Chapter 8

Energy & Enzymes

PHOTOSYNTHESIS

Conversion of sunlight energy to chemical bonds.

RESPIRATION

Breaking of chemical bonds in food to produce useful energy.

AUTOTROPH
(self food)

Do not require food molecules from other organisms. (Typically photosynthetic organisms)

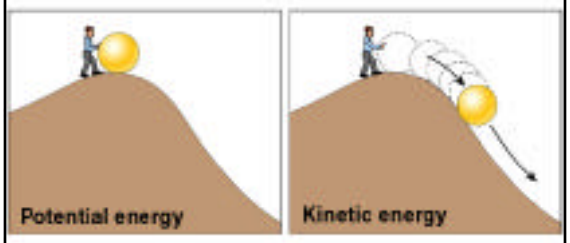
HETEROTROPHS
(other food)

Organisms that can not make their own energy and require food from other organisms.

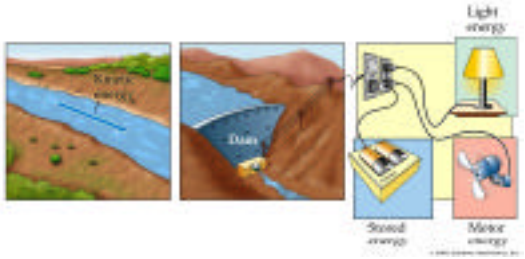
An Energy Source

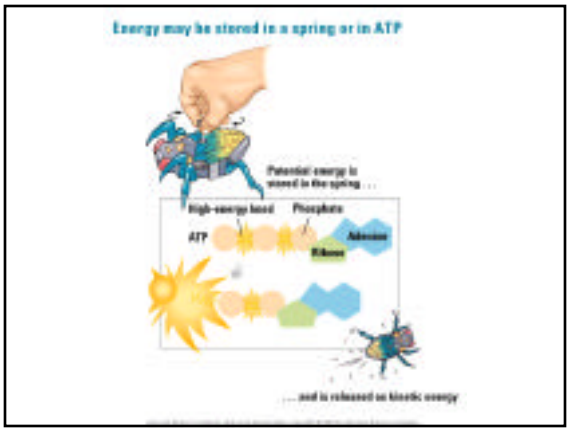
Fig. 8.2

Potential and Kinetic Energy



Kinetic vs potential energy



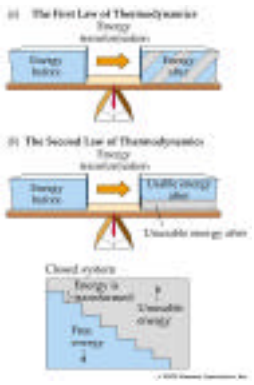


LAWS OF THERMODYNAMICS

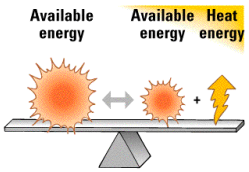
1. Energy can not be created or destroyed.

2. In any change of energy from one form to another some useful energy is converted to useless heat energy.

The Laws of Thermodynamics

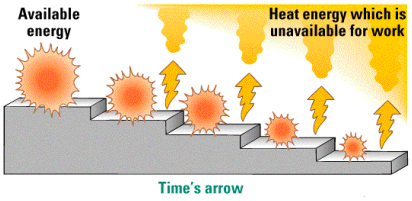


First Law of Thermodynamics



Conservation of energy: energy is neither gained nor lost, it is only transformed.

Second Law of Thermodynamics



Energy available for work decreases as energy is lost to heat.

Gibbs' Free Energy

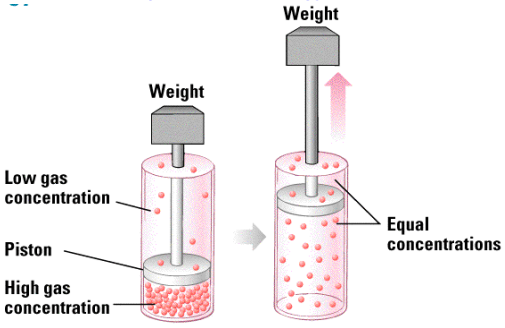
$$\Delta G = G_{products} - G_{reactants}$$

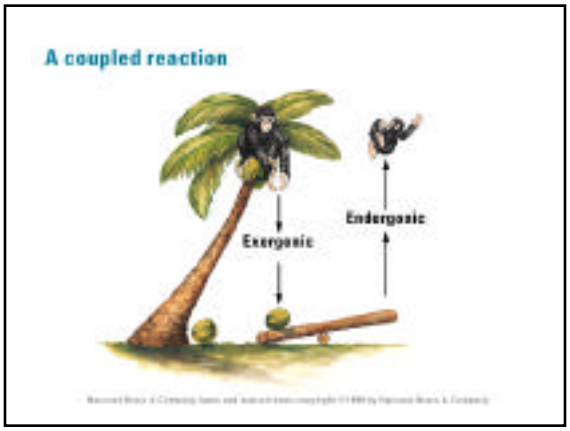
Changes in entropy determine reaction direction

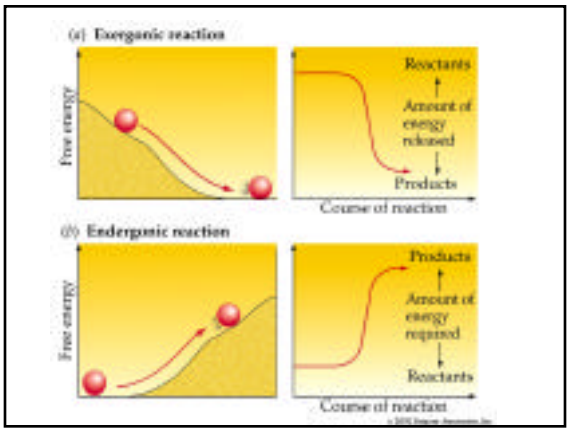
$$\Delta G = \Delta H - T\Delta S$$

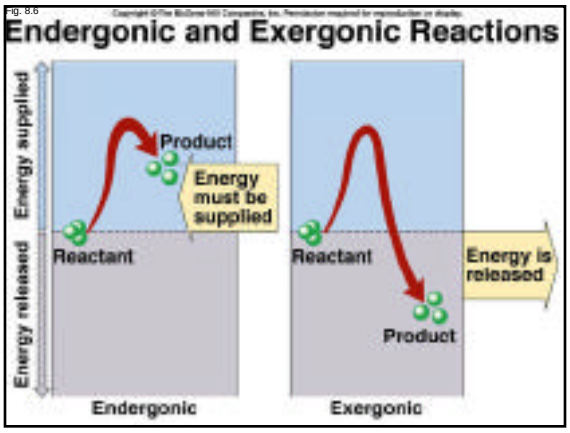
ΔG = change in Gibbs' free energy (useable energy)
ΔH = change in enthalpy (total energy in the system)
ΔS = change in entropy (measure of disorder)
T = absolute temperature (°K)

