

Enzymes



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Introduction



- It is a proteins which act as catalyst.
- They play vital role in function of cell & activities of organism.
- Enzyme shows **maximum activity** b/w **35 – 40 °C**
- **Inactive** at **0 °C** & **beyond 65 °C** denaturated.
- Soluble in water& dil. alcohol. Conc. Alcohol ppts them
- pH of medium & effect is directly proportional to the activity of it.
- Its **activity is reduced** by formaldehyde, free iodine, heavy metals & tannin.
- The enzyme are proteins that range in **molecular weight** from about **13,000** to as much as **840,000 Dalton**.
- Minute quantities of enzymes are required to complete a chemical reaction.

Properties of Enzymes



- 1) **Specificity:** They catalyze only one range of reaction
- 2) They are exceptionally **versatile catalyst:** they give hydrolytic reaction, oxidation-reduction, acyl-transfer reaction, aldol condensation, polymerizations & free radical reaction.
- 3) They are exceedingly efficient under **optimal condition.**
- 4) Enzymatic reactions proceed **8-10 times more rapidly** than non-enzymatic reaction

Chemical nature of enzymes



- Many enzymes are inactive when first produced.
- Such enzymes in inactive form are called **proenzymes** or **zymogens**.
- They are inactive because the active site is not exposed and unable to bind with substrate. Later by the action of essential coenzymes or activator they made active.
- Enzymes often occur in combination with inorganic and organic substances that have an important part in the catalytic action. If these are **non protein organic** compounds known as **coenzymes**. If they are **inorganic** ions, they are referred as **activator**.



- **Coenzymes** are integral part of enzyme system. Several vitamins, thiamine chloride, riboflavin, nicotinic acid are recognized as having a coenzymatic function.
- Both are collectively called **cofactors**. The cofactors bound to the protein part of enzyme tightly. In this case cofactor is termed as prosthetic cofactor or **prosthetic** group.

Classification of Enzyme



1. Classification on the basis of reaction they control (Activity)
2. Classification on the basis of site of action
3. Classification on the basis of nature of substrate

On the basis of their activity



1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases (Synthetase)

On the basis of their activity



- 1. Oxidoreductases:** Catalyzing oxidoreductions between two substances.
e.g. peroxides, glucose 6-phosphate
- 2. Transferases:** Catalyzing a transfer of group, other than hydrogen, between a pair of substrates. e.g. transmethylase, transaminase
- 3. Hydrolases:** Catalyzing hydrolysis of esters, ether, peptide, glycosyl, acid-anhydride, C-C, C-halide or P-N bonds. e.g. carbohydrases, lipases, proteinases

On the basis of their activity



- 4. Lyases:** catalyzing removal of groups from substrates by mechanisms other than hydrolysis, leaving double bond. e.g. Fumerase
- 5. Isomerases:** catalyzing inter conversion of optic, geometric or positional isomers. e.g. G-6-phosphate isomerase
- 6. Ligases (synthetase):** Catalyzing linkage of 2 compounds coupled to the breaking of a pyrophosphate bond in ATP or a similar compound. e.g. Acetyl Co-A carboxylase.

Classification on the basis of site of action



- Enzyme are classified into two groups
- **Endoenzymes:** these enzymes act inside the cells also called intracellular enzymes. These enzyme are responsible for synthesis of cell components, production of energy and metabolism. Examples are synthetase, isomerase and phosphorylase
- **Exoenzyme:**
- They act out side the cell and called extracellular enzymes.
- Such enzymes are digestive in their function i.e. break down complex molecule into small units. Examples proteases, lypases, amylases.

Classification on the basis of nature of substrate



- **The amylolytic Enzyme or carbohydrates :**
- **Diastase and amylase:** term applied to 2 well known amylolytic enzymes salivary diastase or ptyalin and pancreatic diastase or amylopsin are found in digestive tract of animals
- **Malt diastase:** it is formed during the germination of barley grains and converts starch into lactose.
- **Invertase or sucrase:** found in yeast and intestinal juices. It brings hydrolysis of sucrose into glucose and fructose.

