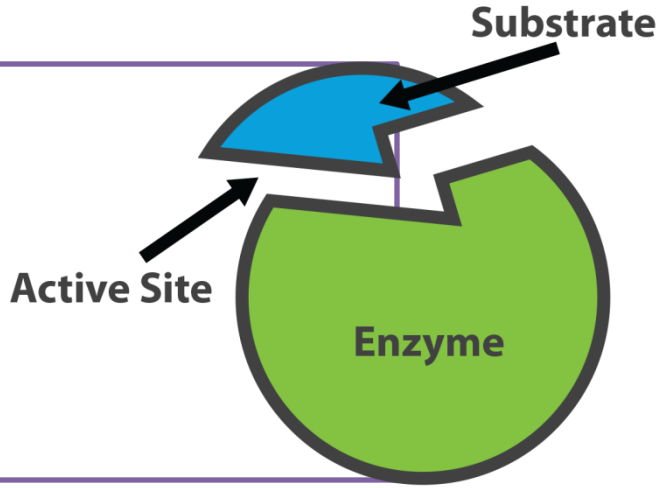
ENZYMES



By

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Once upon a time there was a rich merchant. In his last will and testament, he put aside his 17 white horses to his 3 sons to be shared thus; 1/2 for the 1st son, 1/3 for the 2nd son and 1/9 for the 3rd son. After his death, the sons started to quarrel, as the division could not produce whole number. Then their brotherin-law told them that they should include his black horse also for the sharing purpose. Thus now they had 17 + 1 = 18 horses, and so division was possible; 1st son got one-half or 9 horses; 2nd son got 6 and 3rd son had 2 horses. Now all the 17 white horses were correctly divided among the sons. The remaining black horse was taken back by the brother-in-law. **Catalysts**

are similar to this black horse. The reaction, although theoretically probable, becomes practically possible only with the help of catalysts. They enter into the reaction, but come out of the reaction without any change. Catalysts are substances which accelerate the rate of chemical reactions, but do not change the equilibrium.

What are enzymes?

Enzymes are proteins that help speed up metabolism, or the chemical reactions in our bodies. They build some substances and break others down. All living things have enzymes. Our bodies naturally produce enzymes. But enzymes are also in manufactured products and food.

What are the different types of enzymes?

There are thousands of individual enzymes in the body. Each type of enzyme only has one job. For example, the enzyme sucrase breaks down a sugar called sucrose. Lactase breaks down lactose, a kind of sugar found in milk products. Some of the most common digestive enzymes are:

Carbohydrase breaks down carbohydrates into sugars.

Lipase breaks down fats into fatty acids.

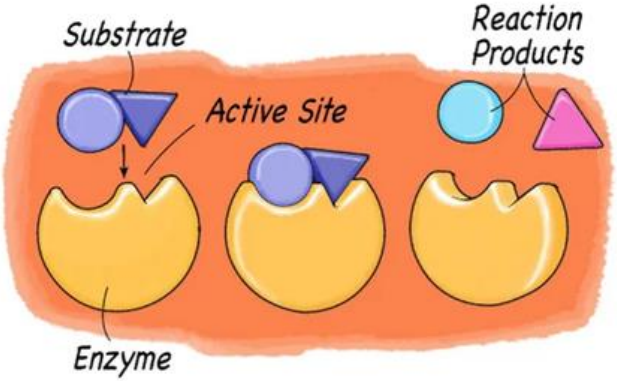
Protease breaks down protein into amino acids.

What are the parts of an enzyme?

Each enzyme has an “active site.” This area has a unique shape. The substance an enzyme works on is a substrate. The substrate also has a unique shape. The enzyme and the substrate must fit together to work.

Enzymes are biocatalysts. Life is possible due to the coordination of numerous metabolic reactions inside the cells. Proteins can be hydrolyzed with hydrochloric acid by boiling for a very long-time; but inside the body, with the help of enzymes, proteolysis takes place within a short-time at body temperature. Lack of enzymes will lead to block in metabolic pathways causing **inborn errors of metabolism**. The substance upon which an enzyme acts, is called the **substrate***.* The enzyme will convert the substrate into the **product** or products.

Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins. They are heat labile, soluble in water, precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid) and contain 16% weight as nitrogen.



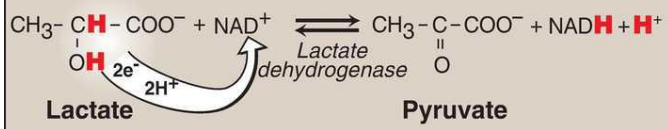
CLASSIFICATION OF ENZYMES

Early workers gave whimsical names such as Pepsin, Trypsin, Chymotrypsin, etc. some of which are still used. Later, enzymes were named by adding the suffix "ase" to the substrate. Thus, enzyme Lactase acts on the substrate lactose. These are known as the **trivial names** of the enzymes. But there may be more than one enzyme acting on the same substrate.

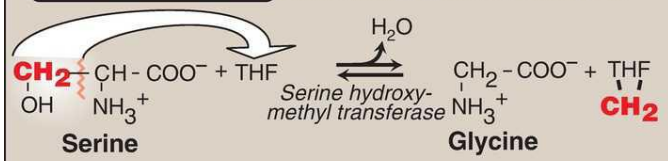
IUBMB System of Nomenclature of Enzymes

International Union of Biochemistry and Molecular Biology (IUBMB) in 1964, (modified in 1972 and 1978), suggested the nomenclature of enzymes. It is complex and cumbersome; but unambiguous. As per this system, the *name starts with EC (enzyme class) followed by 4 digits.* The first digit represents the class; second digit stands for the subclass; third digit is the sub-subclass or subgroup; and the 4th digit gives the number of the particular enzyme in the list. The enzymes are *grouped into 6 major classes* (Table1). For example, Class 1 is called oxidoreductases.

1. *Oxidoreductases* Catalyze oxidation-reduction reactions, such as:

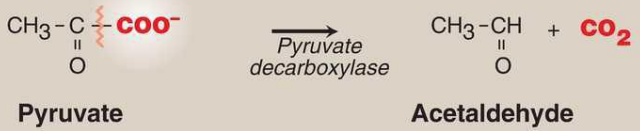


2. *Transferases* Catalyze transfer of C-, N-, or P-containing groups, such as:

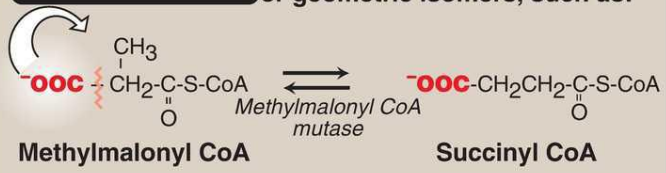


3. *Hydrolases* Catalyze cleavage of bonds by addition of water, such as:

4. *Lyases* Catalyze cleavage of C–C, C–S, and certain C–N bonds, such as:



5. *Isomerases* Catalyze rearrangement of optical or geometric isomers, such as:



6. *Ligases* Catalyze formation of bonds between carbon and O, S, and N coupled to hydrolysis of high-energy phosphates, such as:

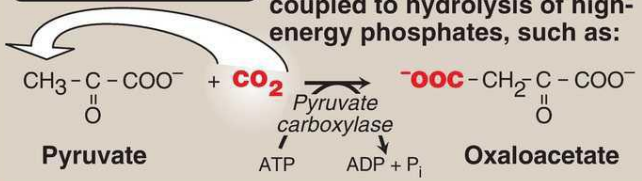


Table 3.1: Classification of enzymes

Class 1. Oxidoreductases: Transfer of hydrogen, e.g. alcohol dehydrogenase.

Class 2. Transferases: Transfer of groups other than hydrogen. (Subclass: Kinase, transfer of phosphoryl group from ATP, e.g. hexokinase).

Class 3. Hydrolases: Cleave bond; adds water, e.g. acetyl choline esterase.

Class 4. Lyases: Cleave without adding water, e.g. aldolase. (Subclass: Hydratase adds water to double bond).

Class 5. Isomerases: Intramolecular transfers. This class includes racemases and epimerases. Example, triose phosphate isomerase.

Class 6. Ligases: ATP dependent condenzation of two molecules, e.g. acetyl CoA carboxylase.

