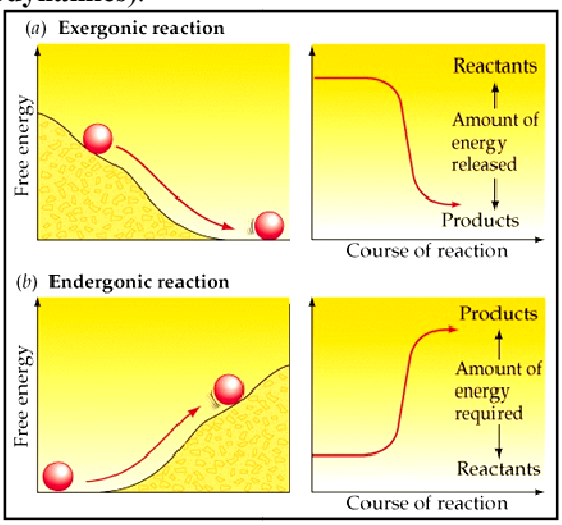
Enzymology

Endergonic and exergonic

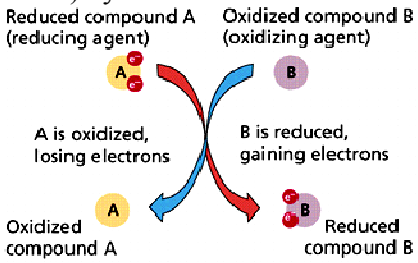
Energy releasing processes, ones that "generate" energy, are termed **exergonic reactions**. Reactions that require energy to initiate the reaction are known as **endergonic reactions**. All natural processes tend to proceed in such a direction that the disorder or randomness of the universe increases (the **second law of thermodynamics**).



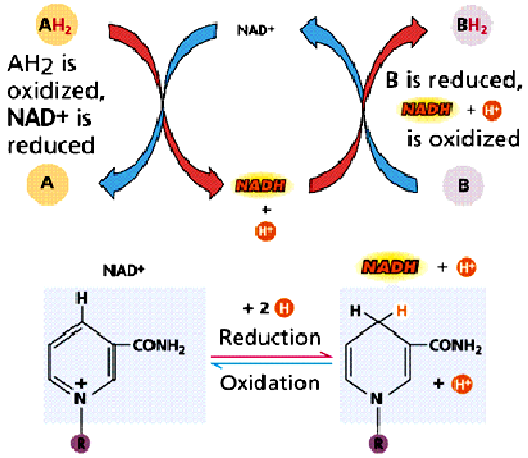
Time-energy graphs of an exergonic reaction and endergonic reaction

Oxidation/Reduction

Biochemical reactions in living organisms are essentially energy transfers. Often they occur together, "linked", in what are referred to as oxidation/reduction reactions. **Reduction** is the gain of an electron. Sometimes we also have H ions along, so reduction also becomes the gain of H. **Oxidation** is the loss of an electron (or hydrogen). In oxidation/reduction reactions, one chemical is oxidized, and its electrons are passed to another (reduced, then) chemical. Such coupled reactions are referred to as **redox reactions.** The metabolic processes **glycolysis, Kreb's Cycle,** and **Electron Transport Phosphorylation** involve the transfer of electrons (at varying energy states) by redox reactions.



Passage of electrons from compound A to compound B. When A loses its electrons it is oxidized; when B gains the electrons it is reduced.



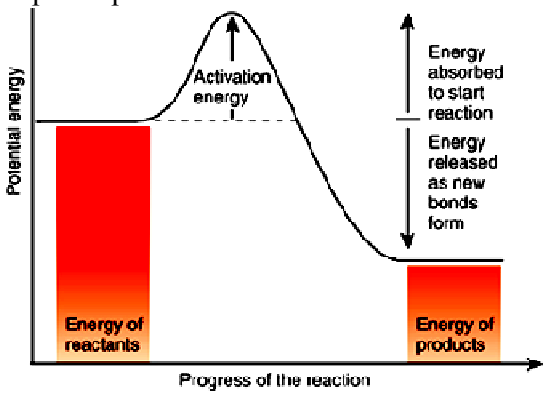
Oxidation/reduction via an intermediary (energy carrier) compound, in this case NAD^+.

