PHARMACOGNOSY

Protein Containing Drugs

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Introduction

Enzymes and proteins have been used as drugs since ancient times and many of them were available before the development of biotechnology. The enzymes and proteins used as therapeutic agent are isolated from plant and animal sources as well as from microorganisms. Some proteolytic enzymes isolated from animals may be advantageous in special fields of application. Plant proteases isolated from pineapple (Bromelin) and the papaya plant (Papaine) have been used in meat tenderizing and chill proofing beer. Useful amylolytic enzymes occur in plant tissues such as barley, rye, potatoes and soybeans. Microorganisms have become increasingly important as producers of industrial enzymes and in fact most enzymes used in industry today are of microbial origin. Microorganisms are attractive as enzyme sources because of their biochemical diversity and the ease with which enzyme concentration may be increased by environmental and genetic manipulations. Genetic engineering and recombinant DNA technologies have been widely applied to produce abnormal amount of enzyme inherent in microorganisms as well as to synthesize foreign proteins derived from animal cells. Proteins and enzymes used as drugs fit into many therapeutic categories. Some of the important products of plant animal and microbial source are given in (Table 1).

Table 1: Enzymes and Proteins from various sources

Enzymes & Proteins	Source	Activity
Papain	Papaya latex	Proteolytic
Diastase	Barley	Amylolytic
Bromelin	Pineapple	Blood clotting
Ficin	*Ficus carica* latex	--
Pepsin	Hog stomach	Proteolytic
Pancreatin	Hog pancreas	Digestive
Hyaluronidase	Human testes	Mucolytic
Urokinase	Human kidney & urine	In pulmonary embolism
Streptokinase	*Streptococci*	Dissolve blood clot
Asparaginase	*E. coli* (r DNA)*	Antileukemic
Somatotropin	*E. coli* (r DNA)*	in Hypothyroidism

* r DNA - Recombinant DNA technology

Proteins are polymers of amino acids joined together by peptide linkage. Enzymes are proteins with catalytic capability and have the molecular weight in the range of 7000 Daltons to over one million Daltons. Peptides have a primary structure with simpler sequence of amino acids while the proteins have secondary and tertiary structures. Secondary structures of proteins involve the folding of polypeptide back bone chain. They are determined by the rotation angles around Cά-N bonds of the peptide chain.

Tertiary structures involves further folding of amino acid chains including the alpha helices and beta - pleated sheets that have been formed as a part of secondary structure and it establishes the conformation of each amino acid side chain. Enzymes generally have tertiary structures, which provide a catalytically active conformation, which is held by hydrogen bonds, disulphide bonds, hydrophobic interactions, and electrostatic charges. The loss of tertiary structure results in denaturation of protein's overall loss of activity. The denaturants that induce denaturation are salt, organic solvents, detergents, or conditions like pH.

pH or hydrogen ion concentration affects the enzyme activity. For example, pepsin works only in acidic medium while trypsin is active in alkaline medium. The maximum activity of the enzyme is at the optimum pH that is 5.0 and 9.0. Heating and freezing generally disrupt the stabilizing forces of the proteins. Some proteins are permanently denatured and in some cases it is temporary and reversible. Temperature is an important parameter which accelerates an enzyme reaction with its rise. However at the same time it causes inactivation of the enzyme due to denaturation of protein. At certain temperature known as optimum temperature, the activity of enzyme is maximum. Rate of reaction of enzyme diminishes with diminishing temperature and at 0^0C, most enzymes are practically inactive.

Enzymes are highly sensitive to radiation such as x., β, or γ rays. High energy radiation causes oxidation of enzymes resulting in loss of enzyme activity. Enzymes consist of active groups such as amino, carbonyl and sulphydryl groups in the side chains of amino acids. These groups along with some others are responsible for union between substrate and enzyme to form enzyme substrate complex. Many enzymes are inhibited by the salts of mercury, silver, gold and heavy metals.

Enzymes are classified on the basis of substrate or the product of the reaction. Suffixes such as 'ase' or 'in' indicate enzymes as in case of streptokinase or papain respectively. The suffix 'olytic' is used to indicate the activity of the enzyme.

The enzymatic catalysis of reaction is essential to living systems. The distinguishing feature of enzyme-catalysed reaction is that it occurs within the confines of a pocket on the enzyme called the active site. The molecule that is bound by the active site and acted upon by the enzyme is called as substrate. The enzyme substrate complex is central to the action of enzyme.

Quality control assessment of proteins and enzymes is done by variety of quality control tests. The exact active confirmation and size of protein is established by polyacrylamide gel electrophoresis. The charge of protein molecule and its lipophilicity can be confirmed by isoelectric focusing and HPLC, respectively. The contaminants detection is performed by using capillary electrophoresis while the biological activity studies are used for confirming their therapeutic potential.