COENZYMES

i. Enzymes may be simple proteins, or complex enzymes, containing a non protein part, called the **prosthetic group.**

ii. The protein part of the enzyme is then named the **apoenzyme***,* the prosthetic group the **coenzyme***;* and these two portions combined together is called the **holoenzyme .**

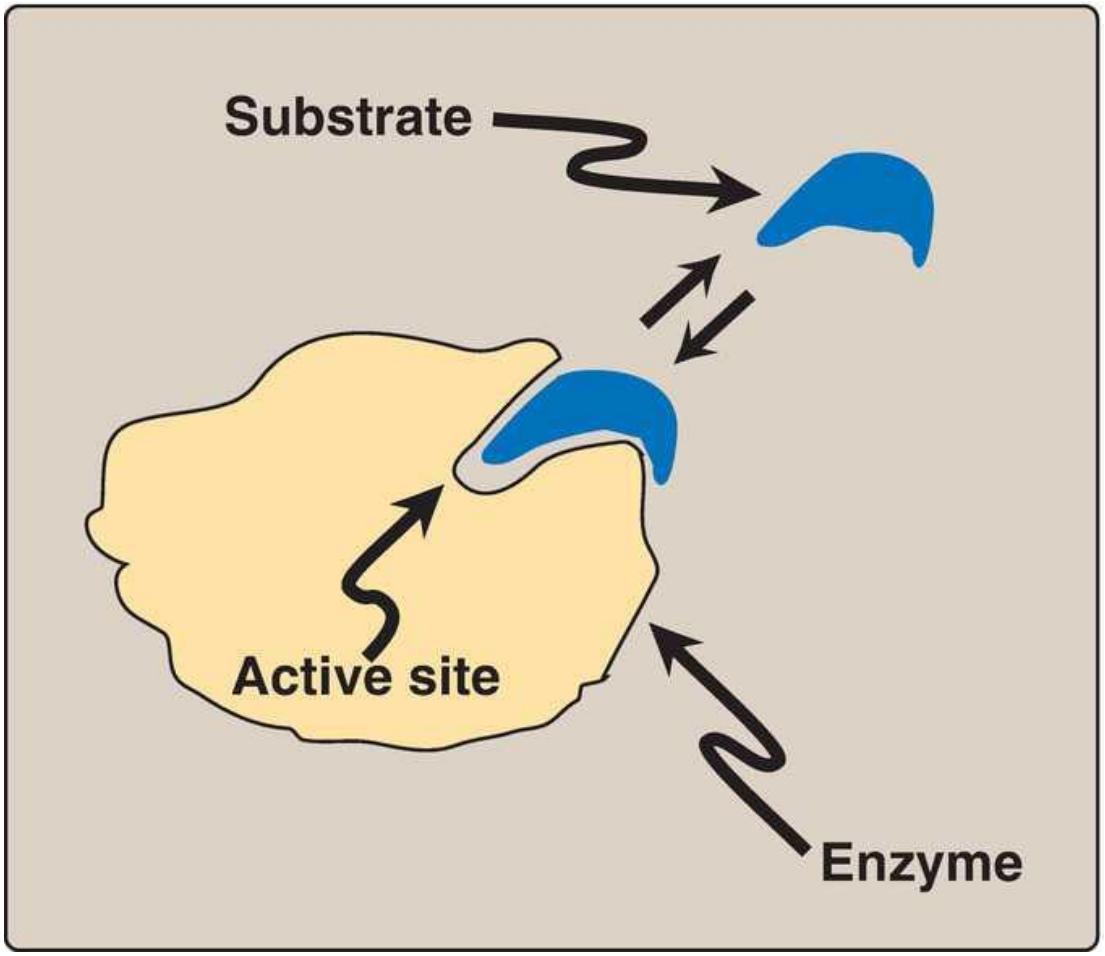
iii. The coenzyme is essential for the biological activity of the enzyme. A coenzyme is a low molecular weight organic substance, without which the enzyme cannot exhibit any reaction. One molecule of the coenzyme is able to convert a large number of substrate molecules with the help of enzyme.

iv. Coenzymes may be divided into: (a) Those taking part in reactions catalyzed by *oxidoreductases* by donating or accepting hydrogen atoms or electrons. (b) Those coenzymes taking part in reactions transferring groups other than hydrogen.

