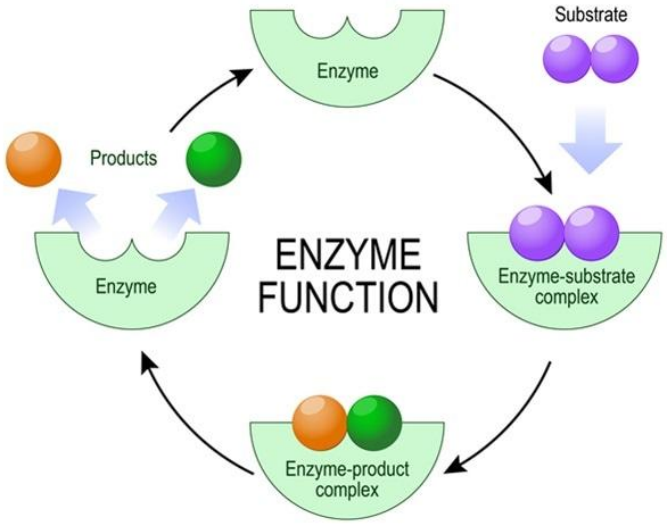
Chapter Two :

Enzymes

Enzymes are biological catalysts. There are about 40,000 different enzymes in human cells, each controlling a different chemical reaction. They increase the rate of reactions by a factor of between 10^{6} to 10^{12} times . Enzymes are proteins they are highly selective catalysts.



The common names for most enzymes derive from their most distinctive characteristic: their ability to catalyze a specific chemical reaction. In general, an enzyme's name consists of a term that identifies the type of reaction catalyzed followed by the suffix-*ase.* For example, dehydrogen*ase*s remove hydrogen atoms, prote*ase*s hydrolyze proteins, and isomer*ase*s catalyze rearrangements in configuration.

the International Union of Biochemists (IUB) enzymes are grouped into six classes.

Listed below are the six IUB classes of enzymes and the reactions they catalyze.

catalyze oxidations and reductions

catalyze transfer of groups such as methyl or glycosyl groups from a donor molecule to an acceptor molecule.

catalyze the hydrolytic cleavage of C-C, C-O, C-N, P-O, and certain other bonds.

catalyze cleavage of C=C, C=O, C=N, and other bonds by elimination, leaving double bonds, and also add groups to double bonds.

:catalyze geometric or structural changes within a single molecule.

catalyze the joining together of two molecules

PROSTHETIC GROUPS, COFACTORS, & COENZYMES PLAY IMPORTANT ROLES IN CATALYSIS

Many enzymes contain small nonprotein molecules and metal ions that participate directly in substrate binding or catalysis. Termed **prosthetic groups, cofactors,** and **coenzymes**

Prosthetic groups are distinguished by their tight, stable incorporation into a protein's structure by covalent or noncovalent forces. Examples include pyridoxal phosphate, flavin mononucleotide (FMN), flavin dinucleotide(FAD), thiamin pyrophosphate, biotin, and the metal ions of Co, Cu, Mg, Mn, Se, and Zn. Metals are the most common prosthetic groups. The roughly one-third of all enzymes that contain tightly bound metal ions are termed **metalloenzymes.**

Cofactors serve functions similar to those of prosthetic groups but bind in a transient, dissociable manner either to the enzyme or to a substrate such as ATP. Unlike the stably associated prosthetic groups, cofactors therefore must be present in the medium surrounding the enzyme for catalysis to occur. The most common cofactors also are metal ions. Enzymes that require a metal ion cofactor are termed **metal-activated enzymes** to distinguish them from the **metalloenzymes** for which metal ions serve as prosthetic groups.

