BIOMOLECULES

Topic: 7

ENZYMES

VERY SHORT ANSWER QUESTIONS

1. What are enzymes?

Ans: Enzymes are the biocatalysts of life. They are defined as biocatalysts synthesized by living cells. They are simple or conjugate protein and specific in action.

2. What are different types of enzymes?

Ans: At present about 300 enzymes are recognized and classified into six classes by International Union of Biochemistry (IUB). They are: Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases. However about ~10% i.e., 300 enzymes are commercially available.

3. What are the functions of enzymes?

Ans: The enzyme facilities a biochemical reaction by providing alternative lower activation energy pathways thereby increasing the rate of reaction. Enzymes being proteins having colloidal nature often get inactivated during reactions and have to be constantly replaced by synthesis in the body.

4. What is 'Holoenzyme'?

Ans: The functional unit of enzymes is called 'Holoenzyme'. This is made up of 'apoenzyme' which is a protein part and a non protein part called prosthetic group. The prosthetic group which is covalently attached with the enzyme is known as cofactor.

$$\text{Apoenzyme} + \text{Co-enzyme} \longrightarrow \text{Holoenzyne}$$

(Protein part) (Non protein part) (Active enzyme)

5. What is co-enzyme?

Ans: The prosthetic groups which get attached to the enzyme at the time of reaction are known as coenzymes.

SHORT ANSWER QUESTIONS

1. What are the general characteristics of enzymes?

Ans: General Characteristics of Enzymes

- Enzymes lower/ reduce the activation energy required to carry out the reaction.
- Enzymes themselves do not undergo any change, means they remain intact at the end of reaction.
- Enzymes are highly specific for their substrates.
- Enzymes are present in very less amount in body and have specific life span.
- Activator is the molecule that enhances the enzyme activity.
- Inhibitors are the molecules that reduces the enzyme activity

2. On what factors the activities of enzymes depend?

Ans : Activity of enzymes depends upon:

- Concentration of specific enzymes
- Concentration of its substrate
- pH of reaction
- Temperature of reaction
- Concentration of salts
- Presence of activator or inhibitor
- Time required to carry out the reaction
- Presence of proteolytic enzymes.

3. What are the applications of industrial enzymes?

Ans; Applications of Industrial Enzymes

a) Detergents

b) Starch hydrolysis and fructose production

c) Drinks

d) Textiles

e) Animal feed

f) Baking

g) Pulp and Paper

h) Leather

i) Special enzymes: Widely used in DNA-Technology

j) Enzymes in analytics: Alkaline phosphatase, glucose oxidase

k) Enzymes in personal care products: Tooth paste, enzyme solution for lens cleaning

l) Enzyme in chemical production: Formate dehydrogenase

The industrial enzyme market is growing at steady rate and requires improved production efficiency such that enzymes are available at low price and can be used in widest applications.

4. What are the advantages of microbial enzymes?

Ans; Advantages of Microbial Enzymes

1) Natural origin and nontoxic

2) Highly specific in nature

3) Work best under mild conditions of moderate temperature, neutral pH

4) Can act at low concentrations and rate of reaction can be controlled by adjusting temperature, pH, and amount of enzyme used

5) Can be easily inactivated once the reaction is completed

Microbial enzymes are also of high clinical value as they are used in surgical removal of foreign matter and dead tissue from wound. Some of them are streptokinase, crystalline trypsin, and chymotrypsin.

For clinical and therapeutic uses the microbial enzymes need to be of highest purity and crystalline in nature. As on date research is on towards utilization of the microbial enzymes at higher volume

LONG ANSWER QUESTIONS

1. **Explain the action of enzymes?**

Ans: Active site of an enzyme binds the substrate and forms Enzyme substrate complex. Active site is a small region on enzyme at which the substrate binds and participates in catalysis. The enzyme substrate complex formed release enzymes and products. Thus the free enzyme is available for reuse.

