Enzymes

proteolytic enzymes (Papain, bromelain, serratiopeptidase, urokinase, streptokinase, pepsin).

Enzymes are organic catalysts produced in the body by living organisms. They perform many complex chemical reactions that make up life processes. Enzymes are lifeless and when isolated, they still exert their characteristic catalytic effect. Their chemical composition varies, and they do show several common properties. They are colloids, soluble in water and dilute alcohol but are precipitated by concentrated alcohol. Most enzymes act best at temperatures between 35 and 40°C; temperatures above 65°C, especially in the presence of moisture, destroy them, whereas their activity is negligible at 0°C. Certain heavy metals, formaldehyde, and free iodine retard the enzymes activity. Their activity is markedly affected by the pH of the medium in which they act or by the presence of other substances in this medium. They are highly selective in their action.

The enzymes are proteins having molecular weight from about 13,000 to 8,40,000. At present they are divided according to their action by a complex system established by the Commission on Enzymes of the International Union of Biochemistry. Six major classes are recognized; each has 4 to 13 subclasses, and each enzyme is assigned a systematic code number (B.C.) composed of 4 digits. The major classes are given in table below.

Enzymes are found in combination with inorganic or organic substances that have an important part in the catalytic action. If these are nonprotein organic compounds, they are known as coenzymes. If they are inorganic ions, they are referred to as activators. Coenzymes are integral components of a large number of enzyme systems. Several vitamins (thiamine, riboflavin, nicotinic acid) have a coenzymatic function.

Enzymes are obtained from plant and animal cells and many have been purified. They are used as therapeutic agents and as controlling factors in certain chemical reactions in industry. Pepsin, pancreatin, and papain are used therapeutically as digestants. Hyaluronidase facilitates the diffusion of injected fluids. Streptokinase and streptodornase dissolve clotted blood and purulent accumulations. Zymase and

rennin are used in the fermentation and cheese industries; and penicillinase inactivates the various penicillins.

Table : International classification of enzymes

No.	Class	Type of reaction catalysed	Examples
1.	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)	Dehydrogenases, oxidases
2.	Transferases	Group transfer reactions	Transaminase, kinases
3.	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	Estrases, digestive enzymes
4.	Lyases	Addition of groups to double bonds or formation of double bonds by removal of groups	Phospho hexo isomerase, fumarase
5.	Isomerases	Transfer of groups within molecules to yield isomeric forms	Decarboxylases, aldolases
6.	Ligases	Formation of C–C, C–S, C–O, and C–N bonds by condensation reactions coupled to ATP cleavage	Citric acid synthetase

The names used to designate enzymes usually end in *-ase* or *-in.* The important enzymes are given hereunder.

Properties of Enzymes

1. Enzymes are sensitive to heat and are denatured by excess heat or cold, i.e. 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 (augmentin, 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 immobilized, without killing them.

3. Enzymes are sensitive to pH, the rate at which they can conduct reaction is dependent upon the pH of where the reaction is taking place, for example, pepsin in the stomach has an optimum pH of about 2, whereas 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, for example, catalase working on hydrogen peroxide, try putting some liver into hydrogen peroxide.

5. Enzymes will only catalyse one reaction, for example, 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 act 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.

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, β-casein and κ-casein. The distinguishing property of all casein 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 groups are important to the structure of the casein micelle. Calcium binding by the individual caseins is proportional to the phosphate content.

Within the group of caseins, there are several distinguishing features based on their charge distribution and sensitivity to calcium precipitation:

Alpha (s1)-casein: (molecular weight 23,000; 199 residues, 17 proline residues).

Two hydrophobia regions, containing all the proline residues, separated by a polar region, which contains all but one of eight phosphate groups. It can be precipitated at very low levels of calcium.

Alpha (s2)-casein: (molecular weight 25,000; 207 residues, 10 prolines).

Concentrated negative charges near N-terminus and positive charges near C-terminus. It can also be precipitated at very low levels of calcium.

β-*casein:* (molecular weight 24,000; 209 residues, 35 prolines).

Highly charged N-terminal region and a hydrophobia C-terminal region. Very amphiphilic protein acts like a detergent molecule. Self association is temperature-dependent; will form a large polymer at 20°C but not at 4°C. Less sensitive to calcium precipitation.

κ-*casein:* (molecular weight 19,000; 169 residues, 20 prolines).

Very resistant to calcium precipitation, stabilizing other caseins. Rennet cleavage at the Phe l05 – Met l06 bond eliminates the stabilizing ability, leaving a hydrophobia portion, para- κ-casein and a hydrophilic portion called κ-casein glycomacropeptide (GMP), or more accurately, caseinomacropeptide (CMP).

