Role of Enzymes in Pharmaceutical and Biotechnology Industries

9

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Abstract

In the modern world, enzymes are not just catalysts but also "tools" that modify the process based on the requirement. Enzymes hold key functions in the reactions where other alternatives fail to deliver the desired outputs. The main features of enzymes are their specificity, reusability, and non-formation of unwanted by-products that may contaminate the product as well as the environment. These features have made enzymes a popular choice in the biopharmaceutical sector. The increased use of enzymes is also attributed to high efficiency and low cost achieved by adapting modern techniques such as strain improvement, mutations, genetic engineering, recombinant DNA technology, etc. Additionally, the enzyme activity can be controlled by physical or chemical methods making them applicable to a wide range of processes. Enzyme application in various fields is possible due to the continuous research in biotechnology. The global market of enzymes in the industrial sector is valued at 4.91 billion USD in 2015 and is expected to reach 9.74 billion USD by 2022 as reported by Stratistics Market Research Consulting Pvt. Ltd. The pharmaceutical and biotechnology sectors contribute to a major share of revenue generation globally. This chapter provides an overview of enzymatic applications in the biopharmaceutical sector along with the current market scenario and expected future trends.

Keywords

Enzymes · Biotechnology · Biocatalysts · Biosensors · Therapeutic enzymes

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9.1 Introduction

Enzymes have been used for therapeutic purpose since many decades. It started since the 1960s as a replacement therapy for genetic deficiencies by de Duve (Vellard 2003). Several decades of research on synthesis and commercialization of enzyme-based products have brought it to the current comprehended status. Enzymes are proteins which are specialized to catalyze biochemical reactions within and/or outside the living cells. They either aid in combining molecules to produce new products or breaking them into smaller parts. Enzymes in pharmaceutical and biotechnology industries are used either as tools in manufacturing active pharmaceutical ingredients or directly for therapeutic purpose as a drug. The market competition is decided mainly by two factors, viz., price and quality of the product. In the beginning, enzyme-catalyzed reactions were very expensive and time-consuming. The research and developments in the field of strain improvement, mutations, genetic engineering, recombinant DNA technology, etc. led to successful development of more active and stable enzymes. This has also led to the development of technology of enzyme immobilization for varied applications in the industrial sector. Consequently enzymes can be immobilized and reused until their activity gets reduced, whereas chemicals get neutralized in the first reaction itself. Enzyme immobilization has also reduced the process cost and hence cost of the final products drastically.

The quality of a product depends on the reactants used as well as the process involved during manufacturing. All chemicals used in industries are not safe for consumption. It has been observed that the patients suffer with side effects mostly when the drugs contain traces of harmful chemicals from the method used for manufacturing. Also chemicals used in a reaction often add by-products to the reaction mixture, thus reducing the quality of the product and introducing the need for more purification. In comparison with the purely chemically synthesized drugs, enzymes are comparatively safer to use. The by-products of enzymatic reactions are not harmful; the regiospecificity of these reactions reduces the purification costs. The requirement of enzymes per unit is also very less due to it's higher reaction efficiency and recyclable nature. Moreover, the same enzymes can catalyze multiple reactions till the activity reduces below its commercial feasibility.

The enzyme committee (EC) of the International Union of Biochemistry and Molecular Biology (IUBMB) which was formed in 1955 sets the guidelines for classification and nomenclature for enzyme based on the reactions they catalyze. The aim of IUBMB is to support the growth and development of biochemistry and molecular biology for the betterment of mankind.

9.2 Therapeutic Enzymes

Therapeutic enzymes are those enzymes which can be used in the medical treatment of various diseases. These enzymes can be used solely or in combination with other therapies for the desired cure/prevention. Using enzyme as preventive or therapeutic

medicine is a modern way of treatment that provides additional advantages of having high accuracy, strong affinity, and specificity for target location with minimum side effects (Mane and Tale 2015).

9.2.1 Sources of Therapeutic Enzymes

There are three sources of the enzymes, *viz*., plant, animal, and microorganism.

9.2.1.1 Plant Enzymes

Enzymes extracted from plants are mostly macromolecule-degrading enzymes. Plant enzymes are used as drugs in the form of capsules containing a mixture of enzymes that help support digestion and specifically formulated for vegan or vegetarian patients. Enzymes are lyophilized (freeze-dried) and packed in a capsule or other forms of wall material. These are stored under dry and cool conditions. These enzymes assist in the degradation of fats, fiber, lipids, proteins, carbohydrates, and other macromolecules which help in releasing vitamins and nutrients from the food to be absorbed through the intestinal walls after ingestion. Plant enzymes also include lactase to support lactose-intolerant patients which help in digestion of dairy products.

For example, NOW Health Group, Inc. has developed a product called "NOW Plant Enzymes." The product contains dietary enzymes to treat patients suffering from indigestion problems. Enzymes like protease, acid-stable protease, amylase, lipase, cellulase, lactase, papain, bromelain, etc. are isolated, purified separately and mixed together in a desired proportion, and packed in a capsule.

