Enzymes

Enzymes define as converting starting molecules (substrates) into different molecules (products). Enzymes are biologic polymers that catalyze the chemical reactions. Generally they are much larger than their substrates and bind with them by means of active sites created by the specific three-dimensional folding of the protein. Deficiencies in the quantity or catalytic activity of key enzymes can result from genetic defects, nutritional deficits, or toxins. Defective enzymes can also result from genetic mutations or infection by viral or bacterial pathogens.

An important function of enzymes is in the digestive systems of animals. Enzymes such as amylases and proteases break down large molecules (starch or proteins, respectively) into smaller ones, so they can be absorbed by the intestines. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. Without enzymes, metabolism would neither progress through the same steps nor be fast enough to serve the needs of the cell. Indeed, a metabolic pathway such as glycolysis could not exist independently of enzymes.

Classification of Enzyme

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*ases* remove hydrogen atoms, prote*ases* hydrolyze proteins, and isomer*ases* catalyze rearrangements in configuration. While many modifiers name the specific substrate involved (xanthine oxidase), others identify the source of the enzyme (pancreatic ribonuclease). Enzymes are grouped into six classes, each with several subclasses. Listed below are the six International Union of Biochemistry (IUB) classes of enzymes and the reactions they catalyze:

1-Oxidoreductases: catalyze oxidations and reductions. Oxidoreductases catalyze the transfer of electrons from one molecule (the oxidant) to another molecule (the reductant) includes the dehydrogenases, reductases, oxidases, dioxidases.

2-Transferases: catalyze transfer of groups such as methyl groups from a donor molecule to an acceptor molecule include kinases, aminotransferases, acetyltransferases

3-Hydrolases: catalyze the hydrolytic cleavage of C-C, C-O, C-N, P-O, and certain other bonds include peptidases, esterases.

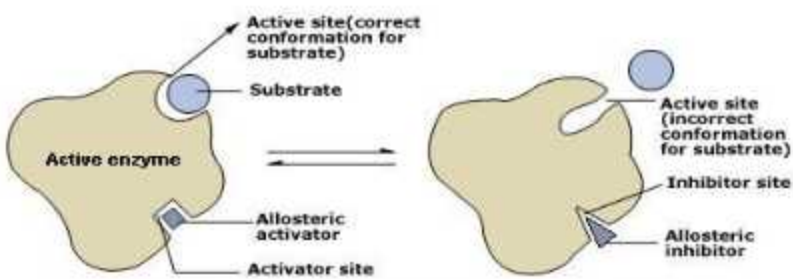
4-Lyases: catalyze cleavage of C-C, C-O, C-N, and other bonds by elimination, leaving double bonds include adenylyl cyclase.

5-Isomerases: catalyze geometric or structural changes within a single molecule include isomerase.

6-Ligases: catalyze the joining together of two molecules by forming a new chemical bond include form C-C, C-O, C-N include synthases.

Allosteric modulation

Allosteric sites are sites on the enzyme that bind to molecules in the cellular environment. The sites form weak, noncovalent bonds with these molecules, causing a change in the conformation of the enzyme. This change in conformation translates to the active site, which then affects the reaction rate of the enzyme. Allosteric interactions can both inhibit and activate enzymes and are a common way that enzymes are controlled in the body



Schematic representation of allosteric enzyme activity

Prosthetic groups, cofactors and coenzyme:

A cofactor is a non-protein chemical compound that is required for the protein's biological activity. These proteins are commonly enzymes, and cofactors can be considered "helper molecules" that assist in biochemical transformations. Cofactors can be subdivided into either inorganic ions or a complex organic molecule called **coenzyme**.

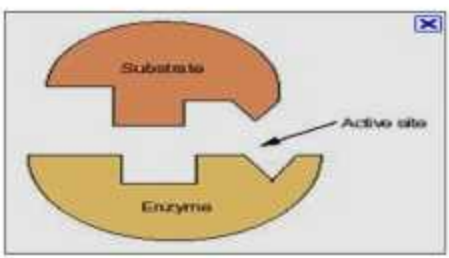
The prosthetic group may be organic (such as a vitamin, sugar, nucleic acid, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids. Prosthetic groups are bound tightly to enzymes and may even be attached through a covalent bond, as opposed to coenzymes.

