Enzymes

Enzymes are pure proteins that act as biological catalysts (biocatalysts). Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products.

Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life. Metabolic pathways depend upon enzymes to catalyze individual steps.

Enzymes are known to catalyze more than 5,000 biochemical reaction types. Like all catalysts, enzymes increase the reaction rate by lowering its activation energy. Some enzymes can make their conversion of substrate to product occur many millions of times faster.

Enzyme activity can be affected by other molecules: inhibitors are molecules that decrease enzyme activity, and activators are molecules that increase activity. Many therapeutic drugs and poisons are enzyme inhibitors. An enzyme's activity decreases markedly outside its optimal temperature and pH, and many enzymes are (permanently) denatured when exposed to excessive heat, losing their structure and catalytic properties. Some enzymes are used commercially, for example, in the synthesis of antibiotics. Some household products use enzymes to speed up chemical reactions: enzymes in biological washing powders break down protein, starch or fat stains on clothes, and enzymes in meat tenderizer break down proteins into smaller molecules, making the meat easier to chew.

Chemical Nature of Enzymes

Enzymes are generally globular proteins, acting alone or in larger complexes. The sequence of the amino acids specifies the structure which in turn determines the catalytic activity of the enzyme.

active site: is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of amino acid residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site).

Apoenzymes: inactive form of enzymes, are still able to bind substrate with an affinity comparable to holoenzyme, but they are not able to transform substrate into product

Holoenzymes: represent the apoenzyme bound to its necessary cofactors or prosthetic groups. Many enzymes require the presence of other compounds - cofactors - before their catalytic activity can be exerted. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) is called the holoenzyme.

Apoenzyme + Cofactor = Holoenzyme

Accordingly the cofactor may be:

1. A coenzyme - a non-protein organic substance which is dialyzable, thermostable and loosely attached to the protein part.

2. A prosthetic group - an organic substance which is dialyzable and thermostable which is firmly attached to the protein or apoenzyme portion.

3. A metal ion activator - these include K^{+}, Fe^{++}, Fe^{+++}, Cu^{++}, Co^{++}, Zn^{++}, Mn^{++}, Mg^{++}, Ca^{++}, and Mo^{+++}.

Classification and Nomenclature

Enzymes can be classified by two main criteria: either amino acid sequence similarity (and thus evolutionary relationship) or enzymatic activity.

Enzyme activity: An enzyme's name is often derived from its substrate or the chemical reaction it catalyzes, with the word ending in –ase, for examples, lactase, alcohol dehydrogenase and DNA polymerase.

Different enzymes that catalyze the same chemical reaction are called **isoenzymes**. The International Union of Biochemistry and Molecular Biology have developed a nomenclature for enzymes, the EC numbers (for "Enzyme Commission"). Each enzyme is described by "EC" followed by a sequence of four numbers which represent the hierarchy of enzymatic activity (from very general to very specific). That is, the first number broadly classifies the enzyme based on its mechanism while the other digits add more and more specificity.

The top-level classification is:

1- **Oxidoreductase**: catalyze oxidation/reduction reactions

2-**Transferase**: transfer a functional group (e.g. a methyl or phosphate group)

3-**Hydrolase**: catalyze the hydrolysis of various bonds

4-**Lyase**: cleave various bonds by means other than hydrolysis and oxidation

5-**Isomerase**: catalyze isomerization changes within a single molecule

6-**Ligase**: join two molecules with covalent bonds.

These sections are subdivided by other features such as the substrate, products, and chemical mechanism. An enzyme is fully specified by four numerical designations. For example, hexokinase (EC 2.7.1.1) is a transferase (EC 2) that adds a phosphate group (EC 2.7) to a hexose sugar, a molecule containing an alcohol group (EC 2.7.1), and the last number was specific code for enzyme working(EC 2.7.1.1).

Specificity of enzymes

Enzymes differ from most other catalysts by being much more specific. Enzymes' specificity comes from their unique three-dimensional structures.

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze.

A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

1-Absolute specificity - the enzyme will catalyze only one reaction.

