8. PHARMACEUTICAL ENZYMES AND PROTEINS

ENZYMES

A living system controls its activity through enzymes. An enzyme is a protein molecule that acts as a biological catalyst with three characteristics. First, the basic function of an enzyme is to increase the rate of a reaction. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme. Second, most enzymes act specifically with only one reactant (called a substrate) to produce products. The third and most remarkable characteristic is that enzymes are regulated from a state of low activity to high activity and vice versa.

Enzymes can be broken down into three categories: metabolic enzymes, digestive enzymes and food enzymes. Metabolic enzymes spark many of the reactions inside the cells of our body. Our organs, tissues and cells would not work without the help and direction of metabolic enzymes. Digestive enzymes secreted by our own pancreas break down the nutrients contained in the food we eat. Pancreatic (animal) enzymes serve the digestive system as well as to promote a healthy and robust immune system. Food enzymes are supplied to us through the foods we eat. Food enzymes help to break down the foods before our digestive enzymes are called upon. Food (plant) enzymes play the role of enhancing the body's vitality and also enhances the efficiency of digestion. Food enzymes are destroyed at temperatures above 118 degrees. All cooked and processed foods are devoid of food enzymes. Enzymes have important roles in medicine.

Enzymes are either obtained from plants, or they are manufactured by the pancreas. Pancreatic enzymes are animal based and only function in our small intestine. If pancreatic enzymes are taken with food they will be destroyed by the acids in our stomach and therefore, they are not nearly as effective as plant enzymes.

Plant enzymes are much more effective because they begin pre-digestion in our mouth, they are not destroyed by the acids in our stomach and they function in both an acid and in an alkaline environment.

Cell culture has opened a way for the production of enzyme from the plant cells. By means of new technology, enzymes can be immobilized on a suitable carrier either in whole plant cells or as the isolated enzyme. The immobilized enzymes can be held in a bead of soft permeable gel or coat the internal surface of a porous solid. These biocatalysts can be repeatedly used in analytical and clinical chemistry, or to effect specific chemical transformations. Advantages of the immobilized enzymes includes, easier purification of the product as the separation of the enzyme beads is not a problem, easy to recover and recycle the enzymes, they remain functional for much longer as it is a gentler process and so they are cheap.

As we age, our body looses its ability to produce its own enzymes and so we have to include them in our diet. There are only two ways to accomplish this. One method is by eating raw organic food and the other method is by taking enzyme supplements. Some good food sources for enzymes are alfalfa, barley grass, chlorella, spirulina, kelp, peppermint and sea vegetables. Most fruits, especially bananas, are also a good source.

Enzyme Classification

1. Oxidoreductases
   - It catalyze oxidation-reduction reactions (dehydrogenases, reductases) eg, *lactate dehydrogenase* (NAD^+), *acyl CoA dehydrogenase* (FAD), *ketoacyl-ACP reductase* ($NADPH/H^+$)
2. Transferases
   - It catalyze functional group transfers (kinases, aminotransferases, thiolases) eg, *glucokinase* (ATP), *aspartate aminotransferase* (PLP), β-*ketothiolase*
3. Hydrolases
   - It catalyze hydrolysis reactions (peptidases, glycosidases, lipases, phosphatases) e.g., *trypsin, amylase, triacylglycerol lipase, fructose-1,6-bisphosphatase*
4. Lyases
   - It catalyze elimination (or addition) of groups to form (or break) double bonds (synthases, decarboxylases, dehydratases) e.g., *citrate synthase, pyruvate decarboxylase* (TPP), *fumarase*.
5. Isomerases
   - It catalyze reactions that alter structure, not composition (optical, geometric, or structural isomers) (isomerases, mutases) e.g., *glucose-6-phosphate isomerase, phosphoglycerate mutase*
6. Ligases
   - It catalyze coupling of two compounds along with hydrolysis of a phosphoanhydride bond (synthetases, carboxylases, polymerases) *eg. glutamine synthetase* (ATP), *pyruvate carboxylase* (biotin), *DNA polymerase*

The enzymes are also classified on the basis of site of action

1. Exoenzymes- They are also known as extracellular enzymes and they are secreted outside the cell. They are mainly used in digestion, eg, proteoses, amylases, lipases, etc.
2. Endoenzymes- They are also known as intracellular enzymes and they act inside the cell. They are responsible for the synthesis of cell components, food reserves and liberation of energy, eg, synthetases, isomerases, etc.