1. Papain

Synonyms **:** Papain, Papayotin

Biological Source: Papain is the dried and purified latex obtained from the milky juice of unripe fruits of *Carica papaya* Linn, family Caricaceae.

Geographical Source : Papaya is indigenous to tropical America and cultivated in almost all parts of the world. On large scale it is cultivated in SriLanka, Tanzania, India, Hawaii, Florida, Philippines, South Africa and Australia. In India it is successfully cultivated in Maharashtra, Bengal, Bihar and Uttar Pradesh. The two major industries Enzochem laboratories Ltd. Yeola, Nasik and Biocon Limited, Bangalore produce large quantities of commercial papain.

Cultivation, Collection and Preparation: *C. papaya* is an herbaceous tree of 10-15 m. height. It is normally dioecious but rarely monoecious. Papaya thrives best on the well-drained soil. The fruits are borne near the top of the trunk, gregariously packed at the base of the leaves (Fig. 1.) The fruits are spherical to cylindrical weighing up to 4.4 kg. Shallow incisions are given on the full grown, green unripe fruits on the four sides. Milky juice flows freely for a few seconds but soon coagulates. Incisions and collection of latex is done at weekly intervals till the fruit exudes latex. The collected coagulated latex is shredded and dried under sun or by artificial heat to yield crude papain. The crude papain is purified by dissolving in water and precipitating with alcohol. Isolation of proteolytic activity based fractions of crystalline papain is best accomplished at pH 5.3 from dried latex. The term papain is currently applied to both the crude dried latex and the purified crystalline proteolytic enzyme.

Fig. 1: Carica papaya plant bearing papaya fruits

Characteristics: Purified papain is white or grayish white, slightly hygroscopic powder. It is completely soluble in water and glycerol, and practically insoluble in most organic solvents. Its potency varies according to process of preparation. Papain can digest about 35 times its own weight of lean mean. The best quality papain digests 300 times its own weight of egg albumin. It should be kept in well-closed containers. The best pH for its

activity is 5.0 but it functions also in neutral and alkaline media. Total ash value should not exceed 1 %.

Chemical Constituents: Papain is referred to as vegetable pepsin as it contains enzymes similar to those in pepsin. The papain molecule consists of one folded polypeptide chain of 212 amino acids with molecular weight upto 23400 dalton. N-terminal amino acid sequence of papain has been illustrated in (Table2). Papain contains several proteolytic enzymes such as peptidase-I, rennin like milk coagulating enzyme, amylolytic enzyme and a clotting enzyme similar to pectase. Peptidase - I has the ability to convert proteins into dipeptides and polypeptides.

I	P	EYV	DWR	QK	GAVT	PV	KNQ	GS	CG	S	CW

Fig. 2 : N- terminal amino acid sequence of papain

Uses: Being proteolytic enzyme papain is used as a digestant for proteins. It shows the proteolytic activity much like pepsin but, unlike pepsin, it can act in acid, neutral or alkaline media. It can be combined with other enzymes such as amylases to produce digestive aids. It is extensively used as a meat-tendering agent in the meat packing industries. Papain (10%) is used in ointment for wound debridement, that is, for the removal of dead tissue. It is also used in the treatment of contact lenses to prolong wearing time in keratoconic patients with papillary conjunctivitis.

2. Bromelin

Synonyms**:** Bromelin, Bromelain

Biological Source: Bromelin is a mixture of proteolytic enzymes isolated from the juice of *Ananas comosus*,pineapple, family Bromeliaceae.

Geographical Source: Pineapple is a native of tropical America. It is grown in almost all parts of the world including India, China, Thailand, USA, Brazil, Philippines, Mexico, Hawaii and Taiwan.

Cultivation, Collection and Preparation: Bromelin is found in pineapple fruit juice and stem. Pineapple 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 (Fig.3). 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.

A **B**

Fig. 3: *Ananas comosus,* A) Fruiting branch, B) Fruit slice

Characteristics: Bromelin is incompletely soluble in water. The bromelin obtained from the fruit is acidic in nature while that derived from the stem tissues is a basic protein. .

Chemical Constituents: Bromelin is a glycoprotein. It has a molecular weight of about 25000-31000. N - Terminal amino acid sequence of the pineapple endopeptidase of stem bromelain and fruit bromelain has been illustrated in (Fig.4). Other pineapple endopeptidases are ananain and comosain.

AVPQSIDWRDYGAVTSVKNQNPCG	ACW

Stem Bromelain

AVPQSIDWRDY G	A	N	Z	NPCG A	C

Fruit Bromelain

Fig. 4: N-Terminal amino acid sequence of Bromelin

Uses: Bromelin has the ability to dissolve fibrin in conditions of inflammatory oedema. It is used for tenderizing meat, chill proofing reagent for beer as a bating reagent for hides and for production of protein hydrolysate.

