Enzymes

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Abstract

Enzymes are biological catalysts needed in all the biochemical reactions in a living system. Enzymes are proteins in nature which can be defined as biocatalysts synthesized by living cells. Under laboratory conditions the hydrolysis of a protein by a strong acid at 100°C takes a couple of days. However, the enzymes in the gastrointestinal tract digest the same protein during a few hours and moreover at a much lower temperature (37°C, body temperature). The substance on which enzymes act is called the 'substrate' . A catalyst is an organic or inorganic substance that accelerates a chemical reaction without affecting the end products of the reaction and without being destroyed in the course of reaction. All chemical reactions occurring in living cells, and therefore, all metabolic processes are mediated by the highly specific biological catalysts, called 'Enzymes'.

Historical Perspective

The word 'enzyme' was first introduced by Kuhne in 1878 (Gr: en= in + zyme = yeast) which means "in yeast" in 1896. In 1850, Louis Pasteur concluded that the fermentation

of sugar into alcohol by yeast is catalyzed by fermentas. He proposed that fermentas is a structure that is present in living yeast cells. In 1897, E. Buchner from Germany discovered that the yeast extract could ferment sugar into alcohol provided the fermentation was promoted by the molecules that continue to function when removed from the cell. Buchner's experiment marked the end of vitalistic theory; this was the time of origin of Bio- Chemistry. Fradric Kuhne later gave the name enzyme to the molecules. The isolation and characterization of the enzyme was increased by the scientist James Sumner in 1926. Sumner found that the urease enzyme crystals consist of proteins and so the enzymes are proteins.

Definition

An enzyme may be defined as a complex biological catalyst, produced by the living organisms in their cells to regulate the various physiological processes of the body. Some enzymes depend for activity only on their structure while others also require one or more non - protein components called co - factor. The co- factor may be metal ion or an organic molecule called a co - enzyme. Co - factors are generally stable to heat, whereas most enzyme proteins lose activity on heating. The catalytically active enzyme co - factor complex is called a HoloEnzyme. When the co - factor is removed the remaining protein is catalytically inactive by itself and is called Apoenzyme. Enzymes requiring metal ions are called Metalloenzymes. Co - enzymes usually function as intermediate carriers of functional groups, specific atoms or

electrons that are transferred in overall enzymatic action. The co-enzyme bonded to the enzyme molecule is usually called the prosthetic group.

Holoenzyme = Apoenzyme + Cofactor

Nomenclature And Classification Of Enzymes

According to International Union Of Biochemistry (IUB) the chemical reaction catalyzed is the specific property which differentiates one enzyme to another. The International Union Of Biochemistry used this criterion as a basis for the classification and naming the enzymes. The major features of this system of classification of enzymes are as follows:

1. The reactions and the enzymes catalyzing them are divided into 6 major classes, each with 4 to 13 subclasses.
2. Each enzyme name has two parts - The first part is the name of the substrate (S) and the second part which ends in the suffix - ase, indicates the type of reaction catalyzed.
3. Each enzyme has been allotted a systemic code number called Enzyme Commission (E.C) Number. The enzyme commission number for each enzyme consists of a series of numbers at 4 places

The first number represents the major class to which the enzyme belongs, the second number indicates the subclass of the enzyme and the third number indicates the sub - subclass of the enzyme. The last place number or the fourth digit represents the serial number of the enzyme which is very specific. Thus E.C. 2.7.1.1 represents class 2 (a

transferase), subclass 7 (transfer of phosphate), sub - subclass 1 (an alcohol group as phosphate acceptor). The final digit denotes the enzyme, hexokinase. This enzyme catalyzes a transfer of phosphate from ATP to the hydroxyl group of carbon 6 of glucose.

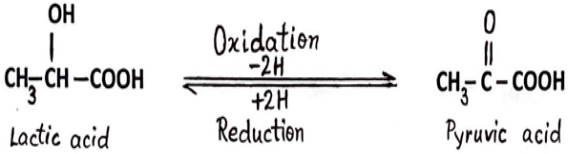
ATP + D - hexose ----------ADP + Hexose -6- phosphate

Classification

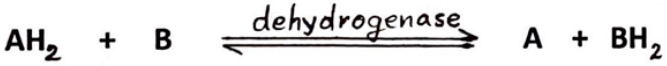
The six major classes of the enzymes are given below.

