

Biochemistry I: Macromolecules

I. Fractionating Life:

Biochemists break up an organism and analyze the individual components.

1) What is the atomic composition of living cells/organisms?

H: 60%

O: 25%

C: 12%

N: ~5%

P, S, Mg, Mn, Se also present in lesser amounts

At certain levels of study, most organisms look the same- especially at the cellular level.

2) What is the molecular composition of cells?

Mostly Water: ~80%

Of remainder weight:

Lipids, fats: 10%

Carbohydrates: 15%

Proteins: 50%

Nucleic Acids: 15%

****Proteins** are key macromolecules (play many structural and functional roles in cells)**

Nucleic Acids (**DNA**, RNA; DNA stores hereditary information in cell)

At some level, chemical forces determine shape of molecules and shape determines function.

What are the forces that give these molecules their properties?

II. Covalent Forces

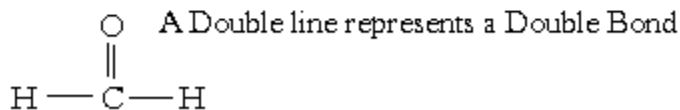
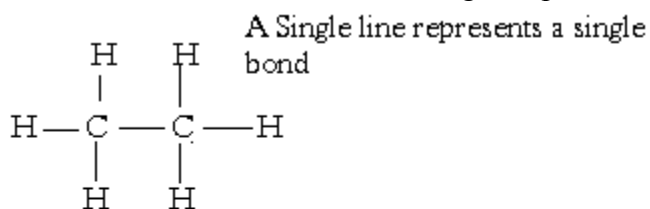
Bonds and chemical interactions hold molecules together:

1) Covalent Bonds

-most important type of bonds

-strongest type of bond – strength ~ 80 kcal/mol

A covalent bond is the sharing of a pair of electrons:



The average thermal (vibrational) energy at room temperature is on average ~ 5 kcal/mol. Since it takes ~ 80 kcal/mol to break a covalent bond, covalent bonds are fairly stable at room temperature.

| <u>Types of covalent bonds:</u> | <u>Strength</u> |
|---|-----------------|
| Single: C-C (one pair of electrons) | 80 kcal/mol |
| Double: C=C (two pairs of electrons) | 150 kcal/mol |
| Triple: C \equiv C (three pairs of electrons) | 200 kcal/mol |

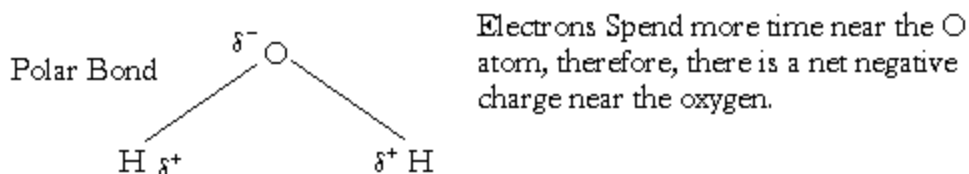
There is free rotation about a single covalent bond, but not about a double or triple bond.

Covalent bonds also have a fixed angle.

Some covalent bonds involve unequal sharing of electrons.

Some atoms hold onto electrons more tightly than other atoms. The tendency to attract electrons is a measure of electronegativity of an atom.

Look at some bonds with unequal sharing of electrons:



Polar bonds have dipole moments; have polarity

Oxygen is a more electronegative atom compared to hydrogen, and thus an O-H bond is considered a polar bond.

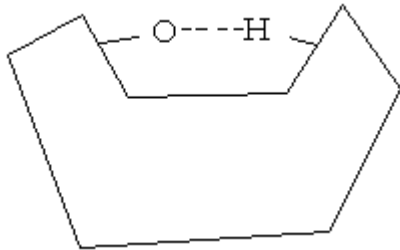
Carbon and hydrogen have similar electronegativities, therefore, a C-H bond is considered nonpolar.

2) Hydrogen Bonds

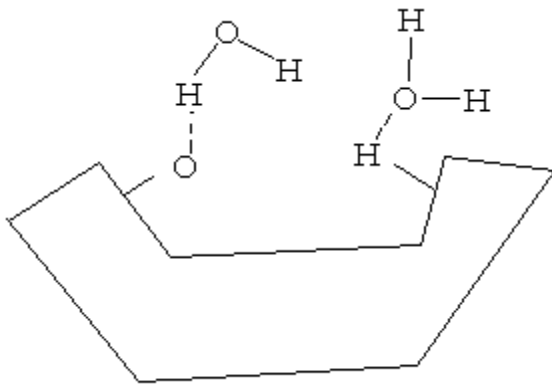
- attraction between a slight positive charge on a hydrogen atom and a slight negative charge on a nearby atom
- strength of bond ~ 5 kcal/mol (relatively weak)
- strongest when the donor, the hydrogen and the acceptor are about 0.25 nm apart
- hydrogen bonds give order and structure to molecules
- a single hydrogen bond is weak, however, most molecules are made up of many hydrogen bonds; leads to overall strength of molecule

Properties of water are determined by hydrogen bonding interactions. Water is highly structured even when liquid. Formation of ice is due to the lattice array of hydrogen bonds.

Hydrogen bonds form between different regions of a protein:



IN an aqueous environment, these regions will form hydrogen bonds with water molecules. These molecules adopt a more favorable conformation when they interact with water.

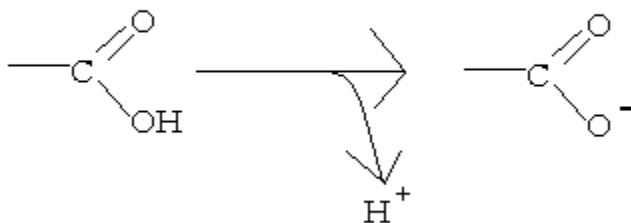


3) Ionic Bonds

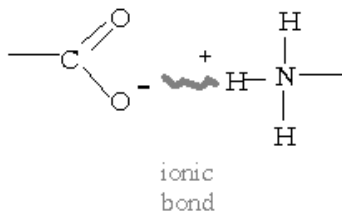
- electrostatic interaction between two oppositely charged groups in a molecule
- limiting cause of unequal sharing of electrons; one atom keeps the electron

$\text{NaCl} \rightarrow \text{Na}^+ + \text{Cl}^-$ unequal sharing of electrons, Cl keeps both electrons.

In solution this group becomes ionized, loses a proton and becomes negatively charged:



Charged atoms can be attracted to oppositely charged atoms:



Charged atoms are held by a force called Coulomb's law:
 $F = \frac{q_1 q_2}{(R)^2}$

Force of attraction is proportional to the charges (q) of the two groups and the distance (R) between them

-strength of ionic bond is about 3-7 kcal/mol; strongest when the two atoms are about 0.28 nm apart

4) Van Der Waals Interaction

- nonspecific attractive force that occurs when any two atoms come in close range
- most favorable when atoms are 0.2-0.3 nm apart
- transient polarity induced between atoms a nonpolar bond leads to attraction with nearby atoms
- very weak interaction – strength is ~1 kcal/mol. However the sum of many Van Der Waals interactions leads to increased strength and stability

Example: A ligand interacting with its receptor is accomplished by many noncovalent interactions such as Van Der Waals interactions.

5) Hydrophobic Interactions/ Entropy

Overall, a molecule is held together by many interactions. A molecule forms a particular shape because it likes to adopt the lowest energy state (minimize entropy)

In adopting this shape, the alternative conformations are selected, and the groups that cannot form hydrogen bonds with water (the hydrophobic ones) tend to cluster on the inside of the molecule (away from water).

Hydrophobic: (“water hating”) uncharged, nonpolar molecules, don’t interact with water

Hydrophilic: (“water loving”): charged or polar molecules; form hydrogen bonds with water

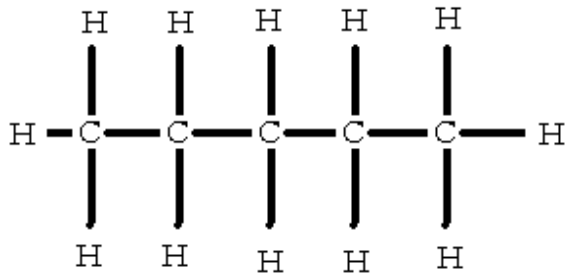
The shape that biological macromolecules adopt is dependent on a large number of molecular interactions.

III. Major Macromolecules

A. Lipids, Phospholipids

Structure of a hydrocarbon:

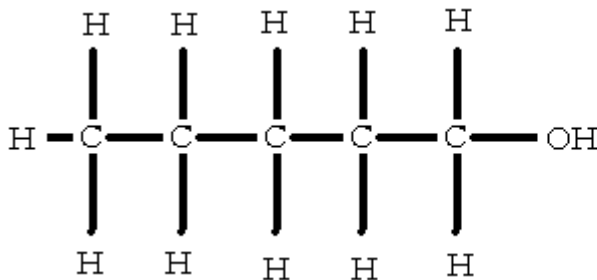




Hydrocarbon:

-nonpolar bonds
-hydrophobic
-insoluble in water (cannot form hydrogen bonds with water)

If you add a Hydroxyl group (OH) to a hydrocarbon you get an alcohol:

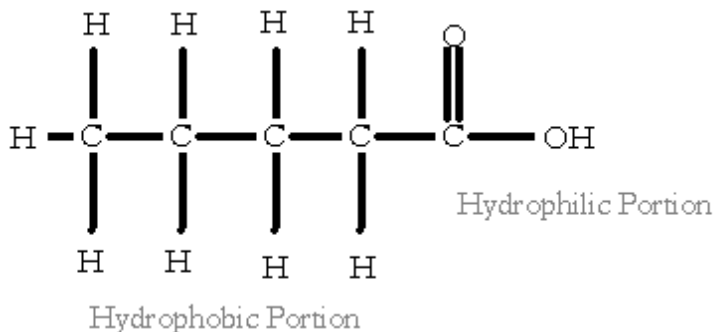
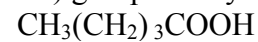


Alcohol:

-hydrophilic
-soluble in water (can form hydrogen bonds with water)

Short chain alcohols are soluble in water.

