**ENZYMES**

Enzymes are biocatalyst which catalyze the reactions of the central metabolic pathways necessary for the maintenance of life. In the absence of the enzymes, metabolic reactions will not proceed at significant rates under physiological conditions. The primary role of enzymes is to enhance the rates of these reactions to make life possible. Enzyme-catalyzed reactions are 103 to 1020 times faster than the corresponding uncatalyzed reactions. A catalyst is defined as a substance that speeds up the attainment of equilibrium. It may be temporarily changed during the reaction but it is unchanged in the overall process since it recycles to participate in multiple reactions.

Properties of Enzymes

1. Enzymes are proteins except for Ribozymes which is RNA acting as an enzyme.
2. Enzymes are highly specific for the reactants, or **substrates**, they act on, but the degreeof substrate specificity varies.
3. Enzymes increase the rate of reaction of a highly specific reaction.
4. Some enzymes combine or couple two reactions that would occur separately. This property allows the energy gained from one reaction to be used in a second reaction. Coupled reactions are a common feature of many enzymes—the hydrolysis of ATP, for example, is often coupled to less favorable metabolic reactions.

History

* The word *enzyme* is derived from a Greek word meaning “in yeast.” It indicates that these catalysts are present inside cells.
* In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments." He postulated that these ferments were inseparable from the structure of living yeast cells; this view, called vitalism, prevailed for decades.
* Then in 1897 Eduard Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells.
* Frederick W Kuhne later gave the name enzyrnes to the molecules detected by Buchner.
* James Sumner in 1926 isolated and crystallized Urease enzyme and proved that it is a protein.

**Classification of Enzymes**

Most of the classical metabolic enzymes are named by adding the suffix -*ase* to the name of their substrates or to a descriptive term for the reactions they catalyze. For example, urease has urea as a substrate. Alcohol dehydrogenase catalyzes the removal of hydrogen from alcohols (i.e., the oxidation of alcohols).

A committee of the International Union of Biochemistry and Molecular Biology

(IUBMB) maintains a classification scheme that categorizes enzymes according to the general class of organic chemical reaction that is catalyzed.

The six categories—

1. Oxidoreductases,
2. Transferases,
3. Hydrolases,
4. Lyases,
5. Isomerases,
6. Ligases

The IUBMB classification scheme assigns a unique number, called the enzyme classification number, or EC number, to each enzyme. IUBMB also assigns a unique systematic name to each enzyme; it may be different from the common name of an enzyme.

1. Oxidoreductase catalyze oxidation–reduction reactions. Most of these enzymes are commonly referred to as **dehydrogenases**. Other enzymes in this class are called oxidases, peroxidases, oxygenases, or reductases.

e.g. Lactate dehydrogenase (EC 1.1.1.27) which catalyzes reversible conversion of L-lactate to pyruvate.

1. **Transferases** catalyze group transfer reactions and many require the presence of coenzymes. In group transfer reactions a portion of the substrate molecule usually binds covalently to the enzyme or its coenzyme. This group includes kinases, enzymes that catalyze the transfer of a phosphoryl group from ATP. Alanine transaminase, whose systematic name is L-alanine:2-oxyglutarate aminotransferase (EC 2.6.1.2), is a typical transferase. It transfers an amino group from L-alanine to -ketoglutarate (2-oxoglutarate).
2. Hydrolases catalyze hydrolysis. They are a special class of transferases with water serving as the acceptor of the group transferred. Pyrophosphatase is a simple example of a hydrolase (EC 3.6.1.1).
3. **Lyases** catalyze addition or removal of a group generating a double bond in nonhydrolytic, nonoxidative, elimination reactions. e.g. Pyruvate Decarboxylase (EC 4.1.1.1) which catalyses lysis of Pyruvate into Acetaldehyde and Carbon Dioxide.
4. **Isomerases** catalyze structural change within a single molecule (isomerization reactions). Because these reactions have only one substrate and one product, they are among the simplest enzymatic reactions. e. g. Alanine racemase (EC 5.1.1.1) catalyzes interconversion of L-Alanine and D-Alanine.
5. **Ligases** catalyze ligation, or joining, of two substrates. These reactions require the input of chemical potential energy in the form of a nucleoside triphosphate such as ATP. Ligases are usually referred to as **synthetases**. Glutamine synthetase, or L-glutamate:ammonia ligase (ADP-forming) (EC 6.3.1.2), uses the energy of ATP hydrolysis to join glutamate and ammonia to produce glutamine.