Structure of Enzymes

Enzymes are composed of many amino acids, joined in long chains, forming proteins, which are manufactured in the cells, or indeed the organism (eg yeast). These long amino chains are then folded in a specific way, so as to make the reactions the enzyme catalyses specific to that particular enzyme, the folded part of the enzyme is called the Active site and it is here that the "reaction" occurs.

Mechanism of Enzymes

The mechanism by which enzymes work is by lowering the activation energy of whatever reaction the specific enzyme is to catalyse. Each enzyme is entirely specific to the reactants; it will only work for one specific reaction, this is due to the unique active site of the enzyme. The enzymes active site is in a unique 3D shape, here the specific substrates, the substances upon which the enzyme works, are temporarily combined with the enzyme, forming an enzyme substrate complex, or ESC, the reaction then occurs and the product of the reaction separates from the emzyme, at which point it may be used.

Properties of Enzymes

1. Enzymes are sensitive to heat and are denatured by excess heat or cold, ie their active site becomes permanently warped, thus the enzyme is unable to form an enzyme substrate complex. This is what happens when you fry an egg, the egg white (augmenten, a type of protein, not an enzyme), is denatured.
2. Enzymes are created in cells but are capable of functioning out side of the cell. This allows the enzymes to be immobilised, without killing them.
3. Enzymes are sensitive to pH, the rate at which they can conduct reaction is dependant upon the pH of where the reaction is taking place, e.g., pepsin in the stomach has an optimum pH of about pH 2. Where as salivary amylase has an optimum pH of about 7.
4. Enzymes are reusable and some enzymes are capable of catalysing many hundreds of thousands of reactions, e.g., catalase working on hydrogen peroxide, try putting some liver into hydrogen peroxide.
5. Enzymes will only catalyse one reaction, e.g.,invertase will only produce glucose and fructose, when a glucose solution is passed over beads of enzyme.
6. Enzymes are capable of working in reverse, this acts as a cut off point for the amount of product being produced. If there are excess reactants the reaction will keep going and be reversed, so that there is no overload or build up of product.

PROTEINS

A protein is a complex, high molecular weight organic compound that consists of amino acids joined by peptide bonds. The word protein is derived from greek "*protos*" meaning "*of primary importance*". Proteins are essential to the structure and function of all living cells. Many proteins are enzymes or subunits of enzymes. Other proteins play structural or mechanical roles, such as those that form the struts and joints of the cytoskeleton, serving as biological scaffolds for the mechanical integrity and tissue signalling functions.

They are obtained from both plant and animal sources. In plants they are stored in the form of aleurone grains.

In animals they are present in structural material in the form of collagen (connective tissue), keratin (hair, wool, hairs, feathers and horns), elastin (epithelial connective tissue), casein (milk) and Plasma proteins. Casein, gelatin, heparin and hemoglobins are pharmaceutically important proteins of animal origin.

Proteins are generally large molecules, having molecular masses of up to 3,000,000 (the muscle protein titin has a single amino acid chain 27,000 subunits long) however protein masses are generally measured in kiloDaltons (kDa). Such long chains of amino acids are almost universally referred to as proteins, but shorter strings of amino acids are referred to as "polypeptides," "peptides" or rarely, "oligopeptides". The dividing line is undefined, though "polypeptide" usually refers to an amino acid chain lacking tertiary structure which may be more likely to act as a hormone (like insulin), rather than as an enzyme (which depends on its defined tertiary structure for functionality).

There are about 20 different amino acids, eight of which must be present in the diet. The eight essential amino acids required by humans are: leucine, isoleucine, valine, threonine, methionine, phenylalanine, tryptophan and lysine. For children, histidine is also considered to be an essential amino acid. Unlike animal proteins, plant proteins may not contain all the essential amino acids in the necessary proportions and so the proteins derived from plants are grouped as incomplete and from animals are grouped as complete. However, a varied vegetarian diet means a mixture of proteins are consumed, the amino acids in one protein compensating for the deficiencies of another.

The structure of protein could be differentiated in to four types:

- **Primary structure:** the amino acid sequence
- **Secondary structure:** highly patterned sub-structures—alpha helix and beta sheet—or segments of chain that assume no stable shape. Secondary structures are locally defined, meaning that there can be many different secondary motifs present in one single protein molecule.
- **Tertiary structure:** the overall shape of a single protein molecule; the spatial relationship of the secondary structural motifs to one another
- **Quaternary structure:** the shape or structure that results from the union of more than one protein molecule, usually called *protein subunits* in this context, which function as part of the larger assembly or protein complex.