Characteristics

The isoelectric point of casein is 4.6. The purified protein is water insoluble. While it is also insoluble in neutral salt solutions, it is readily dispersible in dilute alkalis and in salt solutions such as sodium oxalate and sodium acetate. Casein does not coagulate on heating. It is precipitated by acids and by a proteolytic enzyme (rennet).

Chemical Constituents

Milk consists of 80% of milk proteins (casein). The major constituents of casein are alpha (s1) and alpha (s2)-caseins, β-casein and kappa-casein. These caseins are conjugated proteins with phosphate group(s) which are esterified into serine residues they have a low solubility at pH 4.6.

Uses

It is used in the manufacture of binders, adhesives, protective coatings, plastics (such as for knife handles and knitting needles), fabrics, food additives, and many other products. It is commonly used by bodybuilders as a slow-digesting source of amino acids. There is growing evidence that casein may be addictive for some individuals, particularly those on the autism spectrum or having schizophrenia.

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, belonging to 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 central cell. The central cells are mainly covered with almost cubical shape and secrete pepsinogen and rennin zymogen, whereas 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 –3.5, depending on the isoform. They are reversibly inactivated at about pH 5 and irreversibly inactivated at pH 7–8.

Preparation

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 separates 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.

Description

Pepsin occurs in pale yellow colour, 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.

Uses

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.

BROMELIN

Synonyms

Bromelin, bromelain.

Biological Source

Bromelin is a mixture of proteolytic enzymes isolated from the juice of *Ananas comosus* (pineapple), belonging to family Bromeliaceae.

Geographical Source

Pineapple is a native of tropical America. It is grown in almost all parts of the world including India, China, Thai-land, United States, Brazil, Philippines, Mexico, Hawaii, and Taiwan.

Cultivation, Collection, and Preparation

Bromelin is found in pineapple fruit juice and stem. Pine-apple is perennial, and it does not have a natural period of dormancy. It is propagated through suckers, slips, and crowns. In India it is planted in August, the plant generally flowers in February–March, and the fruit ripens during July–October.

The fruits must be left on the plant to ripen for the full flavour to develop. Dark green unripe fruits gradually change to yellow and finally to deep orange. The fruits are cut off. The enzyme bromelin does not disappear as the fruit ripens. The enzyme from fruit and stem are known as fruit bromelin and stem bromelin, respectively. It is isolated from pineapple juice by precipitation with acetone and also with ammonium sulphide

Characteristics

The optimum pH of bromelain is 5.0–8.0. In solution pH below 3.0 and above 9.5 inactivates the enzyme. The optimum temperature is between 50 and 60°C, still it is effective between 20 and 65°C too. The moisture content should not exceed 6%. It is obtained in light brown-coloured powder.

Chemical Constituents

Bromelain is not a single substance, but rather a collection of enzymes and other compounds. It is a mixture of sulphur-containing protein-digesting enzymes, called proteolytic enzymes or proteases. It also contains several other substances in

smaller quantities, including peroxidase, acid phosphatase, protease inhibitors, and calcium.

Uses

Bromelain is an effective fibrinolytic agent; bromelain inhibits platelet aggregation and seems to have both direct as well as indirect actions involving other enzyme systems in one of the primary uses of bromelain in several foreign countries; it can modify the permeability of organs and tissues to different drugs. The potentiation of antibiotics and other medicines by bromelain may be due to enhanced absorption, as well as increased permeability of the diseased tissue which enhances the access of the antibiotic to the site of the infection. It is also thought that the use of bromelain may provide a similar access to specific and nonspecific components of the immune system, therefore, enhancing the body's utilization of its own healing resources. Bro-melain has been used successfully as a digestive enzyme following pancreatectomy, in cases of exocrine pancreas insufficiency and in other intestinal disorders. Research has indicated that bromelain prevents or minimizes the severity of angina pectoris and transcient ischemic attacks (TIA); it is useful in the prevention and treatment of thrombosis and thrombophlebitis. If administered for prolonged time periods, bromelain also exerts an antihypertensive effect in experimental animals. It may even be useful in the treat-ment of AIDS to stop the spread of HIV. It has no major side effects, except for possible allergic reactions.

SERRATIOPEPTIDASE

Synonym

Serrapeptase, serratiopeptidase.