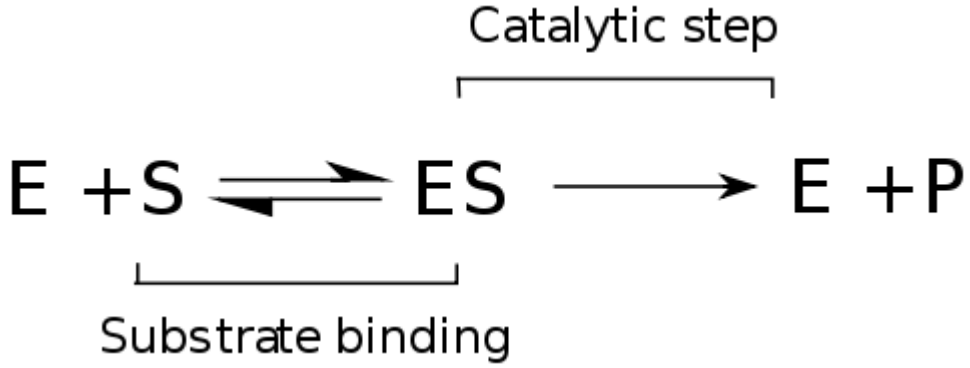
Coenzymes serve group transfer reagents—that transport many substrates from their point of generation to their point of utilization. Association with the coenzyme also stabilizes substrates such as hydrogen atoms or hydride ions that are unstable in the aqueous environment of the cell. Other chemical moieties transported by coenzymes include methyl groups (folates) and acyl groups (coenzyme A)

Specificity

Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereospecificity, regioselectivity and chemoselectivity.

How enzymes work

Enzymes have an **active site**. This is part of the molecule that has just the right shape and functional groups to bind to one of the reacting molecules. The reacting molecule that binds to the enzyme is called the **substrate**.



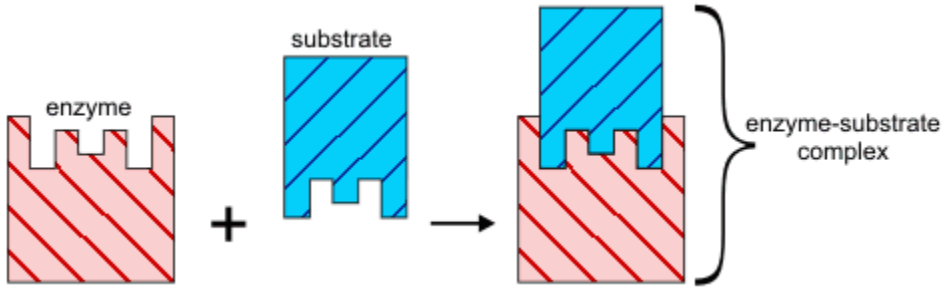
In biochemistry, **Michaelis–Menten kinetics** is one of the best-known models of enzyme kinetics. It is named after German biochemist Leonor Michaelis and Canadian physician Maud Menten. The model takes the form of an equation describing the rate of enzymatic reactions, by relating reaction rate $v$to $[S]$, the concentration of a substrate *S*. Its formula is given by

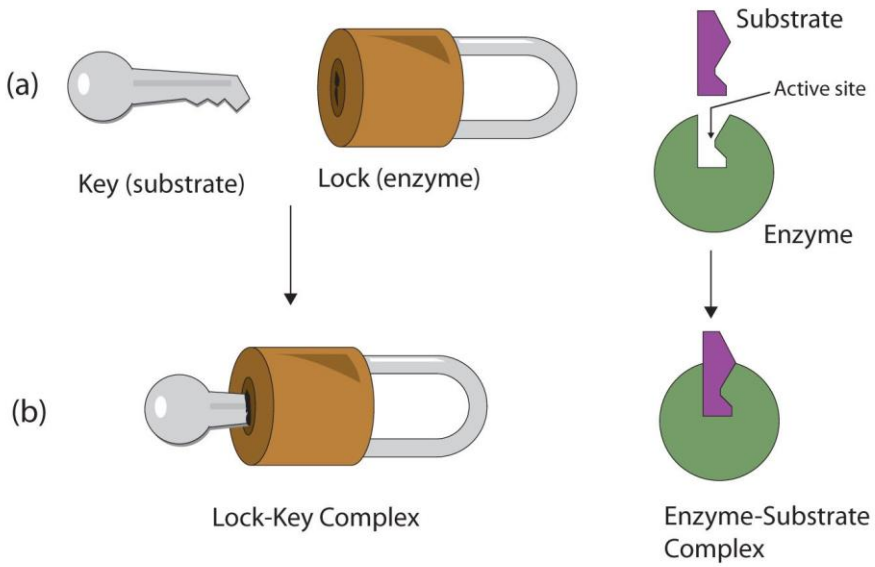
$$v = \frac{d[P]}{dt} = \frac{V_{\max}[S]}{K_m + [S]}.$$