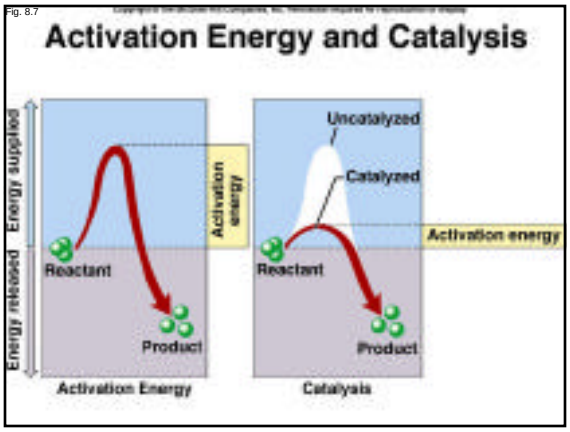
Difference in concentration constitutes potential energy

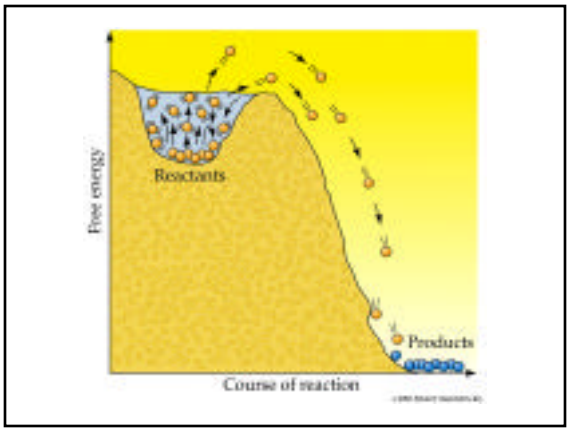




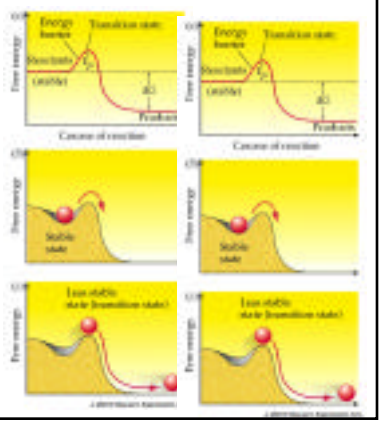




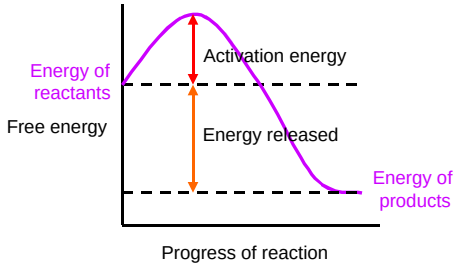




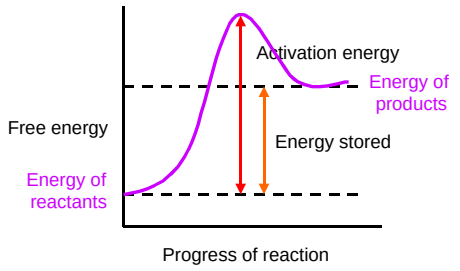
Energy of activation

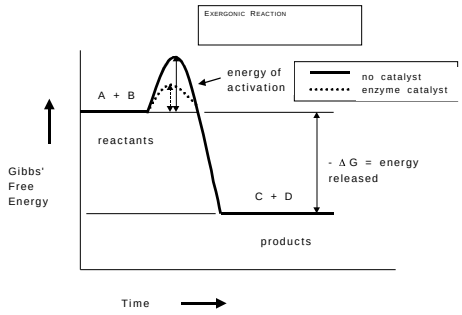


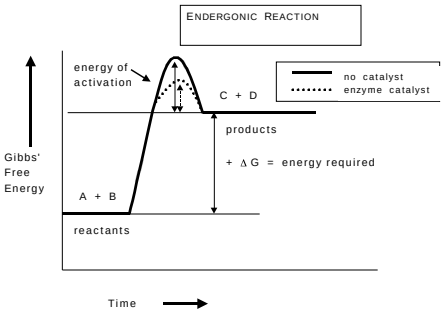
Exergonic reactions

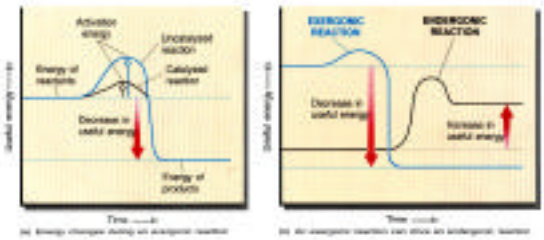


Endergonic reactions

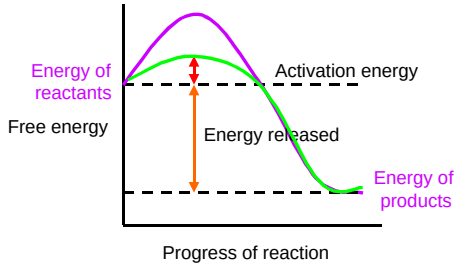


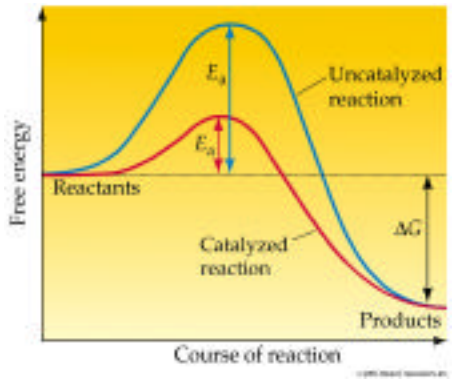






Enzymes lower the activation energy





How do enzyme catalysts reduce activation energy requirements? We need to consider the following equation.