You can make a fatty acid by adding a carboxyl group (COOH) group to a hydrocarbon:

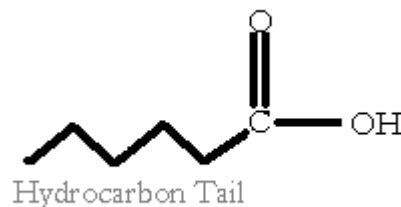


Fatty Acid:

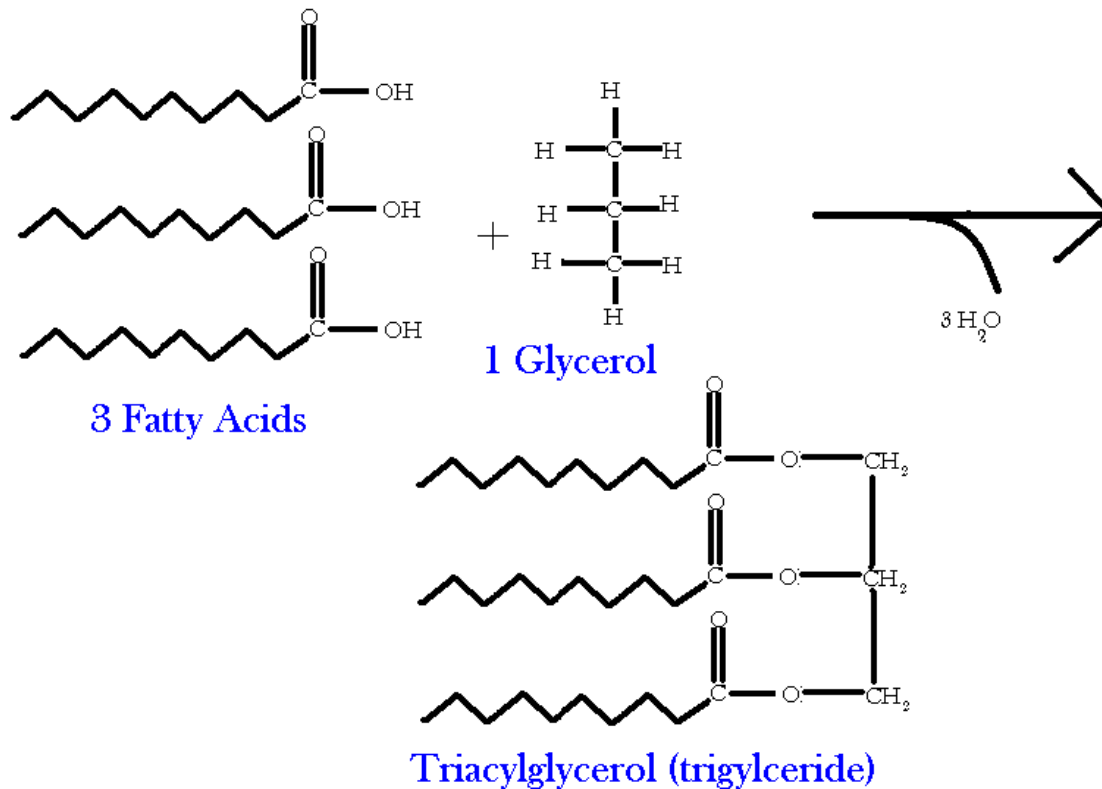
-hydrophilic
-somewhat soluble in water
(can form hydrogen bonds with water)

A fatty acid is an amphipathic molecule – contains both hydrophobic and hydrophilic portions.

A fatty acid can also be represented as:

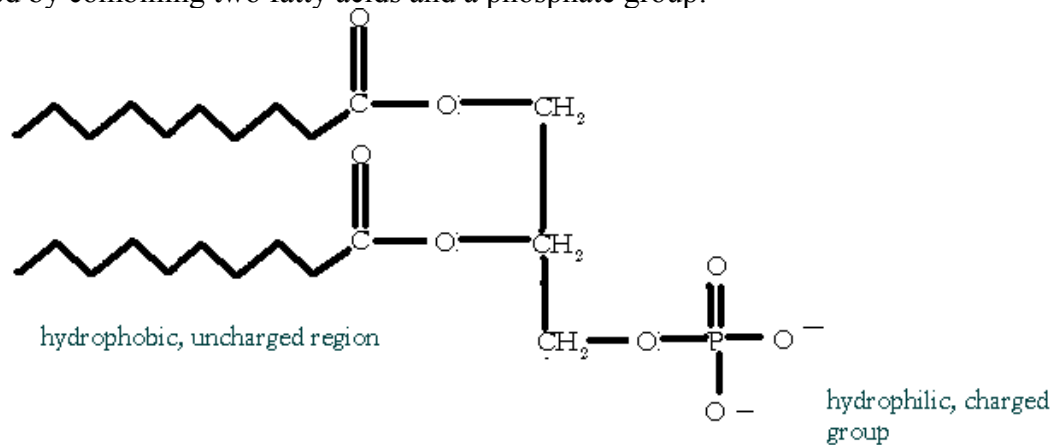


Three fatty acids and one glycerol molecule can be combined in a dehydration synthesis to form a lipid (a triglyceride). Triglycerides are major storage forms of fatty acids inside cells.

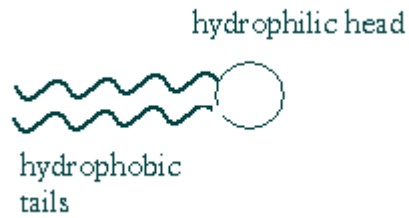


Phospholipids:

A subgroup of lipids that play a key role in cell structure. Phospholipids are formed by combining two fatty acids and a phosphate group:



The phospholipid can also be represented as:



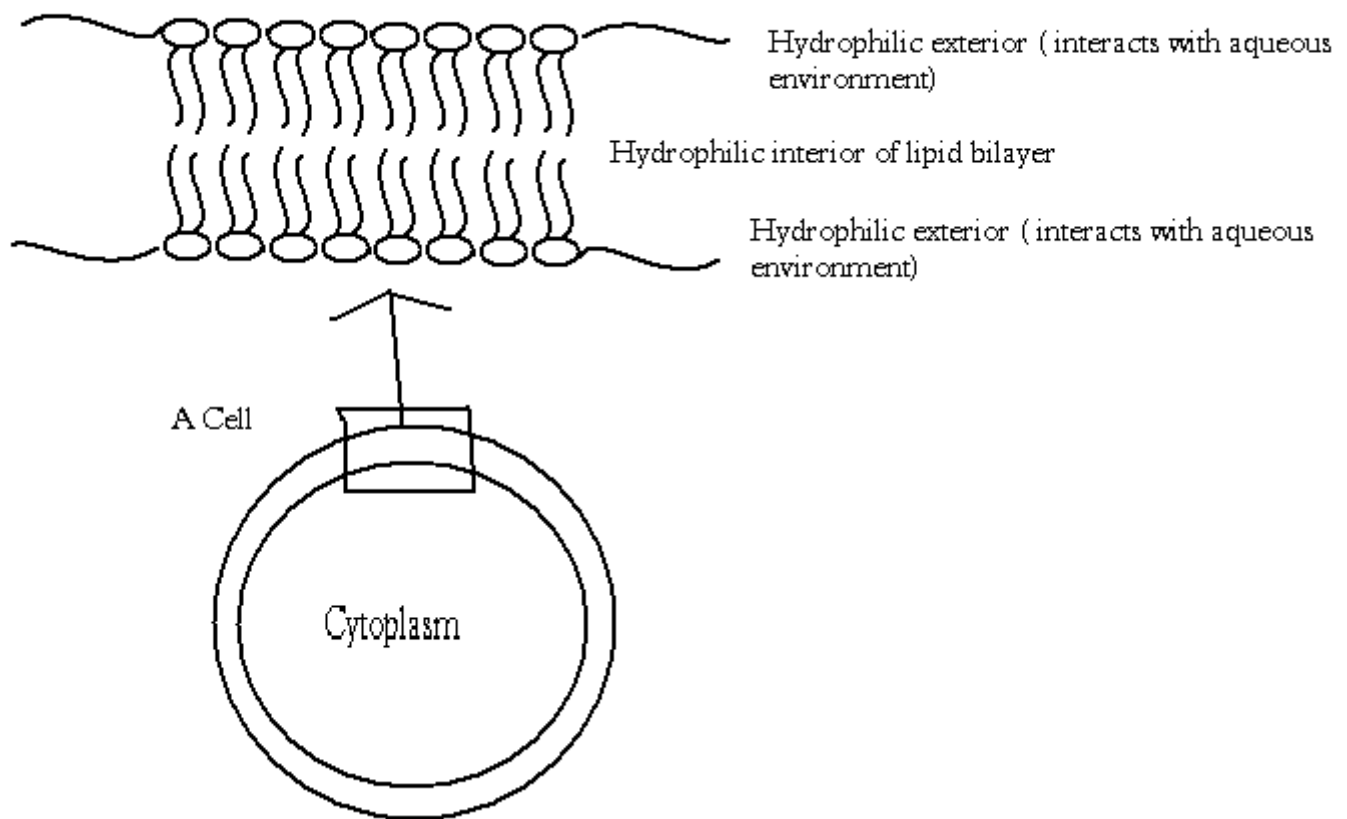
in solution, phospholipids will assemble to form micelles

A micelle:



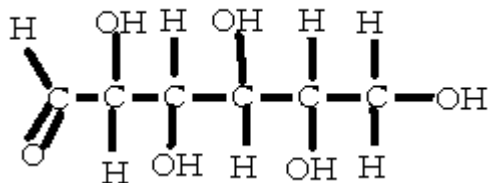
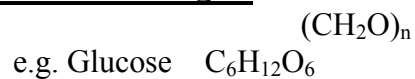
Phospholipids form a lipid bilayer in an aqueous solution. A typical cell is enclosed by a plasma membrane, which is made up of a phospholipid bilayer (2 layers of phospholipid molecules arranged in a lipid bilayer)

The hydrophobic interior of the plasma membrane is impermeable to charged or polar molecule.