3. Malt Extract

Synonym: Diastase, Malt extract

Biological Source: Malt extract is the extract obtained from the dried barley grains of one or more varieties of *Hordeum vulgare* Linne, family Poaceae.

Geographical Source: Barley is widely cultivated throughout the world. The major producers are USA, Russia, Canada, India and Turkey. It is also cultivated in highlands of China and Tibet.

Cultivation, Collection and Preparation: Barley is one of the oldest cultivated cereals. It is an annual erect stout herb resembling wheat. The crop becomes ready for harvest in about four months after sowing. The grains are threshed out by beating with sticks or trampling by oxen. Dried barley grains are artificially germinated by keeping their heaps wet with water in a warm room. When the caulicle of the grains starts protruding out, the germinated grams are dried. Dry germinated barley or dry malt is subjected to extraction. The malt is infused with water at 60^{O}C. An infusion is concentrated below 60^{O}C under reduced pressure and then dried. Less purified malt extract contains sugars, and amylolytic enzymes. Its further purification affords diastase.

Characteristics: Malt extract contains enzymes, which are most active in neutral solution. The acidic conditions destroy the activity. It converts starch into disaccharide maltose. The enzyme is destroyed by heat. Many heat sterilized malt extracts do not contain diastase. It is completely soluble in cold water, more readily in warm water. The aqueous solution shows flocculant precipitate on standing. Limit for arsenic should not exceed 1 part per million.

Chemical Constituents: Malt extract contains dextrin, maltose, traces of glucose and about 8 % of amylolytic enzyme diastase.

Uses: Malt extract and purified diastase, both are used as amylolytic enzymes and as an aid in digesting starch. They are used as bulk producing laxatives.

4. Streptokinase

Synonym: Estreptokinase, Plasminokinase

Biological Source: Estreptokinase, Plasminokinase is a purified bacterial protein produced from the strains of group C Beṭa- haemolytic *S. griseus*

Preparation: Streptokinase is a bacterial derived enzyme of serine protease 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 57^{th} 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 IU.

Characteristics: Streptokinase is a bacterial protein with half-life of 23 minutes. Its anisolylated plasminogen activator complex (APSAC) has a higher half life of 6 hours.

Chemical Constituents: Streptokinase is the purified bacterial protein with about 484 amino acid residues. Its schematic representation is given in (Fig. 5).

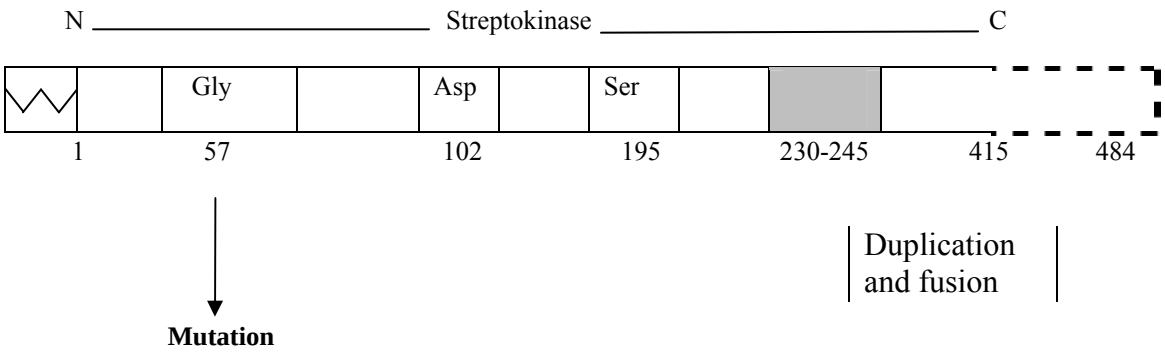

Fig. 5: Scheme for evolution of streptokinase gene and protein

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.

5. 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 adsorbants 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 intravenously in a dose of 4400 units/kg body weight per hr. for twelve hours.

6. Hyaluronidase

Synonym: Spreading factor, Hyalase

Biological Source: Hyaluronidase is an enzyme product prepared from mammalian testes which shows the capability of hydrolysing hyaluronic acid like mucopolysaccharides. Skin is considered as the largest store of hyaluronidase in the body.

Preparation: Hyaluronidase enzyme is found in type-II *Pneumococci*, in group A and C haemolytic *streptococci*, *S. aureus* and *Clostridium welchii*. Hyaluronidase manufacturers define their product in terms of turbidity reducing (TR) units or in viscosity units. Prepared solution for injection usually contains 150 TR units or 500 viscosity units dissolved in 1ml. of isotonic NaCl solution.