Drawbacks of Plant Enzymes

Plants grown for commercial extraction of enzymes such as pineapple and papaya are generally get exposed to harsh chemical contaminants of pesticides, preservatives, and growth enhancers. A variety of harmful pesticides are used in different countries to improve the productivity. In such situations, studies related to the effect of pesticides, preservatives and growth enhancers on the cellular and molecular level are very limited. Additionally, traces of such harmful chemicals in the final product should also be studied as this may be the reason for some patients developing allergic reactions to plant enzymes. Moreover, plant enzymes are not fully active at human body temperature; they generally require higher temperatures to become fully active. In addition to these factors, the purification of enzymes from plant sources is difficult as compared to bacterial sources. This increases the cost of the enzyme-containing formulation.

9.2.1.2 Animal Enzymes

Enzymes, hormones, and proteins derived from animals were widely used before the modern technology was developed. The most common hormone from animal source was insulin used for diabetes type I patients. The most common enzyme derived from animal source was from pancreatic juice containing digestive enzymes. Crude

enzyme extracts and animal tissues such as the pancreatic tissue of pigs were collected at the slaughter houses and purified for any further uses. Recombinant DNA technology has simplified certain difficulties. The human genes responsible for coding of enzymes or hormones are isolated with the help of molecular scissors and incorporated in the host cell system which is generally bacteria or yeast. Despite having this technology, some companies still harvest animal enzymes and market them in the form of digestion aids and other products. However, microbial- or plant-source enzymes have several additional advantages when compared to animal enzymes. The following is a list of animal enzymes used in the industries earlier; now most enzymes have been replaced by microbial enzymes (Table 9.1).

Drawbacks of Animal Enzymes

Enzymes from animal sources are not preferred by patients of certain ethnicity. The activity and availability of animal-based enzymes are very limited and hence cannot fulfill the requirement of patients. The quality of enzymes is not consistent due to difference in health conditions of the source, i.e., animals. Moreover, high level of impurities calls for a purification steps, making the final product expensive.

9.2.1.3 Microbial Enzymes

This is the era of microbial enzymes. Around 88% of enzymes used in the industries are from microbial sources. The microbial-enzyme market share is expected to increase up to 98% in the coming years. Apart from enzymes, microbes are the first choice for synthesis of recombinant hormones, proteins, antioxidants, secondary metabolites, and other macromolecules. Increasing research on different microorganisms at various institutions across the world indicate the future trend of pharmaceutical and biotechnology industries.

Microbes are preferred as a primary source of enzymes due to the low manufacturing cost. This is due to ready availability of raw material for fermentation and lower time of manufacturing. Microbial enzymes are more predictable, controllable, and highly specific, and the process is easy to understand as compared to enzymes from other sources. Additionally genetic manipulation on microbes can be

Table 9.1 List of animal-derived enzymes

Animal enzymes	Source organ	Application	Function
Catalase	Liver	Food industry	Reduces hydrogen peroxide in food items
Serine protease	Pancreatic juice	Food, leather	Serine protease enzyme used in the production of hydrolyzed proteins from vegetable and animal sources
Lipase	Pancreatic juice	Food (fat and oil industries)	Hydrolysis, transesterification, esterification
Rennet	Abomasum	Cheese	It is a protease enzyme that curdles the casein in milk
Trypsin	Pancreatic juice	Biotech	Used to convert proinsulin to insulin

easily performed for overproduction of enzymes. In recombinant DNA technology, the microbial host cells are induced to produce the same enzyme that is produced in the human body. This technology is explained in detail in Sect. 9.4. In comparison with microbial system, plant and animal cells are more complex to handle and contain potentially harmful materials including phenol compounds (from plants), endogenous enzyme inhibitors, and proteases. The most commonly used microbial enzymes are digestive enzymes such as proteases for digesting protein present in the food, lipases for digesting fats, and amylases for digesting some carbohydrates. These digestive enzymes are also commercially available in the form of recombinant pancreatic enzyme products such as aristozyme (diastase and pepsin liquid), Aristo Pharmaceuticals Pvt. Ltd. Different types of microbial enzymes used in industries are listed in Table 9.2.

9.3 Why Enzymes Are Very Specific?

Enzymes are produced for a specific task and produced only when required by a living cell. This production is a complex biochemical reaction that occurs with highest accuracy. Some enzymes are secondary metabolites which need to be triggered by external factors like chemical, physiological, or mechanical. The process of enzyme (secondary metabolite) production starts with the availability of substrate and contact made with the cell receptor externally. The signals are then sent to DNA which contains code for the specific enzyme production. Now the cell initiates the process of transcription, translation, and posttranslational modification. After posttranslational modification, the enzymes or proteins enters in to the Golgi apparatus from endoplasmic reticulum where Golgi apparatus sorts the macromolecules (enzymes, hormones, and proteins) and sends them to their destination. If any faulty protein is identified, then it is tagged and sent to lysosome for degradation which is known as autophagy. The process of enzyme production is designed in such a way that only the active enzymes can successfully move out of Golgi apparatus and faulty enzymes are recycled. Thus the specific enzyme is only made available for the reaction.