Oxidoreductase

Enzymes which catalyzed the biological oxidation and reduction, the removal or addition of hydrogen atoms. Ex. – Dehydrogenases,Peroxidases.

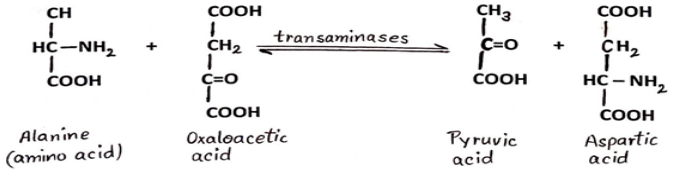


A dehydrogenase or reductase is an enzyme that catalyzes the dehydrogenation or removal of hydrogen atom from the substrate A, transferring them to another substrate B



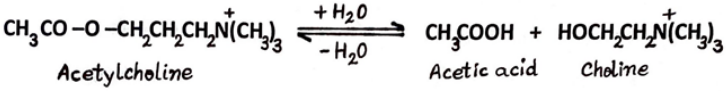
Transferases

These are the enzymes that catalyze the transfer of the chemical group from one molecule to another. Ex. – Transaminase, Trans Phosphorylases and Transduction.



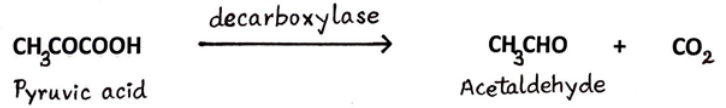
Hydrolases

These are the enzymes that function the hydrolysis of a variety of compounds by water. Ex. – Acetylcholinesterase which catalyzes the cleavage of acetylcholine to choline and acetic acid.



Lyases

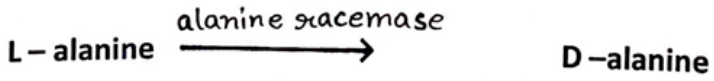
These are a group of enzymes that reversibly catalyze the removal of groups from substrates non hydrolytically. Ex. – pyruvic acid decarboxylase accelerates the following reaction.



Isomerases Or Mutases

These are the enzymes that catalyze the

interconversion of a compound to one of its isomers. Ex. – alanine racemase catalyzes this reaction.

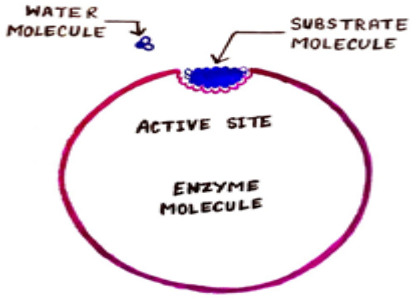


Ligases Or Synthetases

These are the enzymes that catalyze the linking together of two separate molecules, coupled with the breaking of a pyrophosphate bond. A triphosphate nucleotide, such as ATP presumably participates in the reaction.

This enzyme is usually called acetic thiokinase but the systematic name would be acetate: CoA-SH-ligase(AMP).

Characteristics



Colloidal Nature

Enzyme molecules are very large in size as compared

to the substrates. Due to their large size, the enzyme molecules possess extremely low rates of diffusion and form colloidal systems in water.

Catalytic Nature Or Effectiveness

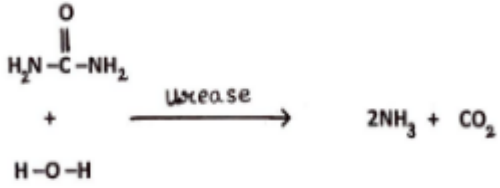
Enzymes act catalytically and accelerate the rate of chemical reactions occurring in animal and plant tissues. They do not participate in the reaction and are recovered as such at the end of the reaction without undergoing any qualitative or quantitative changes. That is why small amounts of enzymes are capable of transforming large quantities of substrate. The catalytic power of an enzyme is measured in terms of turnover number (the number of substrate molecules converted into products per unit time).