$\Delta G = \Delta H - T\Delta S$

The change in Gibbs' Free Energy is equal to the change in Enthalpy minus the quantity of absolute Temperature (°K) times the change in Entropy.

The Temperature, the Enthalpy and the Entropy all affect how enzymes are able to reduce activation energy requirements.

Temperature is a measure of the average kinetic energy of a system. Kinetic energy is the energy of motion. All atoms and molecules are in motion with some moving faster and others moving more slowly. If we raise the temperature of a system, then on average the atoms and molecules move faster.

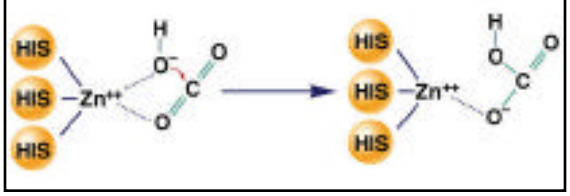
Change in Enthalpy (Δ H) approximates the change in chemical bond energies while the change in Entropy (Δ S) measures the amount of disorder (+ Δ S), or order (- Δ S), introduced into the system by breaking chemical bonds and forming new ones.

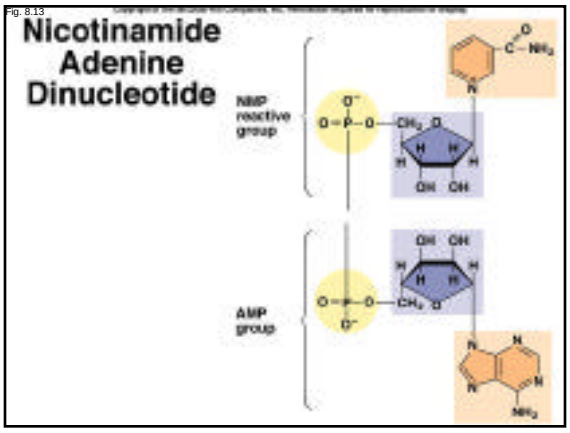
In going from reactant molecules to product molecules, the reactant molecules must collide with each other with enough kinetic energy and with the correct orientation to break target bonds between reactant molecule atoms and to form new bonds between atoms of the product molecules. Without catalysts (enzymes are usually protein catalysts) many collisions may occur before there is the right amount of energy and the proper orientation to permit formation of product molecules.

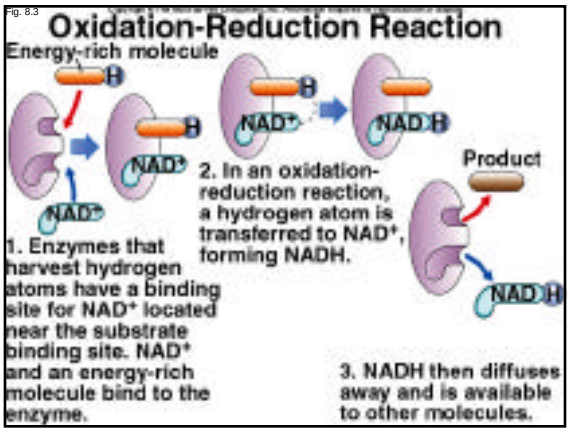
Enzymes speed up reactions in the following manner. The active site is a pocket of certain amino acid R groups with a particular shape. Since all molecules are moving, the pocket is bombarded continually. However, its size and shape restrict access to reactant molecules (or similarly shaped molecules). In the process key amino acid R groups both position the reactant molecules correctly and begin to break target bonds between reactant atoms in the molecules. This ordered system reduces the amount of kinetic energy needed to drive the reaction over the energy of activation hump.

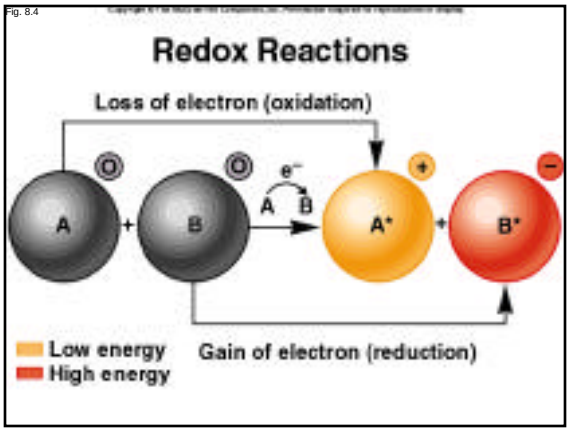
$CO_2 + H_2O \rightarrow H_2CO_3^- + H^+$