Here, $V_{\max}$represents the maximum rate achieved by the system, at maximum (saturating) substrate concentrations. The Michaelis constant K_m is the substrate concentration at which the reaction rate is half of $V_{\max}$. Biochemical reactions involving a single substrate are often assumed to follow Michaelis–Menten kinetics, without regard to the model's underlying

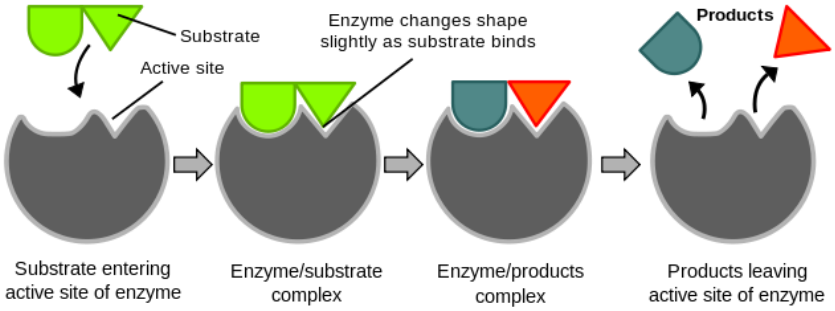
Lock and key model

Enzymes are very specific, and it was suggested by Emil Fischer in 1894 that this was because 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. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve.



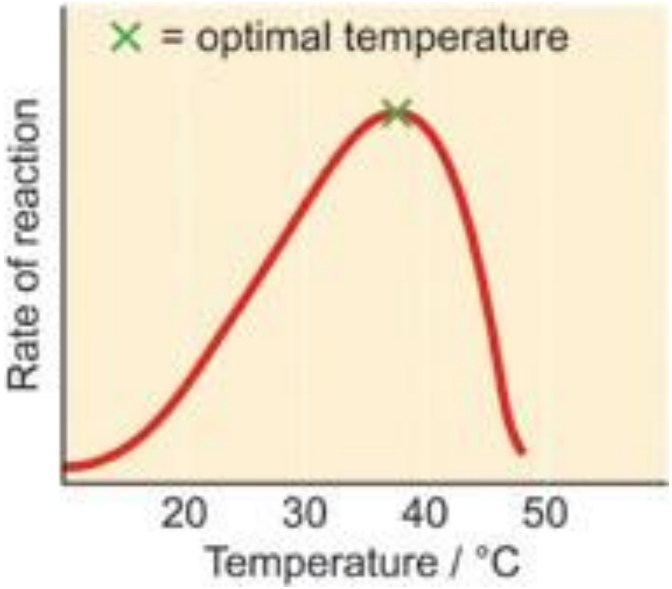


In this model the enzyme molecule changes shape as the substrate molecules gets close. The change in shape is 'induced' by the approaching substrate molecule. This more sophisticated model relies on the fact that molecules are flexible because single covalent bonds are free to rotate.



Factors affecting catalytic activity of enzymes

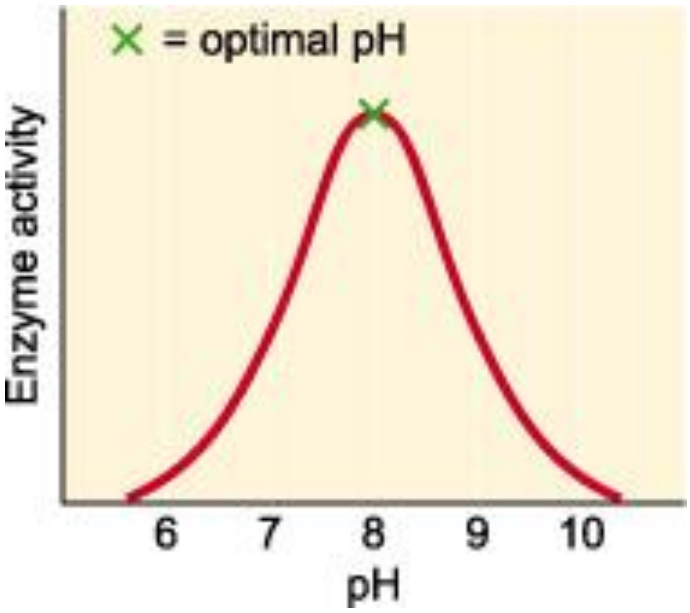
Temperature:



As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (37.5 ^{o}C) for the enzymes in human cells.