**Structure of Enzymes**

Most enzymes are protein except few catalytic RNA. Many enzymes are only protein and they do not require any other chemical group for their catalytic activity other than amino acid residues. However some enzymes require additional groups called Cofactors for catalysis activity. A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a holoenzyme

There are two types of cofactors: **essential ions** (mostly metal ions such as Mg2+, Cu2+, Mn2+, Fe3+) and organic compounds known as **coenzymes. Both inorganic and organic cofactors become essential portions of the active sites of certain enzymes.**

 **Mechanism of Enzyme Action**

The enzyme catalyzed reaction take place at an active site on enzyme where substrate binding takes place. Substrate is the molecule which binds to the active site and on which enzyme acts. The surface of the active site is lined with amino acid residues with substituent groups that bind the substrate and catalyze its chemical transformation.



Binding of substrate to active site of enzyme

The binding of Enzyme-substrate to form [ES complex] is the first step in an enzyme catalyzed reaction. The enzyrne-substrate complex, existence was first proposed by Charles-Adolphe Wurtz in 1880, is central to the action of enzymes.

The function of enzymes and other catalysts is to lower the activation energy, for a reaction and thereby enhance the reaction rate. The equilibrium of a reaction is unaffected by the enzyrne.

A simple reaction is written as

E+S 🡨--🡪 ES <---🡪 EP 🡨--🡪 E+P

where E= Enzyme; S= Substrate; P=Product; product; ES and EP are transient complexes of the enzyme with the substrate and with the product.

Enzyme increases the rate of reaction without affecting the equilibrium.

In every biological system there exists free energy which is denoted G- Gibbs Free energy.

In reaction conversion of S--🡪P the starting point of either forward or reverse reaction is called **ground state**. There exists an energy barrier between substrate and product.



In the above shown the free energy of the ground state of P is lower than that of S, so G'' for the reaction is negative and the equilibrium favors P. The position and direction of equilibrium are not affected by any catalyst.

There exist an energy barrier between S and P. To undergo reaction all the molecules of S must overcome the energy barrier and reach to a higher energy level called **transition state** where all the molecules have equal probability to form product.

The difference between the energy levels of the ground state and the transition state is the activation energy. This affect the rate of reaction, higher Activation energy corresponds to slower rate of reaction.

Enzymes lower the activation energy thus increasing the rate of reaction.



Enzyme catalyses the reaction by binding to the molecule in transition state to form reaction intermediates. As mentioned in this fig ES and EP are the reaction intermediates. G for uncatalysed reaction is higher than the catalyzed reaction.

**How enzyme lowers the activation energy?**

As mentioned above in order to catalyse a reaction the enzyme must be compatible with substrate in *transition state*. This means that optimal interaction between substrate and enzyme occur only in the *transition state*.

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In an enzyme catalyzed reaction, there is rearrangement of covalent bonds between enzyme and substrate. Catalytic functional groups on an enzyme may form a transient covalent bond with the substrate and activate it for reaction. Covalent interactions between enzymes and substrates lower the activation energy (and thereby accelerate the reaction) by providing an alternative, lower-energy reaction path.

Also the ES complex is stabilized by formation of several weak non covalent interactions such as hydrogen bonds, ionic and hydrophobic interactions. Formation of each weak interaction in the ES complex is accompanied by release of a small amount of free energy that stabilizes the interaction. The energy derived from enzyme substrate interaction is called binding energy.

Binding energy is the major source of energy used by enzyme to lower the activation energy of an enzyme catalyzed reaction.

There are two theories that describe the binding of enzymes: 1) Lock and Key Theory and 2) Induced Fit Theory.

1) Lock and Key Theory: The shape of the enzyme's active site is complementary to that of its substrate

2) Induced Fit Theory: The active site has a flexibility of shape, thus when an appropriate substrate comes in contact with the enzyme's active site, the shape of the active site would change to fit with the substrate. Mechanism postulated by Daniel Koshland in 1958. Induced fit serves to bring speci-fic functional groups on the enzyme into the proper position to catalyze the reaction. The conformational

change also permits formation of additional weak bonding interactions in the transition state