Specificity Of Enzyme Action

The enzymes are highly specific in action. The degree of specificity varies with respect to the types of substrates and chemical reactions. There are four patterns of enzyme specificity:

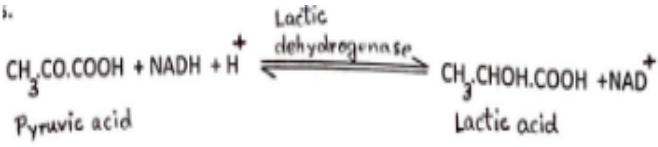
1. **Absolute Specificity**

Some enzymes are capable of acting on only one substrate. e.g. Urease acts only on Urea to produce ammonia and carbon dioxide.



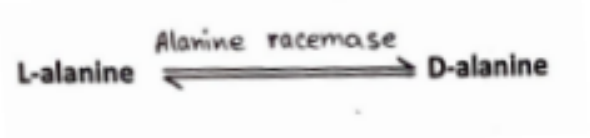
2. Group Specificity

Some other enzymes are capable of catalyzing the reaction of a structurally related group of compounds. e.g. Lactic dehydrogenase (LDH) catalyzes the interconversion of pyruvic and lactic acids.



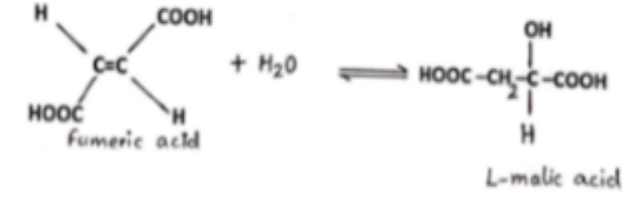
3. Optical Specificity

Enzymes exhibit optical specificity. However, some enzymes interconvert the two optical isomers of a compound. e.g. alanine racemase catalyzes the interconversion between L- and D- alanine.



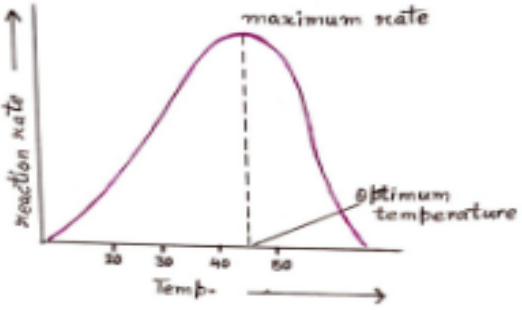
4. Geometrical Specificity

Some enzymes exhibit specificity towards the cis- and trans- forms. e.g. Fumarase catalyzes the interconversion of fumaric acid and malic acid.



Temperature

The rate of enzyme action increases with increase in temperature. This increase is upto certain temperature. A very high temperature tends to fall in the rate of reaction due to denaturation of enzymes. Each enzyme shows maximum activity at a certain temperature called Optimum Temperature.



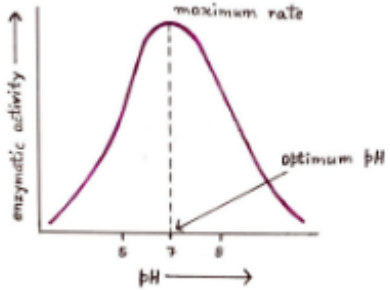
Thermolability

Thermolability is sensitiveness towards heat. Enzymes are proteinaceous in nature and so are highly sensitive to heat. Increase in temperature due to heat decreases the enzymatic activity after a certain temperature due to the denaturation of enzymes. The effect of heat also manifests itself in the preservation of enzyme activity during storage. The best preservation of enzyme preparations is by refrigeration and by quick freezing.

Ph-Sensitivity

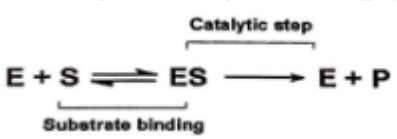
Enzymes normally function more efficiently at particular hydrogen ion concentration. Each enzyme reduces its activity outside its own optimum pH range. This decrease in activity of enzymes is because the hydrogen ion

concentration changes the ionization and solubility of enzyme susceptibility to heat.



Reversibility Of Reaction

The enzymes are capable of bringing reverse reactions.



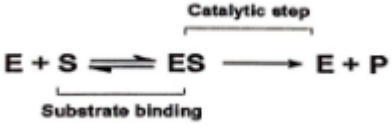
The direction of the reaction depends upon

1. pH of the cell.
2. presence of reacting substrate.
3. accumulation of end products.