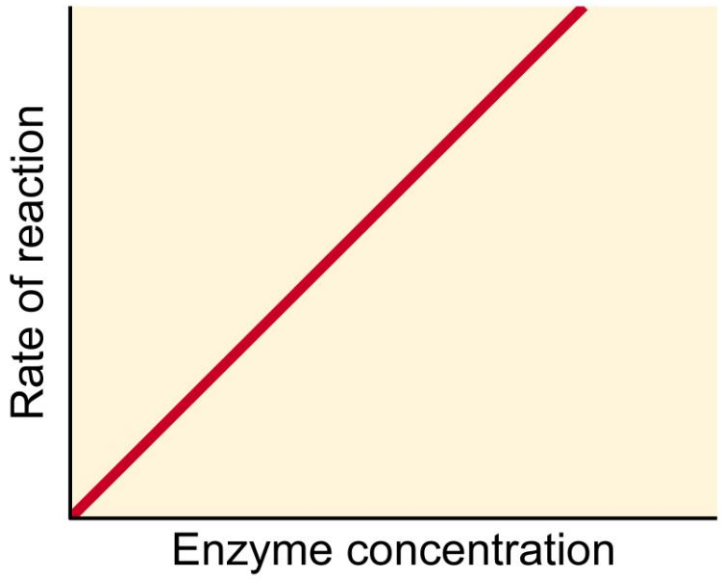
Above this temperature the enzyme structure begins to break down (denature) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

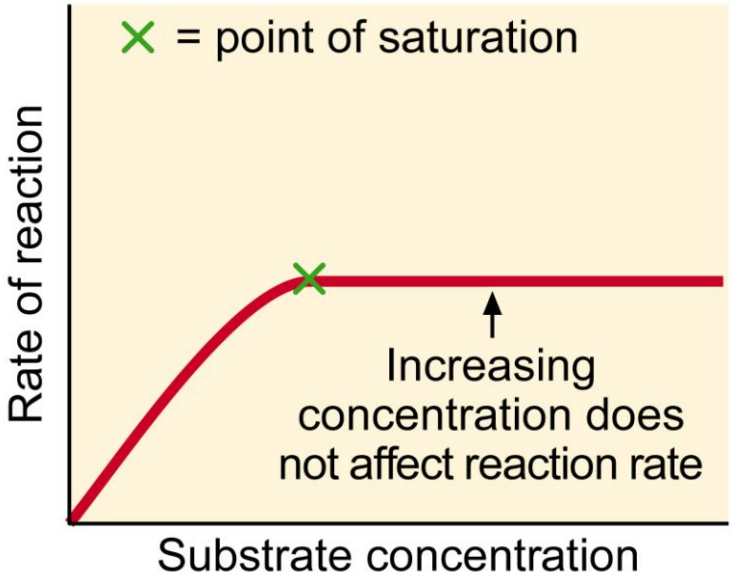
pH:



Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.

Concentration of enzyme and substrate:



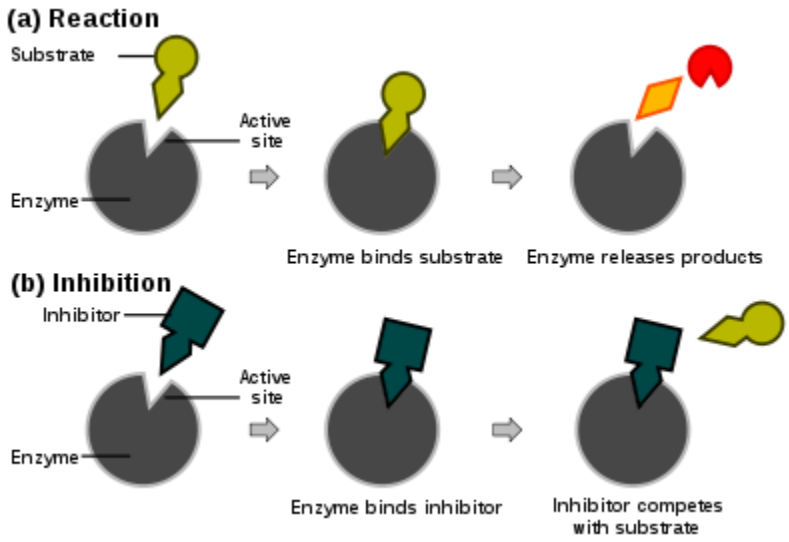


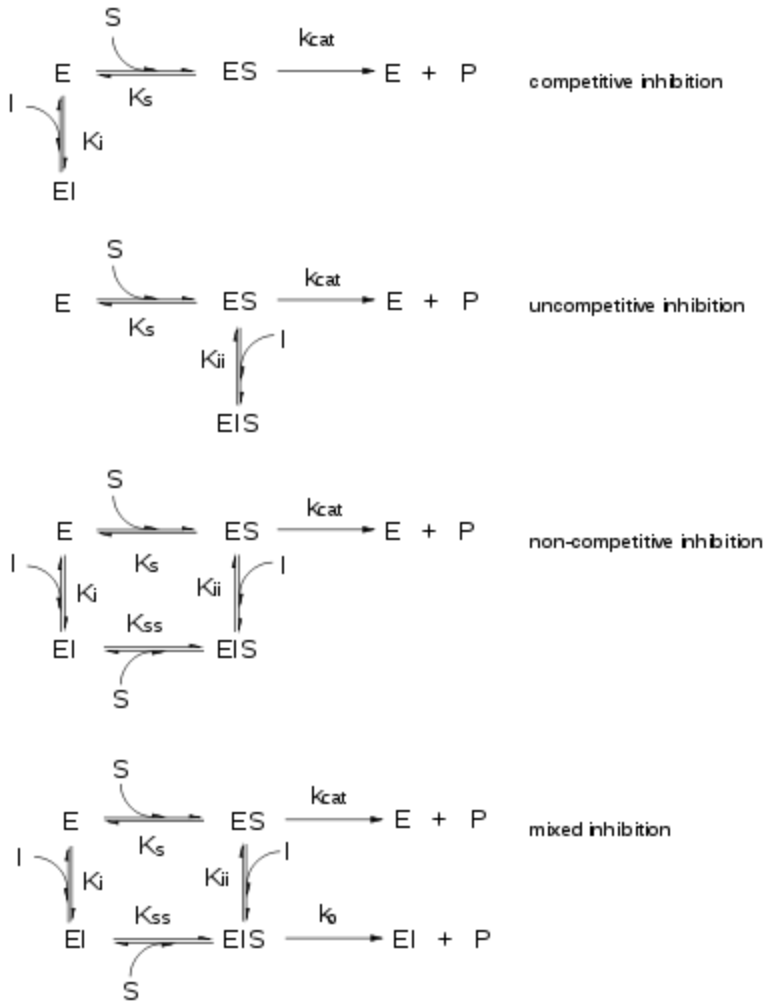
The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate .

Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration.

Inhibition

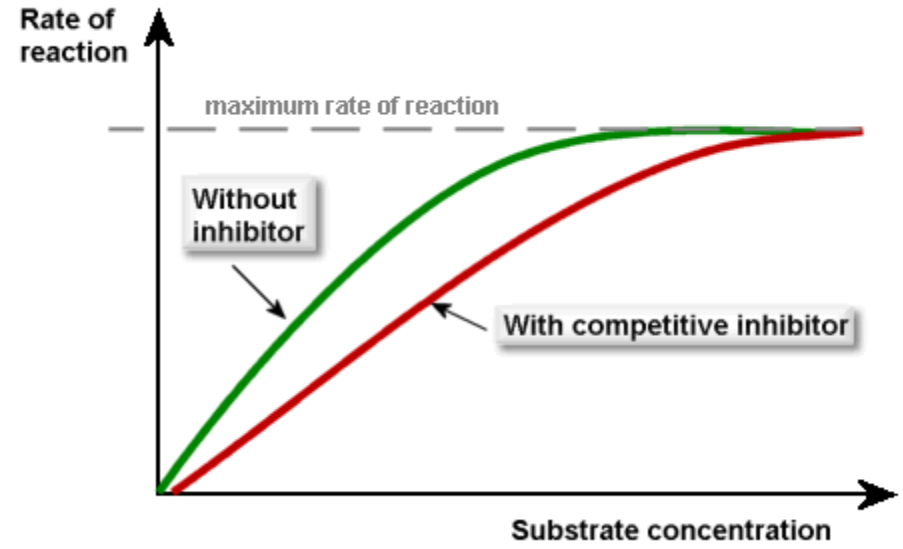




Types of inhibition.