Proteins are sensitive to their environment. They may only be active in their native state, over a small pH range and under solution conditions with a minimum quantity of electrolytes. A protein in its native state is described as folded and that is not in its native state is said to be denatured. Denatured proteins generally have no well-defined secondary structure. Many proteins denature and will not remain in solution in distilled water also they are denatured due to heat, changes in pH, treatment of organic solvents or by ultra violet radiation.

Proteins are essential for growth and repair. They play a crucial role in virtually all biological processes in the body. All enzymes are proteins and are vital for the body's metabolism. Muscle contraction, immune protection and the transmission of nerve impulses are all dependent on proteins. Proteins in skin and bone provide structural support. Many hormones are proteins. Protein can also provide a source of energy. Generally the body uses carbohydrate and fat for energy but when there is excess dietary protein or inadequate dietary fat and carbohydrate, protein is used. Excess protein may also be converted to fat and stored.

DIASTASE

Synonym: Amylase

Biological Source: It is an amylolytic enzymes present in the saliva (salivary diastase or ptyalin and pancreatic diastase or amylopsin) found in the digestive tract of animals and also in malt extract.

Diastase hydrolyzes starch, glycogen and dextrin to form in all three instances glucose, maltose and the limit-dextrins. Salivary amylase is known as ptyalin; although humans have this enzyme in their saliva, some mammals, such as horses, dogs and cats, do not. Ptyalin begins polysaccharide digestion in the mouth; the process is completed in the small intestine by the pancreatic amylase, sometimes called amylopsin. The amylase of malt digests barley starch to the disaccharides that are attacked by yeast in the fermentation process.

Description:

Color: Yellowish white

Nature: Amorphous powder

Odour: Characteristic

Solubility: Forms a colloidal solution with water, it precipitates in alcohol.

Thermolabile and denatures at a temperature above 45°C and a pH less than 4

Best active at temperature 35-40°C and pH of 6-7.

Uses: It is used as a digestant, used in the production of predigested starchy foods & also for the conversion of starch to fermentable sugars in fermentation.

PAPAIN

Biological Source: It is a mixture of proteolytic enzymes derived from the latex of unripe fruits of tropical melon tree, *Carica papaya* Linn.

Family: Caricaceae

Preparation:

The fruit latex is collected in aluminium trays after making vertical incision and to the latex potassium metabisulphate is added in a proportion of 5gm per kg of latex. Extraneous matters are cleaned by passing it through sieve and the latex is dried in vacuum at a temperature of 55-60°C to obtain papain.

Description:

Color: Light brown or white coloured

Odour: Characteristic

Taste: Characteristic

Best active: pH of 5-6

Solubility: Soluble in water and glycerine.

Chemical nature: It is a mixture of papain and chymopapain.

Uses: It is used in the clarification of beverages, as a meat tenderiser, in cheese manufacture as a substitute of renin. Acts as anti-inflammatory agent, it relieves symptoms of episiotomies. It's also used in various industries like thé textile industry for the degumming of silk fabrics and in leather industry for dehairing of skin.

PEPSINOGEN

Pepsinogen is an inactive pro-enzyme secreted by the chief cells in the stomach of animals eg, hog, *Sus scrofa.*

Family: Suidae

Pepsinogens are secreted in a form such that the activation peptide assumes a compact structure that occludes the active site. On exposure to an acidic (pH < 4) environment such as occurs in the lumen of the stomach, the activation peptide unfolds, allowing the active site to clip it off, yielding mature, catalytically active pepsin. The pH of the stomach at rest is about 5.5. When protein enters the stomach, the chief cells begin to concentrate hydrochloric acid. It takes between 30 to 60 minutes for enough hydrochloric acid (HCl) to form in the stomach to convert pepsinogen to pepsin. The pH within the stomach by this time is at about 2.0 - 3.0 which is very acidic - enough to burn through metal.

PEPSIN

Biological Source: It is the enzyme prepared from the mucous membrane of the stomach of various animals like pig, sheep or calf. The commonly used species of pig is *Sus scrofa* Linn

Family: Suidae.