PROPERTIES

A. Active site

Enzyme molecules contain a special pocket or cleft called the active site. The active site, formed by folding of the protein, contains amino acid side chains that participate in substrate binding and catalysis (Fig. below). The substrate binds the enzyme non covalently, forming an enzyme substrate (ES) complex. Binding is thought to cause a conformational change in the enzyme (induced fit model) that allows catalysis. ES is converted to an enzyme–product (EP) complex that subsequently dissociates to enzyme and product.



Schematic representation of an enzyme with one active site binding a substrate molecule.

B. Efficiency

Enzyme-catalyzed reactions are highly efficient, proceeding from 10^3 to 10^8 times faster than un-catalyzed reactions. The number of substrate molecules converted to product per enzyme molecule per second is called the turnover number, or k_{cat}, and typically is 10^2–10^4 s^{-1}. [Note: k_{cat} is the rate constant for the conversion of ES to E + P.

C. Specificity

Enzymes are highly specific, interacting with one or a few substrates and catalyzing only one type of chemical reaction. The set of enzymes made in a cell determines which reactions occur in that cell.

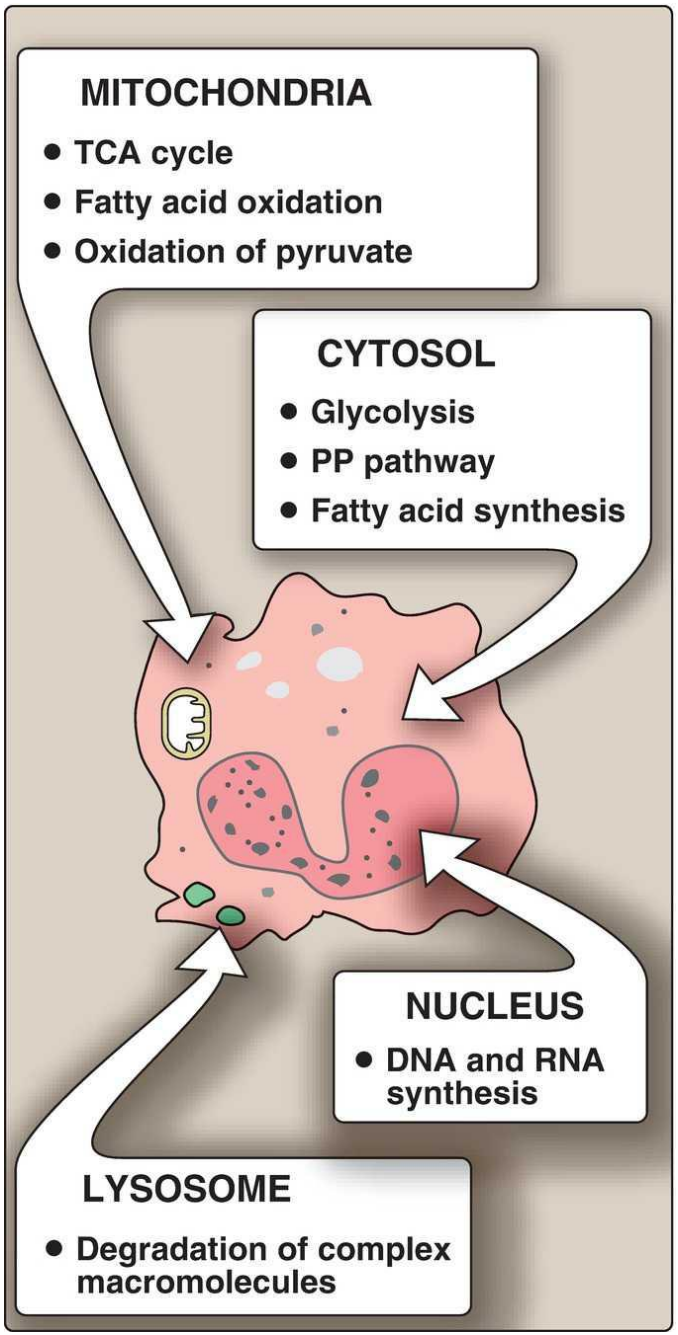
E. Regulation

Enzyme activity can be regulated, that is, increased or decreased, so that the rate of product formation responds to cellular need.