9.4 Recombinant Enzymes

Recombinant DNA technology is a branch of science which deals with the modification of DNA molecules by using laboratory techniques of genetic recombination. The idea of recombinant DNA was first proposed by Peter Lobban, a graduate student from Biochemistry Department at Stanford University Medical School (Kayser and Müller 2005). DNAs from two different species are combined together to form a recombinant DNA molecule (also called chimeric DNA) to produce a desired product from the host cell that is of value to scientific research in the field of medicine, agriculture, environment, and industry. Enzymes are proteins, and proteins can be modified by understanding the coding of DNA sequence. Natural

Table 9.2 List of microbial enzymes

Enzyme	Organism	Application
Bacterial enzymes		
α-Amylase	*Bacillus*	Enzymatic hydrolysis of starch
Asparaginase	*Escherichia coli*	Asparaginase prevents formation of acrylamide and some heterocyclic amines in baked foods
		Asparaginase helps in starving cancer cells from L-asparagine
Glucose isomerase	*Bacillus*	Used in production of high-fructose corn syrup (HFCS)
Penicillin amidase	*Bacillus*	Penicillin and cephalosporin biosynthesis
Protease	*Bacillus*	Used in detergents and biotech industries
Pullulanase	*Klebsiella*	Maltose syrup, glucose syrup production, and cyclodextrin production
Fungal enzymes		
α-Amylase	*Aspergillus*	Used for ethanol production where enzyme breaks starch into fermentable sugars
Aminoacylase	*Aspergillus*	Treatment of neurological disorders
Catalase	*Aspergillus*	To eliminate hydrogen peroxide from food
Cellulase	*Trichoderma*	Used for ethanol production where enzyme breaks waste cellulose into fermentable sugars
Dextranase	*Penicillium*	Used to eliminate dextrin from cane juice
Glucose oxidase	*Aspergillus*	Used to identify free glucose in blood serum
Lactase	*Aspergillus*	Used in dairy industry
Lipase	*Rhizopus*	Used in oil and food industries
Rennet	*Mucor miehei*	It is a protease enzyme that curdles the casein in milk for cheese production
Pectinase	*Aspergillus*	Used in pulp and paper industries
Pectin lyase	*Aspergillus*	Used in pulp and paper industries
Protease	*Aspergillus*	Detergent and biotech industries
Yeast enzymes		
Invertase	*Saccharomyces*	Used in chocolate industries to break sucrose
Lactase	*Kluyveromyces*	Used in dairy industry
Lipase	*Candida*	Used in oil and food industries
Raffinase	*Saccharomyces*	Used to remove the stachyose and raffinose from soybean milk

enzymes have certain limitations with respect to stability, activity, efficiency, yield, etc. In case of recombinant enzymes, the DNA encoding a highly efficient enzyme from any source is combined with the DNA of host cell which has the ability to produce it in a higher yield. Genetic recombination can be done with the help of vectors which can carry the foreign DNA molecule into the host cell and place it in between the host DNA molecule. The demand for recombinant enzymes is rapidly increasing in the industrial sector. Industries have started adopting recombinant

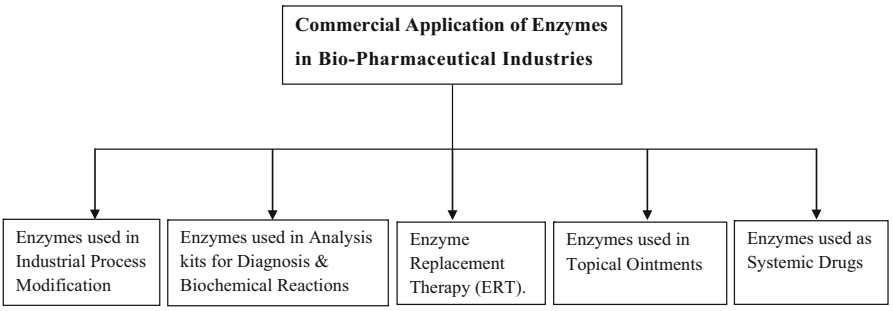

Fig. 9.1 Classification of enzymes used in biopharmaceutical Industries

enzymes over natural enzymes for their high activity, lower reaction time, and ease of reaction control. Though the recombinant enzymes are expensive but continual research and market competition will reduce the cost in future.

9.5 Enzymes Used in Bio-Pharmaceutical Industries

The enzymes used in bio-pharmaceutical industries can be classified on the basis of their application as shown in Fig. 9.1.

9.5.1 Enzymes Used in Industrial Process Modification

9.5.1.1 Proinsulin to Insulin

Insulin is a human hormone used for the treatment of diabetes type I patients. This is now produced from microbial sources, i.e., *Escherichia coli* and *Saccharomyces cerevisiae.* During the synthesis of insulin, the step of conversion of proinsulin to insulin is catalyzed by enzymes like trypsin and carboxypeptidase. They are used for removing unwanted side chains by cleaving peptide bonds.

9.5.1.2 Trypsin

Trypsin (EC 3.4.21.4) was discovered in 1876 by Wilhelm Kühne. It is also known as serine protease found in the intestine of many vertebrates, where it hydrolyzes the proteins. Digestion aided by trypsin is a very important step as too large protein cannot be absorbed through the walls of the intestine. Trypsin is produced in the pancreas in the form of proenzyme trypsinogen which is released in the small intestine to activate to trypsin. Now recombinant trypsin is produced through fed-batch fermentation using recombinant *Escherichia coli* at the biotechnology production plants. Trypsin cleaves peptide bonds at the carboxyl end of the amino acids lysine or arginine, except when these amino acids are followed by proline. It is widely used in the modification of proteins such as proinsulin to insulin. This biotechnological process is known as trypsin proteolysis or trypsinization.