Catabolism and Anabolism

Anabolism is the total series of chemical reactions involved in synthesis of organic compounds. Autotrophs must be able to manufacture (synthesize) all the organic compounds they need. Heterotrophs can obtain some of their compounds in their diet (along with their energy). For example humans can synthesize 12 of the 20 amino acids, we must obtain the other 8 in our diet. **Catabolism** is the series of chemical reactions that breakdown larger molecules. Energy is released this way, some of it can be utilized for anabolism. Products of catabolism can be reassembled by anabolic processes into new anabolic molecules.

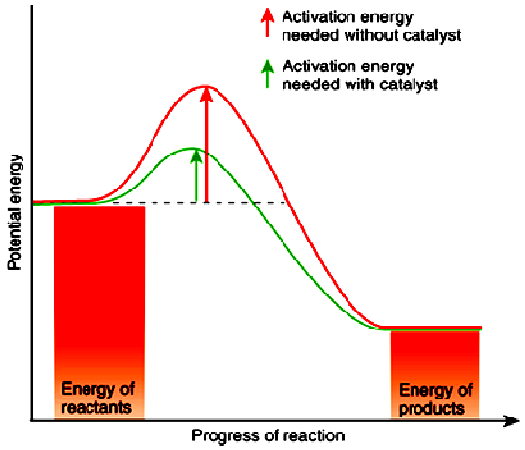
Enzymes: Organic Catalysts

Enzymes are catalysts. Most are **proteins**. (A few **ribonucleoprotein enzymes** have been discovered and, for some of these, the catalytic activity is in the RNA part rather than the protein part.) Enzymes bind temporarily to one or more of the **reactants** of the reaction they catalyze. In doing so, they lower the amount of **activation energy** needed and thus speed up the reaction.



Progess of Non Catalyzed Reaction

By bringing the reactants closer together, chemical bonds may be weakened and reactions will proceed faster than without the catalyst.



The use of enzymes can lower the activation energy of a reaction (E_a)

Examples of enzymes include: **Catalase** catalyzes the decomposition of **hydrogen peroxide** into **water** and **oxygen**.

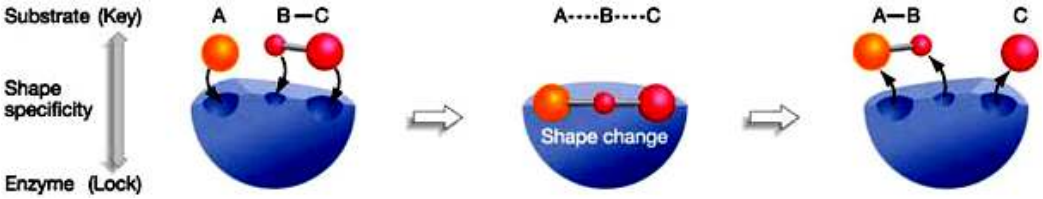
$$2H_2O_2 \rightarrow 2H_2O + O_2$$

One molecule of catalase can break 40 million molecules of hydrogen peroxide each second. **Carbonic anhydrase** is found in red blood cells where it catalyzes the reaction

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

It enables red blood cells to transport carbon dioxide from the tissues to the lungs. One molecule of carbonic anhydrase can process one million molecules of CO_2 each second.

