Dr. Rima Kumari: Date: 13/07/2020

Online class and e- content for M.Sc. II semester students

Date and Time	Online class medium	E. content topic
13/07/2020 1:30 p.m to 2.30 p.m	Via Google meet Link: Meeting URL: https://meet.google.com/vxu-hocp-fyz	Basic concept of Enzymes, Classification of enzymes

M.Sc. Semester II

MBOTCC-7 Physiology and Biochemistry

Enzymes: Basic concept of Enzymes, Classification of enzymes

Enzymes

Enzymes are central to every biochemical reaction within all living cells of an organism and are called the catalysts of biological systems (biocatalysts). They in organized sequences catalyse the hundreds of stepwise reactions by which nutrient molecules are degraded, chemical energy is conserved and transformed, and biological macromolecules are made from simple precursors. Through the action of regulatory enzymes, metabolic pathways are highly coordinated to yield a harmonious interplay among the many different activities necessary to sustain life. Enzymes catalyse an enormous diversity of biochemical reactions due to their capacity to specifically bind a very wide range of molecules. Enzymes bring substrates together in an optimal orientation, to undergo making and breaking chemical bonds. They catalyse reactions by stabilizing transition states, the highest energy-species in reaction pathways. By selectively stabilizing a transition state, an enzyme determines which one of several potential biochemical reactions actually takes place.

Until 1980s, all enzymes were believed to be proteins. Then, Tom Cech and Sidney Altman independently discovered that certain RNA molecules may function as enzymes may be effective biocatalysts. These RNA biocatalysts have come to be known as **ribozymes.**

Enzymes:

Definition: Enzymes are commonly proteinaceous substances which are capable of catalysing chemical reactions of biological origin without themselves undergoing any change. Therefore, they are called biocatalysts. Enzymes are synthesised by living cells.

The term ‘enzyme’ was coined by **Kuhne** (1878) for catalytically active substances previously called ferments. **Enzymes were actually found out by Buchner** (1897) with the accidental discovery that fermentation of sugar is not only caused by living yeast cells but also yeast extract. The extract obviously possessed biocatalysts required for the process. Buchner (1903) also isolated the first enzyme. He was awarded Nobel Prize in the same year, 1903. There are numerous enzymes as every biochemical reaction is catalysed by a separate enzyme. It is estimated that a cell contains over 5000 chemicals. The number of chemical reactions is many times more.

Enzymes functional outside the living cells are called exo-enzymes, e.g., enzymes present in digestive juices, lysozyme of tears. Enzymes functional inside living cells are known as endoenzymes, e.g., enzymes of Krebs cycle (inside mitochondria), enzymes of glycolysis (inside cytoplasm).

Enzymes are mainly functional inside the living cells. As found out by Buchner they can be extracted from the cells and made to catalyse reactions outside the living cells. In nature some enzymes are secreted by living cells and made to perform extracellular catalysis. Digestive enzymes belong to this category. Several enzymes of medical and chemical importance are now available in the market, e.g., rennet tablets (from rennin of calf stomach) for coagulating milk protein casein during preparation of cheese and other milk products.

The biochemical which is acted upon by an enzyme is known as substrate. In case two biochemicals are involved in a reaction, the same are called reactants. The chemicals formed after the completion of a reaction are termed as **products**. The final products are also called **end products**. **Part of enzyme that takes part in catalysing biochemical reaction is called active site.**

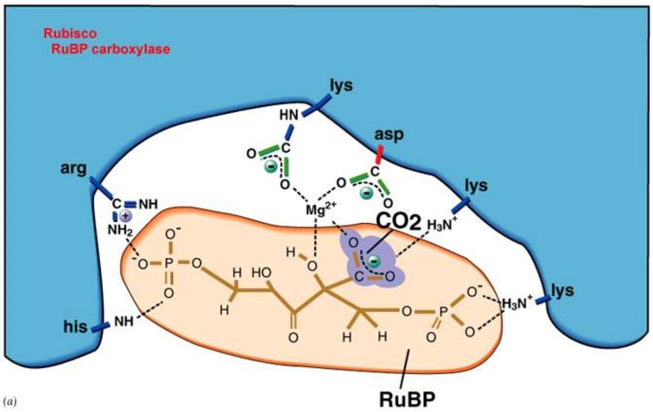
The protein nature of enzyme was first discovered by James Sumner in 1926 when he purified the enzyme urease and obtained it in crystalline form. For this, Sumner was awarded Nobel Prize in 1946.

Substrate in active site

Enzyme Structure

Enzymes are proteins, and their function is determined by their complex structure. The reaction takes place in a small part of the enzyme called the **active site**, while the rest of the protein acts as "scaffolding". This is shown in this diagram of a molecule of the enzyme **trypsin**, with a short length of protein being digested in its active site. The amino acids around the active site attach to the substrate molecule and hold it in position while the reaction takes place. This makes the enzyme **specific** for one reaction only, as other molecules won't fit into the active site – their shape is wrong.