Catalysis—Carbonic Anhydrase









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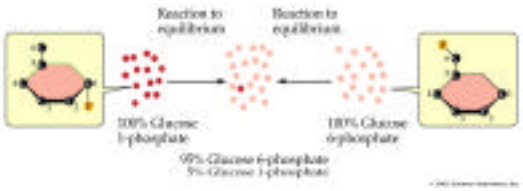
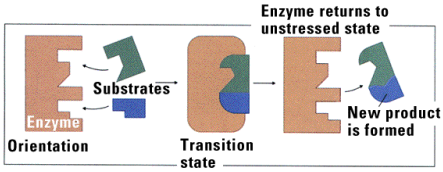
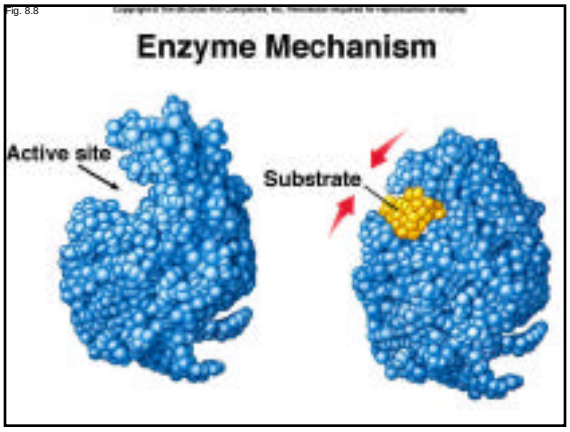
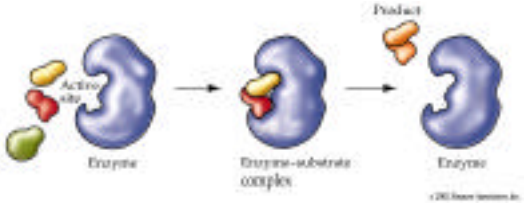
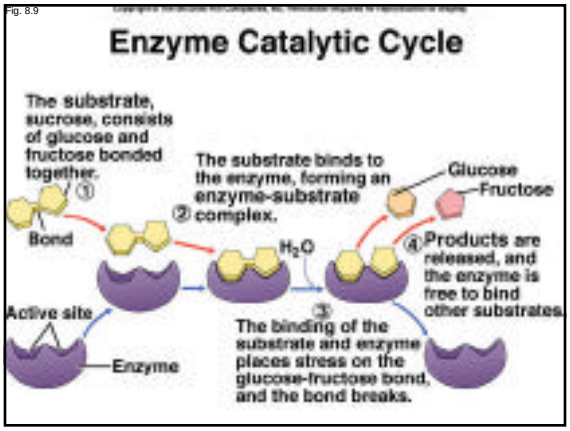


Biological reactants are called substrates







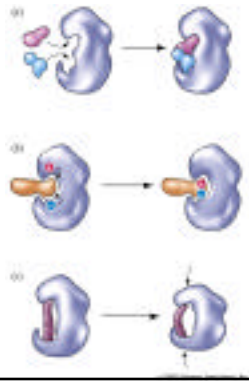


Substrate-enzyme interaction

Orientation

Charges

Physical strain



Enzymes

- ✓ Biological catalysts
- ✓ Are most often proteins.
- ✓ Lower the activation energy of a reaction.
- ✓ Speed up the rate of reactions which they catalyze by bringing substrates closer together.
- ✓ Do not alter the equilibrium of the reaction.
- ✓ Are efficient, specific, and controllable
- ✓ Are not consumed by the reactions they catalyze.
- ✓ Are usually influenced by pH and temperature

Speed or velocity of enzyme catalyzed Rxn

- ✓ Amount of substrate
- ✓ Enzyme concentration
- ✓ Temperature
- ✓ pH
- ✓ Presence or absence of inhibitors

- ✓ Max velocity or Vmax
  - ✒ Excess substrate = enzyme is working as fast as possible

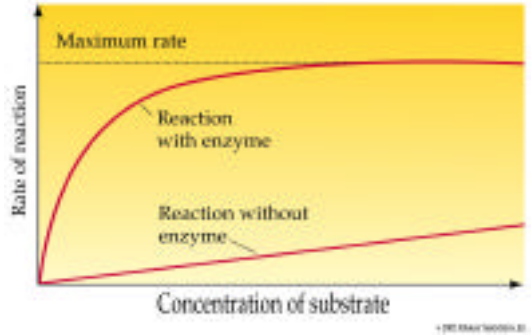
Michaelis-Menton

✓ E + S ⇆ ES ⇆ E + P

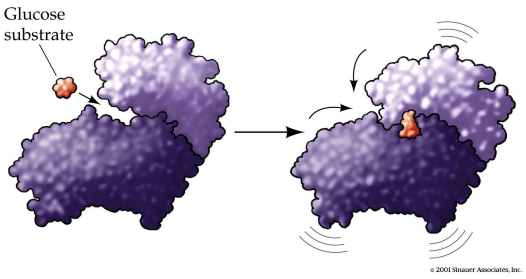
$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

✓ Km is the substrate concentration at 1/2 Vmax

Increase in reaction rate



Induced fit of the enzyme



Features of the active site

- ✓ The active site is a relatively small part of the enzyme.
- ✓ The active site is 3-dimensional.
- ✓ Substrates are bound to enzymes by multiple weak attractions.
- ✓ The active site is a nonpolar cleft or crevice.
- ✓ Specificity of binding in the active site depends on the arrangement of atoms in the active site.

Ribonuclease

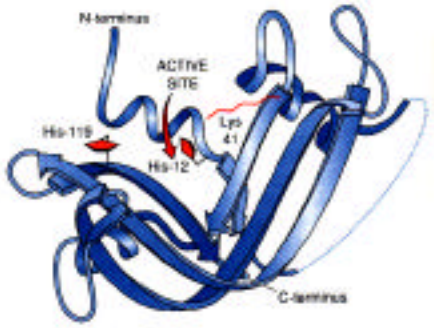


Fig. 8.10

Pyruvate Dehydrogenase Enzyme

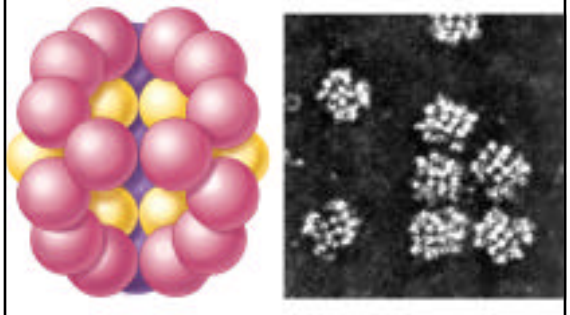
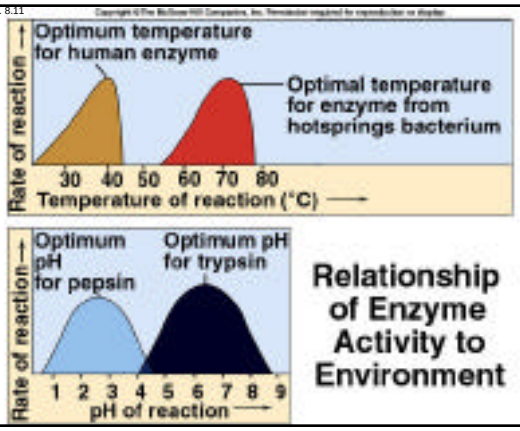