B. Sugars, Carbohydrates

1) General formula for a sugar:



In all sugars, n-1 of the carbons has a hydroxyl (OH) group and the C-1 carbon has a carbonyl (C=O) group. The location of the carbonyl group and the orientation of the hydroxyl groups determine the type of sugar.

If the carbonyl group is at the end (an aldehyde group) then it is an aldose (e.g. glucose)

If the carbonyl is in the middle (a ketone group) then it is a ketose (e.g. fructose)

Six carbon sugars are called hexoses (e.g. glucose)

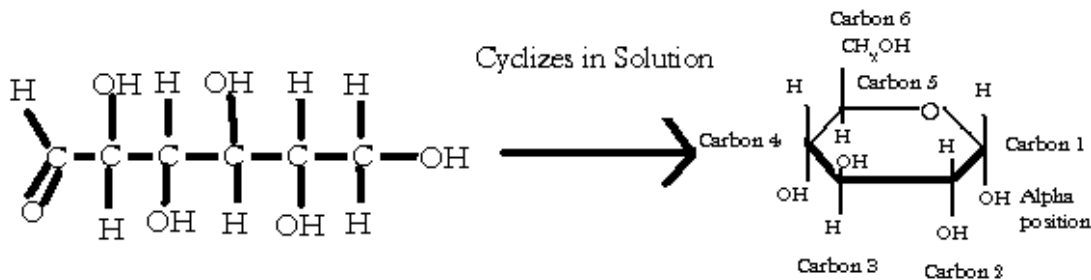
Five carbon sugars are called pentoses (e.g. ribose)

Three carbon sugars are called trioses (e.g. glyceraldehyde)

2)Conformation of sugars

a)Monosaccharides

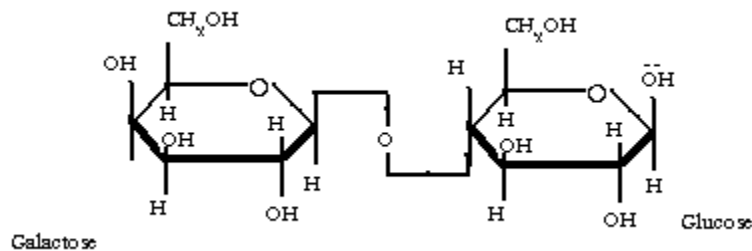
Glucose is more often found in a ring form in solution:



The Orientation of the OH group on the C-1 carbon can be either in the alpha (below the plane of the ring) or beta (above the plane of the ring) position

b)Disaccharides

Disaccharides consist of two Monosaccharides linked by a covalent bond:



Lactose (form)
(Galactose (1→4) Glucose)

The enzyme lactase breaks down lactose to glucose and galactose. Many adult individuals stop synthesizing lactase enzyme. As a result a large percent of certain populations becomes lactose-intolerant.

c) Polysaccharides

Polysaccharides consist of many monosaccharide units (usually glucose monomers) linked together to form long chains.

e.g. starch, glycogen, cellulose

Polysaccharides are used as a form of storage of energy and also for structural roles.

Starch is an unranked polymer of Glucose (1→4) linkage

Cellulose – plays an important structural role in plants; one of the most abundant molecules on earth.. it is an unbranched polymer of glucose in (1→4) linkage.

The alternating units of glucose enable adjacent cellulose molecules to form hydrogen bonds with each other. The hydrogen bonding capability of cellulose leads to the strength of the cell walls and also fibers such as wood.

Biochemistry II: Proteins

Proteins

- have many functions in the cell
- structural and functional roles
- 10^5 different kinds of proteins made in eukaryotic cells

Proteins are polymers of building blocks known as amino acids

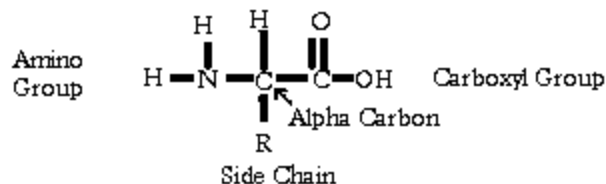
20 different amino acids → can make 20^n combinations of proteins length n

Nucleic Acids

- store and transfer genetic material
- 4 different building blocks called nucleotides → can make 4^n combinations of nucleic acids length n

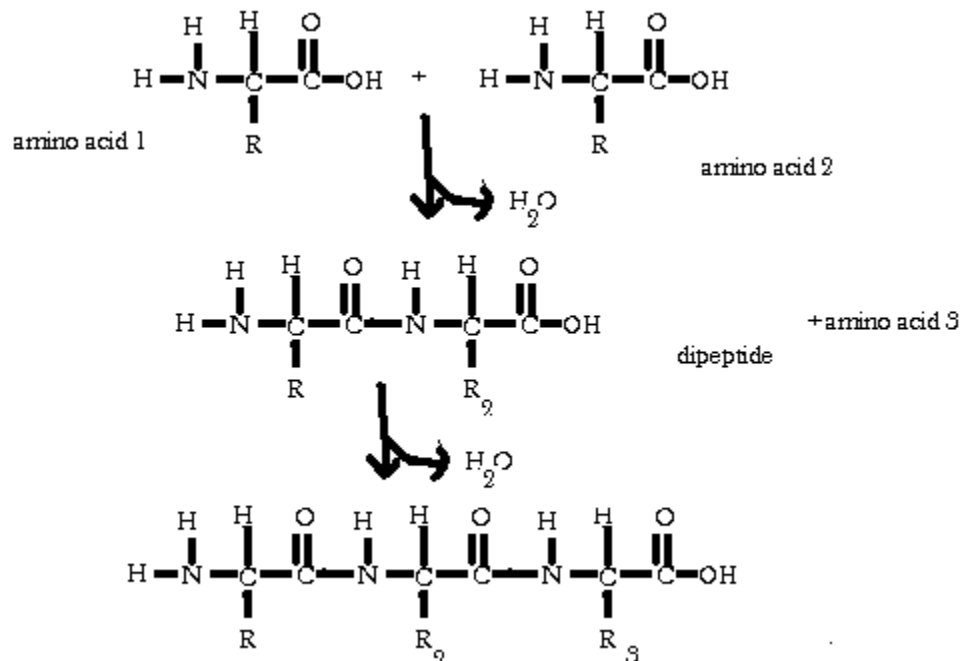
§1. Amino Acids & Peptide Bonds

General Formula for Amino Acids:

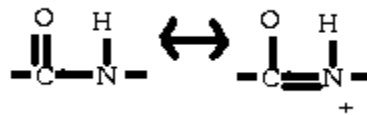


R group (side chain) varies among the 20 different amino acids. 20 different amino acids make up all proteins.

Peptides are oligomers of amino acids formed: via a dehydration reaction when the - carboxyl group of one peptide is linked to the amino group of a second amino acid



The peptide bond is planar, has partial double bond character; no rotation about the amide bond. The actual structure of the peptide bond is a hybrid of the two forms below:



There is however, rotation about the bonds adjacent to the peptide bond.

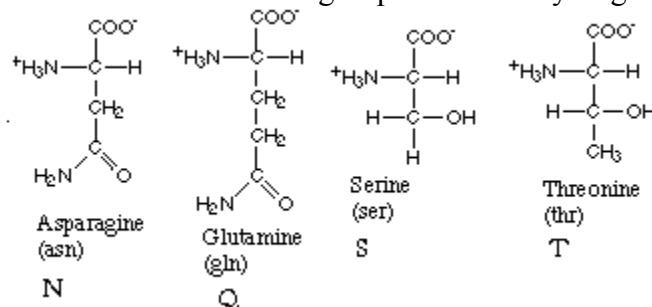
A peptide unit is a planar arrangement of four atoms.

A long polypeptide made up of many amino acids is called a protein. Each protein has a specific order of amino acids and adopts a particular shape – which is determined by the sequence of amino acids.

§2. The Twenty Different Amino Acids

a. Side chains that are polar but uncharged:

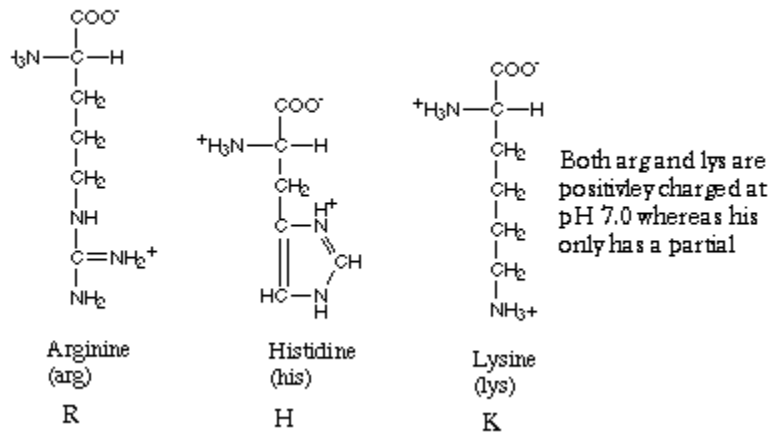
The side chains of these groups can form hydrogen bonds with other polar groups