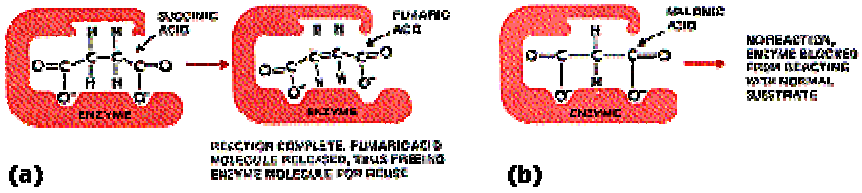
Acetylcholinesterase catalyzes the breakdown of the **neurotransmitter acetylcholine** at several types of **synapses** as well as at the **neuromuscular junction,** the specialized synapse that triggers the contraction of skeletal muscle. One molecule of acetylcholinesterase breaks down 25,000 molecules of acetylcholine each second. This speed makes possible the rapid "resetting" of the synapse for transmission of another nerve impulse. Enzyme activity can be analyzed quantitatively. Some of the ways this is done are described in the page **Enzyme Kinetics**. In order to do its work, an enzyme must unite, even if ever so briefly with at least one of the reactants. In most cases, the forces that hold the enzyme and its substrate are **noncovalent**, an assortment of **hydrogen bonds, ionic interactions** and **hydrophobic interactions**. Most of these interactions are weak and especially so if the atoms involved are farther than about one angstrom from each other. So successful binding of enzyme and substrate requires that the two molecules be able to approach each other closely over a fairly broad surface. Thus the analogy that a substrate molecule binds its enzyme like a **key in a lock**.This requirement for complementarity in the configuration of substrate and enzyme explains the remarkable **specificity** of most enzymes. Generally, a given enzyme is able to catalyze only a single chemical reaction or, at most, a few reactions involving substrates sharing the same general structure.



Theory of Lock-and-Key (Induced-Fit) Hypothesis of enzyme action

Competitive Inhibition

The necessity for a close, if brief, fit between enzyme and substrate explains the phenomenon of competitive inhibition.



Competitive Inhibition

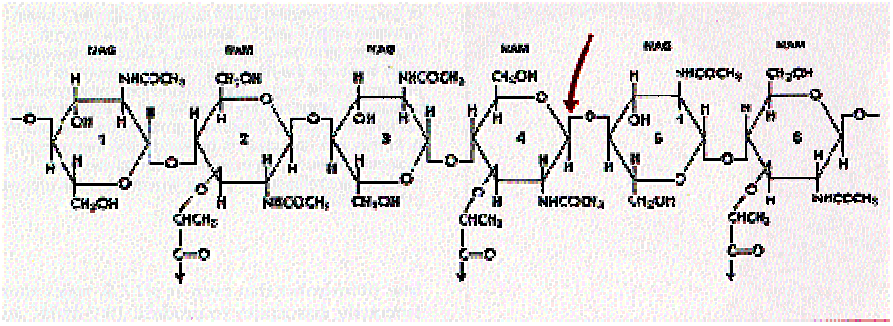
One of the enzymes needed for the release of energy within the cell is **succinic dehydrogenase**. It catalyzes the oxidation (by the removal of two hydrogen atoms) of succinic acid **(a)**. If one adds **malonic acid** to cells, or to a test tube mixture of succinic acid and the enzyme, the action of the enzyme is strongly inhibited. This is because the structure of malonic acid allows it to bind to the same site on the enzyme **(b)**. But there is no oxidation so no speedy release of products. The inhibition is called **competitive** because if you increase the ratio of succinic to malonic acid in the mixture, you will gradually restore the rate of catalysis. At a 50:1 ratio, the two molecules compete on roughly equal terms for the binding (catalytic) site on the enzyme.

Enzyme Cofactors

Many enzymes require the presence of an additional, nonprotein, cofactor. Some of these are metal ions such as Zn^{2+} (the cofactor for carbonic anhydrase), Cu^{2+}, Mn^{2+}, K^{+}, and Na^{+}. Some cofactors are small organic molecules called **coenzymes**. The B vitamins-thiamine (B1), riboflavin (B2) and nicotinamide are precursors of coenzymes. Coenzymes may be covalently bound to the protein part (called the **apoenzyme**) of enzymes as a **prosthetic group**. Others bind more loosely and, in fact, may bind only transiently to the enzyme as it performs its catalytic act.