Fig. 8.11

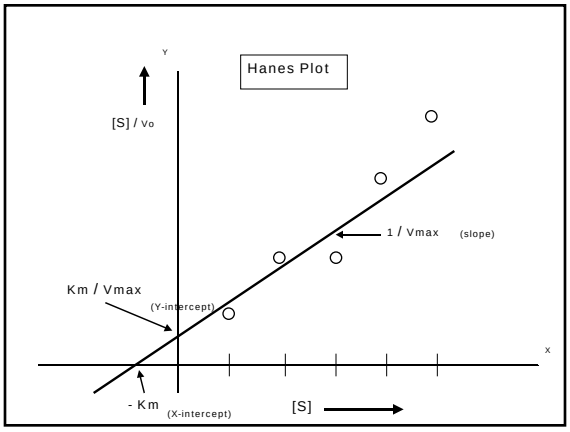


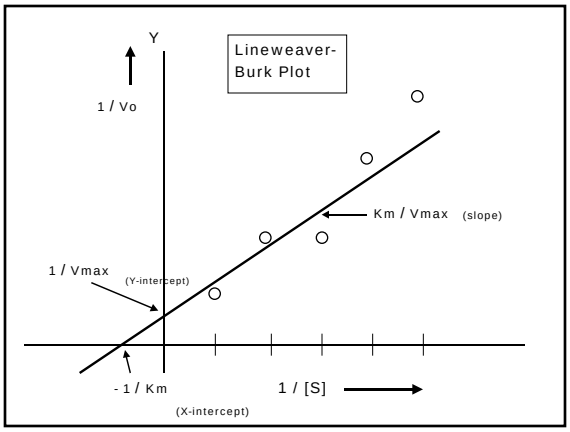
If there is more enzyme such that only some active sites are processing substrate, then the reaction velocity is proportional to the substrate concentration, ie V = Vmax [S] / ([S] + Km).

The Michaelis constant Km is the substrate concentration [S] at which half of the enzyme active sites are processing substrate and here V = 1/2 Vmax.

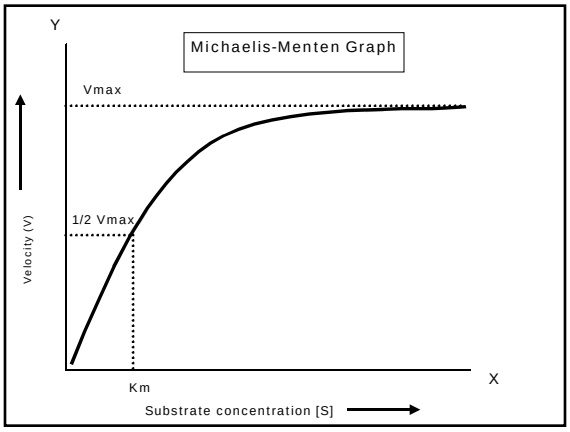
The Vmax and Km terms are used to construct the Michaelis-Menten graph of enzyme behavior. Before the high-powered computers and mathematical programs of today, calculation of the hyperbolic Michaelis-Menten graph directly from the data was very difficult. A strategy called the single reciprocal (Hanes) plot or the double reciprocal (Lineweaver-Burk) plot was used to determine in linear fashion the values of Km and Vmax.

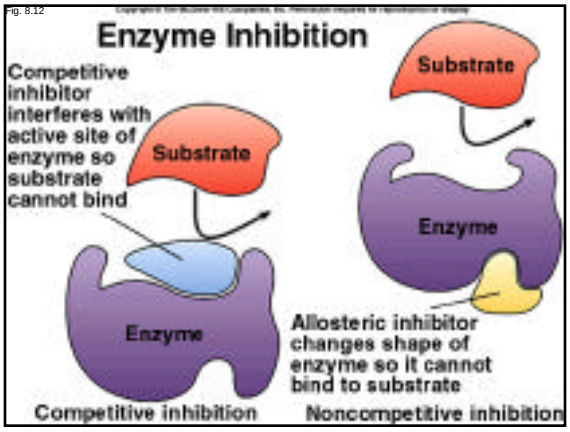
[Different substrate concentrations [S] which are less than the Km substrate concentration are reacted with small amounts of enzyme and the reactions are quickly stopped to avoid reactions going backwards. Rate of product formation is the velocity (Vo) for each different substrate concentration.]



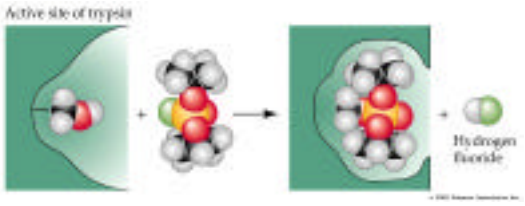


Once the values of Km and Vmax have been determined (estimated), then the Michaelis-Menten graph can be constructed. Vmax is the horizontal asymptote (Y = Vmax). The origin and the point (Km, 1/2 Vmax) are the vertices of a straight line that curves to the right as it approaches the Vmax asymptote. [The velocity of the enzymatic reaction is approximately linear at substrates less than Km.]