Classification on the basis of nature of substrate



- **Maltase:** cause conversion of maltose into glucose.
- **Zymase:** fermentation enzyme cause conversion of monosaccharides into alcohol and carbon dioxide.

1) Diastase



- ❑ **Syn:** Maltin, Diastase of malt, Amylase
- ❑ **B.S.:** It is mixture of amylolytic enzymes obtained from malt.
- ❑ **Description:**
- ❑ **Color:** Yellowish white
- ❑ Amorphous powder, obtained from infusion of malt
- ❑ **Odour:** faint characteristic

Preparation of diastase



- ❑ **Barley** is the dried grain of one or more varieties of ***Hordeum vulgare*** Linne (Family. **Gramineae**).
- ❑ Barley is grown throughout the world wherever the climate is favorable.
- ❑ Malt or malted barley is dried, artificially germinated barley grain.
- ❑ To prepare malt. heaps of barley grain are kept wet with water in a warm room and allowed to germinate until the caulicle(small stalk) protrude.
- ❑ The grain is then quickly dried. The enzyme diastase in the moist warm grains converts the starch to maltose, thereby stimulating the embryo to growth. The embryo is killed when the grain is dried.

Preparation of diastase



- Dry malt resembles barley, but is more crisp, has an agreeable odor, and has a sweet taste. It contains 50 to 70% of the sugar, maltose; 2 to 15% of dextrans; 8% of proteins; diastase; and a peptase enzyme.
- Malt is used extensively in the brewing and alcohol industries.
- It is the product obtained by extracting Malt, the partially and artificial germinated grain of one or more varieties of *Hordeum vulgare*.

Use



- 1. as a digestant
- 2. in the production of predigested starchy foods and also for conversion of starch to fermentable sugars in fermentation & brewing industries.

Identification test



- **1) Arsenic:**
- 0.25 gm of diastase is placed in a platinum, quartz, or porcelain crucible.
- In that 10ml of **Mg nitrate** in **ethyl alcohol** is added & **ignite** it.
- Then reduced to **ash** by heating at **450-550°C**.
- If carbeneous matter persist, it is wetted with minute amount of **Nitric acid**, which is further treated and heat at 450-550°C.
- After cooling, **3 ml HCl** is added to residue, which is dissolved by heating in a water bath.
- When this test is carried out with arsenic it should **NMT 4ppm**.

2) Heavy Metals



- Take 0.5 g diastase in porcelain, carbonized by heating.
- After it add 2 ml of HNO_3 & 5 drops of H_2SO_4 . heated until white smoke is disappear.
- Which is reduced to ash by further heating at $450\text{-}550^\circ\text{C}$.
- After cooling, 2 ml of HCl is added which is evaporated to dryness in a water bath.
- 3 drops of HCl & 10 ml hot water is added to residue, heated for 2 min.
- After cooling 1 drops of Phenolphthalein indicator is added, then ammonia solution is added until the color of solution become Pale red.



- This resulting solution is transferred into Nessler cylinder by rinsing with water.
- 50 ml test solution prepared by 2 ml of dil. Acetic acid & water.
- For heavy metals NMT 40ppm
- Color sol.: 2 ml HNO_3 , 5 drops H_2SO_4 , 2 ml HCl and evaporate to dryness in crucible.
- 3 drops of HCl added to residue, which transfer in to another Nessler cylinder.
- Finally, 2 ml of lead standard sol., 2 ml Dil. Acetic acid, water added and makeup to 50 ml.

3) Lead



- ❑ 0.8 g Diastase slowly carbonised by heating, temp. below 550°C.
- ❑ 20 ml of dil. HNO₃ is added to ash, then boil for 5 min. , filter, residue is wash with water. Make up volume 50 ml with water.
- ❑ Detection of lead NMT 10ppm.
- ❑ **4) Coliform group:**
- ❑ Diastase tested by Microbe Test Method for in General Test Method for Food Code.
- ❑ It should contain 30 or less per 1 g of product.
- ❑ **5) Salmonella:**
- ❑ Diastase tested by Microbe Test Method for in General Test Method for Food Code.
- ❑ It should be negative.

