**Unit 6: Enzymes** **(4 lectures)**

**Structure of enzyme: holoenzyme, apoenzyme, cofactors, coenzymes and prosthetic group; Classification of enzymes; Features of active site, substrate specificity, mechanism of action (activation energy, lock and key hypothesis, induced - fit theory), Michaelis – Menten equation, enzyme inhibition and factors affecting enzyme activity.**

Enzymes are commonly known as **biocatalyst** are unique and highly specific proteinaceous substances. These are produced by living system and have great ability to catalyse all the biochemical reactions in the living tissue with high degree of specificity and efficiency.

**Definition** – Enzyme can be defined as **“a substance of biological origin, which can alter the velocity of the chemical reactions without themselves undergoeing any apparent change during the course of its action.”** An enzyme can be extracted from the parent cell and it can perform similar catalytic reaction outside the biological system if similar condition is maintained.

**History** – the term enzyme was introduced by **Kuhne** in 1878. Edward Buchner, a german chemist, in 1897 while preparing for yeast extract could isolate as enzyme called Zymase that could breakdown of hexose sugars in fermentation, for which he was awarded **Nobel** prize in1907.

**Structure of enzyme: holoenzyme, apoenzyme, cofactors, coenzymes and prosthetic group**

Enzymes are proteinaceous in nature. They are giant molecules and their molecular weight rangesbfrom 35000 (pepsin) to 483000 (urease). Enzymes may entirely consist of protein (e.g. amylase, pepsin) or may contain a non-protein part. If an enzyme consists only protein, it is called simple protein enzyme and if it contains another group it is called conjugated protein enzyme. Euler (1932) proposed that conjugated enzymes showing complete activity be called ***holoenzymes***. These consist of two portions. The protein part of enzyme is called ***apoenzyme*** and the non-protein part is called ***prosthetic group*** or ***cofactor***. Thus both these apoenzymes and prosthetic groups together are called holoenzymes.

Depending upon the nature of prosthetic group, conjugated enzymes are of two categories

1. If the prosthetic group is an *inorganic ion* then it is called *cofactors*. e.g. ascorbic acid.
2. The prosthetic group is an *organic compound*, though at times inorganic ions may be present. The organic prosthetic group are called *coenzymes*. A coenzyme constitutes about one percent of the entire enzyme molecule. This part of the enzyme is more or less easily separable, usually heat resistant, often containing cyclic traces which may also accompanied by ribose and phosphate molecules. They have activating effects on enzymes.

Sometimes a nonprotein substance is required at the active centre for enzyme activity which is bound tightly to the enzyme protein by covalent linkages and is known as prosthetic group. The prosthetic group may consists of a) organic compound or b) simple metal ions such as Cu, Zn, Mn, Mo etc. the organic compounds acting as prosthetic groups are usually (i) flavin compound such as FMN (Flavin Mono Nucleotide), FAD (Flavin adenine Dinucleotide) (ii) iron-porphyrin or (iii) biotin etc.

**Nomenclature**

With the development of enzyme chemistry there has been a confusion in its terminology. **Duclaux** for the first time introduced a system of enzyme naming in 1883 on the basis of substrate on which it acts, followed by the suffix- **ase**, though there are many exceptions. In this system suffix **ase** is added to the root word to the substrate of the enzyme. For example, the name **sucrase** refers to an enzyme that causes breakdown of **sugar, sucrose**, similarly **cellulase** acts on **cellulose**, **protease** acts on **proteins** and so on. In another system of nomenclature of enzyme a suffix- **lytic** is added to the substrate such as **proteolytic** enzyme that catalyses **proteins**.

Enzymes are also named after the name of the **source from where they are extracted**. For example, **Papain** from **papaya**, **Bromalin** from **pineapple (Family- Bromaliacea**) etc. some enzyme are also named according to the **reaction they catalyse**. Thus, the enzyme which is responsible for **hydrolytic** **reaction** is known as **hydrolase**, **dehydrogenaqse** for **succinic dehydrogenase**.

This naming system was haphazard, therefore a systematic approach of naming the enzyme has been recommend by the **Commission on Enzyme of the International Union of Biochemistry (1961)** according to which the various enzyme are designated by **code numbers** of **four digit**.

The main features of the new system of classification of the enzymes as recommended by the **Commission on Enzyme of the International Union of Biochemistry (1961)** are as follows-

1. All the known enzymes have been grouped into 6 major classes.
2. Each major class has been divided into sub-classes.
3. Each sub-class has been furt her sub divided into sus-sub-classes.
4. Each enzyme has been assigned a specific code number consisting of four digit. The first digit indicates the major class, the second indicates the sub-class, the third digit indicates its sub-sub-class while the fourth digit denotes the systematic specific name of the enzyme the first part of which indicates the name of the substrate and the second part the nature of the reaction.

**Major class**

1. **Oxidoreductase**- Catalyse oxidation reduction reaction
2. **Transferase**- Catalyse reaction which involve group transfer
3. **Hydrolases**- Catalyse hydrolytic reactions
4. **Lyases**- catalyse reaction in which either double bond is established due to the removal of a group, or a group added to the double bond
5. **Isomerase**-catalyse isomerisation reactions
6. **Ligases**- catalyse those reactiob in which linking of two molecule is coupled with the breakdown of pyrophosphate bond of ATP or similar triphophate.