Lysozyme: A Model of Enzyme Action

A number of lysozymes are found in nature; in human tears and egg white, for examples. The enzyme is antibacterial because it degrades the polysaccharide that is found in the cell walls of many bacteria. It does this by catalyzing the insertion of a water molecule (a glycosidic bond). This **hydrolysis** breaks the chain at that point.



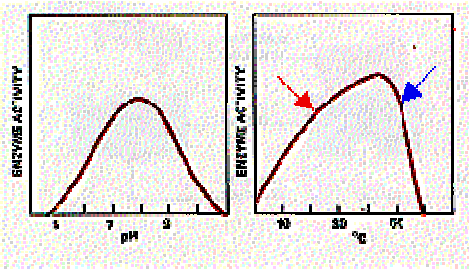
The bacterial polysaccharide consists of long chains of alternating amino sugars: **N-acetylglucosamine** (**NAG**) and **N-acetylmuramic acid** (**NAM**). These hexose units resemble glucose except for the presence of the side chains containing amino groups. Lysozyme is a globular protein with a deep cleft across part of its surface. Six hexoses of the substrate fit into this cleft. With so many oxygen atoms in sugars, as many as 14 hydrogen bonds form

between the six amino sugars and certain amino acid R groups such as **Arg-114**, **Asn-37**, **Asn-44**, **Trp-62**, **Trp-63**, and **Asp-101**. Some hydrogen bonds also form with the C=O groups of several **peptide bonds**. In addition, hydrophobic interactions may help hold the substrate in position. X-ray crystallography has shown that as lysozyme and its substrate unite, each is slightly deformed. The fourth hexose in the chain (ring #4) becomes twisted out of its normal position. This imposes a strain on the C-O bond on the ring-4 side of the oxygen bridge between rings 4 and 5. It is just at this point that the polysaccharide is broken. A molecule of water is inserted between these two hexoses, which breaks the chain. Here, then, is a structural view of what it means to lower activation energy. The energy needed to break this covalent bond is lower now that the atoms connected by the bond have been distorted from their normal position.

As for lysozyme itself, binding of the substrate induces a small (~0.75Å) movement of certain amino acid residues so the cleft closes slightly over its substrate. So the "lock" as well as the "key" changes shape as the two are brought together. (This is sometimes called "induced fit".) The amino acid residues in the vicinity of rings 4 and 5 provide a plausible mechanism for completing the catalytic act. Residue 35, glutamic acid (**Glu-35**), is about 3Å from the -O- bridge that is to be broken. The free carboxyl group of glutamic acid is a hydrogen ion donor and available to transfer H^+ to the oxygen atom. This would break the already-strained bond between the oxygen atom and the carbon atom of ring 4. Now having lost an electron, the carbon atom acquires a positive charge. Ionized carbon is normally very unstable, but the attraction of the negatively-charged carboxyl ion of **Asp-52** could stabilize it long enough for an -OH ion (from a spontaneously dissociated water molecule) to unite with the carbon. Even at pH 7, water spontaneously dissociates to produce H^+ and OH^- ions. The hydrogen ion (H^+) left over can replace that lost by **Glu-35**. In either case, the chain is broken, the two fragments separate from the enzyme, and the enzyme is free to attach to a new location on the bacterial cell wall and continue its work of digesting it.