2-Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

3-Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

4-Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

Enzyme Kinetics: The Enzyme Substrate Complex

A theory to explain the catalytic action of enzymes was proposed by the Swedish chemist Savante Arrhenius in 1888. He proposed that the substrate and enzyme formed some intermediate substance which is known as the enzyme substrate complex. The reaction can be represented as:

$$S + E \longrightarrow ES$$

Substrate Enzyme Enzyme substrate complex

If this reaction is combined with the original reaction equation , the following results:

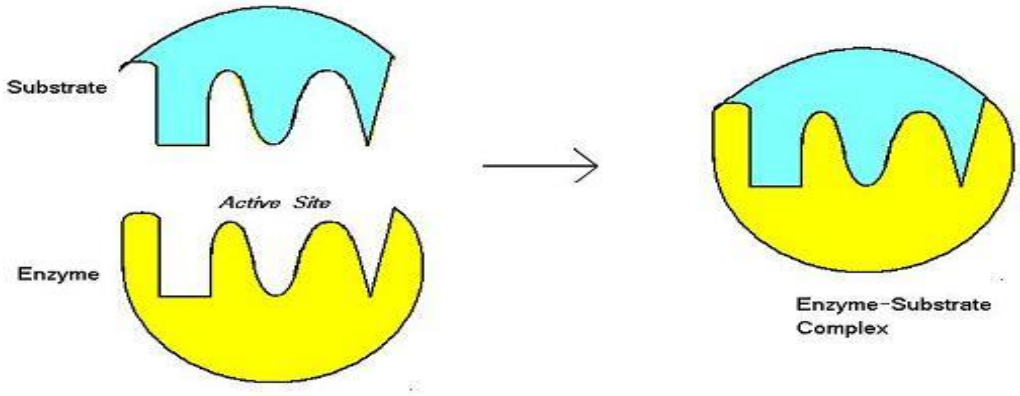
$$S + E \longrightarrow ES \longrightarrow P + E$$

Substrate Enzyme Enzyme substrate complex Product Enzyme

There are two hypotheses to explain the binding of the enzyme to the substrate

1-Lock and key model

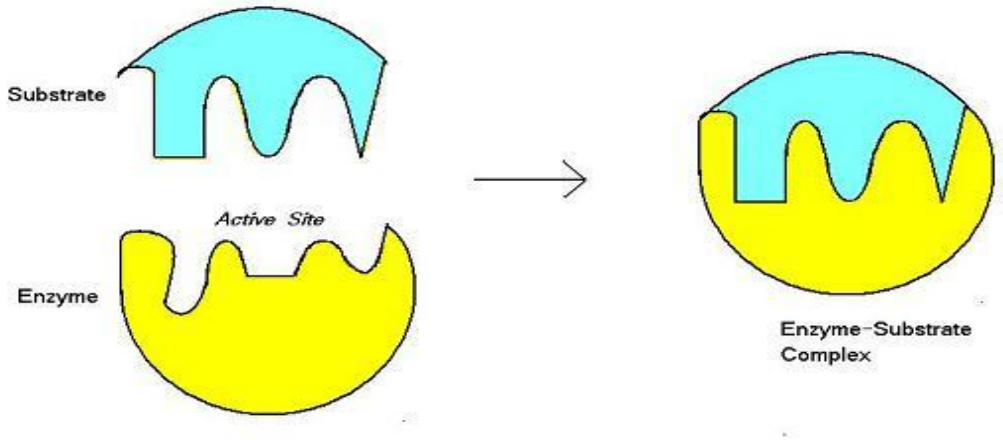
To explain the observed specificity of enzymes, in 1894 **Emil Fischer** proposed that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. This early model explains enzyme specificity, but fails to explain the stabilization of the transition state that enzymes achieve.



Lock-and-key Model.- The substrate and enzyme active site have complementary shapes

2-Induced fit model

In 1958, **Daniel Koshland** suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge distribution is determined. Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via the conformational proofreading mechanism.



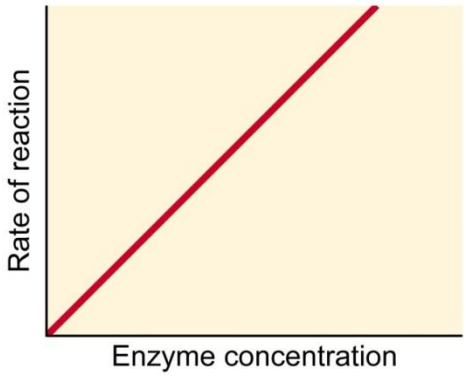
Induced-fit Model. - The enzyme active site forms a complementary shape to the substrate after binding.

Factors Affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect the rate at which enzymatic reactions precede - enzyme concentration, substrate concentration, temperature, pH, , and the presence of any inhibitors or activators.