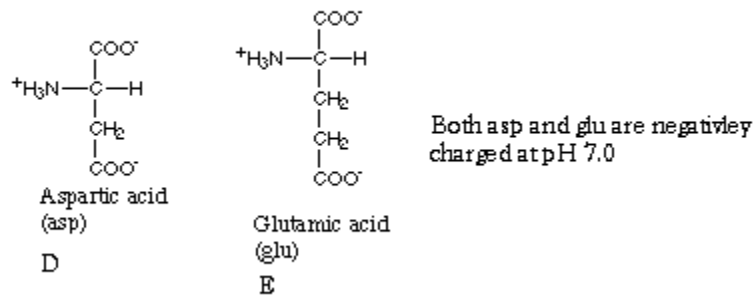
b. Side chains that are polar and charged

The side chains of these amino acids can form hydrogen bonds or ionic bonds

1. Positively Charged:

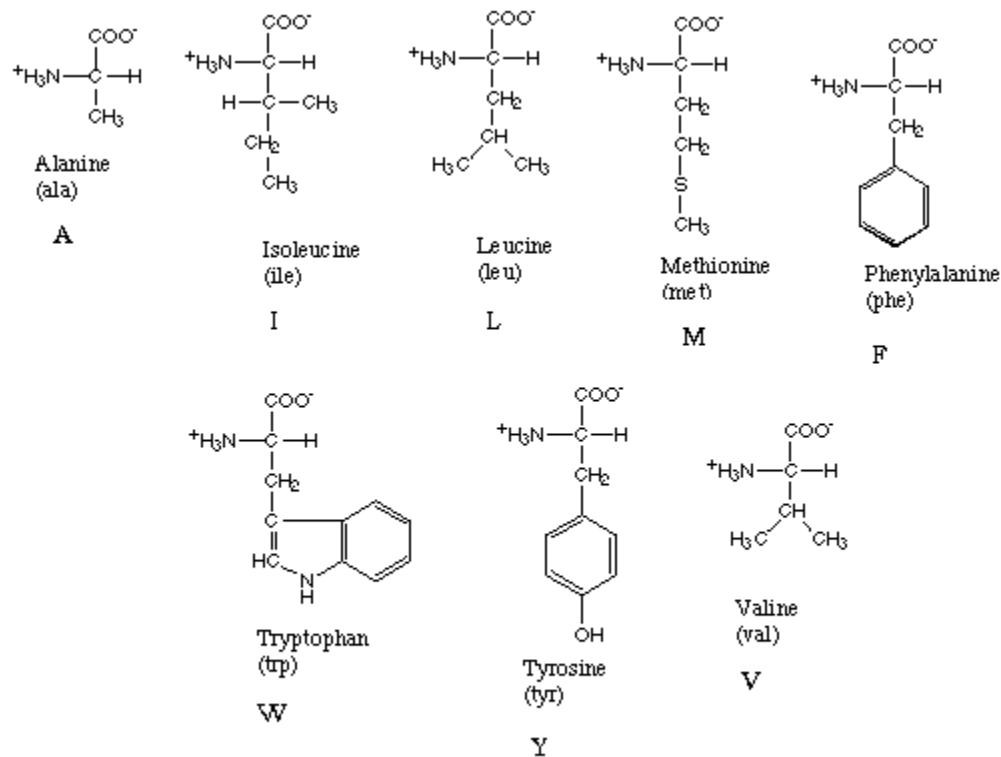


2. Negatively charged:



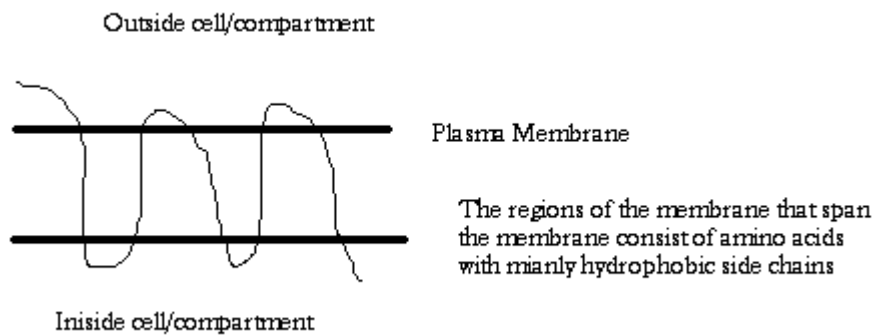
c. Side Chains that are hydrophobic (nonpolar, uncharged)

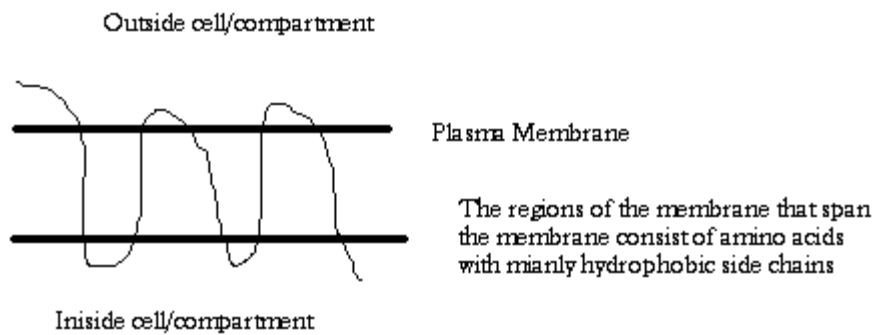
The side chains of most of these amino acids cannot form hydrogen bonds or ionic bonds and prefer to be in a nonpolar environment. These amino acids prefer to be in the interior of proteins in an aqueous solution.



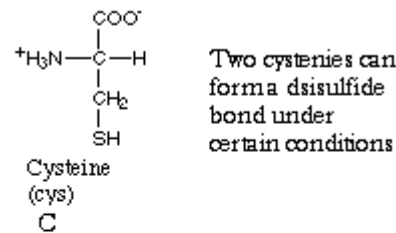
Although Tyrosine is largely nonpolar, its side chain does have a polar hydroxyl group which can form hydrogen bonds.

Some proteins are called transmembrane proteins. A region of the transmembrane protein crosses the membrane of the cell or the cellular compartment in which they are found

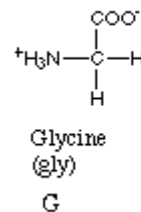
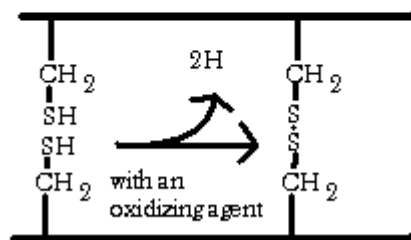




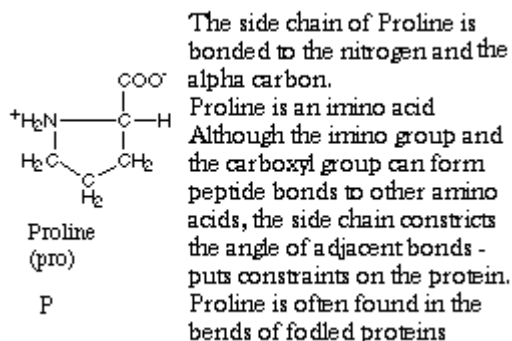
d. Special cases



Two cysteines can form a disulfide bond under certain conditions



The Side chain of Glycine is small and flexible but it does not participate in chemical interactions



The side chain of Proline is bonded to the nitrogen and the alpha carbon. Proline is an imino acid. Although the imino group and the carboxyl group can form peptide bonds to other amino acids, the side chain constricts the angle of adjacent bonds - puts constraints on the protein. Proline is often found in the bends of folded proteins

§3. Protein Structure.

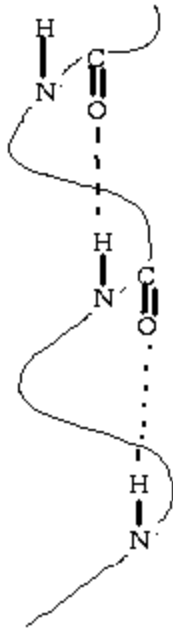
Understanding protein structure – a combination of forces determines structure:

- hydrophobic effect
- ionic bonds
- hydrogen bonds
- disulfide bonds

-1930's: Linus Pauling proposed that the NH group in one amino acid and the C=O group in another amino acid can interact to form a hydrogen bond.

-He predicted that these groups would interact to result in a polypeptide structure termed a -helix (alpha helix). IN an α helix, the CO group of amino acid n is hydrogen bonded to the NH group of amino acid $(n+4)$.

Structure of -helix:

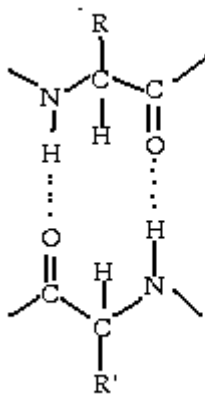


This hydrogen bonding occurs within one polypeptide chain.

3.6 aa per turn of the helix

Each aa contributes 0.15 nm (1.5 Å) to the rise of the helix

Pauling also predicted another polypeptide structure called the -pleated sheet (beta pleated sheet). In a pleated structure, there are intermolecular attractions between two or more polypeptide chains:



Hydrogen bonds form between two antiparallel polypeptide chains.

Levels of Protein Structure:

a) Primary Structure:

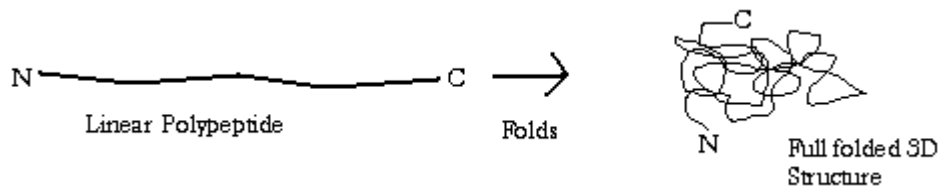
-The linear sequence of amino acids (e.g. NH_3^+ ...met-cys-leu-lys-glu... COO^-)

b) Secondary Structure

-The local arrangement of amino acids that are close together in the linear chain to form structures that include α -helices, β -pleated sheets and random coils and loops.

c) Tertiary Structure

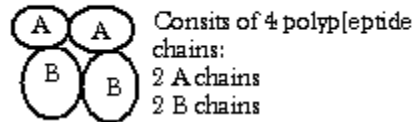
-Spatial arrangement of amino acids that are far apart in the linear polypeptide chain to form the full 3-dimensional (folded) structure of the protein. Also includes disulfide bonds:



d) Quaternary Structure

-Interaction of more than one polypeptide chain; association between different proteins to form complexes such as dimers, trimers, tetramers:

e.g.
Hemoglobin
(oligomeric
protein)



§4. Protein Structure and function

-Protein structure determines protein function.