Factors Affecting Enzyme Action

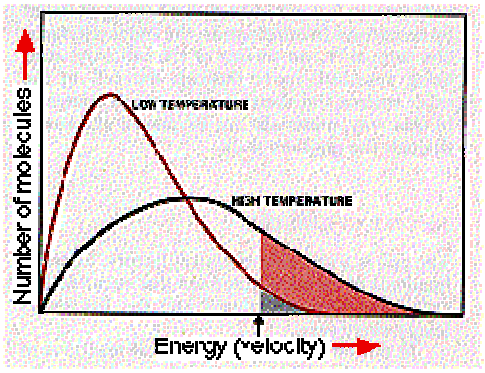
The activity of enzymes is strongly affected by changes in **pH** and **temperature**. Each enzyme works best at a certain pH and temperature, its activity decreasing at values above and below that point. This is not surprising considering the importance of **tertiary structure** (i.e. shape) in enzyme function and noncovalent forces, e.g., ionic interactions and hydrogen bonds, in determining that shape.



Effects of pH and Temperature on enzyme activity

Examples include the protease **pepsin** works best as a pH of 1–2 (found in the stomach) while the protease **trypsin** is inactive at such a low pH but very active at a pH of 8 (found in the small intestine as the bicarbonate of the pancreatic fluid neutralizes the arriving stomach contents). Changes in pH alter the state of ionization of charged amino acids (e.g., Asp, Lys) that may play a crucial role in substrate binding and/or the catalytic action itself.

Without the unionized -COOH group of **Glu-35** and the ionized -COO^- of **Asp-52**, the catalytic action of lysozyme would cease. Hydrogen bonds are easily disrupted by increasing temperature. This, in turn, may disrupt the shape of the enzyme so that its affinity for its substrate diminishes. The ascending portion of the temperature curve reflects the **general effect of increasing temperature** on the rate of chemical reactions. The descending portion of the curve reflects the loss of catalytic activity as the enzyme molecules become **denatured** at high temperatures.



Effects of temperature increase on enzyme activity

Regulation of Enzyme Activity

Several mechanisms work to make enzyme activity within the cell efficient and well-coordinated.

Anchoring Enzymes in Membranes

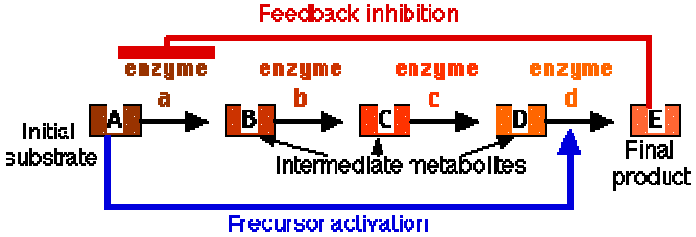
Many enzymes are inserted into cell membranes, e.g. the plasma membrane, the membranes of mitochondria and chloroplasts , the endoplasmic reticulum and the nuclear envelope. These are locked into spatial relationships that enable them to interact efficiently.

Inactive Precursors

Enzymes, such as proteases, that can attack the cell itself are inhibited while within the cell that synthesizes them. For example, pepsin is synthesized within the **chief cells** (in gastric glands) as an inactive precursor, **pepsinogen**. Only when exposed to the low pH outside the cell is the inhibiting portion of the molecule removed and active pepsin produced.

Feedback Inhibition and Precursor Activation

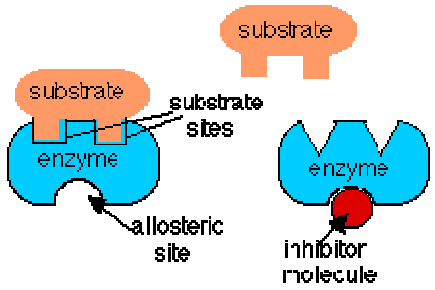
If the product of a series of enzymatic reactions, e.g., an amino acid, begins to accumulate within the cell, it may specifically inhibit the action of the first enzyme involved in its synthesis. Thus further production of the enzyme is halted.