Enzyme reaction rates can be decreased by various types of enzyme inhibitors.

Competitive inhibitors bind reversibly to the enzyme, preventing the binding of substrate. On the other hand, binding of substrate prevents binding of the inhibitor. Substrate and inhibitor compete for the enzyme.
(i.e., they can not bind at the same time). Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate. In competitive inhibition the maximal rate of the reaction is not changed, but higher substrate concentrations are required to reach a given maximum rate, increasing the apparent K_m.



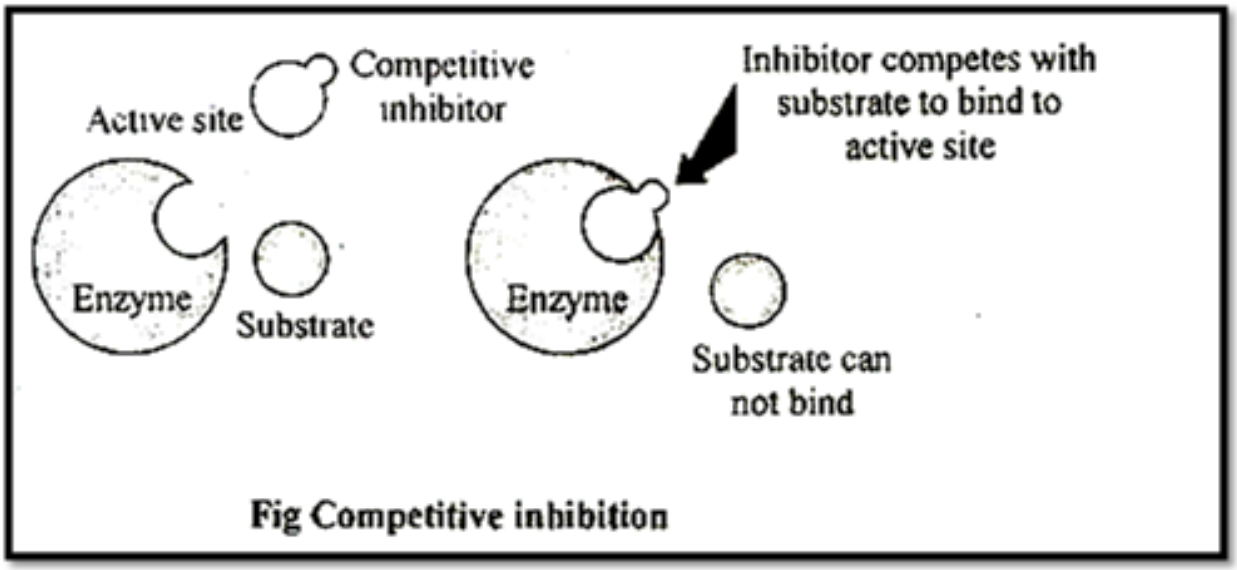
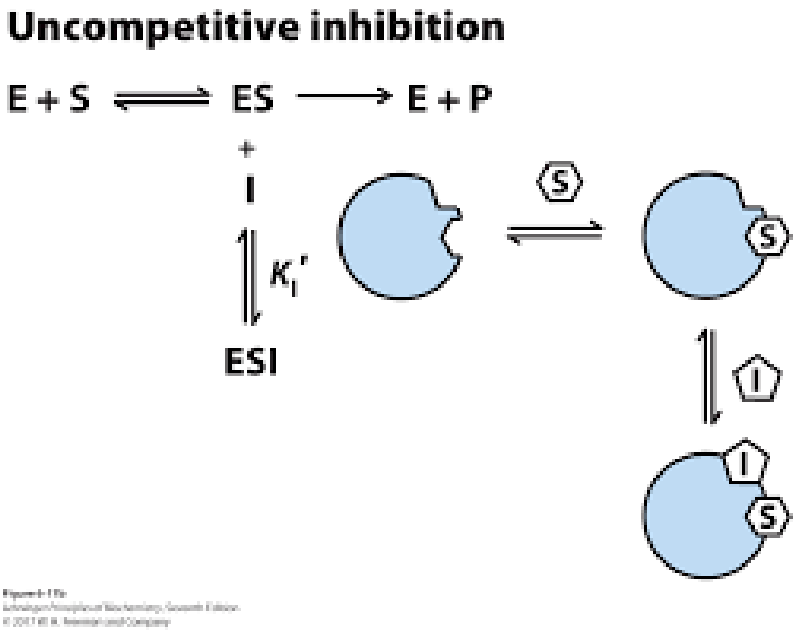