An inactive enzyme without the cofactor is called an **apoenzyme**, while the complete enzyme with cofactor is called **a holoenzyme.**

Some enzymes or enzyme complexes require several cofactors. For example, pyruvate dehydrogenase at the junction of glycolysis and the citric acid cycle requires organic cofactors and one metal ion: loosely bound thiamine pyrophosphate (TPP), covalently bound flavin adenine dinucleotide (FAD), and the cosubstrates nicotinamide adenine dinucleotide (NAD^+) and coenzyme A (CoA), and a metal ion (Mg^{2+}).

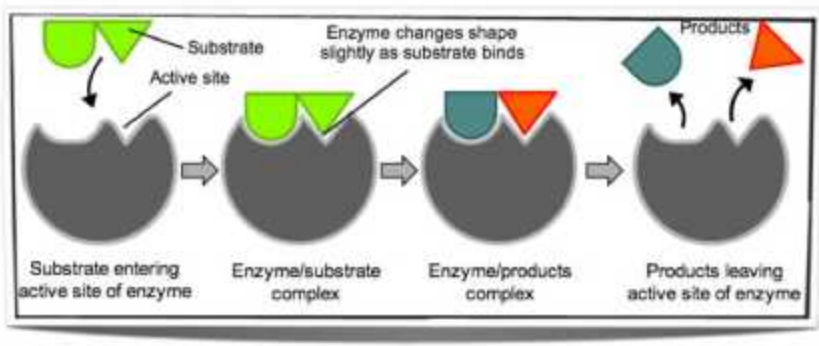
Substrates induce conformational changes in enzyme

Emil Fischer proposed **lock and key model** that mean both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another.



The **induced fit model** suggested by Daniel Koshland in 1958 is the more accepted model for enzyme-substrate complex than 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. The active site continues to change until the substrate is completely bound, at which point the final shape and charge distribution is determined.



Isozymes

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. (eg. hexokinase and glucokinase). Also LD is a tetramer which can be assembled from two subunits, H (for heart) and M (for muscle). The five forms are therefore H4, H3M, H2M2, HM3 and M4 which can be separated by electrophoresis and shown to have different affinities for their substrates, lactate and pyruvate, and for analogues of these two compounds.

Enzyme-Linked Immunoassays

Enzymelinked immunoassays (ELISAs) use antibodies covalently linked to a "reporter enzyme" such as alkaline phosphatase or horseradish peroxidase, enzymes whose products are readily detected.

Clinical Applications Of Cellular Enzymes

All of the hundreds of enzymes present in the human body are synthesized intracellularly, and most of them function within the cell in which they were formed. These are the enzymes responsible for catalyzing the metabolic reactions (either catabolism and anabolism) occurring in each cell. Some are secreted in an inactive form and are rendered active in the extracellular fluids where they function. Those that function in the bloodstream are called plasma-specific enzymes. Increases of concentrations of cellular enzymes can be good indicators because the intracellular concentration of enzymes is hundreds or thousands of times greater than in blood .

Enzymes Kinetics

Biochemical importance

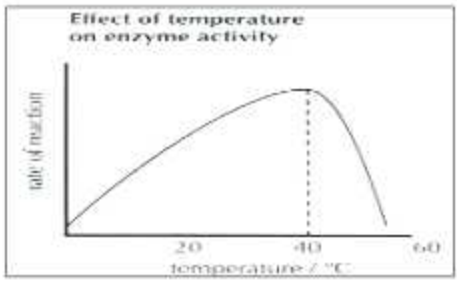
Enzyme kinetics is the investigation of how enzymes bind substrates and turn them into products is the field of biochemistry concerned with the quantitative measurement of the rates of enzyme catalyzed reactions and the systematic study of factors that affect these rates. Kinetic analyses permit scientists to reconstruct the **number** and **order** of the individual steps by which enzymes transform substrates into products.