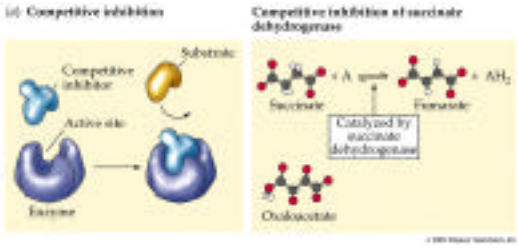
Irreversible inhibition



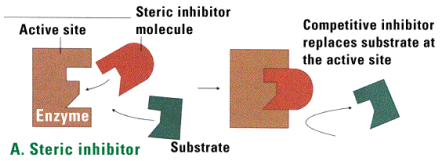
Diisopropylphosphofluoridate (DIPF) bind to the OH on the side chain of serine in the active site

Reversible inhibition

Competitive inhibition will compete for the active site

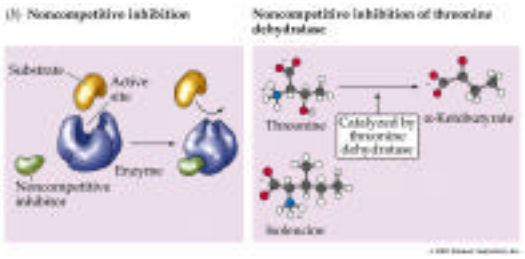


Competitive inhibition

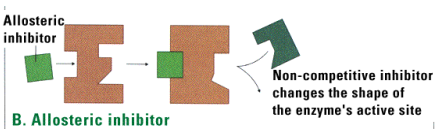


Reversible inhibition

Uncompetitive alters the enzyme by binding to site other than the active site-this alters the shape of the enzyme
Allosteric enzymes are multi-subunit enzymes

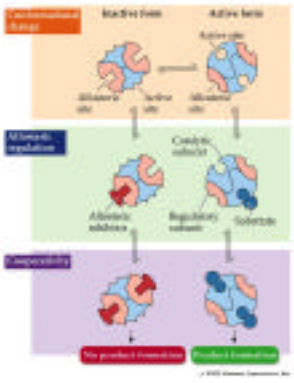


Allosteric inhibition



un-competitive inhibition

Allosteric regulation



Types of Inhibition
Competitive inhibition is where the rate at which the substrate binds to the active site is affected.

Uncompetitive inhibition is a descriptive term for effects that slow down the rate of catalysis. This may occur by molecules, atoms or ions binding to sites on the enzyme other than to the active site affecting enzyme conformation and hence its catalytic ability. Often such binding affects both the catalytic rate of the enzyme and its ability to bind the substrate. Such effects are termed mixed inhibition.

Biochemists, particularly enzyme kineticists, do not use the term noncompetitive inhibition. (In reality noncompetitive inhibition is a special case of mixed inhibition where $Ki_{comp} = Ki_{uncomp}$ so that Km remains unchanged but Vmax is affected.)

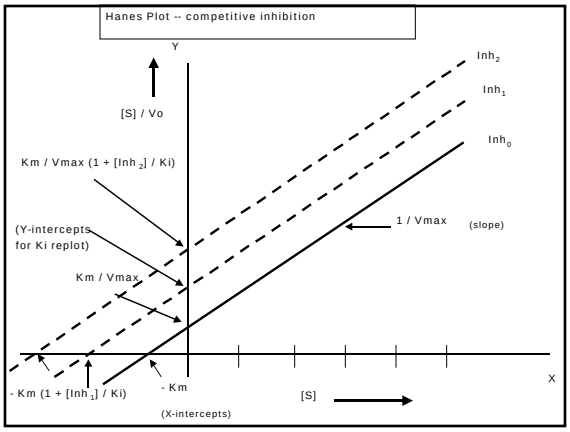
In irreversible inhibition, the enzyme is modified at the active site or elsewhere so that it either works at a much slower rate or not at all.

Allosteric enzymes are generally composed of two or more polypeptides. They have an acitve site and one or more effector sites where molecules, atoms or ions can bind to speed up the catalytic process or to slow it down. Often these enzymes are involved in negative feedback regulation of biochemical (metabolic) pathways at critical junctures to more efficiently control production levels of key endproducts essential to maintaining metabolic processes of living organisms in homeostasis.

In competitive inhibition, the inhibitor mimics substrate and competes with the substrate for the active site. It slows down the reaction but does not affect the rate of catalysis. This happens because when the inhibitor is in the active site, no substrate can be acted upon by that enzyme.

As substrate concentrations increase, there is a greater chance that substrate instead of inhibitor molecules will occupy the active sites of the enzymes so that the reaction rate increases. Competitive inhibitors have their effect on substrate binding to the active site increasing the apparent Km. Apparent Km = Km (1 + [Inh] / Ki).

In the graph below, note that the slopes of the lines with inhibitor are parallel to the control without inhibitor. This means that the catalytic rates remain the same. However, note the apparent shift to the left of the apparent Km. This shows that competitive inhibitors affect substrate binding at the active site.

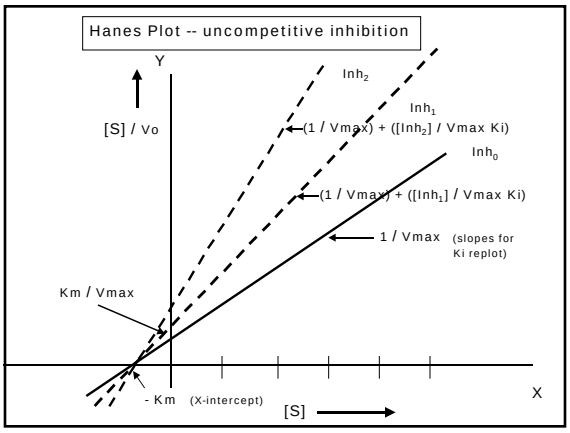


Uncompetitive inhibition is usually found in two substrate enzyme systems with two products. Uncompetitive inhibitors do not bind the active site. Instead the inhibitors bind to the enzyme at another site changing enzyme conformation such that the catalytic rate is slower. There is no effect on the Km.

Increasing substrate concentrations cannot remove this slowing effect on the velocity of the enzyme rate of reaction.

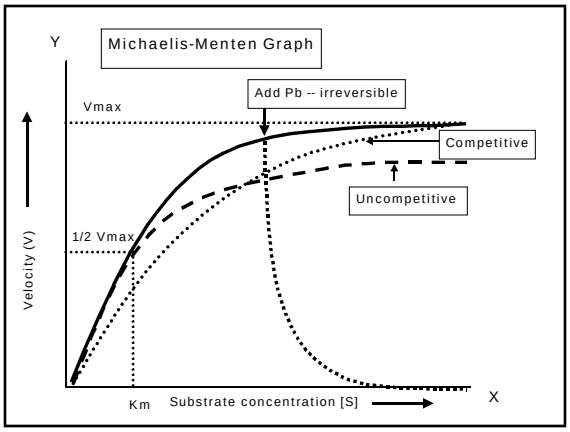
Uncompetitive inhibitors have their effect on the rate of catalysis decreasing the apparent Vmax. Apparent Vmax = Vmax / (1 + [Inh] / Ki).