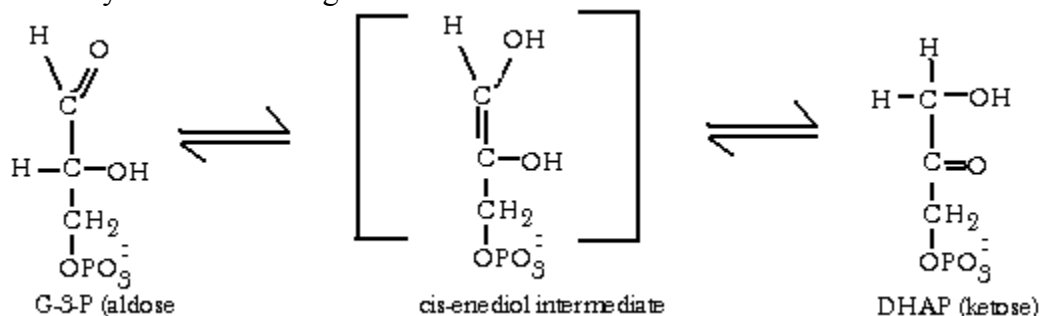
-Look at an example of a protein that catalyzes a biochemical reaction.

Triose phosphate isomerase (TPI)

TPI is a protein dimer that is made up of two polypeptide chains that are each 247 amino acids in length. The polypeptide folds to form a basket shape enzyme with a well in the center.

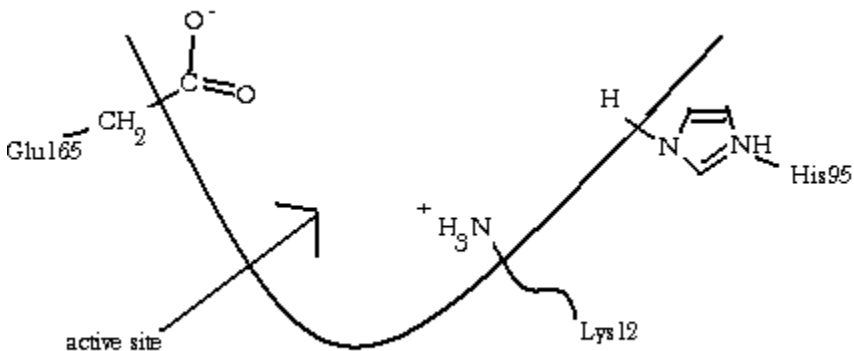
ONLY THE DIMER IS ENZYMATICALLY ACTIVE!

TPI catalyzes the following reaction:



Although this protein is 247 amino acids in length, only three of these amino acids (Glu165, His95 and Lys12) are important in the catalytic function of TPI. The catalytic (active) site is formed when the protein folds into its full 3-D shape and the three amino acids are brought into close proximity.

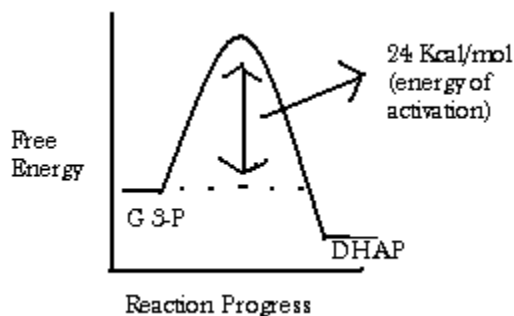
Look at the active site of TPI:



Mechanism:

- 1) G 3-P binds to active site of TPI (N from His95)
- 2) Lys12 stabilizes G 3-P in the active site
- 3) Glu165 acting as a base catalyst (B⁻) picks up a H⁺ from the C₂ group (the carbon next to the carbonyl) of G 3-P
- 4) His95 donates a H⁺ to the C₁ group (the carbonyl) of G 3-P to make the OH group on C₁ (and facilitates the formation of the enediol intermediate)
- 5) Glu165 picks up the H⁺ to the C₁ group of the intermediate.
- 6) His95 picks up the proton from the OH group on the C₂ group on the intermediate

TPI stabilizes (lowers the energy of the intermediate) of the highly unfavorable intermediate, which has a much more positive free energy than either G 3-P or DHAP.



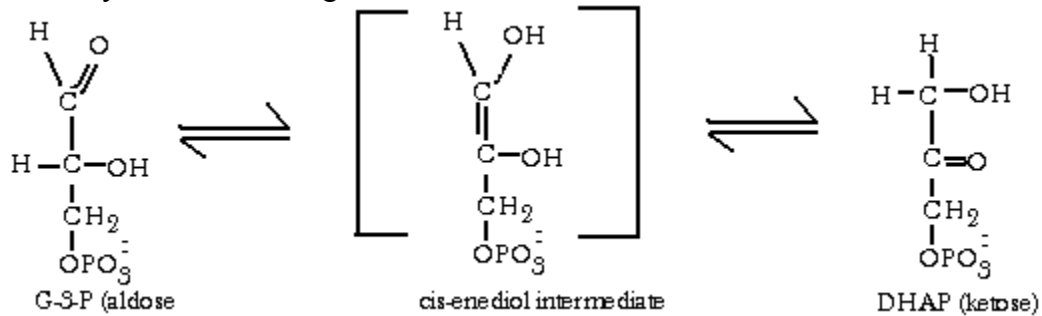
TPI also speeds up the reaction 10-fold. The amino acid that stabilizes the transition state is Glu165. If you substitute Glu165 in TPI with Aspartic Acid (which is only 1 carbon shorter in length than glu) you reduce the activity of the enzyme by 1000-fold (TPI becomes 1000 times less effective)

ARTICLES FROM WASHINGTON POST, 5/21/93 pA3 "Influenza's Burglary Tools" by Boyce Rensberger and

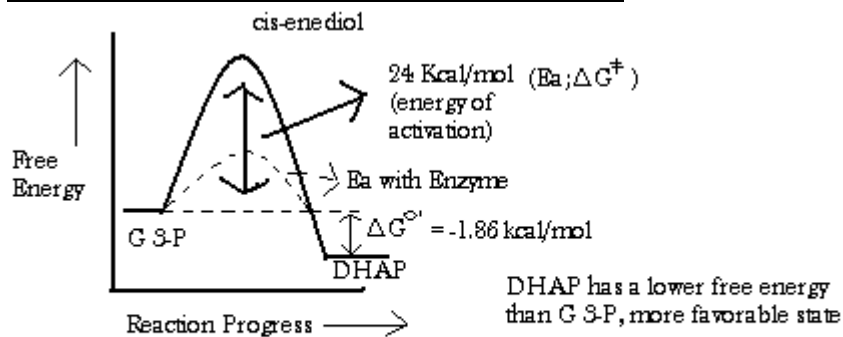
NY TIMES, 5/21/93 "Breakthrough on Flu Viruses Reported by Warren E. Leary

Biochemistry III: Free Energy and Reaction Kinetics

TPI catalyzes the following reaction:



Free Energy Diagram for the above reaction:



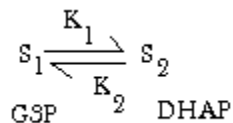
TPI lowers the energy of activation (E_a) for the reaction.

TPI does this by stabilizing the transition state (cis-enediol)

TPI (as with all enzymes) do not affect the ΔG° values for a chemical reaction.

§1. Reaction Equilibrium, K_{eq} and Free Energy

At equilibrium, molecules tend to be in their lowest energy state. Two states, S_1 and S_2 with difference in energy between the two expressed as ΔG° = change in free energy under standard conditions.



The equation that relates ΔG to the concentrations of S_1 and S_2 :

$$\Delta G = \Delta G^\circ + RT \ln \frac{[S_1]}{[S_2]}$$

ΔG = Free Energy difference in Reaction

ΔG° = Free Energy at specific standard conditions

RT = gas constant x T (in Kelvins)

$[S_1]/[S_2]$ = ratio of products to reactants

At equilibrium for the reaction, $G = 0$ (no net reaction)

$$\Delta G^{\circ} = -RT \ln \frac{[S_1]}{[S_2]}$$

$RT = 0.59 \text{ kcal/mol}$ at 25°C , $RT = 0.61 \text{ kcal/mol}$ at 37°C so $RT \sim 0.6$

$$\frac{-\Delta G^{\circ}}{RT} = \ln \frac{[S_1]}{[S_2]} \rightarrow \frac{[S_1]}{[S_2]} = e^{\frac{-\Delta G^{\circ}}{RT}} = Keq \quad Keq = \text{equilibrium constant}$$

$$Keq = e^{\frac{-\Delta G^{\circ}}{RT}}$$

$Keq = \text{ratio of constants}$

$$Keq = \frac{K_1}{K_2} \quad (\text{net rate of forward reaction} = \text{net rate of reverse reaction})$$

$$\text{rate of forward reaction} = K_1[S_1]$$

$$\text{rate of reverse reaction} = K_2[S_2]$$

$$K_1[S_1] = K_2[S_2]$$

$$\frac{K_1}{K_2} = \frac{[S_2]}{[S_1]} = Keq$$

Example: $\text{G3P} \leftrightarrow \text{DHAP}$ ($\Delta G^{\circ} = -1.86 \text{ kcal/mol}$)

$$Keq = \frac{[\text{DHAP}]}{[\text{G3P}]} = e^{\frac{-1.86 \text{ kcal/mol}}{0.6 \text{ kcal/mol}}}$$

$$\text{At equilibrium } \frac{[\text{DHAP}]}{[\text{G3P}]} = e^{3.1} = 22$$

$$Keq = 22$$

At equilibrium there will be 22x more DHAP than G3P

Given pure G3P: The reaction proceeds \rightarrow DHAP (net synthesis of DHAP)

Given pure DHAP: The reaction proceeds \leftarrow to form G3P (net synthesis of G3P)

When $\text{G3P} = \text{DHAP}$ $G_{\text{Rxn}} = 0$ (no net change)

Is 22:1

$\sim 7:1$

The Reaction proceeds to the right \rightarrow DHAP

The direction of a reaction depends on:

- 1) the intrinsic energy difference between the products and the reactants
- 2) the concentrations of the reactions and products

$$\text{This is described in the equation: } \Delta G = \Delta G^{\circ} + RT \ln \frac{[\text{PRODUCTS}]}{[\text{REACTANTS}]}$$

If ΔG of rxn < 0 , rxn will proceed forward \rightarrow (exergonic reaction)
 If ΔG of rxn > 0 , rxn will proceed in reverse \leftarrow (endergonic reaction)
 If $\Delta G = 0$, rxn is at equilibrium, there is no net reaction.