Fig Competitive inhibition

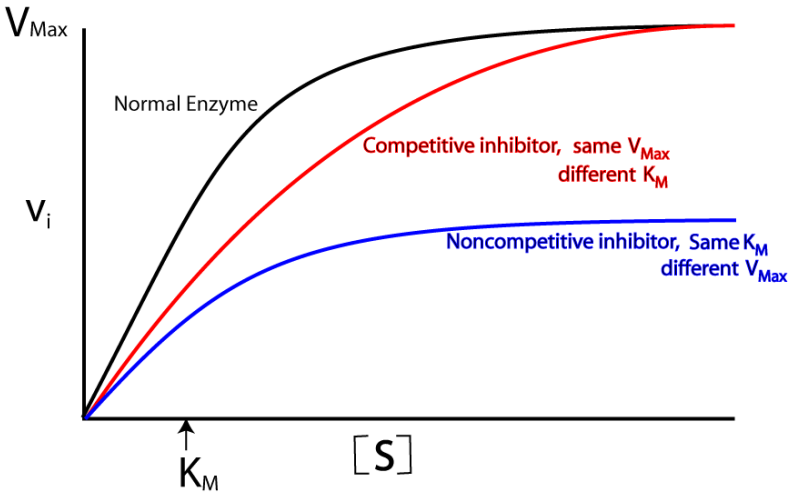
Uncompetitive inhibition

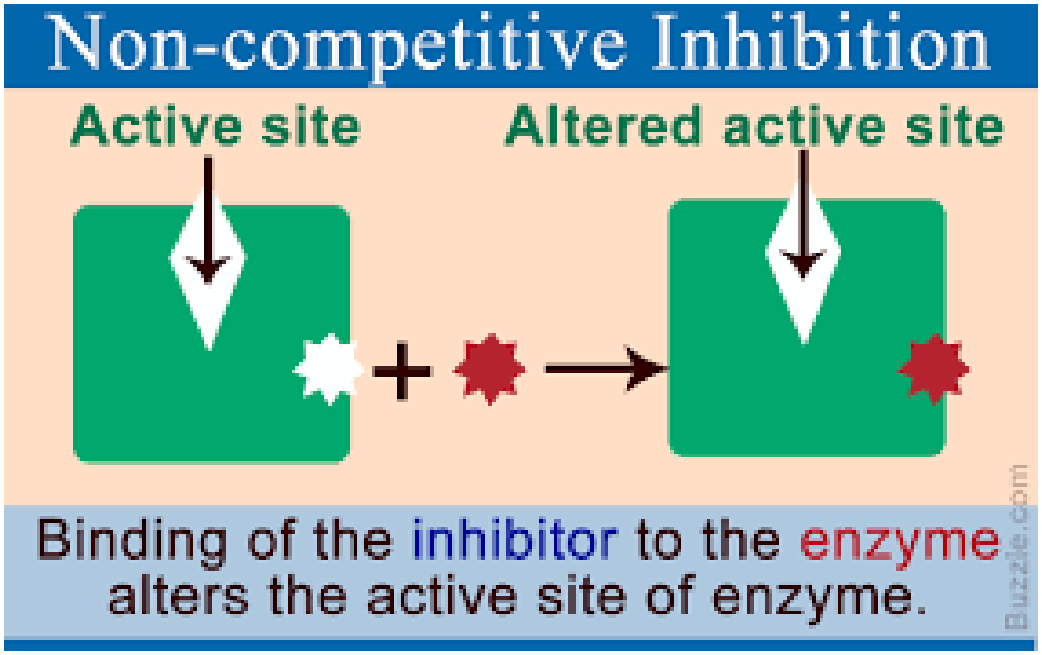
In uncompetitive inhibition, the inhibitor cannot bind to the free enzyme, only to the ES-complex. The EIS-complex thus formed is enzymatically inactive. This type of inhibition is rare.