9.5.1.3 Carboxypeptidase B (CPB)

Carboxypeptidase B (EC number 3.4.17.2) is also known as protaminase, pancreatic carboxypeptidase B, peptidyl-L-lysine, and L-arginine hydrolase. This enzyme has the ability to hydrolyze peptide chain at the carboxy-terminal end of a protein or peptide chain. Carboxypeptidase B is used in the production of human recombinant insulin and monoclonal antibody (IgG1) processing. Carboxypeptidase is highly active in the pH range 5–12 and maximum temperature up to 60 °C to act on amino acids such as arginine and lysine.

9.5.1.4 Semisynthetic Penicillin

Penicillin acylase enzyme falls under the family of hydrolases, which act on carbon-nitrogen bonds other than peptide bonds. This enzyme is used in the process of production of semisynthetic penicillin by catalyzing the reaction of penicillin G and H_2O as a substrate to produce carboxylate and 6-aminopenicillanate. After gaining resistance to penicillin by microbes, semisynthetic penicillin was developed which has extended its effectiveness against a wide range of infectious microorganisms such as *streptococcal* and *staphylococcal* species, aerobic gram-negative, and many anaerobic organisms (Oshiro 1999). Semisynthetic penicillin has flexibility features such as mode of administration, combination with other drugs, and low production cost as compared to other antibiotics.

9.5.1.5 Pretreatment for Extraction of Medicinal Compounds

Enzymatic pretreatment for the extraction of medicinal compounds from plant tissues has shown enhancement in aroma recovery. Most ayurvedic medicines are prepared from plants and herbs which are sensitive to heat. Traditional methods like drying at low temperature and extraction of medicinal compounds from fresh herbs are time-consuming. In such cases, flavors and oils can be efficiently extracted by giving enzymatic pretreatment. Enzymes such as cellulases, hemicellulases, and pectinases and a combination of these have been used for the pretreatment of various plant materials (Grumezescu 2017). These enzymes efficiently damage the plant cell wall and hydrolyze them which increase the permeability resulting in higher yield of the desired bioactive compounds. Enzyme application in the field of flavor extraction from plant materials is a relatively new area, which demands extensive research and development to establish itself as a promising technique.

9.5.2 Enzymes Used in Analysis kits for Diagnosis & Biochemical Reactions

9.5.2.1 Diabetes Diagnostic Kits

Diabetes is a disease which is affecting hundred millions of people globally. To control diabetes, patients need to maintain proper diet and monitor blood glucose level. Glucose oxidase is an enzyme which is widely used for the quantification of free glucose in the body fluids. This enzyme is also known as oxidoreductase that catalyzes the oxidation of D-glucose to D-glucono-1,5-lactone and hydrogen

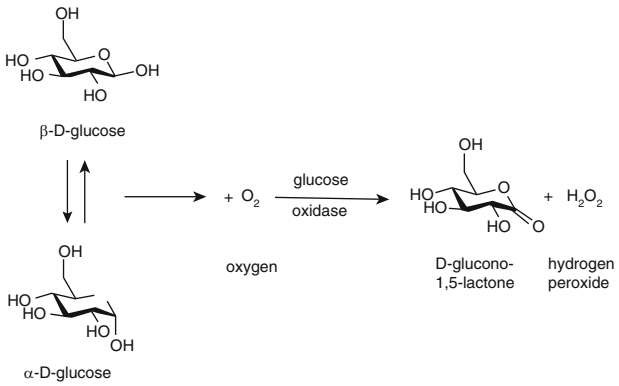

Fig. 9.2 Reaction catalyzed by glucose oxidase

peroxide. It was first isolated and purified from *Aspergillus niger* by Detlev Muller in 1928. It also has other applications in the field of biotechnology, including biosensors in nanotechnology (Fig. 9.2).

9.5.2.2 ELISA Kit

Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a technique that is used to determine the presence of an antigen or an antibody in the sample. Antibodies are Y-shaped proteins produced by the body (plasma cells) in response to harmful external substances called antigens. Enzymes such as horseradish peroxidase (HRP) and alkaline phosphatase (AP) are usually used in these tests.

The technology of ELISA has been widely used for preparing diagnostic tools in the medicine sector and as a quality control check in food industries. Various techniques of ELISA are used depending on the nature of sample. The most commonly used techniques in ELISA are explained in the diagram (Fig. 9.3).

9.5.2.3 PCR Kit

Polymerase chain reaction (PCR) is also known as thermocycler or DNA amplifier used in the making of multiple copies of a particular region of DNA by using existing strand known as template DNA. This DNA region can be a gene of interest or a genetic marker used by forensic investigators to match crime scene DNA with suspects. Amplified DNA can be used for sequencing, identification through visualizing the gel electrophoresis, or gene incorporation into a plasmid (vector) for gene expression in the host cell. PCR also has application in the areas like medicine, molecular biology research, medical diagnostics, forensic sciences, etc.

DNA polymerase enzyme is added into the reaction which utilizes the free nucleotides and synthesizes a new strand of DNA by using existing strand as template. The DNA polymerase is also called Taq polymerase isolated from heat-resistant bacterium *Thermus aquaticus*.