The enzymatic reaction proceeds this four stages.

i) Formation of complex between enzyme and substitute (ES)

ii) Conversion of this complex to in enzyme-intermediate complex (EI)

iii) Further conversion to a complex between enzyme and product (EP)

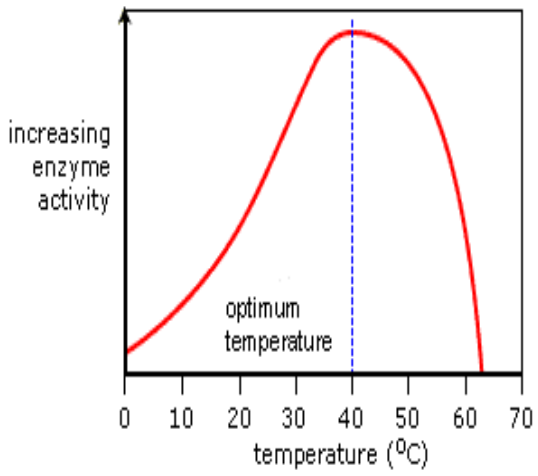
iv) Dissociation of the enzyme - product complex, leaving the enzyme unchanged.

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

(Enzyme) + (Substrate) — (Enzyme - Substrate complex) — (Enzyme) + (Products)

2. **Explain the effect of temperature on enzymes.**

Ans**:** **Effect of Temperature in Enzymes:** Due to pertinacious nature, enzymes are very sensitive to heat. The rate of an enzyme action increases with rise in temperature, the rate of action increases 2 to 3 times for a rise in temperature of 10°C, i.e., the value of temperature quotient or Q_{10} is 2 to 3. But at higher temperatures, the value of coefficient does not remain constant and decreases rapidly. Above 60°C, the enzymes coagulate and thus become inactivated due to irreversible change in their chemical structure.



It has been observed that the initial velocity of reaction increases steadily with increase in temperature. After a certain temperature is passed, the cessation of activity comes earlier and fewer products are formed.

So the optimum temperature for enzyme activity is that at which two factors, increased initial rate and decreased active life of the enzyme are balanced to produce the most product in a reasonable time.

At 60°C to 70°C, in a liquid medium the enzymes are inactivated and destroyed. This destruction of enzymes at high temperature results in coagulation and denaturation. Thus as the temperature is raised, the reaction rate increases upto a certain limit and above that the enzymes get denatured. The temperature, at which the rate of reaction in maximum, is known as optimum temperature.

In general, the optimum temperature range for most enzymes varies between 30° and 45°C. Since enzymes are globular proteins, most are thermolabile and begin to denature at temperature between 45° and 50°C.

Mammalian enzymes often have optimum temperatures in the range 35°-45°C; where as the enzymes from the bacteria that live in volcanic hot springs may have optima of 80°C.

The enzymes of dry tissues like seeds and spores can endure still higher temperatures of about 100° to 120°C.

3. **Explain the nomenclature of enzymes.**

Ans: Enzyme Nomenclature or Naming of Enzyme: Numerous enzymes are known and being discovered and the list is endless, hence it was considered to have proper naming architecture for enzymes. Initially enzymes were named on source of production, location, type of reaction catalyzed, substrate on which they act, and product formed. It ended in endless list of enzymes in various living organisms irrespective of their order in animal or plant kingdom.

Hence International Union of Biochemist association framed Enzyme Commission and decided to classify enzymes based on some specific criteria and accepted that each enzyme should end by a suffix –ase. This was done keeping in view of increased number of enzymes and evolution of separate functional biomolecular class.

Scheme of Enzyme classification by IEC

The IEC gives a code and each letter contains its own significance in relation to that particular enzyme. During classification every enzyme is prefixed by **EC**, followed by the digits.