F. Location within the cell

Many enzymes are localized in specific organelles within the cell (Fig. below). Such compartmentalization serves to isolate the reaction substrate or product from other competing reactions. This provides a favorable environment for the reaction and organizes the thousands of enzymes present in the cell into purposeful pathways.

The intracellular location of some important biochemical pathways. TCA tricarboxylic acid; PP = pentose phosphate.



MECHANISM OF ENZYME ACTION

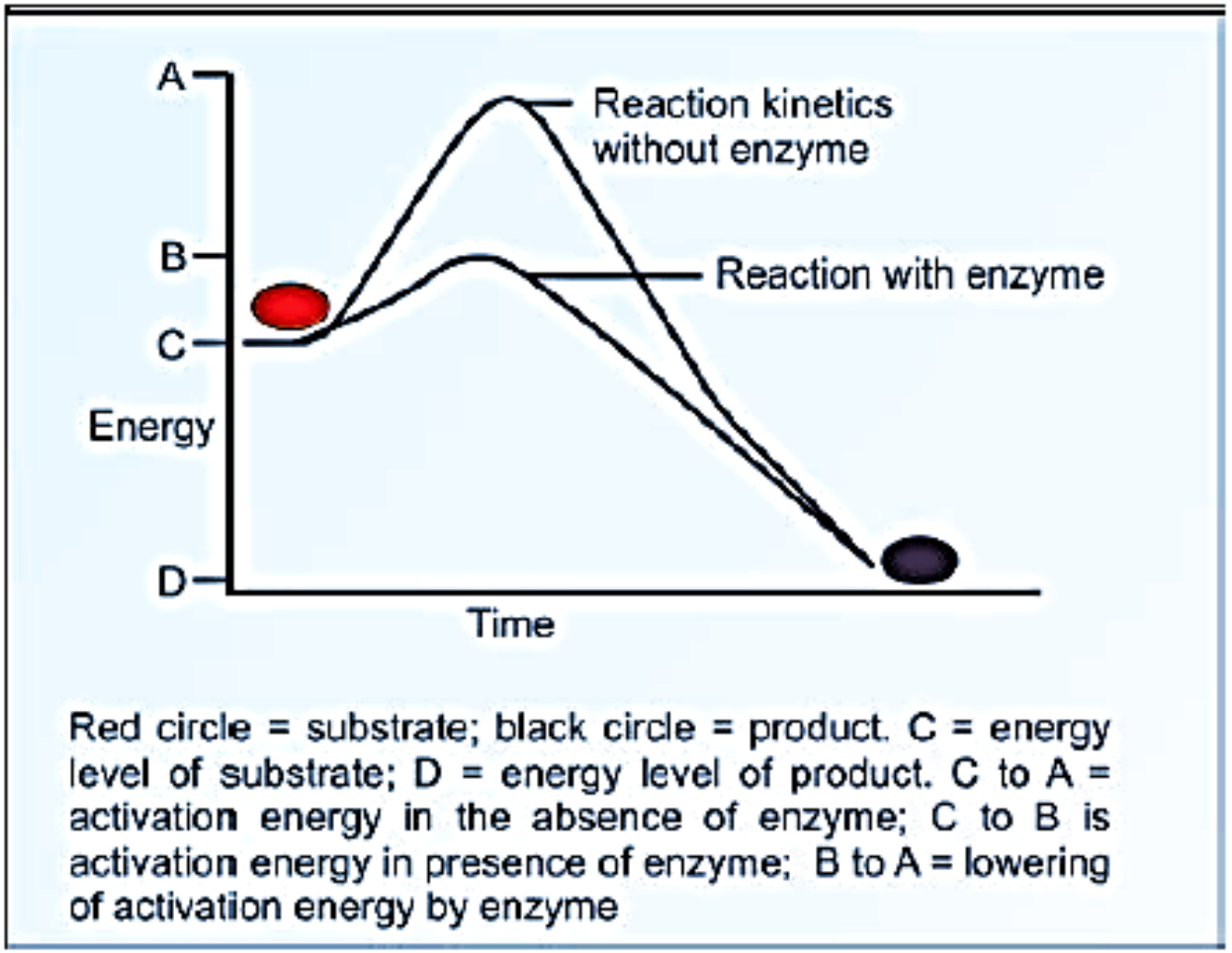
The mechanism of enzyme action can be viewed from two different perspectives. The first treats catalysis in terms of energy changes that occur during the reaction. That is, enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction. The second perspective describes how the active site chemically facilitates catalysis.