Enzyme Action

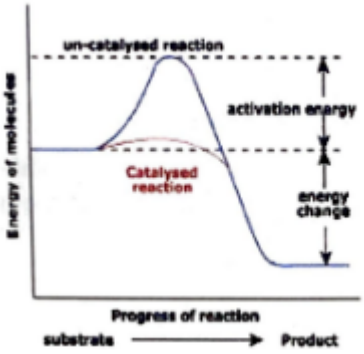
Mechanism Of Action Of Enzymes

A simple enzymatic reaction is given by

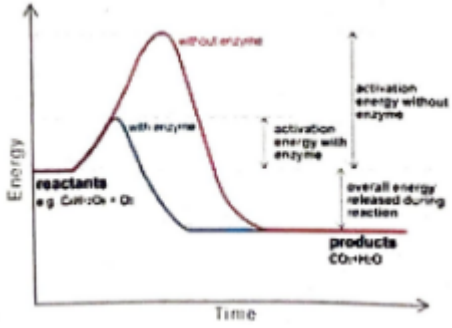


where, E, S and P represent the enzyme, substrate

and product respectively. ES represents the enzyme - substrate complex and EP represents the enzyme with product. To undergo the reaction, the reactants must overcome the barrier or energetic hill. This is called the 'transition state'. The difference between the energy levels of ground state and transition state is called Gibbs free energy of activation and is represented by ΔG.

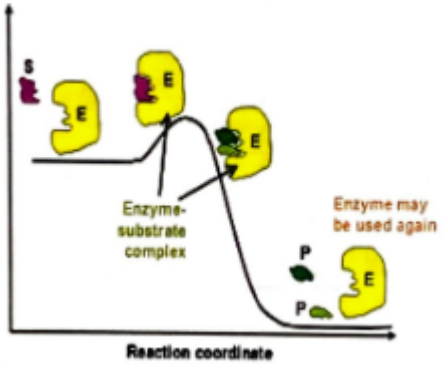


The rate of reaction reflects this activation energy. Higher activation energy corresponds to slower reaction. Reaction rates increase by increase in temperature is due to increase in the number of molecules with higher energy.



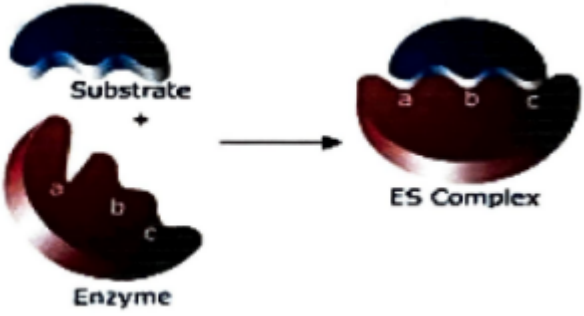
Catalysts enhance the rate of reaction by lowering the

activation energy. Enzymes change the reaction coordinate. The rate of reaction is determined by the step with maximum energy.



Fischer's Lock And Key Theory Of Action

This is also called 'Template Model'. This theory was proposed by Email Fischer in 1898. According to this model, the union between substrate and enzyme takes place at the active sites in a manner in which a key fits a lock and result in the formation of an enzyme- substrate complex.



In fact, the enzyme substrate union depends upon a reciprocal fit between the molecular structure of the enzyme

and the substrate. The two molecules are involved in this process, so it is also known as the concept of inter molecular fit. The enzyme substrate complex is highly unstable and immediately decomposes to produce the products and free enzyme. The enzyme substrate union results in the release of energy. This raises the energy level of the substrate molecule and induces the activated state.

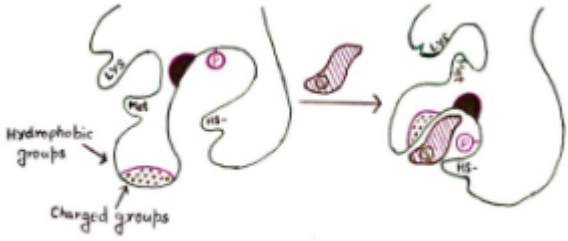
Evidences For Proving The Existence Of ES Complex

1. ES complexes are directly observed by electron microscopy and X- ray crystallography.
2. Physical properties of enzymes change on the formation of ES.
3. Stereospecificity is exhibited in the ES formation.
4. At constant concentration of enzyme , the rate of reaction increases with increase in substrate concentration until the maximum velocity.