The stomach consists of an outer muscular layer and an inner mucous layer. The inner surface is covered with a single layer of epithelial cells which also lines the piths present on them. The piths are about 0.2 mm in diameter and each pith has two to three narrow tubular ducts opening at the base. The epithelial layer is made of either the parietal cell or the centeral cell. The centeral cells are majorly covered with almost cubical shape and secrete pepsinogen and rennin zymogen, while the parietal cells are round or oval shaped cells and they secrete the hydrochloric acid to activate the zymogen to produce rennin and pepsin. Pepsin is the first in a series of enzymes that digest proteins. Pepsin binds with protein chains and breaks it up into small pieces. Pepsin cleaves proteins preferentially at carboxylic groups of aromatic amino acids such as phenylalanine and tyrosine but does not cleave at bonds containing amino acids like valine or alanine. Pepsin mainly cleaves C-terminal to F, L and E and it does not cleave at V, A or G terminals. Structurally, the active site is located in a deep cleft within the molecule. Optimal activity of pepsin is at pH of 1.8 to 3.5, depending on the isoform. They are reversibly inactivated at about pH 5 and irreversibly inactivated at pH 7 to 8.

Preperation: The mucous membrane is separated from the stomach either by the process of stripping or it is scrapped off and it is placed in acidified water for autolysis at 37°C for 2 hours. The liquid obtained after autolysis consist of both pepsin and peptone. It is then filtered and sodium or ammonium salts are added to the liquid till it is half saturated. At this point only the pepsin seperates out and the peptone remains in the solution. The precipitates are collected and subjected to dialysis for the separation of salts. Remaining amount of pepsin if any in the aqueous solution is precipitated by the addition of alcohol into it. The pepsin is collected and dried at low temperature.

General description: Pepsin occurs in pale yellow color, they are odourless or with very faint odour, translucent grains and slightly bitter in taste. It is soluble in dilute acids, water and physiological salt (NaCl) solution. It is best active at a temperature of 40°C with pH 2- 4.

Pepsin is unstable above pH 6. The enzyme gets denatured at a temperature of 70°C and in the presence of alcohol and sodium chloride. Pepsin can be stored for 1-2 years at 2-8°C.

Use: It is used in the deficiency of gastric secretion. Pepsin is also used in the laboratory analysis of various proteins; in the preparation of cheese and other protein-containing foods.

TRYPSIN

Biological Source; Trypsin is a proteolytic enzyme produced by Ox pancreas, *Bos taurus*.

Family: Bovidae

It is one of the three principal digestive proteinases which along with other proteinases like pepsin and chymotrypsin break the dietary protein molecules to their amino acids and peptide component. Trypsin cleaves proteins at the carboxyl side like "C-terminals" of the basic amino acids lysine and arginine. Trypsin is an endopeptidase which cleavage occurs within the polypeptide chain and not the terminal amino acids located at the ends of polypeptides. The aspartate residue located in trypsin is responsible for attracting and stabilizing positively charged lysine and/or arginine.

Production: Trypsin is produced by pancreas in the form of trypsinogen. Trypsin is then transported to the small intestine, where the proteins are cleaved into polypeptides and amino acids. As trypsin is an autocatalytic enzyme, it by itself catalyses the conversion of trypsinogen to trypsin. Another enzyme (enterokinase) is also required in small amount to catalyze the initial reaction of trypsinogen to trypsin.

Process of digestion by trypsin gets started in stomach and is continued to the small intestine where the environment is slightly alkaline. Trypsin has maximam enzymatic activity at pH 8.

Chemical Composition: It has a similar structure as that of other pancreatic proteinase like chymotrypsin and also has the similar mechanisms of action. They differ only in their specificity. Trypsin is active against peptide bonds in protein molecules that have carboxyl groups donated by amino acids like the arginine and lysine while chymotrypsin are active against the carboxyl group denoted by tyrosine, phenylalanine, tryptophan, methionine and leucine. Trypsin is considered the exceptional of all other proteolytic enzyme due to its attack on restricted number of chemical bonds. Trypsin is widely employed as a reagent for the orderly and unambiguous cleavage of proteins in which aminoacid sequence is to be determined.

Storage: Trypsin should be stored at very cold temperatures (between 2° and 8°C) to prevent autolysis (self-cleavage). Autolysis may also be prevented by storage of Trypsin at pH 3. When the pH is adjusted back to pH 8 activity returns.

Uses: In a tissue culture lab, trypsin is used to re-suspend cells adherent to the petri dish wall during the process of harvesting cells. It is also used to harvest corn and oats. Trypsin is vital in a cow's diet, without it they would not be able to digest the grass they eat.

YEAST

Biological Source: It consists of unicellular fungal microorganisms *Saccaromyces cerevisiae* Meyen, along with some other species like *S. carlsbergensis* Hansen and *S. monacensis* Hansen.