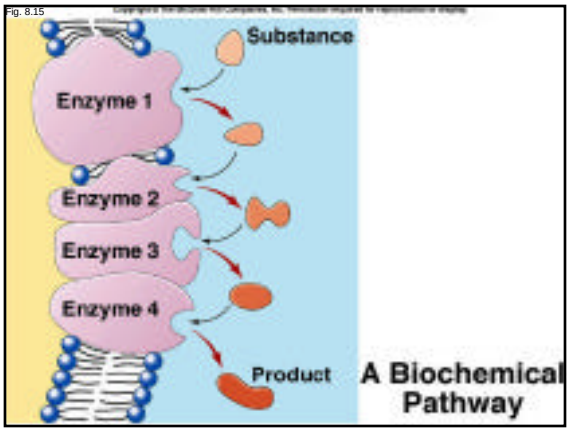
In the graph below, note that the slopes of the lines with inhibitor are less than the slope of the line for the control (no inhibitor). [In a single reciprocal plot, the steeper the slope, the smaller its value.] This means that the catalytic rates are slower for the ones with inhibitor. Also note that the inhibitor lines intersect with Km. This shows that uncompetitive inhibitors do not affect substrate binding at the active site.



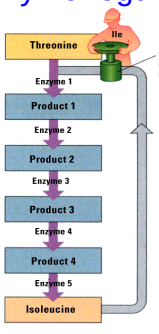
Note that for the double reciprocal (Lineweaver-Burk) plots of competitive and uncompetitive inhibition, the graphs are just the opposite of the Hanes (single reciprocal) plots shown above.

We want to examine the effects of competitive, uncompetitive and irreversible inhibition on the Michaelis-Menten graph below.

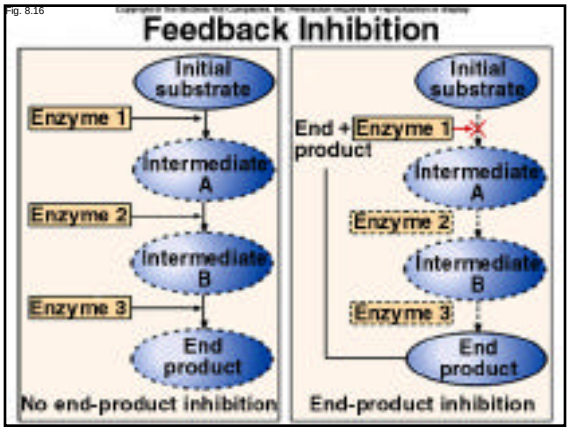




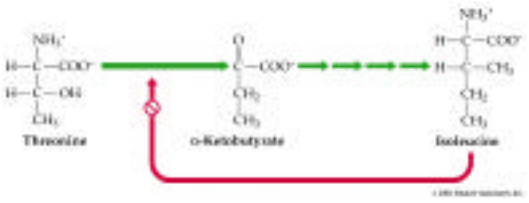
Enzyme regulation



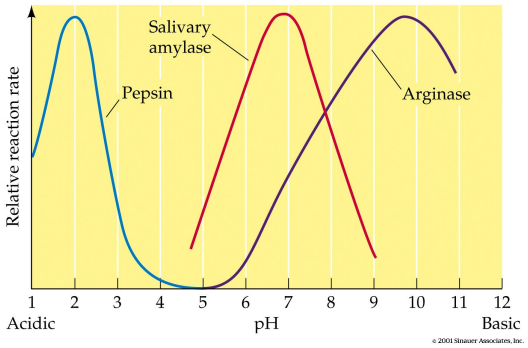
- ✓ Production of enzymes is often regulated by the concentration of the end-product.
- ✓ This is called feedback inhibition.



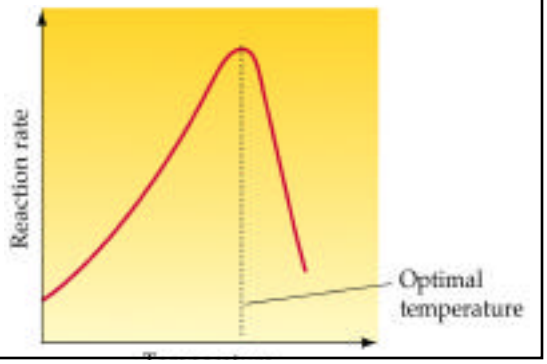
Feed back inhibition (allosteric)



pH effects



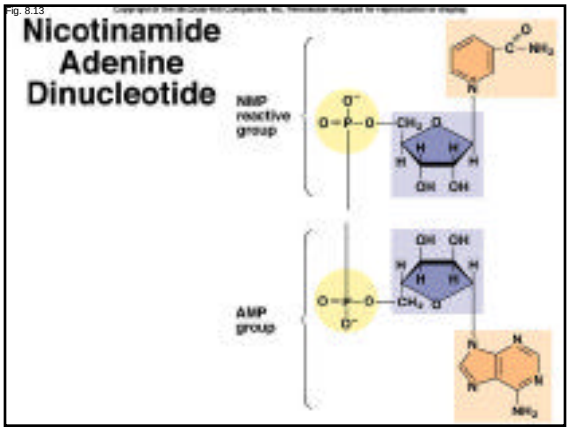
Temperature effects

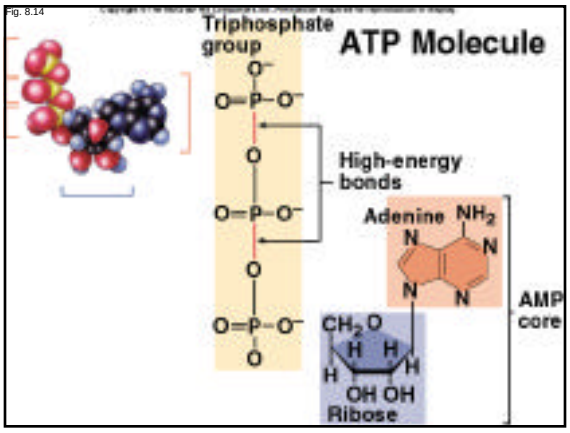


- ✓Coenzymes
  - ✒Organic molecules derived from vitamins that assist enzymes
- ✓Cofactors
  - ✒Metal ions that assist enzyme function
- ✓Prosthetic groups
  - ✒Covalently bonded to the protein or enzyme