Koshland's Induced Fit Model

In Fisher's hypothesis the enzymes are presumed to be reshaped to fit the substrate. This shows the rigidity of active sites. To overcome the above limitation Koshland modified Fischer's model in 1958 and gave his induced fit model theory. According to this model, the enzyme molecule does not retain its original shape and structure but the contact of substrate induces some centrifugal changes in the active site of the enzyme molecule. This helps the enzyme molecule to fit completely the centrifugal and active centres of the substrate. At the same time, other amino acid residues may become buried in the interior of the molecule. This hypothesis

is confirmed by Lipscomb.



The hydrophobic and charged groups both are involved in substrate binding. In the absence of substrate the substrate binding and catalytic groups are far apart from each other. But the approach of substrate induces a conformational change in the enzyme molecule aligning the both substrate binding and catalytic groups.

A phosphoserine (-P) and the -SH group of cysteine residue are involved in catalysis and known as catalytic groups. During the conformational changes there may be three possibilities

1. The enzymes may first undergo conformational change, then bind with the substrate.
2. The substrate may first be bound and then conformational changes occur in the enzymes.
3. Both the processes may occur simultaneously.

Organic Modifiers Of Enzyme Activity

Enzyme Inhibition

The chemical compounds which convert the enzyme in its inactive form and so adversely affect the rate of

enzymically catalyzed reactions are called Enzyme Inhibitors and the process is called Enzyme Inhibition. There are two classes of enzyme inhibitors, Reversible and Non - reversible, depending on whether the enzyme inhibitor complex is dissociated rapidly or very slowly.

Reversible Enzyme Inhibition

A reversible inhibitor dissociates very rapidly from its target enzyme because it gets very loosely bound with the enzyme molecule. There are three general types of reversible inhibition:

1. Competitive Inhibition.
2. Non - competitive inhibition.
3. Uncompetitive Inhibition

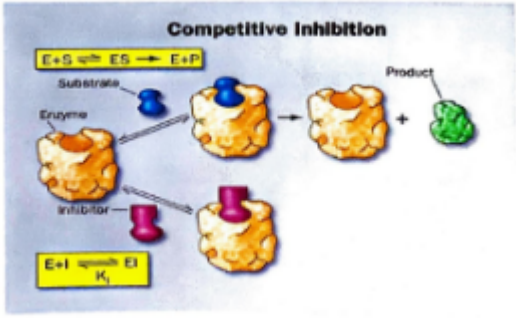
Three conditions necessary for enzyme inhibition are

1. whether the inhibition is or is not overcome by increasing the concentration of the substrate.
2. whether the inhibitor binds at the active site or allosteric site.
3. whether the inhibitor binds either with the free enzyme only, or with the enzyme- substrate complex molecule.

Competitive Inhibition Or Substrate Analog Inhibition

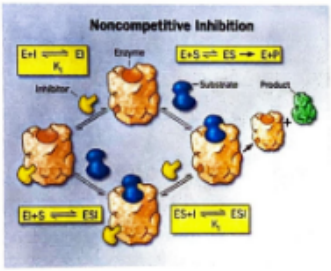
This type of competition occurs at the active site and the substrate shares the structure of inhibitor(I) closely resembling the substrate molecule (S). So it may combine with the enzyme molecule (E) and form an Enzyme - Inhibitor complex (EI) rather than Enzyme - Substrate complex (ES). The inhibitor is complete with the substrate molecule to combine with the enzyme. The degree of inhibition is

dependent on the relative concentration of the enzyme and substrate molecule and also the inhibitor molecules. By increasing the substrate concentration and keeping the inhibitor concentration constant the amount of inhibition decreases and the inhibitor concentration is increased. So the amount of enzyme substrate inhibitor complex is increased.