For example*:* oxidoreductases EC 1.1.1.1

(a)The first digit denotes "**Class**" of the enzyme

(b)The second digit indicates "**Sub-class**" of the enzyme

(c)The third digit gives "**Sub sub-class**" of the enzyme

(d)The fourth digit in the code is "**Serial number**" of the enzyme

The classification if as follows:

Group Name	Type of Reaction catalysed	Example
Oxidoreductases	Oxidation-reduction reactions	Alcohol oxidoreductase (EC 1.1)
Transferases	Transfer of functional groups	Methyltransferase(EC 2.1)
Hydrolases	Hydrolysis reactions	Lipase (EC 3.1)
Lyases	Addition to double bonds or single bonds	Decarboxylases (EC 4.1)
Isomerases	Isomerization reactions	Epimerases and Racemases (EC 5.1)
Ligases	Formation of bonds with ATP cleavage	Enzymes forming carbon-oxygen bonds (EC 6.1)

4. Explain the classification of enzymes.

Ans: Enzyme Classification

Class 1 Oxidoreductases: Enzymes involving in oxidation and reduction reactions in a metabolic process and enzymes representing this class are named as donor:acceptor oxidoreductase, or can alternately named as reductase and even as oxidase if oxygen is electron acceptor.

Class 2 Transferases: Enzymes catalyzing transfer of specific functional group. Named as acceptor group transferase or donor: acceptor group transferase.

$$X\text{-}Y + Z = X + Z\text{-}Y$$

Class 3 Hydrolases: Enzymes involved in hydrolysis of C-O, C-N, C-C by splitting of water molecule.

Class 4 Lyases: Enzymes involved in splitting of C-C, C-O, C-N without involving water molecule.

Class 5: Isomerases: Enzymes involved in interconversion of biomolecule from one isomeric form to the other. These enzymes can be referred as racemases, epimerases, cis-trans-isomerases, isomerases, tautomerases, mutases or cycloisomerases.

Class 6: Ligases: Eznymes capable of catalyzing the joining together 2 molecules with hydrolysis of diphosphate bond in ATP.

Enzymes are under use as since mankind has started. They were used in cheese manufacturing using yeasts and bacteria in food manufacturing. Enzymes which were isolated were first used in detergents earlier. The protein nature was discovered later and slowly the industrial applications also increased.

The world is slowly recognizing the industrial enzyme business and is under steady growth due to improved production technologies, engineered enzyme properties and new application fields. Most of the industrial enzymes are produced by microorganisms in biological reactors called as fermentors.

Basically large volumes of industrial enzymes are usually not purified but are often commercialized as concentrated liquids or granulated dry products. It is of higher importance that enzymes used in diagnostics or DNA-technology need to be highly purified. Enzymes which are isolated from biological sources are with several applications chemical industry.

5. **Explain the effect of P^H on enzymes.**

Ans: Effect of Ph on Enzymatic Activity: The pH represented as acidity or alkalinity of a medium or of a system has direct impact on the functioning of enzymes. Ph of a system affects the ionization state of the amino acids in the protein which can be acidic or basic in nature. It is known that acidic amino acids have carboxyl functional groups and basic amino acids possess amine functional groups as the side chains. As ionization of amino acids get altered the ionic bonds determining the 3-D shape of the protein also get altered. It results in differential recognition of substrates by enzymes and in turn slows down the active nature of enzymes.

It is proven that changes in pH affect the shape of enzyme but also differ with the charge of the substrates such that substrate is not able to bind to the active site and results in autocatalysis.

Variations in pH level to high or low can cause complete or total loss of the enzymatic activity and also the stability.

Regulation of Enzyme Activity with Ph

As explained above enzymes are particular regarding the pH changes in the environment and on analysis it is observed that they have specific optimum pH requirement at which they show maximum activity and in turn maximum conversion of substrate to product takes place.

Example of Enzymes with optimum pH

Lipase (Pancreas)	8.0
Lipase (stomach)	4.0-5.0
Lipase (Castor oil)	4.7
Pepsin	1.5-1.6
Trypsin	7.8-8.7
Urease	7.0
Invertase	4.5
Maltase	6.1-6.8
Amylase (Pancreas)	6.7-7.0
Amylase (Malt)	4.6-5.2
Catalase	7.0

From the above table it is obvious that different enzymes have different optimum pH levels at which they show optimum activity. The optimum range of pH for enzymes can range acidic to neutral and towards basic.