**Sub class**

1.1 Oxidoreductase, acting on the CH.OH group donor

2.1 Transferase, transferring one carbon groups

3.1 Hydrolases, acts on ester links

4.1 Lyases, acts on C-C bond

5.1 Isomerise, acts as racemase and epimerase

6.1 Ligases, , forms C-O bonds

**Sub- sub- class**

1.1.1 Oxidoreductase, acting on the CH.OH group donor, with coenzyme NAD or NADP as acceptor

2.1.1 Transferase, transferring one carbon groups, and a methyl transferase

3.1.1 Hydrolases, acts on carboxylic ester links,

4.1.1 Lyases, acts on C-C bond, carboxylase

5.1.1 Isomerise, acts as racemase and epimerase on amino acids and derivatives

6.1.1 Ligases, , forms C-O bonds, amino acid-RNA ligase

Foe example

|  |  |  |
| --- | --- | --- |
| Code no | Systematic Name | Trivial Name |
| 1.1.1.1 | Alcohol NAD: Oxidoreductase | Alcohol dehydrogenase |

**Classification of enzymes**

Enzymes are classified into six main classes on the basis of their reaction specificity by the International Union of Biochemistry (IUB).

1. **Oxido-reductase** – These enzymes are concerned with biological oxidation and reduction. They are further classified into the following types-
2. **Dehydrogenase** - These enzymes are responsible for removal of hydrogen atom from one substrate to another substrate. Eg. **Alcohol dehydrogenase**.

Dehydrogenase

**AH2 A + 2[H] B**

 **BH2**

1. Oxidases – The enzymes that catalyse the transfer of hydrogen to molecular oxygen are termed as oxidases.

Oxidase

**AH2 + ½ O2 A + H2O**

1. Oxygenase – These enzymes catalyse the incorporation of oxygen to the substrate.
2. **Transferase** – Enzymes which are concerned with the transfer of a group of atoms from one molecule to another are called transferases. The transferase enzymes ususlly transfer carbon aldehyde or ketonic residues e.g. **Creatine phosphoryl transferase** of muscle cell. It transfer the energy rich phos[hate group from creatine phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP).
3. **Isomerases** – these enzymes are concerned with the catalytic reaction of intramolecular rearrangement of atoms in their substrate i.e. they catalvse different types of isomerisation reactions. E.g. Phosphohexose isomerise.
4. **Hydrolases** – these enzymes are concerned with the hydrolysis of complex molecule into simpler forms reacting with water. These are usually named on the basis of substrate they hydrolase. Thus, these are classified into following types-
5. **Proteases** – these are concerned with breaking down of peptide bond of protein into peptides to convert them into amino acid.
6. **Carbohydrases** – These catalyse the hydrolysis of carbohydrate into simpler forms. E.g. **Maltose, Lactose, Salivary amylase** etc.
7. **Esterases** – These enzymes catalyse hydrolysis of ester linkage. E.g. Lipases, Phosphatases etc.
8. **Lyases** – Lyases are concerned with the breakdown of complex substances into simpler forms but without hydrolysis e.g. **deoxycarboxylase**.
9. **Ligase or synthetase** – these catalyse the linkage of two separate molecules. Eg. **Acetyl CoA carboxylase**.

**Chemical properties of enzyme**

1. **Catalytic properties** – Enzymes are biocatalyst. They have enormous ability to catalyse. A small amount of enzyme can catalyse large amount of substrate but at the end of the reactions it remains unchanged.
2. **Specificity of enzyme** – An enzyme is a specific in its action i.e. a particular enzyme can act only upon certain substrate or a group of substrates thus, maltase acts on maltose no other substrate, this means that there should be separate enzymes for separate substances. The specificity of an enzyme determined by its configuration which will fit only the configuration of a particular type of substrate molecule in the same way that a key fits only one type of lock.
3. **Solubility** – Enzymes are soluble in water, alcohol, saline and also in dilute glycerine.
4. **Reversibility in action** – Enzymes can accelerate the biochemical reactions in neither directions.
5. **Sensitivity of enzyme** –
6. The enzymes are very sensitive to heat i.e., they are thermolabile. They are inactivated at very low temperatures. At very high temperatures 60-70°C usually they are destroyed (denatured). Low molecular weight enzymes are comparatively more heat stable.
7. Enzymes are also sensitive to inhibitors. While some inhibitors may partially inhibit their activity, other inhibitors like poisons destroy them permanently and inhibit their activity.
8. Enzymes also show great sensitivity to pH. Change in pH causes decreases of enzyme activity. Enzymes have optimum pH range for their best activities. For example, ptyalin or salivary amylase can perform its best activities in slightly acidic medium in buccal cavity but its acivity is lost in the stomach due to high acidic pH.
9. **Colloidal properties** – Enzymes are proteins, hence, they form colloidal solution in water. For their large size, the enzymes can diffuse but very slowly, therefore enzymes can easily be separated by dialysis.