Many enzymes need **cofactors** (or **coenzymes**) to work properly. These can be metal ions (such as Fe^{2+}, Mg^{2+}, Cu^{2+}) or organic molecules (such as haem, biotin, FAD, NAD or coenzyme A). Many of these are derived from dietary vitamins, which is why they are so important. The complete active enzyme with its cofactor is called a **holoenzyme**, while just the protein part without its cofactor is called the **apoenzyme**.



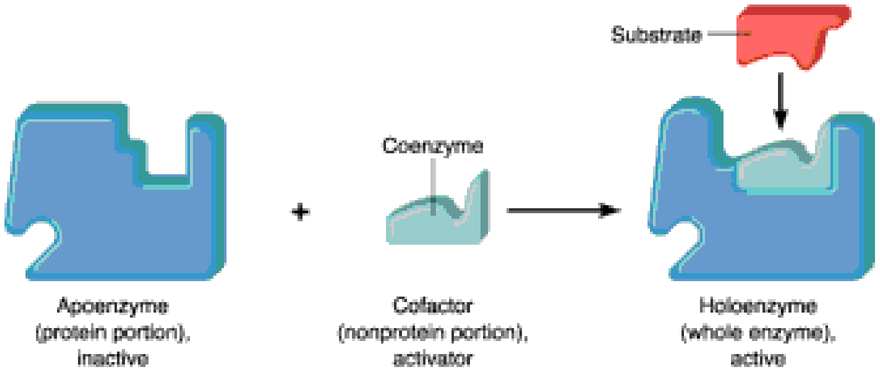
The active site of RUBISCO, the key enzyme in photosynthesis, contains just 6 amino-acids.

Important terminologies:

Holoenzyme and Apoenzyme: An enzyme that requires a helper group and isn't complete without it. Once attached, it's called a holoenzyme. A **holoenzyme** is an enzyme with any co-factor (metal ions or coenzymes) attached to it that is now ready to catalyze a reaction. An enzyme without a cofactor is called an **apoenzyme**. **An apoenzyme and its cofactor together constitute the holoenzyme.**

Cofactors

Cofactors are non-proteinous substances that associate with enzymes. A cofactor is essential for the functioning of an enzyme that increases the rate of reaction. Cofactors are not proteins but rather help enzymes, although they can also help non-enzyme proteins as well.



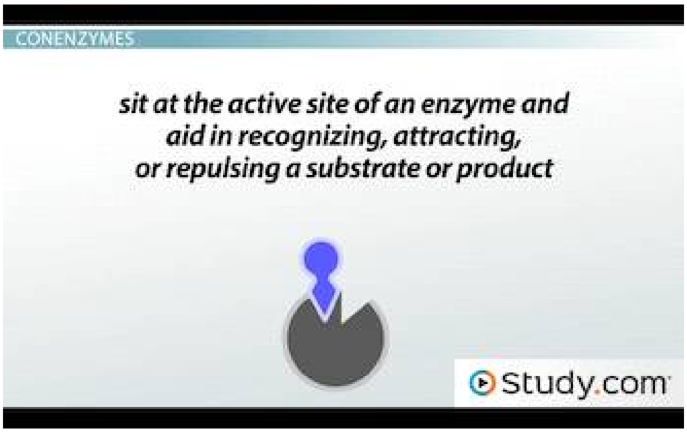
There are three kinds of cofactors present in enzymes:

- **Prosthetic groups**: These are cofactors tightly bound to an enzyme at all times. A fad is a prosthetic group present in many enzymes.
- **Coenzyme**: Coenzymes are defined as organic molecules, small, non-protein which are also termed as cosubstrates. They act as carriers. coenzyme is bound to an enzyme only during catalysis. At all other times, it is detached from the enzyme. Some examples of coenzymes are **vitamin-b**, coenzyme A, biotin, NAD^+ etc.
- **Metal ions**: For the catalysis of certain enzymes, a metal ion is required at the active site to form coordinate bonds. Iron and Zn^{2+} is a metal ion cofactor used by a number of enzymes.

Coenzymes

A specific type of cofactor, **coenzymes**, are organic molecules that bind to enzymes and help them function. The key here is that they're organic. Organic molecules are simply molecules that contain carbon. Coenzymes are not really enzymes. As the prefix 'co-' suggests, they work with enzymes. Many coenzymes are derived from vitamins.

Coenzyme is bound to an enzyme only during catalysis. These molecules often bound near the active site of an enzyme and aid in recognizing, attracting, or repulsing a substrate or product. Remember that a **substrate** is the molecule upon which an enzyme catalyzes a reaction. Coenzymes can also shuttle chemical groups from one enzyme to another enzyme. Coenzymes bind loosely to enzymes, while another group of cofactors do not.