2) Pepsin



- **B.S.:** Pepsin is protolytic enzyme obtained from the glandular layer of the fresh stomach of the hog(Pig), *Sus scrofa* Linne var. *domesticus* Gray. (Sheep, calf)
- **Family:** Suidae
- The generic name *Sus* is from the Greek, meaning Hog; *scrofa* is latin and means breadding ; and *domesticus* is latin and means the house hold.
- **Preparation:**
- **Mucous memb.** Is scraped from stomach, and placed in **acidified** water at **37°C. for 2 hrs.** (Pepsin & Peptone)
- Filtered & add **Sodium** or **ammonium** salt till it become half saturated.(pepsin separated, pepton remain)
- Ppts by addition of **alcohol**, collect it, dried it.



- **Identification test:**

- Based on proteolytic action of it.
- Coagulated egg albumin digested with a pepsin sol. & residue albumen tested with the reference.

- **Description:**

- Pepsin occur as **lustrous, transparent, or translucent** scales, as granular or spongy masses ranging in color from light **yellow** to light **brown**, or as fine **white** or **cream** colored **amorphous** powder. It is free from offensive odor and has a **slightly acid** or **saline taste**.



□ **Use:**

- **1)** it is component of rennet used to curdle milk during the mfg. of Cheese.
- **2)**Used to modify & provide whipping qualities to soy protein and gelatin.
- **3)** to make precooked cereals into instant hot cereals.
- **4)** for flavoring food & beverages.
- **5)** in leather industry to remove hair & residual tissue from hide.
- **6)** in preparation of F2 fragment from antibodies.

Trypsin



- ❑ **SYN:** Protinase, tripsin, Procine, Peptidyl peptide hydrolase.
- ❑ **B.S.:** it is proteolytic enzyme obtained from mammalian pancreas of *ox Bos taurus*.
- ❑ **Family:** Bovidae
- ❑ **Description:**
 - ❑ It is available in amorphous powder.
- ❑ **Color:** white to whitish yellow
- ❑ **Solubility:** in water & insoluble in alcohol, chloroform, ether
- ❑ **Taste:** Acrid

Preparation



- It is readily available from many sources.
- Commercial sources contaminated with other pancreatic enzymes.
- Recombinant trypsin has been expressed many systems, including *E.Coli*, *Saccharomyces cerevisiae*, & *Pichia pastoris*.
- Purification occurs by affinity chromatography on benzamidine or aprotinin.

Identification Test



- 1) dil. 1 ml of solution S (Dissolve 0.10 gm in carbon monoxide free water & dilute 10 ml with same solvent) to 100 ml with water.
- In a depression in a white spot-plate, mix 0.1 ml of this solution with 0.2ml of tosylarginine methyl ester hydrochloride solution.
- A **reddish-violate** color develop within 3 min.
- 2) dil. 0.5 ml of solution S to 5ml of sol. water R .
- Add 0.1 ml of a 20g/l solution of tosyl-lysyl-chloromethane hydrochloride.
- Adjust pH 7 , shake for 2 hr. & dil. 50 ml with water.....As above....but **negetive** test is shown.

Use



- Trypsin give to people who lack enzymes needed for **digestion**.
- Also given with combination with bromelain & rutin for treatment of **osteoarthritis**.
- Apply directly on **wounds & ulcer** to remove dead tissue & improve healing.
- **Spray-on** product that used for **healing mouth ulcers**.(trypsin, peru balsam, castor oil)

Papain



- **Syn:** Papayotin, Vegetable pepsin, Arbuz, Nematolyt, Caroid, Tromasin, velardon, Vermizym
- **B.S.:** it is green & dried leaves of *Carica papaya* L.
- Cultivated in Shri Lanka, Tanzania, Hawaii & Florida.
- Plant 5-6 m in height, bearing 30 cm in length & 5 kg in wt.
- **Family:** Caricaceae