The study of enzyme kinetics also represents the principal way to identify potential therapeutic agents that selectively enhance or inhibit the rates of specific enzyme-catalyzed processes. An understanding of enzyme kinetics thus is important for understanding how physiologic stresses such as anoxia, metabolic acidosis or alkalosis, toxins, and pharmacologic agents affect that balance.

Multiple factors affect the rates of enzyme catalyzed reactions:

1-Temperature

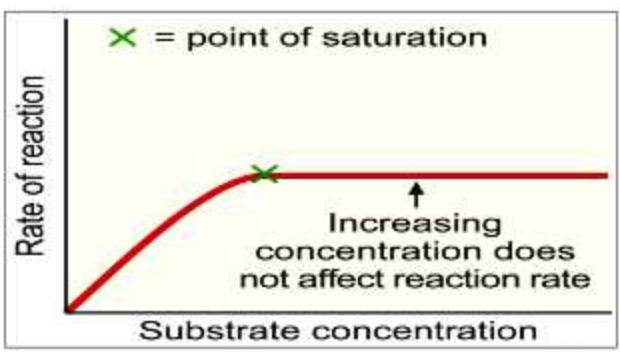
A ten degree Centigrade rise in *temperature* will increase the activity of enzyme by 50 to 100% but many enzymes are denaturated by high *temperatures*.



Temperature concentration affect on rates of reaction

2-Substrate concentration

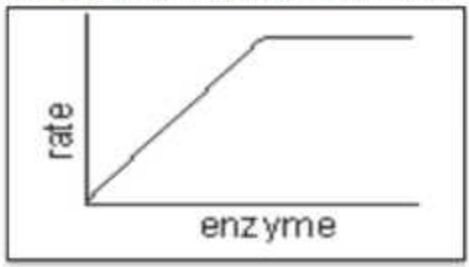
For a typical enzyme, as substrate concentration is increased, vi increases until it reaches a maximum value of rate (V_{max}) . When further increases in substrate concentration do not further increase vi, the enzyme is said to be "saturated" with substrate.



Substrate concentration affect on rates of reaction

3-Enzyme concentration

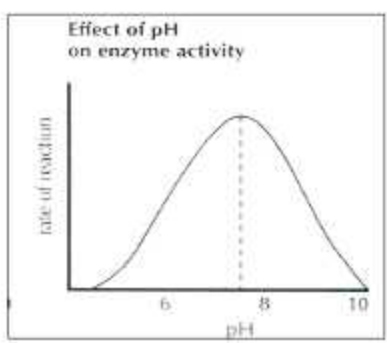
Increasing Enzyme Concentration will increase the rate of reaction, as more enzymes will be colliding with substrate molecules



Enzyme concentration affect on rates of reaction

4-Hydrogen Ion Concentration (PH)

The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration. Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.



Effect of pH affect on rates of reaction

Michaelis-Menten Equation

The general reaction of an enzyme is E+S⇌ES⇌E+P and the velocity of the reaction is given by the Michaelis-Menten equation:

$$v_i = \frac{V_{max}[S]}{K_m + [S]}$$

V: velocity

$\mathbf{V_{max}}$ **:** maximum velocity

S: substrate

$\mathbf{K_m}$**:** Michaelis constant

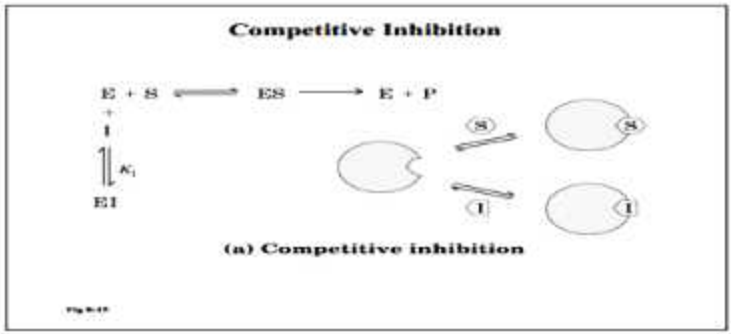
Inhibitors enzymes

Inhibitors of the catalytic activities of enzymes provide both pharmacologic agents as drugs and research tools for study of the mechanism of enzyme action. The strength of the interaction between an inhibitor and an enzyme depends on forces important in protein structure and ligand binding (hydrogen bonds, electrostatic interactions, hydrophobic interactions, and van der Waals forces). Inhibitors can be classified based upon their site of action on the enzyme, or on the kinetic parameters they influence:

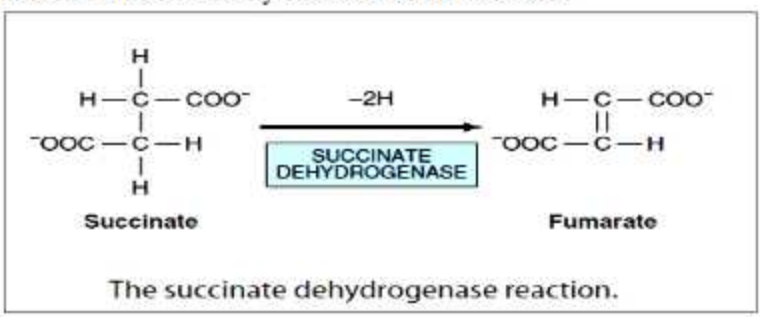
1- Reversible inhibitor: Reversible inhibitors combine non-covalently with the enzyme and can therefore be readily removed by dialysis can be classified as:

A-Competitive Inhibitors

In competitive inhibition, the inhibitor and substrate compete for the enzyme and decreasing the number of free enzyme molecules available to bind substrate to form ES:



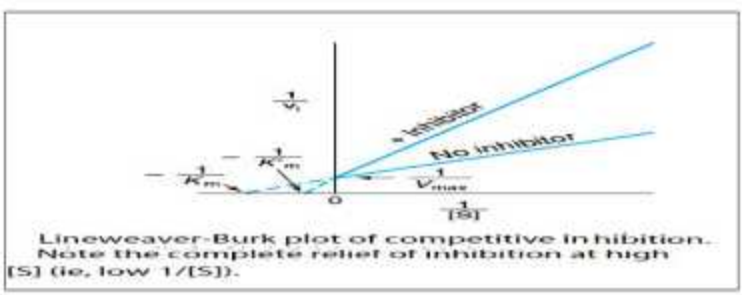
Succinate Dehydrogenase catalyzes the removal of one hydrogen atom from each of the two methylene carbons of succinate .



The succinate dehydrogenase reaction.

Lineweaver-Burk plot as described below:

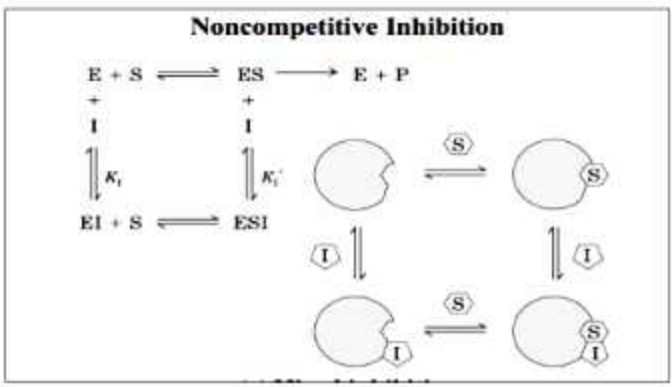
Since the *y* intercept is equal to1/*V*max, this pattern indicates that when 1/[S] approaches 0, *vi* is independent of the presence of inhibitor. a competitive inhibitor has no effect on *V*max but raises *K*'m, the apparent *K*m for the substrate.



Lineweaver-Burk plot of competitive inhibition. Note the complete relief of inhibition at high [S] (ie, low 1/[S]).