Example: IF the ratio of DHAP: G3P was 7.4:1, what would be the ΔG of the rxn and in which direction will it proceed?

$$\Delta G = \Delta G^\circ + RT \ln \frac{[PRODUCTS]}{[REACTANTS]}$$

$$\Delta G = -1.86 \text{ kcal/mol} + .60 \text{ kcal/mol} \ln 7.4$$

$$\Delta G = -0.66 \text{ (} \Delta G < 0 \text{)}$$

Therefore the reaction will proceed forward (\rightarrow) to produce DHAP

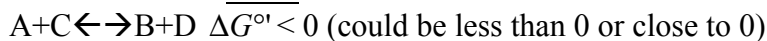
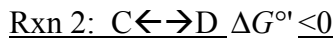
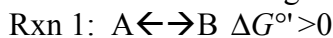
§2. Coupled Reactions

Look at the reaction $A \rightleftharpoons B$

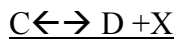
The reaction will tend to proceed in the direction of the molecule with the lower free energy.

How do we drive a reaction forward that has a $\Delta G^\circ > 0$?

You can couple an unfavorable reaction ($\Delta G > 0$) with a favorable reaction ($\Delta G < 0$)
 Sometimes the coupling can still result in unfavorable conditions, but it is usually more favorable than the original, uncoupled reaction.

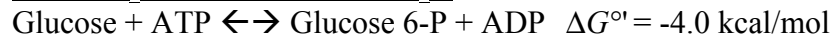
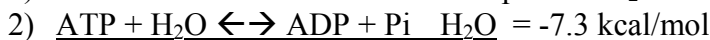


Example:



Cells use this mechanism of coupling unfavorable reactions with favorable ones so that reactions will proceed forward.

Example:



Cells use the energy from the hydrolysis of ATP to drive the energetically unfavorable reaction 1.



ATP is a major source of energy. ATP stores energy by bringing negative charges together in high energy bonds and releases this energy when the bond is broken.

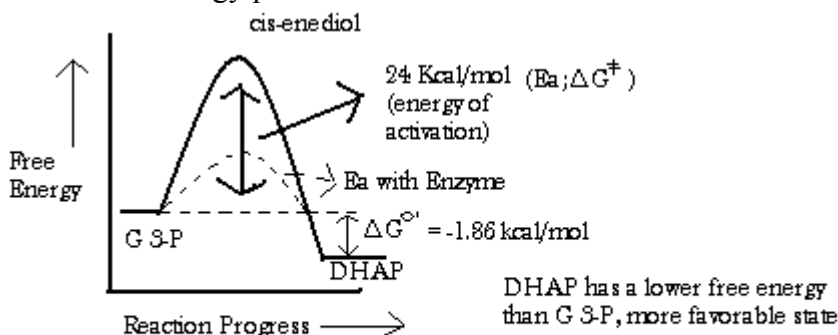
All of the reactions (1000's) that occur in the cell have a $\Delta G < 0$

* Enzymes increase the rate of reaction; however, enzymes do not alter the equilibrium concentrations or the ΔG for the reaction

§3.1 Reaction Rates and the Role of Enzymes

For the reaction $\text{G3P} \leftrightarrow \text{DHAP}$

Look at the energy profile for the reaction



-Energy of activation (E_a) = energy barrier or free energy of activation for a reaction.

-Enzyme (TPI) lowers the E_a by stabilizing (lowering the free energy of) the transition state (intermediate).

In the presence of TPI, the E_a for the reaction is 13 kcal/mol

At equilibrium $\Delta G = 0$, activation energy is 26 kcal/mol

$$\frac{[\text{cis-enediol}]}{[\text{G3P}]} = e^{\frac{-\Delta G^\circ}{RT}} = e^{\frac{-(26 \text{ kcal/mol})}{0.6 \text{ kcal/mol}}} = 1.5 \times 10^{-19} \approx 10^{-19}$$

At equilibrium, there is $\sim 10^{19}$ more G3P than cis-enediol (or 10^{-19} less cis-enediol than G3P).

Therefore the reaction proceeds slowly in the absence of enzyme.

In the presence of enzyme, $\Delta G^\circ = -1.86$ kcal/mol. TPI stabilizes the cis-enediol intermediate, thus lowering its free energy from 26 kcal/mol to 13 kcal/mol.

At equilibrium $\Delta G = 0$; activation energy is 13 kcal/mol:

$$\frac{[\text{cis-enediol}]}{[\text{G3P}]} = e^{\frac{-\Delta G^\circ}{RT}} = e^{\frac{-(13 \text{ kcal/mol})}{0.6 \text{ kcal/mol}}} = 3 \times 10^{-10} \approx 10^{-10}$$

At equilibrium, there is $\sim 10^{10}$ more G3P than cis-enediol (or 10^{-10} less cis-enediol than G3P).

The ratio of [cis-enediol] to [G3P] is 10^9 fold better in the presence of the TPI enzyme.

But how do we know that enzymes lower the E_a for the reaction by binding to the transition state?

-Because transition state analogs (stable molecules) that look like the transition state bind the enzyme better than either the reactant or the product.

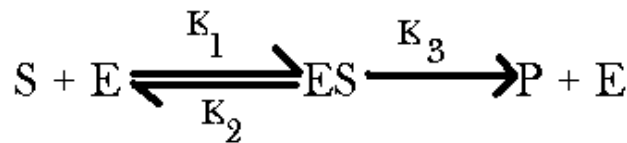
Role of Enzymes In Chemical Reactions:

- A reaction can be exergonic, but have a high E_a ; therefore, you need an enzyme to lower the E_a
- A reaction can be endergonic and cannot proceed unless coupled to an exergonic reaction. An enzyme can catalyze the coupled reaction, therefore allowing the reaction to proceed

Exergonic reactions ($\Delta G < 0$), endergonic reactions ($\Delta G > 0$)

§4. Enzyme Kinetics (Michaelis-Menten Kinetics 1913)

We can model the enzyme reaction mechanism as follows:



S = substrate

E = enzyme

ES = enzyme-substrate complex

P = product

k_1 = Forward reaction rate constant

k_2 = Reverse reaction rate constant

k_3 = forward reaction rate

$$k_1 = ((\# \text{ moles/L of ES formed})/\text{sec})/((\# \text{ moles/L of E})(\# \text{ moles of S})) \\ = 1/(\text{mol/L} \times \text{sec}) \text{ units}$$

$$k_2 = ((\# \text{ moles/L of S formed})/\text{sec})/((\# \text{ moles/L of ES})) \\ = 1/\text{sec} \text{ units}$$

$$k_3 = ((\# \text{ moles/L of P formed})/\text{sec})/((\# \text{ moles/L of ES})) \\ = 1/\text{sec} \text{ units}$$

1) Catalytic Rate

The catalytic rate is equal to the product of the concentrations of ES and K_3
 $V = K_3[ES]$ $V = \text{velocity or reaction rate}$

2) Fraction (f) of enzyme bound to substrate is:

$$f = \frac{[ES]}{[E_T]}$$

$[E_T]$ = Total enzyme concentration

$$f = \frac{[S]}{[S] + K_m}$$

$[ES]$ = Concentration of bound enzyme
 f = fraction of enzyme bound to substrate

$$[ES] = [E_T] \frac{[S]}{[S] + K_m}$$

$$K_m = \frac{k_2 + k_3}{K_1} \text{ Michaelis Constant}$$

$$K_m = \frac{\frac{1}{\text{sec}} + \frac{1}{\text{sec}}}{\frac{l}{(\text{moles})(\text{sec})}} = \frac{\text{moles}}{l} \text{ units}$$

K_m is a measure of the affinity of an enzyme for its substrate

What is the fraction (f) of bound enzyme when:

1) $[S] = K_m$ fraction of bound enzyme = $\frac{1}{2}$

$$f = \frac{[S]}{[S] + K_m} = \frac{[S]}{[S] + [S]} = \frac{[S]}{2[S]} = \frac{1}{2}$$