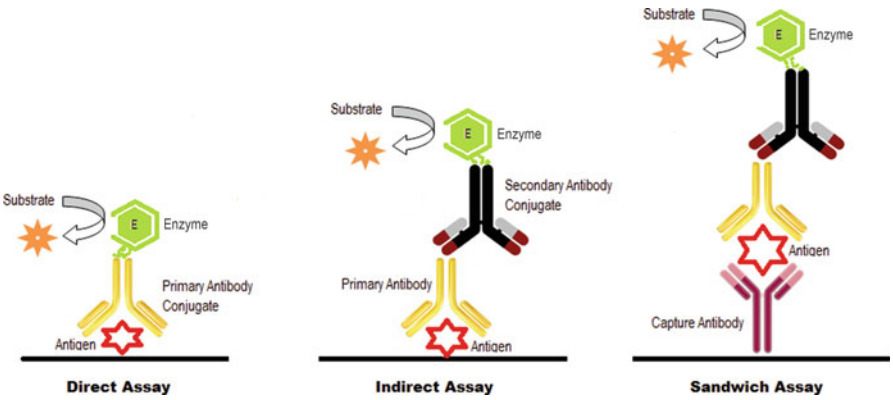

Fig. 9.3 Different methods of ELISA test

Table 9.3 Example of restriction endonuclease type II

Restriction enzyme	Source	Recognition sequence
BamH I	*Bacillus amyloliquefaciens H*	5′ G---GATCC 3′
		3′ CCTAG---G 5′
HindIII	*Haemophilus influenzae*	5′ A---AGCTT 3′
		3′ TTCGA---A 5′

9.5.2.4 Restriction Endonuclease

Restriction endonucleases are also known as restriction enzymes or molecular scissors that cleave the double-stranded DNA at or near specific recognition nucleotide sequence known as restriction sites. Restriction enzymes are classified into types I, II, III, and IV based on their recognition sequence, subunit composition, cleavage position, and cofactor requirements (Sistla and Rao 2004). All restriction enzymes have the ability to make two incisions on each phosphate backbone of the double-helix DNA and glue together with the help of DNA ligase (Table 9.3). Type II restriction endonuclease is most commonly used for molecular biology applications, as they recognize stereotypical sequences and produce a predictable cleavage pattern. The restriction enzymes are named from the cellular strain they are isolated from. The examples of enzymes used in diagnosis and molecular biology kits are listed in Table 9.4.

9.5.3 Enzyme Replacement Therapy (ERT) or Supplements for Patients Deficient for Specific Enzyme

Enzyme replacement therapy (ERT) is a medical treatment which replaces an enzyme that is deficient or absent in the human body. Generally, the mode of drug delivery is by intravenous (IV) injections that contains enzyme and oral capsules for

Table 9.4 List of enzymes used in diagnostic kits

Enzyme	Reaction	Use
Asparaginase	L-asparagine $H_2O \rightarrow$ L-aspartate + NH_3	Leukemia (Verma et al. 2007)
Collagenase	Collagen hydrolysis	Skin ulcers (Sani and Navanietha Krishnaraj n.d.)
Glutaminase	L-glutamine $H_2O \rightarrow$ L-glutamine + NH_3	Leukemia (Sani and Navanietha Krishnaraj n.d.)
Rhodanase	$S_2O_3^{2-} + CN^- \rightarrow SO_3^{2-} + SCN^-$	Cyanide poisoning (Cipollone et al. 2008) (Sani and Navanietha Krishnaraj n.d.)
Ribonuclease	RNA hydrolysis	Antiviral (Moelling et al. 2014)
β-Lactamase	Penicillin → penicillate	Used for penicillin allergy
Streptokinase	Plasminogen → plasmin	Blood clots ("Composition, method, and kit for preparing plasmin" 2009)
Trypsin	Protein hydrolysis	Inflammation (Stolarow et al. 2015)
Uricase	Urate + O_2 → allantoin	Gout (Mulyasuryani and Srihardiastutie 2011)
Urokinase	Plasminogen → plasmin	Blood clots (Blinc et al. 1991)

patients suffering from digestive system disorders. Enzyme replacement therapy is available for some lysosomal storage diseases such as Hurler-Scheie (MPS I), Hunter syndrome (MPS II), Maroteaux-Lamy syndrome (MPS VI), Gaucher disease, Fabry disease, Pompe disease (Ries 2017), and cystic fibrosis. ERT does not cure the disease by repairing genetic defects, but it provides the highly active enzyme or set of enzymes that the patient is deficient to produce (Ries 2017). Severe combined immunodeficiency (SCID) is also treated by ERT which is caused by adenosine deaminase deficiency (Booth et al. 2007).

Alternative treatments for patients with such disease having enzyme or protein deficiencies are gene therapy, bone marrow-derived stem cell transplantation, and substrate reduction therapy (Ries 2017).

9.5.3.1 Lactose Intolerance

Lactose is a sugar dimer made from galactose and glucose which is found in milk and other dairy products. Lactose intolerance is the condition where some people are unable to or have decreased ability to digest lactose. Undigested lactose, when moving through the large intestine, can cause uncomfortable symptoms such as bloating, chronic gas, abdominal pain, nausea, and diarrhea (National Institute of Diabetes and Digestive and Kidney Diseases 2014). The symptoms vary depending upon the concentration of lactose left undigested in the small intestine. Ten percent of Caucasians and 80% of non-Caucasians suffer from lactose intolerance. Some enzyme-based medicines available in the market are mentioned below.