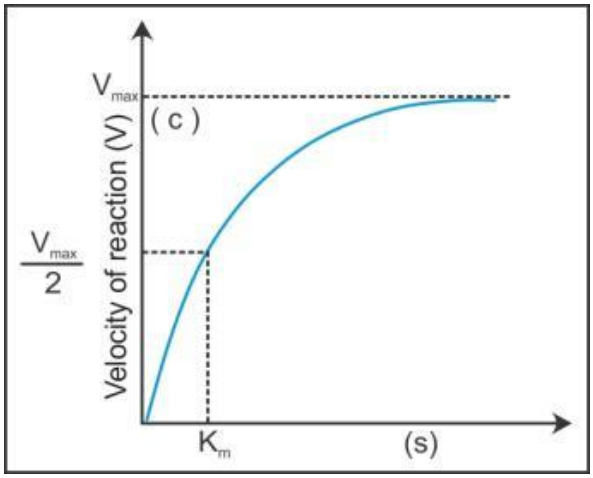
1-Enzyme concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present. Graphically this can be represented as:



2- substrate concentration

It has been shown experimentally that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity.



It is theorized that when this maximum velocity had been reached, all of the available enzyme has been converted to ES, the enzyme substrate complex. This point on the graph is designated V_{max}.

Michaelis-Menten developed a set of mathematical expressions to calculate enzyme activity in terms of reaction speed from measurable laboratory data.

$$v = \frac{v_{max}\,[S]}{K_m + [S]}$$

- v = the reaction rate
- v_{max} = maximum reaction velocity
- K_m = Michaelis–Menten constant
- $[S]$ = substrate concentration

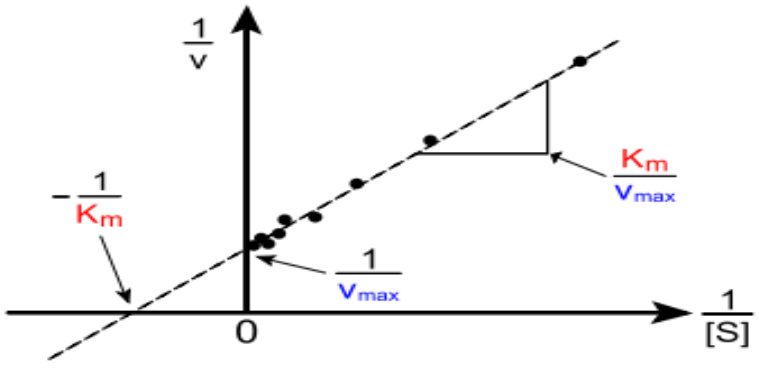
Michaelis constant(K_m) defines the quantity of the substrate when the velocity of the enzymatic reaction is half the maximum velocity.

Michaelis constants have been determined for many of the commonly used enzymes. The size of K_m tells us several things about a particular enzyme.

A small K_m indicates that the enzyme requires only a small amount of substrate to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations.

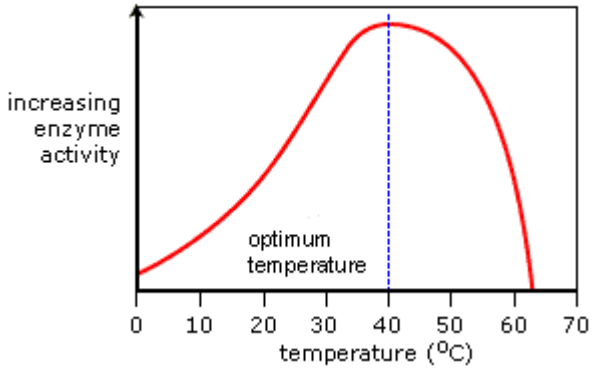
A large K_m indicates the need for high substrate concentrations to achieve maximum reaction velocity.

The **Lineweaver–Burk** plot is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934. The Lineweaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as K_m and V_{max}, before the wide availability of powerful computers and non-linear regression software. The y-intercept of such a graph is equivalent to the inverse of V_{max}; the x-intercept of the graph represents $-1/K_m$. It also gives a quick, visual impression of the different forms of enzyme inhibition.



3-Temperature

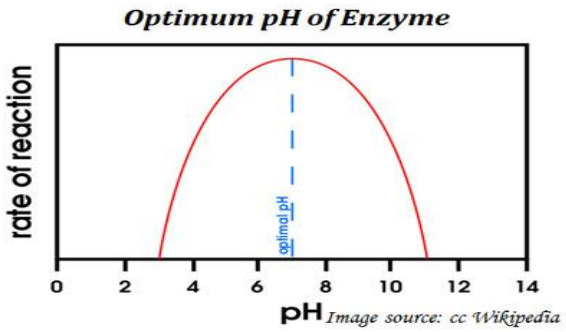
Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A ten degree Centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1 or 2 degrees may introduce changes of 10 to 20% in the results. In the case of enzymatic reactions, this is complicated by the fact that many enzymes are adversely affected by high temperatures. The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.



Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable. Some enzymes lose their activity when frozen.