2) $[S] \ll K_m$ fraction of bound enzyme depends on affinity of enzyme for substrate

$$f = \frac{[S]}{K_m}$$

3) $[S] \gg K_m$ fraction bound is 1 (enzyme is limited – all bound w/ substrate)

$$f = \frac{[S]}{[S]} = 1$$

The Reaction Rate is $V = K_3[ES]$; substitute into equation:

$$[ES] = [E_T] \frac{[S]}{[S] + K_m}$$

$$[V] = k_3[E_T] \frac{[S]}{[S] + K_m}$$

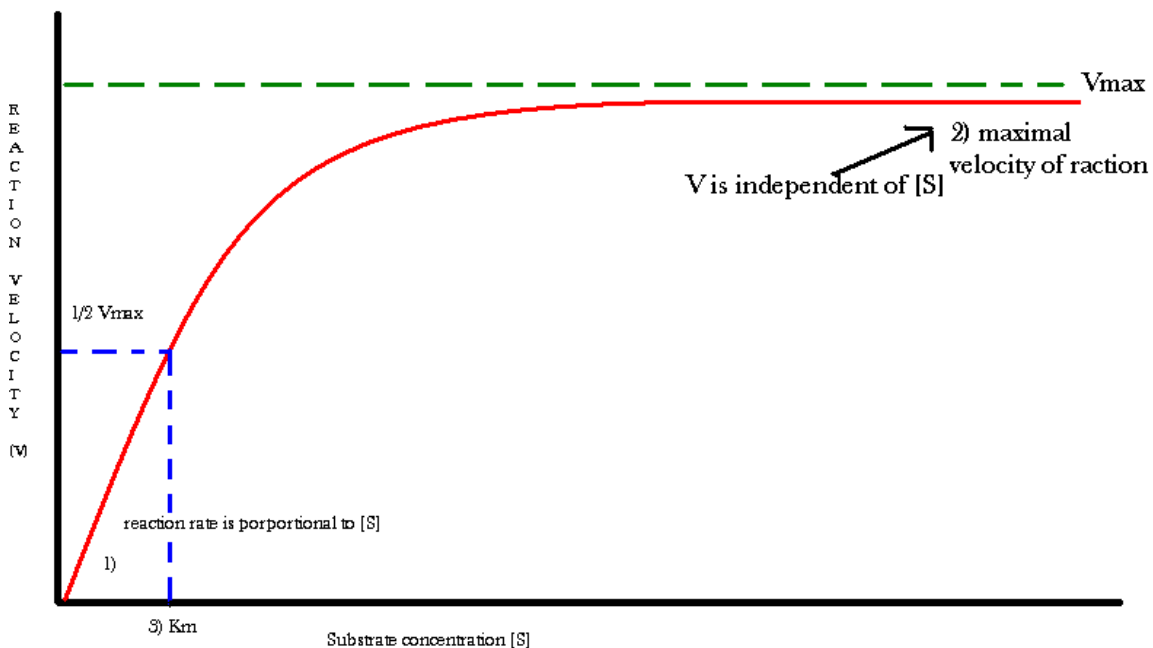
The maximal rate of reaction (V_{\max}) is obtained when the enzyme is saturated with substrate with substrate (when $[S] \gg K_m$) and $\frac{[S]}{[S] + K_m}$ approaches 1 ($f=1$)

$$V = K_3[E_T] \quad (1)$$

$$V_{\max} = K_3[E_T]$$

Thus $V = \frac{V_{\max}[S]}{[S] + K_m} \rightarrow$ Michaelis-Menten Equation

Look at a plot of the reaction velocity as a function of the substrate concentration $[S]$, for an enzyme that obeys Michaelis-Menten kinetics



1) $[S]$ is low $V = \frac{V_{\max}[S]}{K_m}$ ($[S]$ in denominator does not matter anymore)

2) $[S]$ is high $V = \frac{V_{\max}[S]}{[S]}$ (Value of K_m in denominator does not matter) so $V = V_{\max}$

3) $[S] = K_m$ $V = \frac{V_{\max}[S]}{[S] + [S]}$ (since $K_m = [S]$) so $V = 1/2 V_{\max}$

K_m is the $[S]$ at which half the enzyme is bound to substrate

What is the velocity of product formation:

Per $[E]$, per $[S]$

$$[V] = k_3[E_T] \frac{[S]}{[S] + K_m}$$

When $[S]$ is small, most of enzyme is free $[E] = [E_T]$

$$V = \frac{K_3[S][E]}{K_m} \quad K_{\text{cat}} = \text{turnover \#; \# of substrate molecules turned over by enzyme}$$

per time to form product

$$V = K_3 / K_m = K_{\text{cat}}$$

For Triose Phosphate Isomerase:

$$K_1 = 10^8 \text{ l}/(\text{moles} \times \text{sec})$$

$$K_2 = 430/\text{sec}$$

$$K_3 = 430/\text{sec}$$

$$K_3 / K_m = K_{\text{cat}} = \sim (10^8 \text{ products})/(\text{reactants} \times \text{enzyme} \times \text{sec})$$

$$K_m = \frac{K_2 K_3}{K_1} = 4.7 \times 10^{-5} M$$

$$K_3 / K_m = \frac{4300}{4.7 \times 10^{-5} (\text{moles})(\text{sec})/l}$$

-The rate of the TPI reaction is only limited by diffusion; every time TPI encounters G3P, it converts it to DHAP

-The rate of diffusion is $\sim 10^8$ (therefore enzyme rarely encounters substrate)

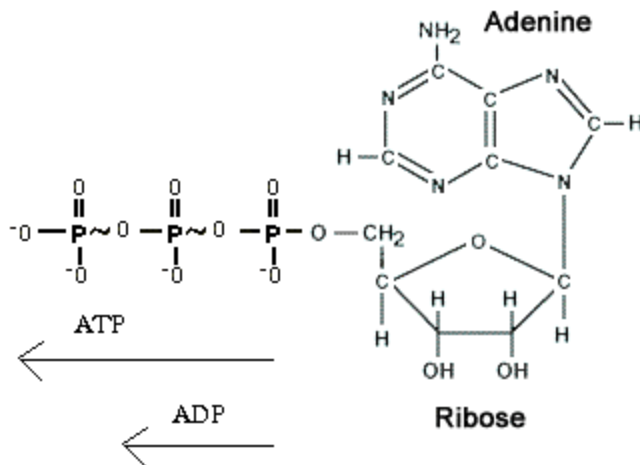
-TPI is, however, kinetically perfect – every time it meets substrate, it converts it to product

7.012 Handout on Enzyme Kinetics (dated 9/10/97).. supplement to Chapter 6 in Purves et al.

Biochemistry IV: Energy Metabolism

Review of Kinetics

Structure of ATP at pH 7.0:



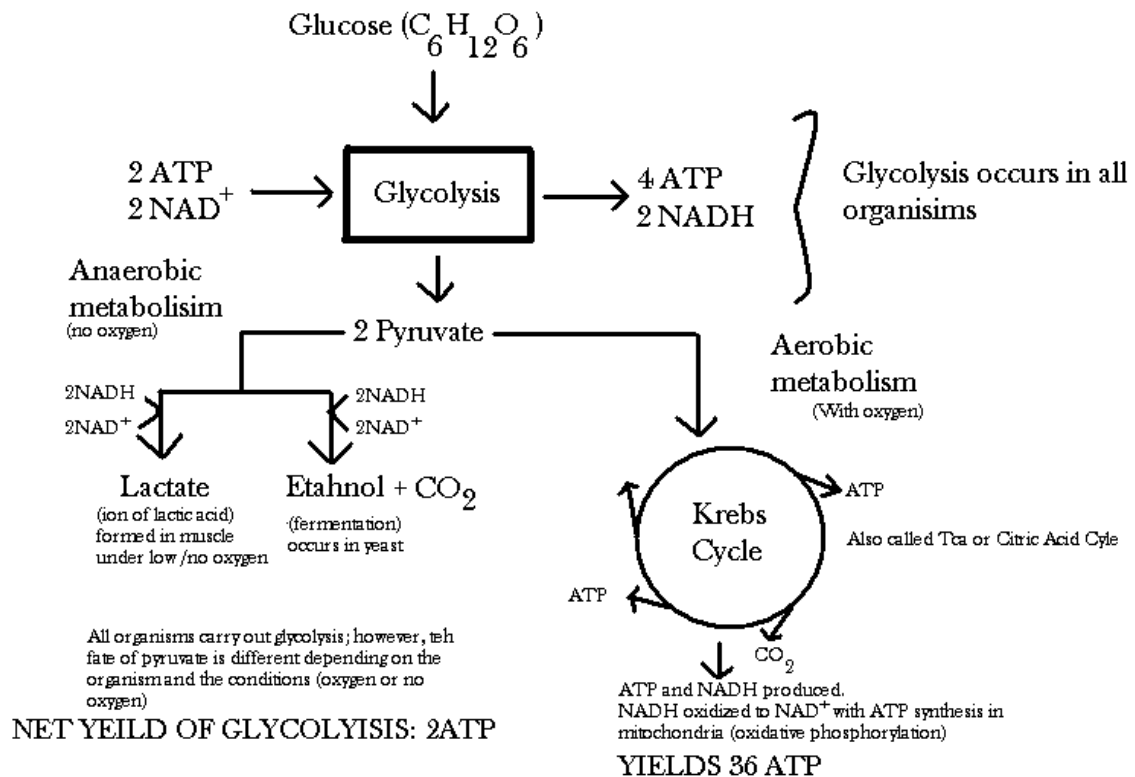
there is a large electrostatic repulsion among the negatively charged phosphate groups.

ATP hydrolysis to ADP + P_i releases free energy and reduces the repulsion

How do you make ATP (energy currency for the cell)?