- Prolactazyme Plus is a marketed name for the product containing combination of the enzymes such as bromelain, lactase, lipase, and papain with *Lactobacillus salivarius* and *Lactobacillus acidophilus*.

- Prolactazyme Forte is another product containing combination of the enzymes such as bromelain, lactase, lipase, papain, and amylase with *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*.

9.5.3.2 Cystic Fibrosis

Cystic fibrosis (CF) is a genetic disorder that affects mostly the lungs, as well as the pancreas, liver, kidneys, and intestine (O'Sullivan and Freedman 2009). It is a chronic disease that severely reduces the patient's life span. Cystic fibrosis is characterized by the formation of thick, sticky mucus which can damage different organs of the body. This abnormal mucus can clog the airways, leading to severe breathing issues and microbial infections in the lungs. In most patients with cystic fibrosis, mucus blocks the ducts of the pancreas, hence reduces the production of insulin and blocks the digestive enzymes to reach the small intestine for digestion of food.

Obstruction of the pancreatic duct results in the deficiency of:

- Protease which makes the patient unable to break down dietary proteins into amino acids.
- Amylase is an enzyme that catalyzes the hydrolysis of carbohydrate molecules and is unable to break down the macromolecules into monomers to be absorbed.
- Lipase that catalyzes the breakdown of lipids prevents the absorption of fat and proteins in the intestinal tract.

Due to lack of these enzymes, the body does not get the necessary nutrients for healthy cell growth and reproduction and hence affects so many organs.

External supplementation of the deficient enzymes can help the patients.

9.5.3.3 Pancrelipase

Pancrelipase is a drug that is classified as a digestant. The preparation contains high concentration of digestive enzymes, protease, amylase, and lipase needed for the digestion of proteins, starch, and fats, respectively. It basically replaces the enzymes that the body is unable to produce which are vital for the breakdown and absorption of nutrients. It prevents malnutrition and further complications due to lack of absorption of necessary nutrients for cell growth and repair. Scientists are separating the lipase, protease, and amylase and purifying them independently to prevent cross-linking of proteins. In this way they can individually perform their functions.

9.5.3.4 DNase

More than 30 years ago, it was believed that the bovine pancreatic DNase I could reduce the increased viscosity caused by high purulent secretions of extracellular DNA which is released by the leukocytes (Shak et al. 1990). Normally the DNase enzyme is found in saliva and pancreatic secretion, but patients suffering from cystic fibroses are unable to produce it. In such cases external supplements of enzymes are

recommended. Pulmozyme (DNase) is an enzyme usually inhaled by a nebulizer, which helps to reduce thickness of mucous and break down the sticky mucus in the lungs so it is easier to cough up and breathe. Due to the advancements in medicine and the pharmaceutical industries, development of digestive enzymes allows most children with cystic fibrosis to be relatively healthy until they reach adulthood. This is a result of the development achived on supplemental enzyme replacement in the form of two pharmaceutical brands, pancrelipase and Pulmozyme DNase.

9.5.3.5 Pompe Disease

Pompe disease, also called lysosomal storage disorders (LSDs) or glycogen storage disease type II, is an inherited enzyme defect that usually occurs in the childhood. The deficiency of the acid α-glucosidase enzyme leads to accumulation of glycogen in the lysosome due to which muscle and nerve cells get damage. This enzyme normally catalyzes reactions that convert glycogen to monosaccharides. The deposition of glycogen in the absence of acid α-glucosidase causes serious damage to various body tissues such as the liver, skeletal, heart muscles, and particularly nervous system. The disease was first identified by the Dutch pathologist J. C. Pompe in 1932 (Zschocke et al. 2016). Lumizyme and Myozyme (alglucosidase alfa) are the marketed products of acid α-glucosidase enzyme. All these drugs are given in the form of intravenous mode. List of diseases that can be treated by enzyme replacement therapy (ERT) is described in Table 9.5.

9.5.4 Enzymes Used in Topical Ointments

Ointments are the smooth oily substance rubbed onto the body surfaces such as a skin or a mucus membrane for a medicinal or a cosmetic purpose. Ointments contain drug(s) that may act on the skin or be absorbed through the skin for systemic action. Enzymes possess debridement, antioxidant, and antimicrobial property because of which they have incorporated in the ointments and creams. Clinical studies have shown that combining enzymes with conventional medicine demonstrates synergistic effects.

9.5.4.1 Collagenase

Wounds can be cured faster only when damaged cells and tissues are removed so that the body can regenerate new healthy tissues. Collagen is a protein that joins and holds cells and tissue together such as the muscle, skin, bone, and tendons. Collagenase is an endopeptidase having debridement activity that digests native collagen in damaged cells and tissues within the skin layers and helps recover severe burns, skin ulcers, etc. Collagenase topical ointment was first commercially available in 1959 isolated from *Clostridium histolyticum.* Collagenase SANTYL ointment is a commercially available, Health Canada-approved active enzymatic therapy that has an ability to remove necrotic tissue from wounds at the microscopic level.