Preparation



- ❑ Mostly found in latex of fruit(stem, leaves, petioles)
- ❑ Latex collected by making 2-4 incisions, about 1/8 inch deep
- ❑ While its become mature, on tree, green
- ❑ Incisions makes early in the morning, at interval 3-7 days.
- ❑ Collected on non metallic container or on cloth.
- ❑ Dry as soon as possible. Either by sun drying or by heating above 38° C.
- ❑ Final product should be creamy white.
- ❑ Sealed in air tight container to prevent loss of activity
- ❑ If 10% common salt & 1% sol. Of formaldehyde is added before drying, activity is maintained for many months.
- ❑ Yield of papain from latex is abt 20% (20-250g per tree).



- Highly active product is obtained by dissolving the commercial product in H_2O , H_2S , ppts with alcohol.
- Commercial papain is adulterated with arrowroot starch, dried milk of cactus, gutta percha, rice flour & pepsin. (**Adulteration**)
- Bleaching is done for improving color, but its affect enzyme activity(lower)
- Papain posses both milk clotting & protein digestion.
- Activity is lost when treated with H_2O_2 , but reduction with H_2S its regained.

Properties



- It is occur as a white or greyish-white, slightly hygroscopic powder.
- It is partialy soluble in water & glycerol. It may digest abt 35 times its wt. of lean meat.
- Best grades digestion of 200-300 times their wt. of coagulated egg albumin in alkaline media.
- Temperature range 60-90° C (optimum point 65°C)
- Best pH is 5.0, but work also in neutral & alkaline media.
- It is activated by reduction (HCN, H₂S), & inactivated by oxidation(H₂O₂).

Chemical Constituent



- Peptidase I, rennin, clotting enzyme pectase, chymopapain.
- It contain 15.5% nitrogen & 1.2% sulphur.
- Crystalline papain is most stable in range of 5-7 pH & readily destroyed at 30°C below pH 2.5 & above pH 12.
- **Identification test;**
- It is reacted with gelatin sol. At 80°C in presence of an activating cysteine chloral hydrate sol. For 1 hr.
- Then sol. Is cooled to 4°C for long time.

Uses



- ❑ To prevent adhesions, in sloughing & infected wounds, internally as protein digestant, as anthelmintic, to relieve episiotomy (incision of vulva) & used for treatment of dyspepsia (digestive), intestinal & gastric disorder.
- ❑ In tmt of diphtheria (respiratory tract infection)
- ❑ Used in digestive mixtures, liver tonic, reducing enlarged tonsils, in preventing of post – operating adhesions, carbuncles (boil, spot) & eschar (dry scar) burns.
- ❑ In prep. of toothpaste, cosmetics, in tanning industries for bating skin & hides.
- ❑ Anti-inflammatory agent
- ❑ Clarification of beverages (beer, fruit juice)
- ❑ in meat tenderizer
- ❑ Degumming of silk fibers in textile industry

Pencreatin



- ❑ **Syn:** Diastase vera, Pandrotanon, Zypanar
- ❑ **B.S.:** it is extracted from **pancreas** of certain **animals** like hog ***sus scrofa var. domesticus*** belonging to or ***ox Bos taurus***.
- ❑ **Family:** Suidae & Bovidae
- ❑ **Description:**
 - ❑ Fine amorphous powder
 - ❑ **Color:** White to cream colored
 - ❑ **Odour:** Faint characteristic odour

Preparation



- ❑ **Pancreatic α -amylase:**
- ❑ It is prepared by fractional precipitation of aqueous ext. from hog or bovine pancreas.
- ❑ The technical enzyme preparation was used principally for textile sizing, because of their low stability.
- ❑ Pancreatic α -amylase is used to prepare pancreatin.
- ❑ **Identification test:**
- ❑ 50 mg of sub. in test tube, add 2-3 mg of resorcinol & 1 ml of H_2SO_4 , shake, heat at $130^\circ C$ for 5 min. & cool.
- ❑ Dilute with water to 5 ml & add hydroxide sol. Drop-wise. To make alkaline sol. Cool & dil. To 10 ml. a greenish blue fluorescence under UV lamp.