Biological Source

Serratiopeptidase is a proteolytic enzyme isolated from nonpathogenic enterobacteria *Serratia* E 15. It is also produced by the larval form of the silk moth.

Preparation

Serratiopeptidase is produced by fermentation technology by using nonpathogenic enterobacteria species such as *Serratia* E 15. The larvae of silk moth produce this enzyme in their intestine to break down cocoon walls. It can thus be obtained from the silk moth larvae.

Characteristics

Serratiopeptidase is very much vulnerable to degradation in the acidic pH. When consumed in unprotected tablet or capsule, it is destroyed by acid in stomach. However enteric coated tablets facilitate its absorption through intestine. One unit of the enzyme hydrolyses casein to produce colour equivalent to 1.0 μmol of tyrosine per minute at pH 7.5 and 35°C.

Chemical Constituents

Serratiopeptidase is a proteolytic enzyme of protease type XXVI. The preparation contains 7.1 units/mg solid.

Uses

Serratiopeptidase is the most widely prescribed antiinflammatory enzyme in developed countries and also in India. It eliminates inflammatory oedema and swelling, accelerate liquefaction of pus and sputum, and enhance the action of antibodies. It is also used as a fast wound healing agent. It is proving to be a superior alternative to the nonsteroidal antiinflammatory drugs traditionally used to treat rheumatoid arthritis and osteoarthritis. It has wide ranging applications in trauma surgery, plastic surgery, respiratory medicine, obstetric and gynaecology.

UROKINASE

Synonym

Uroquinase.

Biological Source

Urokinase is serine protease enzyme isolated from human urine and from human kidney cells by tissue culture or by recombinant DNA technology.

Preparation

Urokinase is a fibrinolytic enzyme produced by recombinant DNA using genetically manipulated *E. coli* cells. It is produced firstly as prourokinase q.v. and then converted to active form by plasmin or kallikrein. Urokinase used medicinally is also purified directly from human urine. It binds to a range of adsorbents such as silica gel or kaolin which can be use to initially concentrate and purify the product. It can be further purified by precipitation with sodium chloride or ethanol or by chromatography. Human urokinase needs sterile filtration, a septic filling and freeze drying.

Characteristics

Urokinase enzyme occurs in two different forms as single and double polypeptide chain forms. It has a half-life of 10–16 minutes after intravenous administration. These enzymes act on an endogenous fibrinolytic system.

Chemical Constituents

Urokinase enzymes are serine proteases that occur as a single low molecular weight (33 kDa) and double, high molecular weight (54 kDa) polypeptide chain forms. They differ in molecular weight considerably. A single chain is produced by recombinant DNA technique and is known as SCUPA.

Uses

Urokinase is used in the treatment of pulmonary embolism, coronary artery thrombosis and for restoring the potency of intravenous catheters. It is generally administered intra-venously in a dose of 4,400 units/kg body weight per hour for twelve hours.

STREPTOKINASE

Synonym

Estreptokinase, plasminokinase.

Biological Source

Estreptokinase, plasminokinase is a purified bacterial protein produced from the strains of group C β-haemolytic *S. griseus.*

Preparation

Streptokinase is a bacterial derived enzyme of serine pro-tease group. The ancestral protease activity lies within the first 230 amino-acid residues at the N-terminal part of the protein that evolves from serine protease due to the replacement of histamine at 57th amino acid by glycine. The amino terminal residue polypeptide chain shows sequence homology to serine protease. Duplication and fusion of gene generate an ancestral streptokinase gene. Streptokinase is produced by fermentation using streptococcal culture and is isolated from the culture filtrate. It is produced in the form of a lyophilized powder in sterile vials containing 2,50,000 to 7,50,000 IUs.

Characteristics

Streptokinase is a bacterial protein with half-life of 23 minutes. Its anisolylated plasminogen activator complex (APSAC) has a higher half-life of six hours.

Chemical Constituents

Streptokinase is the purified bacterial protein with about 484 amino-acid residues.

Uses

Streptokinase is the first available agent for dissolving blood clots. It binds to plasminogen in a 1:1 ratio and changes molecular conformation. Thus, the complex formed becomes an active enzyme and promotes the activity of fibrinolytic enzyme plasmin. Plasmin breaks fibrin clots. Anistreptase or the anisolylated plasminogen streptokinase activator complex (APSAC) can also be used in a similar way for degrading blood clots. Streptokinase and anistreptase are both used in the treatment

of pulmonary embolism, venous, and arterial thrombosis and coronary artery thrombosis. It is also sometimes administered along with heparin to counter act a paradoxical increase in local thrombin.

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