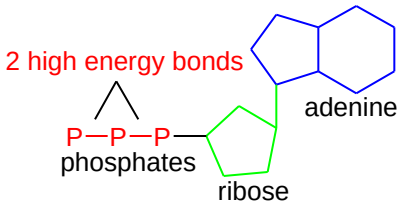
6.1 A Few Examples of Nonprotein Molecular "Partners" of Enzymes

TYPE OF MOLECULE	ROLE IN CATALYZED REACTIONS
Cofactors	
Iron	Oxidation/reduction
Copper	Oxidation/reduction
Zinc	Helps bind NAD
Coenzymes	
Biotin	Carries $-COO^-$
Coenzyme A	Carries $-CH_2-CH_3$
NAD	Carries electrons
FAD	Carries electrons
Prosthetic groups	
Heme	Binds ions, O_2, and electrons; contains iron cofactor
Flavin	Binds electrons
Retinal	Converts light energy

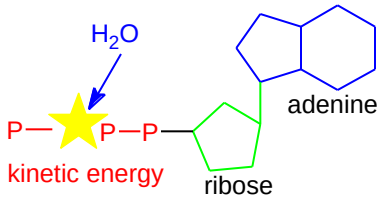


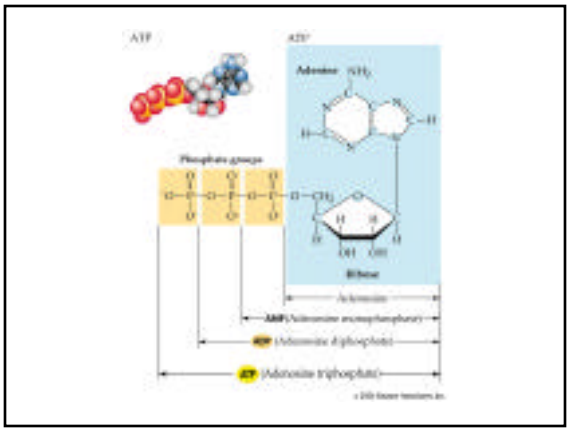


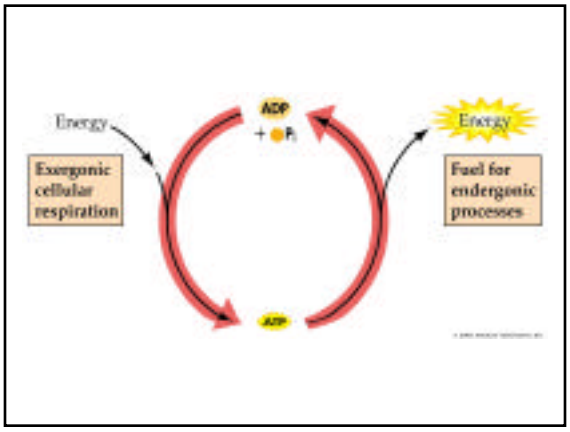
ATP has 2 high energy bonds

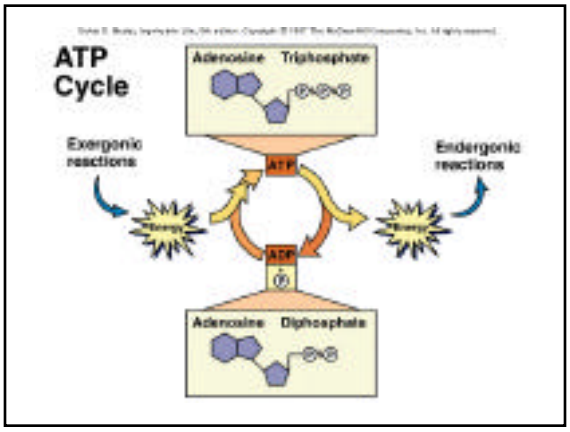


Breaking bonds releases energy









ATP + Water -> ADP + Pi + 7Kcal

ADP + Water -> AMP + Pi + 7kcal

Desert Limo

[This may not be intuitive. More kinetic energy means faster-moving molecules which means more collisions with the concomitant increase in collisions with the proper orientation. Also, with greater speed of collision, there is the possibility that orientation requirements may be less restrictive. So with every 10 degree rise in temperature (C or K), reaction rates double.]

Ask yourself how many baseballs you can catch with a flat board. Probably not very many, but the more chances or thrown baseballs, the more you will catch. Now consider a baseball glove with its pocket as the active site of an enzyme. You will catch most of the baseballs thrown. If there are two pitchers and two catchers where each pair must throw and catch 1,000 baseballs in an hour and one pair only has the flat board and the other pair has the baseball glove, which pair will have caught the most baseballs at the end of the hour?

If the pair with the flat board adds 9 more pitchers and catchers with flat boards each trying to catch 1,000 baseballs, will they catch more baseballs in an hour than the pair with the ball glove? Probably not, but they should catch more than one pair with only a flat board.

Enzymes increase reaction rates some 1,000 to 100 million times. Temperature can increase this effect somewhat until the temperature causes the protein to denature and lose its shape and function.

In reactions catalyzed by enzymes, the reactants are called substrates and the products are still called by that name. The speed or velocity of the reactions depends on several things:
1) amount of substrate, 2) enzyme concentration,
3) temperature, 4) pH and
5) presence or absence of inhibitors.

If there is more substrate than the enzyme active sites can process per unit time, then the enzyme is working as fast as possible and its velocity is at the maximum, ie Vmax.