treatment of disorders of the pancreas, Digest lipids	*Aspergillus oryzae*, *Candida sp.*	[61]
L-Glutaminase	Leukemia	*Escherichia coli*	[64-65]
β-Glucosidase	Anti-tumor	*Aspergillus niger*	[61, 66]
Glucose oxidase	Biosensors, Antimicrobial	*Aspergillus*, *Penicillium Saccharomyces* sp.	[67]
Asparginase	Leukemia	*Escherichia coli*	[11, 68]
Arginase	Antitumor	*Escherichia coli*, *Bacillus subtilis*	[61]
Acid protease	Stomach disorders	*Aspergillus oryzae*, *Aspergillus niger*	[61]
Adenosine deaminase	Rheumatoid arthritis	*Aspergillus terricola*, *Penicillium politans*	[69-71]

5. Conclusion

The use of enzymes from microbial origin increased greatly in pharmaceutical, medical and industrial fields during the past few years. Enzymes are already been used as diagnostic and therapeutic drugs for many diseases and also in clinical test reagents. We expect further development of these fields during the next few years by providing high purity enzymes on medical and industrial scales, especially those enzymes which give positive clinical results, such as streptokinase, thrombolytic, antimicrobial, fibrinolytics, mucolytics and those which are used as coagulants. Recently, the demand for therapeutic enzymes has increased sharply for the treatment of many serious diseases and studies have been conducted to select the proper and efficient microbial resources including terrestrial and marine microorganisms. In the near future, there is a need of deep research on the mechanisms of therapeutic enzymes, and also with their combination with other drugs to alleviate their side effects and improve their action.

6. Conflicts of Interest

The author(s) report(s) no conflict(s) of interest(s). The author along are responsible for content and writing of the paper.

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