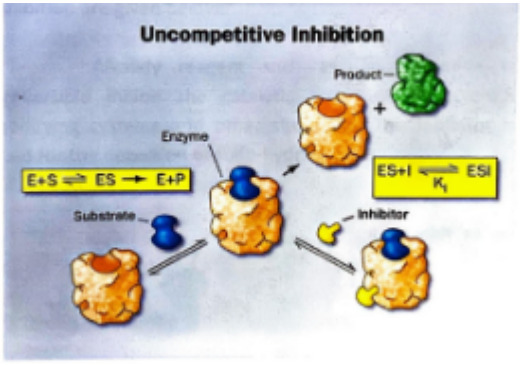
Non Competitive Inhibition

Non- competitive inhibition is present between the substrate and inhibitor (S) (I). The inhibitor has no structural similarity with the substrate and it binds to the enzyme molecules at a place other than the active site, as inhibitor and substrate may combine at different sites. The formation of both enzyme - substrate (ES) and enzyme substrate inhibitor complex (E-S-I) takes place. Both enzyme substrate complex and enzyme substrate inhibitor complex may break down and produce the reaction functional product.



Uncompetitive Inhibition

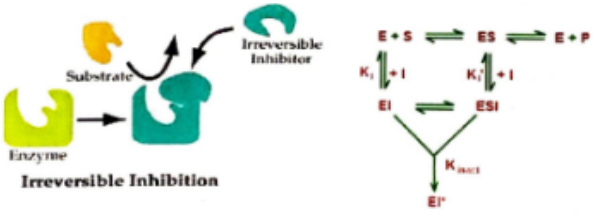
An uncompetitive inhibitor also binds at an allosteric site but the binding takes place only with the enzyme substrate complex and not in the free enzyme molecules. The non-competitive inhibition occurs in a very small quantity in the biological system because most of the reactions (metabolic) are inhibited by negative feedback control.



Irreversible Inhibition

Irreversible inhibitors are those that combine with the enzyme molecule and completely destroy the active sites of the enzyme, which is essential for the activity of enzymes. In fact the irreversible inhibitors dissociate very slowly from the

active site of enzyme and the resulting enzyme molecule is functionless. These inhibitors are attached with the enzyme molecule in the form of bonding that may be covalent or noncovalent. A common example of irreversible inhibition are given below



Applications

The enzymes have many applications in our daily life. Enzymatic processes such as baking, brewing, and tanning are well known. Some other applications of the enzymes are given below

Wine Manufacturing

The early field of enzymology was developed by the scientist Pasture whose work was associated with food, wine and beer industries. Pasture was best known as the father of the wine industry in the French Nation. Because Pasture discovered the process of using 'Papain Enzyme' in the brewing industry as a stabilizer of chill proof beer because it removes small amounts of proteins that cause turbidity in the beer and wine.

Cheese Making

For a long time the animal rennet is used in making

cheese. This enzyme is obtained on a commercial scale from the stomach of buffaloes and cows specially for this purpose. This enzyme helps in coagulating the milk protein (casin). Some preservatives like boric acid, benzoic acid or sodium chloride are added to prevent the decomposition of the enzyme preparation by the bacteria. The enzyme lipase is added in the cheese for imparting flavor into it.

Candy Making

An enzyme invertase helps the prevention of the formation of sugar in the chocolates. Another enzyme lactose helps prevent the formation of crystals in the ice cream.

In Fruit Juices

The enzymes are used in the processing of fruit juices, such as, apple juice and grape juice. These juices are clarified by adding a mixture of pectic enzymes which hydrolysed the pectic substances causing turbidity.

Tendering Meat

Because hydroxy prolyl residues create some bends in collagen protein present in the muscles which contribute to the tough texture of cooked meat.

Desizing Fabrics

The woven fabrics are sized by some special types of enzymes that are called alcalase.

Dehairing Leather

In the manufacturing of leather the dehairing is an important step. This is done by the use of pancreatic enzymes which hydrolyse the protein of hair particles.

Recovering Silver

Pepsin is used to digest gelatin in the process of recovering silver from photographic films.

Correcting Digestion

When the enzymes are present insufficiently in the body the result is certain digestive disorders. Pepsin, Trypsin and Amylase aid in the digestion in the stomach by artificial methods.

Analyzing Biochemicals

Certain enzymes are used in clinical analysis, for example: Uricase and Urease are used in determining the presence of uric acid and urea into the blood.

Wound Healing

Proteolytic enzymes from pig pancreas are used to treat skin diseases and wounds.

Dissolving Blood Clot

The enzyme Urokinase which is manufactured for the treatment of blood clots in the brain for the past five years in Japan.

In Surgery

Techniques using the enzyme trypsin in the cataract surgery.