Table 9.5 List of enzymes used in enzyme replacement therapy

Disease	Enzyme	Enzyme function
Fabry disease	Agalsidase beta	Fabrazyme (agalsidase beta) lowers the amount of a substance called globotriaosylceramide (GL-3)
Fabry disease	Agalsidase alfa	Replagal (agalsidase alfa) degrades glycosphingolipid substrate (Gb3)
Gaucher disease	Imiglucerase	Cerezyme (imiglucerase) breaks down the lysosomal glucocerebroside
	Taliglucerase alfa	Elelyso (taliglucerase alfa) works by catalyzing the hydrolysis of nonfunctional glucocerebroside to glucose, derived from plant (carrot)
Gaucher disease type I	Velaglucerase alfa	VPRIV (velaglucerase alfa) breaks down the hydrolytic lysosomal glucocerebroside
	Alglucerase	Alglucerase helps in breakdown of glucocerebroside
Lysosomal acid lipase deficiency (Wolman disease/CESD)	Sebelipase alfa	Kanuma (sebelipase alfa) breaks down fats (lipids) such as triglycerides and cholesteryl esters
Hurler-Scheie and Scheie disease MPS I	Iduronidase	Aldurazyme (iduronidase) enzyme acts by degeneration of glycosaminoglycans such as dermatan sulfate and heparan sulfate
Hunter syndrome	Idursulfase	Elaprase (idursulfase) and Hunterase (idursulfase beta) that help breakdown glycosaminoglycans (GAGs)
MPS II		
Morquio A syndrome MPS IVA	Elosulfase alfa	BioMarin's Vimizim (elosulfase alfa) replace the missing N-acetylgalactosamine-6-sulfatase enzyme GALNS
Maroteaux-Lamy syndrome	Galsulfase	Naglazyme (galsulfase) increases the catabolism of glycosaminoglycans (GAGs)

9.5.4.2 Papain/Urea Ointment

Papain is an enzyme extracted from papaya fruit which shows anti-inflammatory properties. Papain has also shown beneficial effect against infections, swellings, and edemas. In the ancient period, the Indians and native Americans had a traditional way of healing by applying a slice of papaya fruit and ground seeds on the infected skin. After successful research and development, today we can use ointments containing papain and other enzymes (Simonsohn 2000). Enzyme-based ointments have proved to be very valuable. Enzymes clean the wound bed, support healing, prevent scars, and relieve pain. Papain/urea ointment is a debridement agent and helps in removing damaged or infected tissue in acute or chronic lesions such as pressure ulcers, diabetic ulcers, varicose ulcers, traumatic infected wounds, etc. Allanzyme, Accuzyme, Allanfil, and Ethezyme are the trade names for papain/urea ointment commercially available.

9.5.5 Enzyme-Based Systemic Drugs

Alternative to enzyme replacement therapy (ERT), there are some enzyme-based systemic drugs which are available and cure the disease, whereas ERT only supports the normal life but does not cure the disease. Today enzyme-based drugs are commercially available for only a handful of diseases, but several drugs are under the pipeline and ready to be commercialized.

9.5.5.1 Leukemia

Leukemia is a cancer which starts usually in the bone marrow and enters into the blood. This happens because of the overproduction of abnormal white blood cells which are part of the immune system and are responsible for fighting against antigens ("Leukaemia CARE," n.d.). Asparaginase is an enzyme that is used for the treatment of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and non-Hodgkin's lymphoma by restricting cancer cells from growth and cell division. Asparagine is an amino acid which is not produced in cancer cells and without which that cannot survive (Kate Traynor n.d.). The function of asparaginase is to break down the asparagine into aspartic acid and ammonia. So when free asparagine is not available in the system, cancerous cells starve to death. Medical use of asparaginase was approved in the USA in 1978 (Salzer et al. 2014). Clinical studies have proved that asparaginase treatment on children has shown improvement in childhood acute lymphoblastic leukemia (Pieters et al. 2011). FDA and EUSA Pharma announced the approval of asparaginase or Erwinaze (marketed name), as part of a treatment for acute lymphoblastic leukemia (ALL) in patients who have had hypersensitivity reaction to *Escherichia coli*-derived asparaginase (Kate Traynor n.d.). Elspar is the trade name for asparaginase.

9.5.5.2 Cancer or Tumors

Cancer is caused when abnormal cells divide in an uncontrolled way. The newly produced extra cells form a mass called a tumor. Some cancers may spread into tissues of other parts of the body. Approximately 50% of people in the UK get cancer in their lifetime. It is reported that there are more than 100 different types of cancer and most cancers are named from the place they originate. Ribonuclease (RNase) falls under the class of nuclease that catalyzes the degradation of RNA into smaller components. The antitumor effect of ribonucleases was studied with animal ribonucleolytic enzymes in the past (Matousek 2001). This enzyme binds to negatively charged cell membrane and enters the cell by endocytosis and penetrates into cytosol where they degrade RNA (Ardelt et al. 2009). Antitumor recombinant ribonucleases are small (10–28 kDa) basic proteins. Microbial RNases, especially RNase Sa from *Streptomyces aureofaciens* and binase from *Bacillus intermedius* had shown successful results for antitumor activity (Ardelt et al. 2009) (Table 9.6).