Characteristics: Hyaluronidase for injection consists of not more than 0.25 μg of tyrosine for each USP hyaluronidase unit. Due to its action on hyaluronic acid, it promotes diffusion and hastens absorption of subcutaneous infusions. It depolymerises and catalyses hyaluronic acid and similar hexosamine containing polysaccharides.

Chemical Constituents: Hyaluronidases are a group of enzymes such as 4-lycanohydrolase, hyaluronate 3-glycanohydrolase and hyaluronate lyase. They are mucopeptides composed of alternating N-acetylglucosamine and glucuronic acid residues. Hyaluronidases catalyse the breakdown of hyaluronic acid.

Uses: Hyaluronidase for injection is used in the conditions of hypodermoclysis. It is used as a spreading and diffusing agent. It promotes diffusion, absorption and reabsorption.

7. Serratiopeptidase

Synonym: Serrapeptase, serratiopeptidase

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 hydrolyzes casein to produce colour equivalent to 1.0 μ mol of tyrosine per minute at pH 7.5 and 35^{o}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 anti-inflammatory enzyme in developed countries and also in India. It eleminates inflammatory oedema and swelling, accelerate liquifaction 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 anti-inflammatory drugs traditionally used to treat rheumatoid arthritis and osteoarthritis. It has wide ranging applications in trauma surgery, plastic surgery, respiratory medicine, obstetric and gynaecology.

8. Gelatin
Synonym: Gelatinum

Biological Source: Gelatin is a protein derivative obtain by evaporating an aqueous extract made from bones, skins and tendons of various domestic animals Some important sources are, Ox, *Bos taurus*, and Sheep, *Ovis aries* belonging to family Bovidae, order Ungulata of the class Mammalia.

Preparation: The process of manufacture of gelatin vary from factory to factory.. However, the general outline of the process is given below:
Raw material: Bones, skins and tendons of Bovideans is collected and subjected to liming operation.

Liming Process: The raw material is first subjected to the treatment known as "liming". In this process, the skins and tendons are steeped for fifteen to twenty and sometimes for fourty days in a dilute milk of lime. During this, fleshy matter gets dissolved, chondroproteins of connective tissues gets removed and fatty matter is saponified. The animal skin is further thoroughly washed in running water.

Defattying: In case of bones, the material is properly ground and defatted in close iron cylinders by treatment with organic solvents such as benzene. The mineral and inorganic part of the bone is removed by treatment with hydrochloric acid.

Extraction: The treated material from bones, skins and tendons is boiled with water in open pans with perforated false bottom. This process can also be carried out under reduced pressure. The clear liquid runs of again and again and is evaporated until it reaches to above 45 per cent gelatin content.

Setting: The concentrated gelatin extract is transferred to shallow metal trays or trays with glass bottom. It is allowed to set as a semisolid gelly.

Drying: The gelly is transferred to trays with a perforated wire netting bottom and passed through series of drying compartments of 30^0C to 60 ^{0}C increasing each time with 10^0C. About a month is taken for complete drying.

Bleaching: In case of darker colour, finished product is subjected to bleaching by sulphur dioxide. Bleeching affords a light coloured gelatin.

Characteristics: Gelatin occurs in the form of thin sheets or as shredded flakes or powder. It is nearly colourless or pale yellow devoid of odour and taste. Gelatin is hard and brittle but breaks with short fracture after preliminary bending. It swells in cold water and completely dissolves when heated. It is soluble in acetic acid and glycerin but insoluble in alcohol and organic solvents. A2 per cent hot aqueous solution gelatinizes on cooling. Gelatin reacts with hydrochloric acid to obtain glutin-peptone.

Chemical Tests:

1. When heated with soda lime, it gives out fumes of ammonia.
2. Gelatin is precipitated fro solution on addition of trinitrophenol or tannic acid.
3. It produces white precipitate on addition of mercuric nitrate (Millons reagent). The white precipitate turns brick red on warming.
4. Presence of chondrin in gelatin can be detected by its insolubility in acetic acid and its failure to produce a precipitate with lead acetate, alum, ferric chloride and copper sulphate.

Chemical Constituents: Gelatin consists of a major proportion of protein glutin. Gelatin should be free from protein chondrin which comes from the chondrinogen of connective tissues. It yields about 0.6 to 2 per cent of ash and 12 to 17 per cent of moisture.

Uses: Gelatin is used as a nutrient and as a styptic. It is largely used for the manufacture of hard and soft gelatin capsules. It is also used for the preparation of suppositories, pesseries, pastilles and pastes. It is a component in the bacteriological culture media. Gelatin is also employed in the micro encapsulation of drugs, in injections and perfumes. It is used for the production of absorbable gelatin sponge and gelatin films.

Suggested Reading

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