4-pH effect

Enzymes are affected by changes in pH. The most favorable pH value - the point where the enzyme is most active - is known as the optimum pH.



Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.

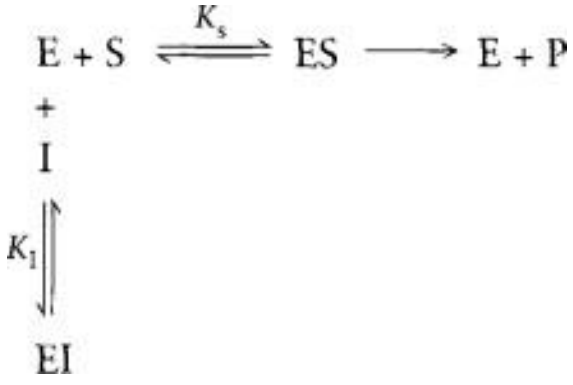
5-Inhibitors

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition.

Most theories concerning inhibition mechanisms are based on the existence of the enzyme-substrate complex ES. As mentioned earlier, the existence of temporary ES structures has been verified in the laboratory.

1-Competitive inhibition occurs when the substrate and a substance resembling the substrate are both added to the enzyme. A theory called the "lock-key theory" of enzyme catalysts can be used to explain why inhibition occurs.

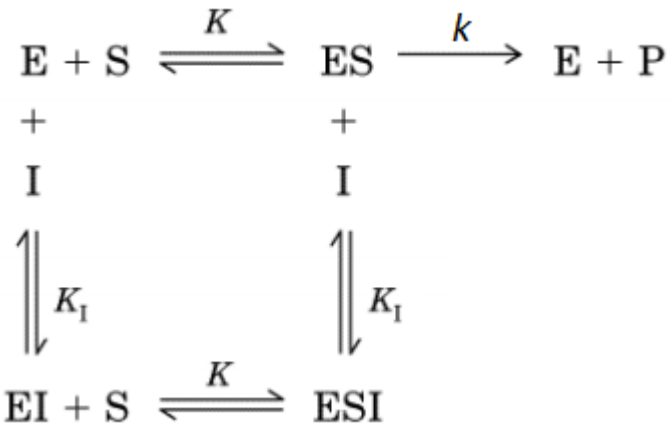
The lock and key theory utilizes the concept of an "active site." The concept holds that one particular portion of the enzyme surface has a strong affinity for the substrate. The substrate is held in such a way that its conversion to the reaction products is more favorable. If we consider the enzyme as the lock and the substrate the key (Figure 9) - the key is inserted in the lock, is turned, and the door is opened and the reaction proceeds. However, when an inhibitor which resembles the substrate is present, it will compete with the substrate for the position in the enzyme lock. When the inhibitor wins, it gains the lock position but is unable to open the lock. Hence, the observed reaction is slowed down because some of the available enzyme sites are occupied by the inhibitor. If a dissimilar substance which does not fit the site is present, the enzyme rejects it, accepts the substrate, and the reaction proceeds normally.



2-Non-competitive inhibitors are considered to be substances which when added to the enzyme alter the enzyme in a way that it cannot accept the substrate.

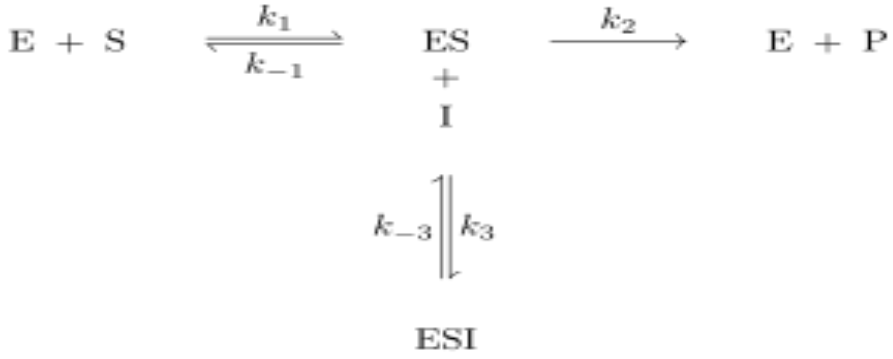
The inhibitor may bind to the enzyme whether or not the substrate has already been bound, but if it has a higher affinity for binding the enzyme in one state or the other, it is called a mixed inhibitor.

Non-competitive inhibition models a system where the inhibitor and the substrate may both be bound to the enzyme at any given time. When both the substrate and the inhibitor are bound, the enzyme-substrate-inhibitor complex cannot form product and can only be converted back to the enzyme-substrate complex or the enzyme-inhibitor complex. Non-competitive inhibition is distinguished from general mixed inhibition in that the inhibitor has an equal affinity for the enzyme and the enzyme-substrate complex.