Feedback Inhibition and Precursor Activation

The accumulation of a substance within a cell may specifically activate an enzyme that sets in motion a sequence of reactions for which that substance is the initial substrate. This reduces the concentration of the initial substrate. In the case of feedback inhibition and precursor activation, the activity of the enzyme is being regulated by a molecule which is **not**

its substrate. In these cases, the regulator molecule binds to the enzyme at a different site than the one to which the substrate binds. When the regulator binds to its site, it alters the shape of the enzyme so that its activity is changed. This is called an **allosteric** effect. In feedback inhibition, the allosteric effect lowers the affinity of the enzyme for its substrate while in precursor activation, the regulator molecule increases the affinity of the enzyme in the series for its substrate.



Allosteric effect

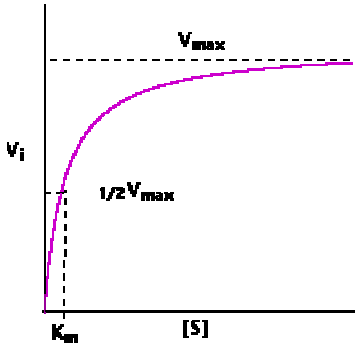
Regulation of Enzyme Synthesis

The four mechanisms described above regulate the activity of enzymes already present within the cell. What about enzymes that are not needed or are needed but not present? Here, too, control mechanisms are at work that regulate the rate at which new enzymes are synthesized. Most of these controls work by turning on or off the **transcription of genes**. If, for example, ample quantities of an amino acid are already available to the cell from its extracellular fluid, synthesis of the enzymes that would enable the cell to produce that amino acid for itself is shut down. Conversely, if a new substrate is made available to the cell, it may induce the synthesis of the enzymes needed to cope with it. Yeast cells, for example, do not ordinarily metabolize lactose and no **lactase** can be detected in them. However, if grown in a medium containing lactose, they soon begin synthesizing lactase by transcribing and translating the necessary genes and so can begin to metabolize the sugar. *E. coli* also has a mechanism which regulates enzyme synthesis by controlling **translation** of a needed messenger RNA.

Enzyme Kinetics

The **concentration** of substrate molecules (the more of them available, the quicker the enzyme molecules collide and bind with them). The concentration of substrate is designated **[S]** and is expressed in unit of molarity. As the **temperature** rises, molecular motion - and hence collisions between enzyme and substrate - speed up. But as enzymes are proteins, there is an upper limit beyond which the enzyme becomes **denatured** and ineffective. The presence of inhibitors also plays a major role in enzymatic kinetics. **Competitive inhibitors** are molecules that bind to the same site as the substrate preventing the substrate from binding as they do so but are not changed by the enzyme. **Noncompetitive inhibitors** are molecules that bind to some other site on the enzyme reducing its catalytic power. The conformation of a protein is influenced by **pH** and as enzyme activity is crucially dependent on its conformation, its activity is likewise affected. The study of the rate at which an enzyme works is called **enzyme kinetics**. Let us examine enzyme kinetics as a function of the **concentration of substrate** available to the enzyme.

We set up a series of tubes containing graded concentrations of substrate, **[S]**. At time zero, we add a fixed amount of the enzyme preparation. Over the next few minutes, we measure the concentration of product formed. If the product absorbs light, we can easily do this in a spectrophotometer. Early in the run, when the amount of substrate is in substantial excess to the amount of enzyme, the rate we observe is the initial velocity of V_i. Plotting V_i as a function of **[S]**, we find that at low values of **[S]**, the initial velocity,V_i, rises almost linearly with increasing **[S]**. But as **[S]** increases, the gains in V_i level off (forming a rectangular hyperbola). The asymptote represents the maximum velocity of the reaction, designated V_{max}. The substrate concentration that produces a V_i that is one-half of V_{max} is designated the **Michaelis-Menten constant**, K_m(named after the scientists who developed the study of enzyme kinetics). K_m is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the K_m, the greater the affinity (so the lower the concentration of substrate needed to achieve a given rate).