Table 9.6 List of enzyme-based systemic drugs

Enzyme	Source	Uses
Acid protease	*Aspergillus niger* and *Aspergillus oryzae*	Stomach disorders
Arginase	*Bacillus subtilis* and *E. coli*	Antitumor
Bacilysin synthetase	*Bacillus subtilis*	Antibiotic
Bacitracin synthetase	*Bacillus licheniformis*	Antibiotic
Glucose oxidase	*Aspergillus*, *Penicillium*, and *Saccharomyces* sp.	Biosensors, antimicrobial
Glucosidase	*Aspergillus niger*	Antitumor
Gramicidin synthetase (carboxyl-activating synthetases)	*Bacillus brevis*	Antibiotic
Nattokinase	*Bacillus subtilis*	Cardiovascular disease
Nonribosomal peptide synthetase	*Aspergillus fumigatus*	Antitumor activity by inhibiting microtubule assembly
Penicillin acylase	*Penicillium* sp.	Penicillin production/broad-spectrum antibiotic production
Peptidase	*Beauveria bassiana* and *Bacillus polymyxa*	Celiac disease, clot formation, inflammation, and repair
Phenylalanine racemase	*Bacillus brevis*	Antibiotic
Protease	*Pseudomonas aeruginosa*	Antibiotic
Rhodanase	*Sulfobacillus sibiricus*	Cyanide poisoning
Ribonuclease	*Saccharomyces* sp.	Antiviral
RNase P ribozyme	*Bacillus subtilis*	Antiviral
Streptokinase	*Streptococci* sp.	Anticoagulant
Tyrosinase	*Streptomyces glaucescens* and *Erwinia herbicola*	Antitumor, treatment of Parkinson's disease
Uricase	*Aspergillus flavus*	Gout
Urokinase	*Bacillus subtilis*	Blood clots
β-Lactamase	*Serratia marcescenes*, *Citrobacter freundii*, and *Klebsiella pneumoniae*	Used in the treatment of antibiotic allergy

9.6 Side Effects of Enzyme-Based Drugs

Side effects are generally undesirable secondary symptoms caused during and/or after the period of medication. Side effects may vary for each individual depending on the person's disease state, ethnicity, gender, age, weight, and general health condition. Side effects of enzyme-based drugs are very rare, but few secondary effects have been reported in the past, so it is strictly recommended that one should always consult a healthcare specialist or doctor for medical advice.

Side effects are classified into minor and major secondary symptoms which depend upon individual patient and their medication. Minor symptoms can led to inconvenience for short time period till the course of drug completes. Some patients may experience life-threatening allergic reactions during the course of medication. In such cases, patients should stop their course immediately and seek emergency medical help. The symptoms such as inconvenience while talking and breathing; nosebleed; heartburn; chest tightness; inflammation of the tongue, mouth, lips, face, or throat; and itching and rashes throughout the body.

9.6.1 Side Effects of Digestive Enzyme

The common side effects suffered by patients are headache, nausea, or vomiting. Some patients may also experience stomach pain which leads to diarrhea. Prolonged dosage may also lead to cough, sore throat, dizziness, and stuffy nose. Some digestive enzymes are extracted from pork; therefore, patients who are allergic to pork should avoid using such products.

9.7 Global Market for Enzymes

The demand for enzyme-based drugs is increasing globally. This is the reason why pharmaceutical giant industries have started investing in the setting up of huge bioreactors for fermentation. Stringent guidelines and high investments are still a barrier for many small- and medium-scale industries. Government in some countries is supporting the biopharmaceutical manufacturing units by providing subsidies to overcome the burden of manufacturing cost. According to Stratistics Market Research Consulting Pvt. Ltd., the global industrial enzymes market is valued at $4.91 billion in 2015 and is expected to reach $9.74 billion by 2022. The pharmaceutical and biotechnology sectors hold a major share in the revenue. Factors responsible for market growth include recent developments in R&D in enzyme technology, consumer demand, advancement in medical treatment, etc. In 2016, the Asia-Pacific region has contributed huge business and is expected to grow at the highest Compound Annual Growth Rate (CAGR). The business in the developed countries is stagnant, whereas developing countries of Asia-Pacific, the Middle East, and African regions are emerging as rapidly growing markets for therapeutic enzymes. The business in underdeveloped countries totally depends on the cost of the product, though there is a requirement for therapeutic enzymes, but the common people cannot afford to buy. So market competition is very important for reducing the cost and increasing the sales.

9.8 Future Prospects

Research and development on enzyme technology has brought the revolution in the medicine sector. The scope of enzymes is continuously being explored by universities and R & D centers. Researchers have continuously accepted the challenges to improve enzymes in every possible way. Now researchers have more complete detailed understanding of the molecular and cellular functions which is heading toward innovative approaches to treatment. By observing current trend of macromolecules, out of which antioxidants, vaccines, and enzymes will be the future of medicine field, it is expected these macromolecules will be incorporated in every possible medicine and food product line. Antioxidants and vaccines prevent disease and enzymes are used to cure disease. Since demand is rapidly increasing for highly purified enzymes, various biopharmaceuticals are making huge investments in the setting up of production plant for enzymes (O'Sullivan and Freedman 2009).

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