CONENZYMES

sit at the active site of an enzyme and
aid in recognizing, attracting,
or repulsing a substrate or product

Coenzymes bind loosely to the active enzymes.

Prosthetic Groups

Prosthetic groups are cofactors that bind tightly to proteins or enzymes. As if holding on, they are not easily removed. They can be organic or metal ions and are often attached to proteins by a covalent bond. The same cofactors can bind multiple different types of enzymes and may bind some enzymes loosely, as a coenzyme, and others tightly, as a prosthetic group. Some cofactors may always tightly bind their enzymes. It's important to note, though, that these prosthetic groups can also bind to proteins other than enzymes.

PROSTHETIC GROUPS

Prosthetic groups

cofactors that bind tightly to proteins or enzymes

Prosthetic groups can be either organic or metal ions.

Isozymes: Multiple forms of enzymes that differ in amino acid sequence but **catalyze the same chemical reaction.** Although they have the same catalytic activity, they are physically distinct and differ in chemically, immunologically, electrophoretic mobility and liability to inhibitors.

Example of isoenzymes

Many enzymes are present in isoenzyme form:

1. Lactate dehydrogenase
2. Creatine kinase
3. Acid phosphatase
4. Alkaline phosphatase
5. Lactate dehydrogenase

Multi-enzyme Many enzymes in living cells catalyses chains of reaction in a sequential order either in a biosynthetic or a catabolic pathway. The series of enzymes catalyzing such chains of reactions are said to form a multi-enzyme system.

The enzymes act as independent entities each interacting with its own substrate, forms product. The product formed from each reaction is acted upon by another enzyme of the sequence. Some of the multi-enzyme systems may operate in a different way, when the enzymes are closely associated with each other to form a multi-enzyme complex.

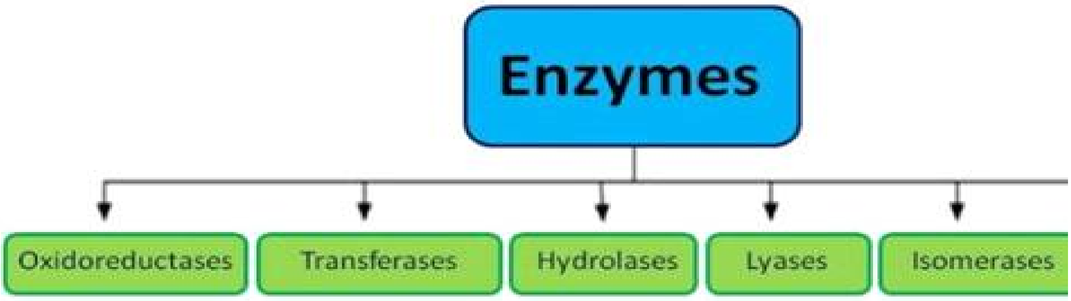
The fatty acid synthetase of yeast provides an example of a multi-enzyme complex. It consists of seven different enzymes which form a tightly bound cluster. Each enzyme of the complex catalyzes a different reaction, ultimately producing a long- chain fatty acid.

A B C D E F end product is F

Enzymes involved in chain reactions are

A-------B (enzyme 1)
B-------C (enzyme 2)
C-------D (enzyme 3) **Multi-enzymes**
D-------E (enzyme 4)
E-------F (enzyme 5)

Enzymes Classification



According to the International Union of Biochemists (I U B), enzymes are divided into six functional classes and are classified based on the type of reaction in which they are used to catalyze. The six types of enzymes are oxidoreductases, hydrolases, transferases, lyases, isomerases, ligases.

Following are the enzymes classifications in detail:

Types	Biochemical Property
Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.
Transferases	The Transferases enzymes help in the transportation of the functional group among acceptors and donors molecules.
Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process.

Oxidoreductases

These catalyze oxidation and reduction reactions,e.g. pyruvate dehydrogenase, which catalyzes the oxidation of pyruvate to acetyl coenzyme A.

Transferases

These catalyze the transfer of a chemical group from one compound to another. An example is a transaminase, which transfers an amino group from one molecule to another.

Hydrolases

They catalyze the hydrolysis of a bond. For example, the enzyme pepsin hydrolyzes peptide bonds in proteins.

Lyases

These catalyze the breakage of bonds without catalysis, e.g. aldolase (an enzyme in glycolysis) catalyzes the splitting of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Isomerases

They catalyze the formation of an isomer of a compound. Example: phosphoglucomutase catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate (transfer of a phosphate group from one position to another in the same compound) in glycogenolysis (conversion of glycogen to glucose for quick release of energy.

Ligases

Ligases catalyze the joining of two molecules. For example, DNA ligase catalyzes the joining of two fragments of DNA by forming a phosphodiester bond.