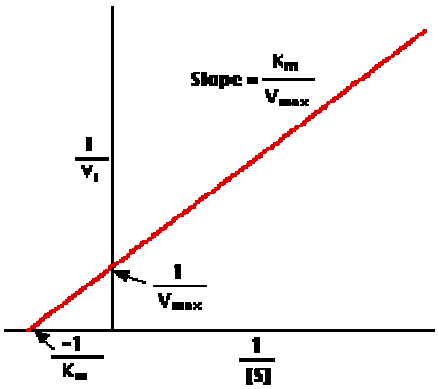


Where

V_i = initial velocity (moles/time)
[S] = substrate concentration (molar)
V_{max} = maximum velocity
K_m = substrate concentration when V_i is one-half V_{max} (Michaelis-Menton constant)

Basics of enzyme kinetics

Plotting the reciprocals of the **same data points** yields a "double-reciprocal" or **Lineweaver-Burk plot**. This provides a more precise way to determine V_{max} and K_m. V_{max} is determined by the point where the line crosses the $1/V_i = 0$ axis (so the **[S]** is infinite). Note that the magnitude represented by the data points in this plot **decrease** from lower left to upper right. K_m equals V_{max} times the slope of line. This is easily determined from the intercept on the X axis.



Lineweaver-Burk plot

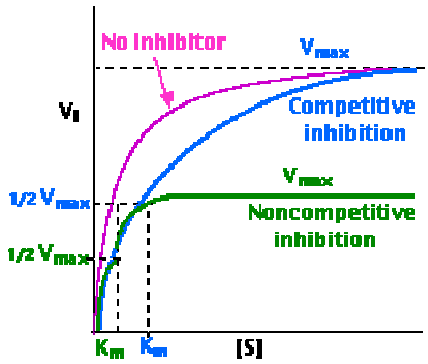
The Effects of Enzyme Inhibitors

Enzymes can be inhibited **competitively**, when the substrate and inhibitor compete for binding to the same active site or **noncompetitively**, when the inhibitor binds somewhere

else on the enzyme molecule reducing its efficiency. The distinction can be determined by plotting enzyme activity with and without the inhibitor present.

Competitive Inhibition

In the presence of a competitive inhibitor, it takes a higher substrate concentration to achieve the same velocities that were reached in its absence. So while $\mathbf{V_{max}}$ can still be reached if sufficient substrate is available, one-half $\mathbf{V_{max}}$ requires a higher **[S]** than before and thus $\mathbf{K_m}$ is larger.

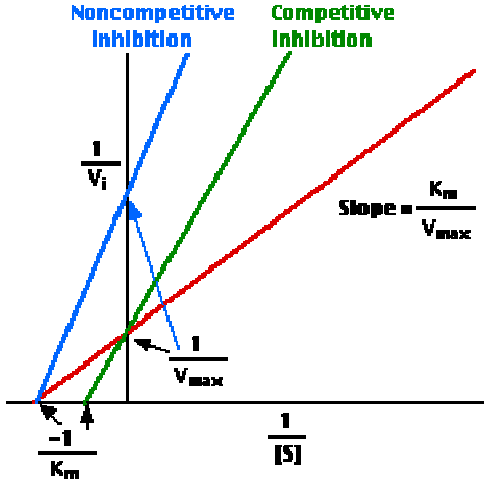


Effects of Enzyme inhibition

Noncompetitive Inhibition

With noncompetitive inhibition, enzyme molecules that have been bound by the inhibitor are taken out of the game so enzyme rate (velocity) is reduced for all values of **[S]**, including $\mathbf{V_{max}}$ and one-half $\mathbf{V_{max}}$ but $\mathbf{K_m}$ remains unchanged because the active site of those enzyme molecules that have not been inhibited is unchanged.

This Lineweaver-Burk plot displays these results.



Lineweaver-Burk plot of enzyme inhibition

e.g. when a slice of apple is exposed to air, it quickly turns brown. This is because the enzyme **o-diphenol oxidase** catalyzes the oxidation of **phenols** in the apple to dark-coloured products. A similar enzyme, **tyrosinase**, converts **tyrosine** to **melanin**.