1. Lowering of Activation Energy

i. Substrates are remaining in an energy trough, and are to be placed at a higher energy level, where upon spontaneous degradation can occur. Suppose, we want to make a fire; even if we keep a flame, the wood will not burn initially; we have to add kerosene or paper for initial burning. Similarly, the activation energy is to be initially supplied.

ii. **Activation energy** is defined as the energy required to convert all molecules in one mole of a reacting substance from the ground state to the transition state.

iii. Enzymes reduce the magnitude of this activation energy. This can be compared to making a tunnel in a mountain, so that the barrier could be lowered (Fig. below). For example, activation energy for hydrolysis of sucrose by H^+ is 26,000 cal/mol, while the activation energy is only 9,000 cal/mol when hydrolyzed by sucrase.

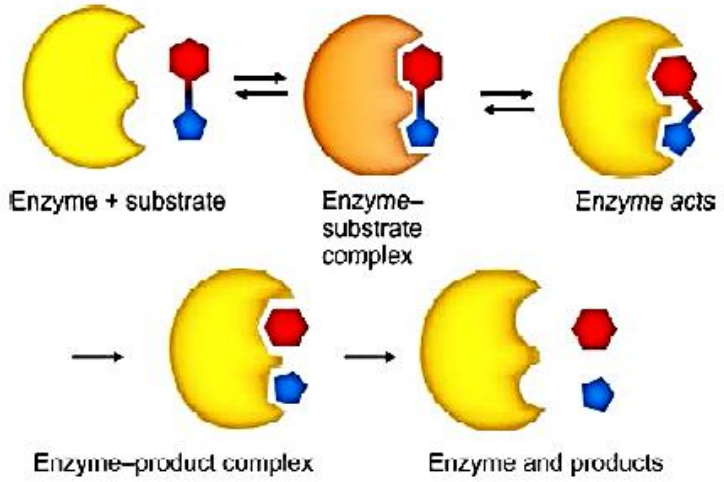


Lowering of activation energy by enzymes

2. Michaelis-Menten Theory

Lenor Michaelis and Maud Menten (1913) put forward the **Enzyme-Substrate complex theory**. The enzyme (E) combines with the substrate (S), to form an enzyme-substrate (ES) complex, which immediately breaks down to the enzyme and the product (P) (Fig. below).

$$E + S \longrightarrow E\text{–}S\ Complex \longrightarrow E + P$$



3. Fischer's Template Theory

i. It states that the three dimensional structure of the active site of the enzyme is complementary to the substrate. Thus *enzyme and substrate fit each other* (Fig. below).

ii. The explanation is that substrate fits on the enzyme, similar to **lock and key**. The key will fit only to its own lock.

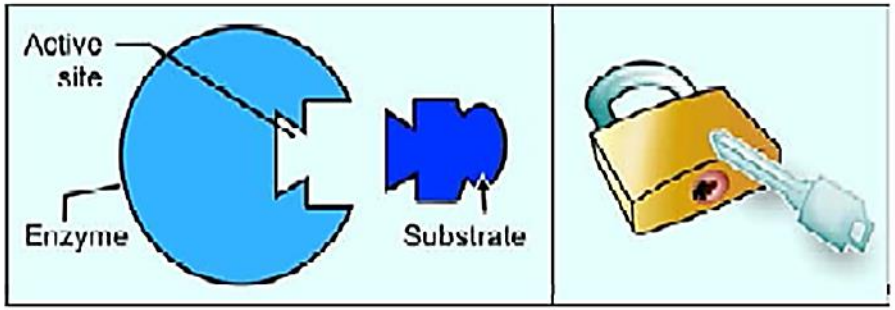
4. Koshland's Induced Fit Theory

i. Conformational changes are occurring at the active site of enzymes concomitant with the combination of enzyme with the substrate.

ii. At first, substrate binds to a specific part of the enzyme, and this leads to more secondary binding and conformational changes.

iii. The *substrate induces conformational changes in the enzyme*, such that precise orientation of catalytic groups is effected.

iv. When substrate analog is fixed to the enzyme, some structural alteration may occur; but reaction does not take place due to lack of proper alignment. Allosteric inhibition can also be explained by the hypothesis of Koshland.