Uses



- 1) Pancreatin is used to treat pancreatic exocrine insufficiency(PEI) attributed to cystic fibrosis, chronic pancreatitis.
- 2) in nutritional formula like tablets, capsules or powders.
- 3) in preparation of leather hides & meat tenderizer (added to meat to make more tender(sensitive, tasty) before cooking)

Bromalein



- ❑ **Syn:** Stem Bromelain, Pineapple stem bromelain
- ❑ **B.S.:** it is proteolytic enzyme obtained from the stem & ripened fruit of Pineapple plant. *Ananas comosus*
- ❑ **Family:** Bromeliaceae
- ❑ **Description:**
- ❑ **Odour:** odourless
- ❑ **Color:** Buff colored powder
- ❑ **Taste:** Acrid

Preparation



- Fresh stem & fruits were collected, washed with H₂O₂ sol. , peeled off, cut into small pieces.
- Juice is collected from fresh pineapple stem & fruit by homogenization, in the presence of sodium acetate buffer solution & filtered.
- 500 ml of filtrate is collected .
- Benzoic acid/sodium benzoate was added as a preservative at a conc. Of 1gm/kg.
- Filtrate obtained was called as crude extract. In that bromelain is added then by centrifugation for 10 min. at 2000rpm, 4000 rpm & 6000rpm at 4 °C, after supernant was collected.



□ **Identification test:**

- 0.2 g of sample add 1g of anhydrous sodium carbonate, & heat gently to carbonize.
- Cool, add 5 ml of H₂O stir & filter.
- Acidify slightly the filter with dil.HNO₃, heat in water bath for 5 min. & cool.
- Now add silver nitrate which yields light yellow ppts. Which insoluble in dil. HNO₃ or ammonia.
- Separate ppts & strong ammonia with shaking.
- Separate liquid & acidified with Dil. HNO₃, yield white turbidity.

Uses



- Angina, Dysmenorrhea & other CVS disorder.
- Arthritis
- Athletics injuries
- Bronchitis
- Burn debridement
- Cancer
- Dermatological condition
- Digestive disorder
- Pancreatic insufficiency
- Thrombophlebitis

Ficin



- ❑ **Syn:** Ficus proteinase, ficus protease
- ❑ **B.S.:** found from latex of plant Ficus. Commercial it is latex of the fig (with fruit/shrub) tree, *Ficus glabrata* or *Ficus carica*.
- ❑ **Family:** Moraceae
- ❑ **Description:**
 - ❑ In the form of micro granular powder.
 - ❑ White to yellowish in color
 - ❑ Optimum temp. is 50-55°C & optimum pH is 5.0-7.5
 - ❑ Partially sol. In water & insol. In Organic solvent.

Preparation



- ❑ Crystalline Ficin was prepared by Walti(1938). These fraction is formed by self –digestion of the enzymes during fractionation.
- ❑ The crude enzyme was inactivated by sodium tetrathionate, & after gel filtration.
- ❑ Jones & Glazer separate latex in 5 component which is quit similar in moi. Wt. 25,000-26,000 (Lucien).
- ❑ Figs (with fruit/shrub) contain protease. Average green fig (10-15gm) contain 100-150 mg of commercial ficin.
- ❑ Sundried figs retain about 12% of their original protease activity.(while oven drying have no activity)



- **Identification test:**
- Neutralize 1 ml of sample with NaOH & 2 drp. of sample, 1 ml of FeCl_3 -----= **deep reddish orange** , change in **yellowish orange** on the addition of mineral acid
- **Uses:**
- in beer & alcohol industry
- Hydrolization of proteins & meat processing
- Baking industry
- Ophthalmology for cleaning contact lens
- Antineoplastic agent, digestive aid, treat arthritis.

Urokinase



- **Syn:** U-plasminogen, Urinary plasminogen activator.
- **B.S.:** obtain from human urine. Now a days also obtained from *E.Coli* by rDNA technique.
- **Description:**
- Available in the form of lyophilised white powder which is soluble in water.
- It convert plasminogen in to plasmin.
- Degrade fibrin & other plasma protines.