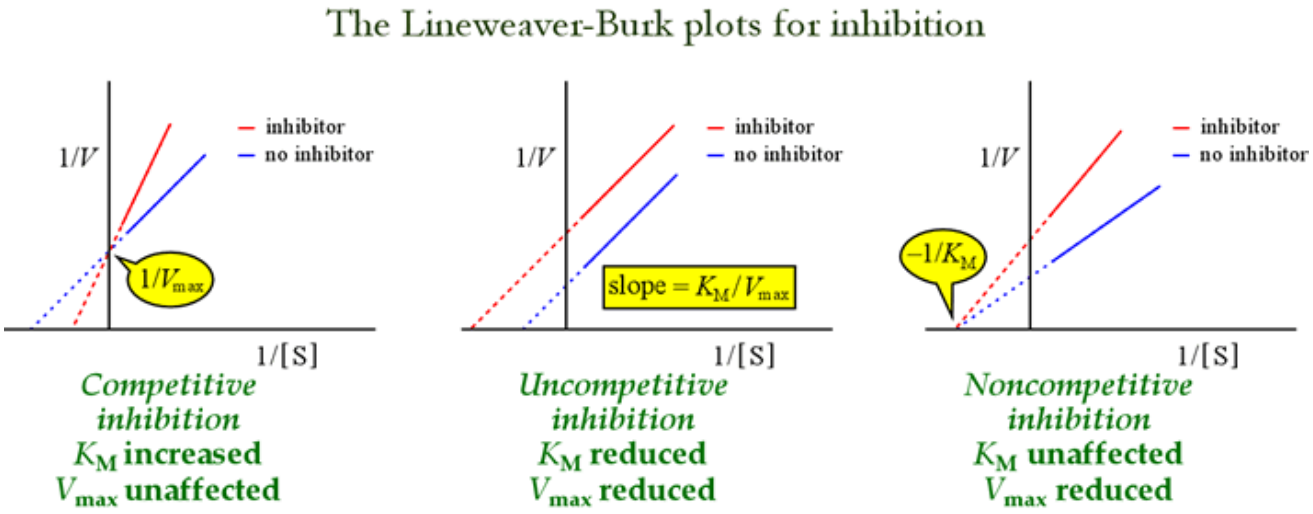


3-Uncompetitive inhibition, also known as anti-competitive inhibition, takes place when an enzyme inhibitor binds only to the complex formed between the enzyme and the substrate (the E-S complex). Uncompetitive inhibition typically occurs in reactions with two or more substrates or products. While uncompetitive inhibition requires that an enzyme-substrate complex must be formed, non-competitive inhibition can occur with or without the substrate present.

Uncompetitive inhibition is distinguished from competitive inhibition by two observations: first uncompetitive inhibition cannot be reversed by increasing [S] and second, as shown, the Lineweaver–Burk plot yields parallel rather than intersecting lines.



In general, uncompetitive inhibition works best when substrate concentration is high. An uncompetitive inhibitor need not resemble the substrate of the reaction it is inhibiting. At no concentration of substrate will the activity of the enzyme be higher when an uncompetitive inhibitor is present, but at low concentrations of substrate the enzyme activity difference will be negligible.

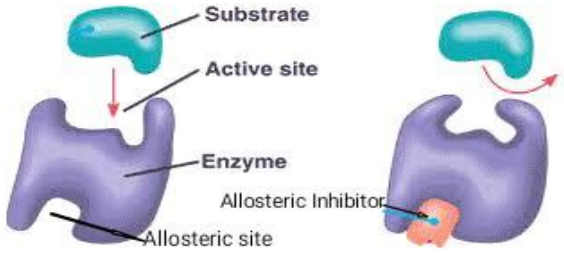


Allosteric Enzyme

Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector (allosteric modulator) which results in an apparent change in binding affinity at a different ligand-binding site. This "action at a distance" through binding of one ligand affecting the binding of another at a distinctly different site, is the essence of the allosteric concept. Allosteric regulation plays an important role in

many basic biological processes, including but not limited to cell signaling and metabolism regulation. In biochemistry, allosteric regulation (or allosteric control) is the regulation of a protein by binding an effector molecule at a site other than the enzyme's active site call.

The site to which the effector binds is termed the **allosteric site**. Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change involving protein dynamics. Effectors that enhance the protein's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors.



Allosteric enzymes produce a sigmoid shaped graph which shows a rapid increase/decrease over a narrow range of Substrate change.