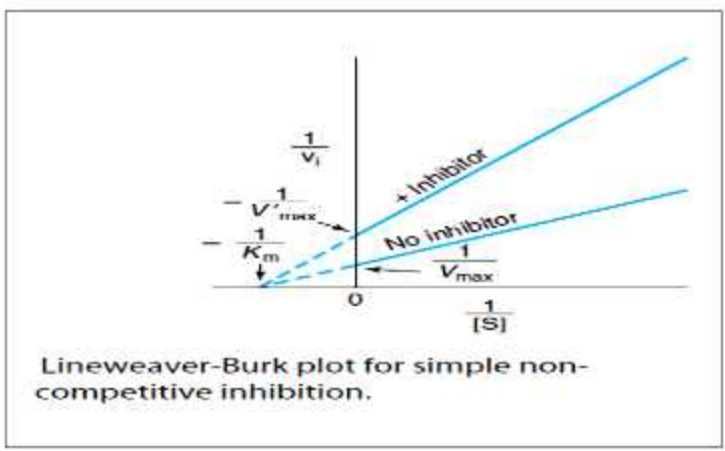
2-Noncompetitive Inhibitors

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).



Lineweaver-Burk plot:

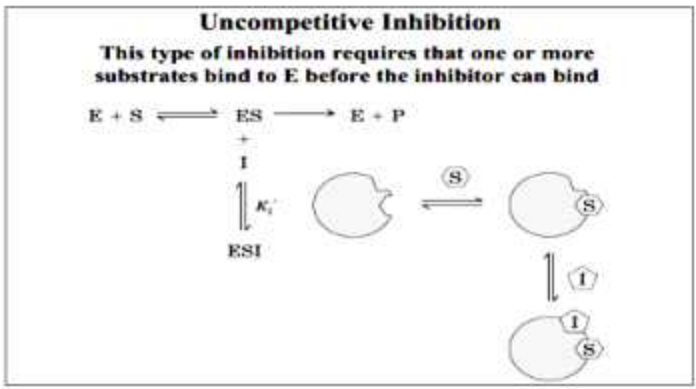
However, while the enzyme-inhibitor complex can still bind the substrate, its efficiency at transforming substrate to product, reflected by *V*max, is decreased, inhibitor has no effect on *K'*m.



Lineweaver-Burk plot for simple non-competitive inhibition.

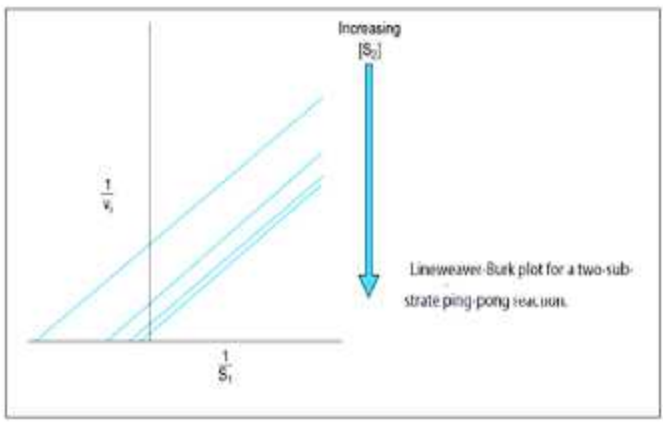
C-Uncompetitive inhibition

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, but may occur in multimeric enzymes.



For Lineweaver-Burk plot:

Reflected by *V*max, *K'*m.is decreased.



4-Irreversible Inhibitors "Poison" Enzymes

Active enzyme can be recovered simply by removing the inhibitor from the surrounding medium. However, a variety of other inhibitors act *irreversibly* by chemically modifying the enzyme. These modifications generally involve making or breaking covalent bonds with aminoacyl residues essential for substrate binding, catalysis, or maintenance of the enzyme's functional conformation. Since these covalent changes are relatively stable, an enzyme that has been "poisoned" by an irreversible inhibitor such as a heavy metal atom or an acylating reagent remains inhibited even after the removal of the remaining inhibitor from the surrounding medium. These compounds include eflornithine a drug used to treat the parasitic disease sleeping sickness. Penicillin and Aspirin also act in this manner. With these drugs, the compound is bound in the active site and the enzyme then converts the inhibitor into an activated form that reacts irreversibly with one or more amino acid residues

Uses of inhibitors

Since inhibitors modulate the function of enzymes they are often used as drugs. A common example of an inhibitor that is used as a drug is aspirin, which inhibits the enzymes that produce the inflammation messenger prostaglandin