Enzyme and substrate are specific to each other. This is similar to key and lock (Fischer's theory).

ACTIVE SITE OR ACTIVE CENTER

That area of the enzyme where catalysis occurs is referred to as active site or active center. For example, Serine is the important amino acid at the catalytic site of Trypsin. Salient features of the active sites of the enzymes are:

1. Although all parts are required for keeping the exact three dimensional structure of the enzyme, the reaction is taking place at the active site.
2. The active site occupies only a small portion of the whole enzyme. Generally active site is situated in a crevice or cleft of the enzyme molecule. The amino acids or groups that directly participate in making or breaking the bonds (present at the active site) are called catalytic residues or catalytic groups.
3. To the active site, the specific substrate is bound. The binding of substrate to active site depends on the alignment of specific groups or atoms at active site.
4. During the binding, these groups may realign themselves to provide the unique conformational orientation so as to promote exact fitting of substrate to the active site.
5. Proteolytic enzymes having a serine residue at the active center are called **serine proteases**, e.g. Trypsin,

THERMODYNAMICS

1. Exergonic or Exothermic Reaction

Here energy is released from the reaction, and therefore reaction essentially goes to completion, e.g. **urease** enzyme:

$$\text{Urea} \longrightarrow \text{ammonia} + CO_2 + \text{energy}$$

At equilibrium of this reaction, the substrate will be only 0.5% and product will be 99.5%. Such reactions are generally irreversible.

2. Isothermic Reaction

When energy exchange is negligible, and the reaction is easily reversible, e.g.

$$\text{Glycogen} + \text{Pi} \longrightarrow \text{Glucose-1-phosphate}$$

At equilibrium of this reaction, 77% glycogen will be unutilized and 23% glucose-1-phosphate will be formed.

3. Endergonic or Endothermic Reaction

Energy is consumed and external energy is to be supplied for these reactions. In the body this isusually accomplished by coupling the endergonic reaction with an exergonic reaction, e.g. **hexokinase reaction**

$$\text{Glucose} + \text{ATP} \longrightarrow \text{Glucose-6-Phosphate} + \text{ADP}$$

Inhibition

To ensure that the body's systems work correctly, it is sometimes necessary to slow down enzyme function. For instance, if an enzyme makes too much of a product, then the body needs a way to reduce or stop the production.

Several factors can limit enzyme activity levels, including:

Competitive inhibitors: This inhibitor molecule blocks the active site so that the substrate has to compete with the inhibitor to attach to the enzyme.

Non-competitive inhibitors: This molecule binds to an enzyme somewhere other than the active site and reduces how effectively it works.

Uncompetitive inhibitors: This inhibitor binds to the enzyme and substrate. The products leave the active site less easily, which slows the reaction.

Irreversible inhibitors: This is an irreversible inhibitor, which binds to an enzyme and permanently inactivates it.

Examples of specific enzymes

Thousands of enzymes in the human body exist to perform around 5,000 different functions. A few examples include:

Lipases: This group of enzymes help digest fats in the gut.

Amylase: In the saliva, amylase helps change starches into sugars.

Maltase: This also occurs in the saliva, and breaks the sugar maltose into glucose.

Trypsin: These enzymes break proteins down into amino acids in the small intestine.

Lactase: Lactase breaks lactose, the sugar in milk, into glucose and galactose.

Acetylcholinesterase: These enzymes break down the neurotransmitter acetylcholine in nerves and muscles.

Helicase: Helicase enzymes unravel DNA.

DNA polymerase: These enzymes synthesize DNA from deoxyribonucleotides.