Non-competitive inhibition

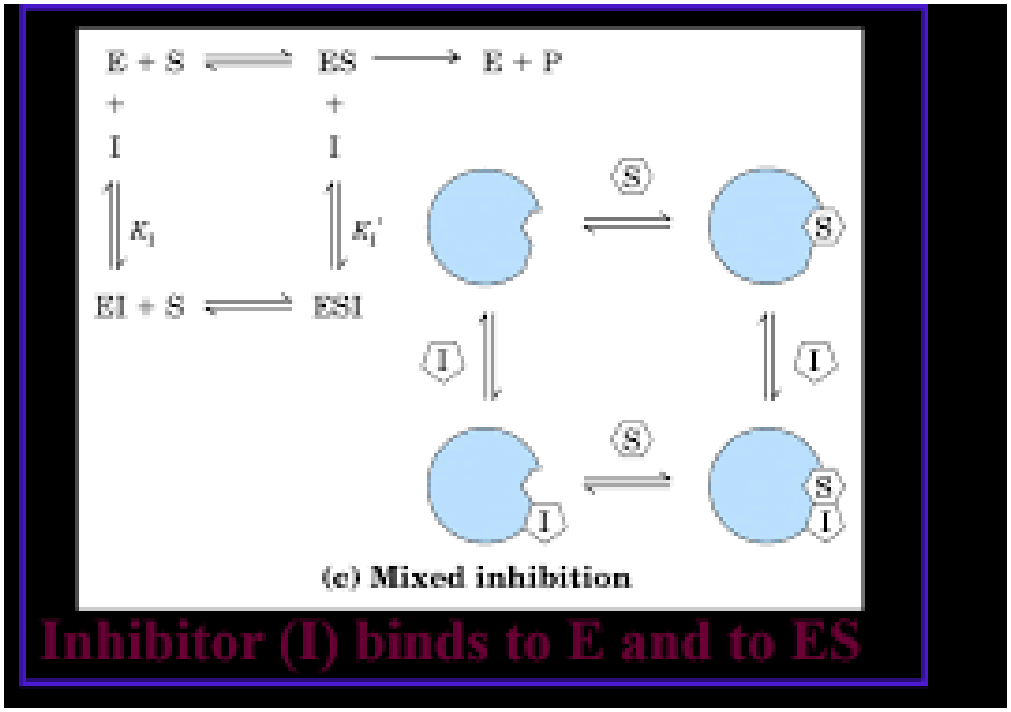
Non-competitive inhibitors can bind to the enzyme at the binding site at the same time as the substrate,but not to the active site. Both the EI and EIS complexes are enzymatically inactive. Because the inhibitor can not be driven from the enzyme by higher substrate concentration (in contrast to competitive inhibition), the apparent V_{max} changes. But because the substrate can still bind to the enzyme, the K_m stays the same.





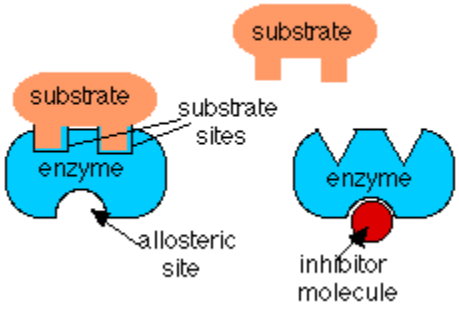
Mixed inhibition

This type of inhibition resembles the non-competitive, except that the EIS-complex has residual enzymatic activity.This type of inhibitor does not follow Michaelis-Menten equation.



Allosteric enzyme:

Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector, 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. **Allostery** plays a crucial role in many fundamental biological processes, including but not limited to cell signaling and the regulation of metabolism. Allosteric enzymes need not be oligomers as previously thought. and in fact many systems have demonstrated allostery within single enzymes



Isoenzymes:

Isozymes (also known as **isoenzymes** or more generally as **Multiple forms of enzymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different K_M values), or different regulatory properties. for example lactate dehydrogenase (LDH)). In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes. In many cases, they are coded for by homologous genes that have diverged over time.

Feedback Inhibition

If the product of a series of enzymatic reactions, e.g., an amino acid, begins to accumulate within the cell, it may specifically inhibit the action of the first enzyme involved in its synthesis . Thus further production of the enzyme is halted.