Through a process known as Glycolysis (“glyco” – sugar, “lysis” – breaking)

In glycolysis, 1 molecule of glucose is converted into 2 molecules of pyruvate



Energy efficiency of this process:

With Oxygen: $C_6H_{12}O_6 + 6H_2O - 6CO_2 + 6CO_2 + 6H_2O$ yields -686 kcal/mol

36 ATPs

Without Oxygen: $C_6H_{12}O_6 \rightarrow 2 \text{ lactate} \rightarrow 2 \text{ ATPs}$

$C_6H_{12}O_6 \rightarrow 2 \text{ ethanol} + CO_2 \rightarrow 2 \text{ ATPs}$

Glycolysis takes place in the cytoplasm of the cells, does not require oxygen and occurs in all organisms

~3.5 billion years ago: not enough oxygen – so organisms carried out anaerobic metabolism

~2.5 billion years ago: enough oxygen available to carry out aerobic metabolism

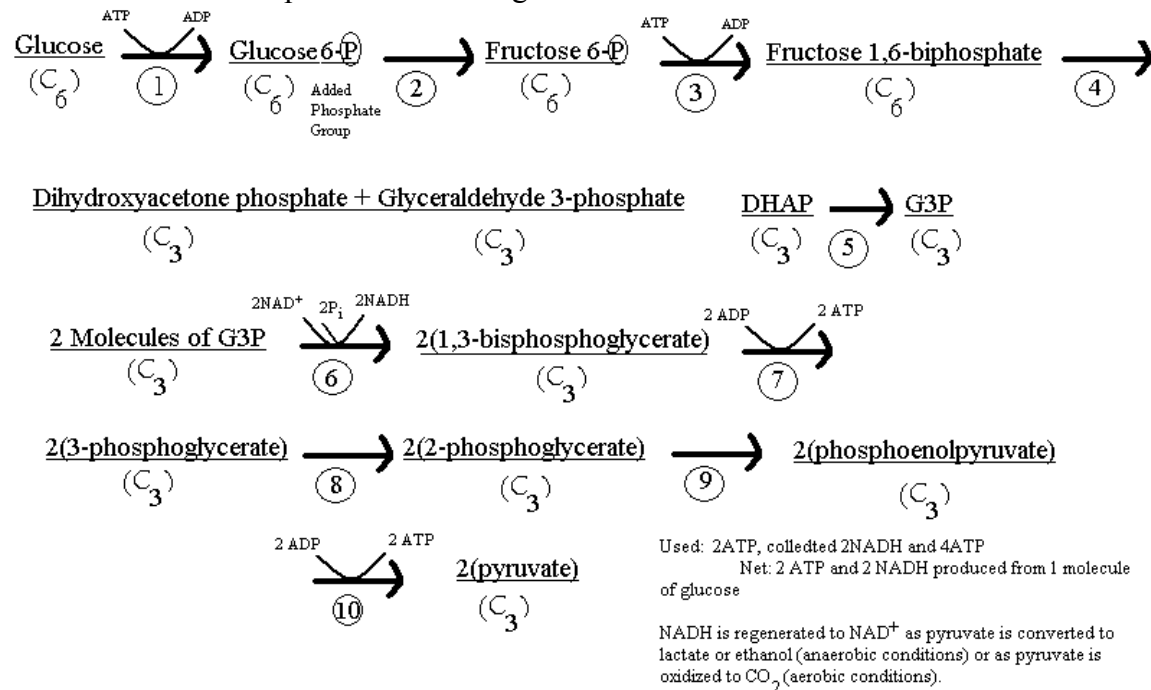
Glycolysis can be carried out in yeast extracts (i.e. does not living yeast – enzymes only required. Enzyme means “in yeast”)

Glycolysis has two phases:

- 1) Energy Investment phase (uses ATP)
- 2) Energy Generation phase (makes ATP)

Summary of Glycolysis Reactions:

Consists of 10 steps: 1 6-carbon sugar is converted to 2 3-carbon molecules.



One important note:

The reaction $G3P \leftrightarrow DHAP$ has a $G^\circ = -1.86 \text{ kcal/mol}$

However, in glycolysis DHAP is converted to G3p, therefore the G° of this reaction is $+1.86 \text{ kcal/mol}$!

How does the reaction proceed in cells??

Because G3P is used up in further reactions, the $[G3P]$ decreases which drives the reaction to the right, creating more G3P.

Even though the $G^\circ > 0$, the G value < 0 due to the ratio of products/reactants

$$\Delta G = \Delta G^\circ + RT \ln \frac{[PRODUCTS]}{[REACTANTS]}$$

In the cell, all reactions proceed forward because $\Delta G < 0$ (achieved by maintaining optimum products to reactants ratio)

TABLE OF ΔG° and ΔG values of the reactions in glycolysis.

Note that many $\Delta G^\circ > 0$, however, all the ΔG values are < 0

Therefore, the reaction goes to forming pyruvate!

A Kinase is an enzyme that adds a phosphate group to a molecule.

Regulation of Pathways

The purpose of glycolysis is to make ATP. The ATP made is used for other reactions in the cell (biosynthetic reactions, transport, etc.)

- Cell senses ratio of ATP/ADP
e.g. when muscles are working hard ATP is converted to ADP
- if [ATP] is high, glycolysis is low
- if [ATP] is low, glycolysis is turned on (increased ADP activates glycolysis)

You can change the reaction velocities (v) of enzymes in glycolysis by changing the

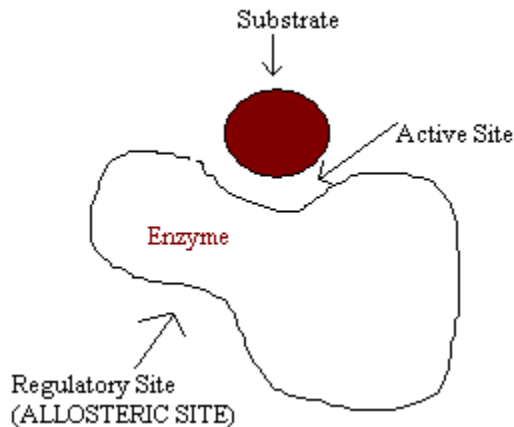
variables in the equation: $[V] = k_3[E_T] \frac{[S]}{[S] + K_m}$

To Increase Rate of Glycolysis:

- 1) Increase $[E_T]$; make more enzyme (new synthesis)
e.g. after eating lots of carbohydrates – levels of pyruvate kinase increase
- 2) Increase $[S]$; if you increase the amount of substrate than you increase the fraction (f) of enzyme that is occupied with substrate: $f = \frac{[S]}{[S] + K_m}$
- 3) If K_m is changed – can affect rate of reaction
 - if K_m is higher \rightarrow slower rate
 - if K_m is lower \rightarrow faster rate

You can change the K_m of an enzyme by changing the shape of the active site (catalytic site) of the enzyme

Example:



The substrate binds at the active site and is converted to product.

Molecules can also bind to the allosteric site.

Binding at the allosteric site causes the enzyme to adopt a different shape.

"allo" - other
"steric" - shape

The binding of regulators at the allosteric site leads to a change in the shape of the enzyme – which affects the K_m of the enzyme.

-If you decrease $K_m \rightarrow$ increase the rate (v) because f will also increase

-If you increase $K_m \rightarrow$ decrease the rate (v) because you decrease f

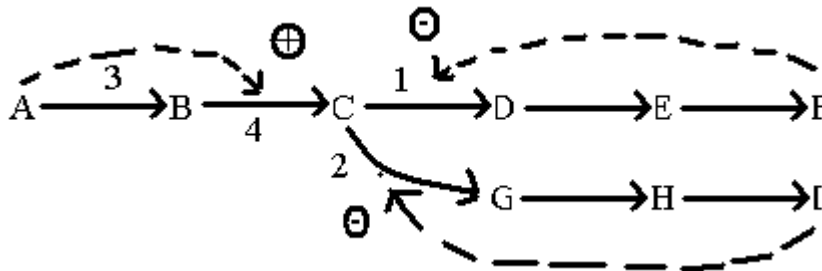
Binding of regulators at the active site – causes the active site to become changed – the position of some amino acids change by a few angstroms – enough to inactivate the enzyme

Regulation of Pathways:

Feedback and Feed-Forward Regulation

Most enzymes that catalyze reactions in biochemical pathways are regulated.

Example Pathway



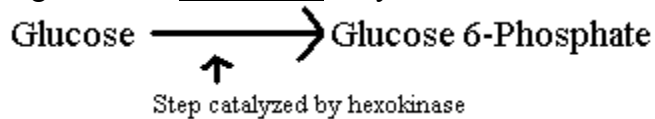
- 1) F can act as a feedback inhibitor of the enzyme that catalyzes the $C \rightarrow D$ step (1st committed step in the synthesis of F)
- 2) I can act as a feedback inhibitor of the enzyme that catalyzes the $C \rightarrow G$ step to slow the rate of that step
- 3) Both I and G together or alone can act as a feedback inhibitor of the $A \rightarrow B$ step
- 4) A can act as a feed forward inhibitor of the $B \rightarrow C$ step to increase the rate of that reaction

E.g. if you have too much A (A is building up) can go to the rate-limiting step, decrease the K_m of the enzyme and speed up the reaction

In general, the end product of a pathway usually regulates the 1st committed step of the pathway (or first enzyme requiring step)

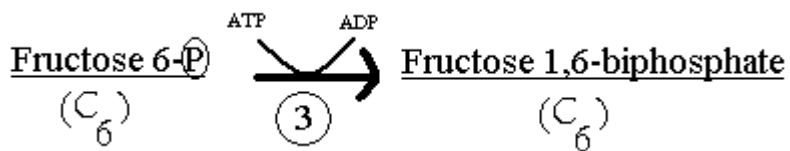
In glycolysis, certain enzymes are regulated so that the rate of glycolysis can be increased or decreased depending on the energy requirements of the cell.

Regulation of hexokinase enzyme:



G6-P inhibits hexokinase if glycolysis is blocked or slowed down.

Regulation of phosphofructokinase enzyme (PFRK enzyme)



-ATP acts as a negative regulator of PFK enzyme.

-At high [ATP], ATP binds to the allosteric site and changes the catalytic activity of PFK (increases the K_m)

-At low [ATP], ATP binds to the active site on PFK and activates PFK

-High [ADP] activates PFK