Family: Saccharomycetaceae

Preparation: Several species are been used in the industries for the production of yeast of which the most common is the *Saccaromyces cerevisiae.* Yeast is grown in saccharine fluid consisting of suitable proportions of nitrogenous nutrients like ammonium sulphate and super phosphate. The equipments are properly sterlized to prevent any contamination due to microorganisms or bacteria. The yeast is added to the liquor at 20 to 25°C and fermented for 11 hours. The yeast is skimmed from the surface, washed with water and the remaining liquor is passed through sieve. Several washing is done and the yeast is allowed to settle, which later on is removed through filter press and dried. The moisture content of yeast is maintained at 75 % and it is filled in containers and stored at low temperature.

Description:

Colour: Whitish powder or colorless

Odour: Characteristic (resembling beer)

Size: Unicellular microorganism measuring less than 4-6-8 μ in diameter.

Shape: Round or ovoid

Chemical constituents: 60-85% of moisture, nitrogenous compounds, glycogen, fat, vitamins & ash. The enzymes invertase, zymase, diastase and maltase are present in yeast. Vitamins reported are thiamine, nicotinic acid, riboflavin, biotin, pantothenic acid.

Uses: It is used in the manufacture of alcohol, beer, various wines & in the bread industry to raise dough, as source of vitamin and the dead cells of yeast are used in making compressed tablets of yeast.

GELATIN

Biological Source: It is a protein derivative obtained by partial hydrolysis of aqueous extract of collagenous tissue like skin tendons, ligaments & bones of various domestic animals like *Bos taurus* Linn, or *Ovis aries* Linn.

Family: Bovidae

Preparation: Though the process of preparation of gelatin varies in many of the industries, the general process for its preparation is as follows. The raw materials first subjected to the process of "liming" by placing the material of skin and tendon in a dilute milk of lime. The process of liming dissolves the unwanted materials like the fleshy matters, chondro proteins and saponified fat present in the connective tissue. The skins, etc, are then washed with water.

If the raw material used is bone, then it is first grounded and then defatted with any organic solvents like benzene in an iron cylinder. The defatted material is then treated with mineral acid like hydrochloric acid.

The treated material (skin, tendon or bone) is then heated in an open pan or under pressure in perforated false bottoms. The fluid obtained is then evaporated under pressure to get a gelatin concentration of 45-50 %. The concentrated fluid is then spreaded on glass tray to form a jelly. The jelly is removed and passed through wire netting and then dried at various temperatures for a month at an increasing rate of 10°C each time from 30°C to 60°C. Bleaching with sulphur dioxide is also done to obtain gelatin with lighter colour.

Description:

Shape: As thin sheets or as powder

Colour: Yellow to amber

Odour: Odourless

Taste: Tasteless

Nature: Hard and brittle

Solubility: Swells in cold water and gets dissolved on heating, also soluble in acetic acid, glycerin, insoluble in alcohol, ether, etc. It forms glutin-peptone hydrochloride on boiling with dilute hydrochloric acid.

Constituents: The main constituent present in gelatin is glutin.

Chemical tests:

- It evolves ammonia when heated with sodalime, indicating the presence of 18 % of nitrogen.
- It gives a white precipitate with mercuric nitrate (Millons reagent) which on warming turns to brick red color.
- Gelatin when treated with trinitrophenol gives yellow precipitate.
- Solution of tannic acid when mixed with gelatin solution gives white precipitate.
- Gelatin when boiled with lead acetate and caustic potash should not produce any black color. It indicates the absence of sulphur, a constituent of albumin.
- Gelatin should be free from chondrin and its absence can be ensured by the following test:
  - Gelatin solution should not produce any precipitate with chemicals like: lead acetate, ferric chloride, copper sulphate.

Uses: It is mainly used in manufacture of hard & soft capsule shells, for micro encapsulation of drugs, as a vehicle in some injections, in the preparation of bacteriological culture media, as a basis for glycerin suppositories, preperation of pessaries, pastilles.

CASEIN

Biological Source: Casein is a proteolytic enzyme obtained from the stomachs of calves. It is extracted from the proteins of the milk; in the milk, casein is structured in voluminous globules. These globules are mainly responsible for the white colour of the milk. According to various species, the casein amount within the total proteins of the milk varies.

The casein content of milk represents about 80% of milk proteins. The principal casein fractions are alpha(s1) and alpha(s2)-caseins, ß -casein and kappa-casein. The distinguishing property of all caseins is their low solubility at pH 4.6. The common compositional factor is that caseins are conjugated proteins, most with phosphate group(s) esterified to serine residues. These phosphate