Preparation



- Produced by *E.Coli* by rDNA technique.
- 1st – prourokinase than converted in to plasmin(kallikrein)
- Directly found by purification of human urine.
- Adsorbent used- silica gel or kaolin
- Further purification – pptts by NaCl or ethanol or by chromatography.
- Human urokinase needs sterile filtration, septic filling & freeze drying.

Use



- Coronary thrombosis
- Myocardial Infraction
- Pulmonary embolism
- Venous thrombosis

Streptokinase



- **B.S.:** culture filtrates of β -haemolytic *Streptococci* Grp C.
- **Activity:** human plasminogen in to plasmin
- **Description:**
- Shows its activity on plasminogen into a proteolytic enzyme.
- E.g. plasmin (carris out degradation of fibrin clots, fibrinogen, plasma proteins.)
- It is purified bacterial protein with about 484 amino acid residue.

Preparation



- ❑ Available as sterile, friable solid or white powder.
- ❑ Water Soluble at pH 7 (maximum activity)
- ❑ Higher conc. Sol. Stable for 6 hrs. at 4°C.
- ❑ **Identification test:**
- ❑ It is bacterial protein with half life of 23 min. its anisoylated plasminogen activator complex(APSAC) has a higher half life of 6 hrs.
- ❑ Its activity is determine by Silverstein.
- ❑ It is based on the observation that streptokinase-human plasma complex can hydrolyzed the artificial subtract.



□ **Use:**

- As a fibrinolytic agent to help in removal of fibrin thrombi (thrombus clot) from the arteries.
- In thromboembolic disorder, lysis of arterial thrombus, acute coronary artery thrombosis, deep vein thrombosis, pulmonary emboli.

Hyaluronidase



- **Syn:** Spreading factor
- **B.S.:** it is observed in the testes & semen.
- It specifically depolymerizes hyaluronic acid, thereby enhancing the permeability of the connective tissues.
- It also act the disperse the cells of corona radiate about the newly ovulated ovum, thus largely facilitating entry of the sperm.
- **Description:**
- It reduce viscosity of tissue cement & increase rate of IM & SC injectables in humans.
- It is grp of enzymes like 4-lykanohydrolase, hayaluronate 3-3 glycano hydrolase & hyluronate lyase.
- They are mucopeptides composed of alternating N-acetyl glucosamine & glucuronic acid.

Preparation



- It is sterile, dry, soluble enzyme preparation, which is prepared from testes & semen by fractional precipitation of aq. Extract & further by dialysis.
- Sterilization by filtration & lyophilization.
- It is odourless, white to yellow in color & highly soluble in water, but insoluble in organic solvent.
- **Identification test**
- Acid mucopolysaccharide, histochemically using colloidal iron stain.
- Determine by digestion of a serial section with hyaluronidase prior to staining.



- **Use:**
- Enhance absorption rate
- Reduce discomfort caused by intramuscular or subcutaneous injections.
- Also used in hypodermolysis (in SC & parental cases)

Penicillinase



- **Syn:** β -lactamase
- **B.S.:** it is bacterial enzyme, *bacillus* species & certain strain of staphylococcus.
- **Description:**
 - It contain β -lactam ring .
 - It includes penicillin derivatives.
 - It inhibit bacterial cell wall synthesis
- **Preparation:**
 - Obtained from *B.subtilis* & *B.cereus*



- It is divided in to 2 class
- 1) Penicillin amidase or acylase
- 2) β -lactamase
- Amidase attack on acyl grp attached to the nucleous thats why its called acylase also
- This enzyme more specific with Pen –V & K
- β -lactamase act on basic nucleus itself,
- This enzyme more specific with Pen –G & X & lesser with Pen-V.



- **Identification Test:**

- Each strip emarginated with benzylpenicillin & pH indicator, bromocresol purple.

- Positive produce- penicilloic acid

- These cause fall in pH

- Purple to yellow

- **Use:**

- Inactivation of penicillin

- Antigen-antibody reaction.