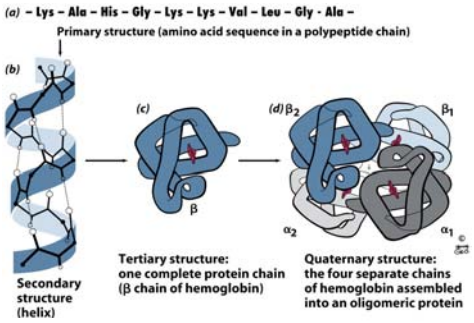
Exam II Review
(10 / 21 / 2008)

TOPICS

- **Protein Structure**
- **Myoglobin/Hemoglobin**
- **Enzymes**
- **Enzyme Kinetics**

Example of each level of protein structure



Fibrous Proteins

α Keratin - A Coiled Coil

Nails, hair, horns and feathers
α or β-forms
30 variants, tissue specific
type I and type II
acidic negative charge basic positive charge

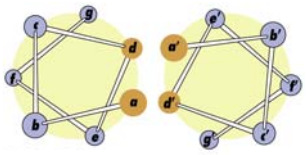
α keratin
- **hair- 20 μM diameter**
- **macrofibril 2000 Å parallel to hair**
- **microfibril 80 Å and high sulfur content protein**
- **can break -S-S- with mercaptans and reconnect (i.e. can give hair a "permanent" wave)**

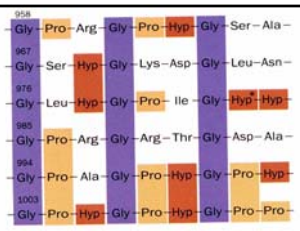
α keratin proteins are helical
but spacing differs from a regular α-helix
a 5.1 Å vs. **5.4** Å pitch.
This change in pitch forms closely associated pairs of helices.
Each pair consists of a type I and type II protein
Left-handed coil coiled-coil
310 AA residues 7-residue pseudo repeat.
Helical wheel - 3.6 residues/turn 360° = 100° per residue
a - b - c - d - e - f - g - a repeat on side of helix
↑ ↑ ↑

View down the coil axis

Helical wheel diagram

a and ***d*** residues are nonpolar.

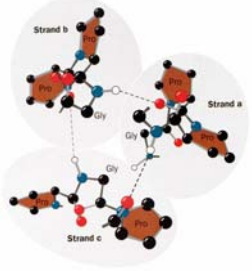




Collagen triple residue repeat:
Gly-X-Y X often Pro Y often Hyp
like a poly Gly or poly Pro helix

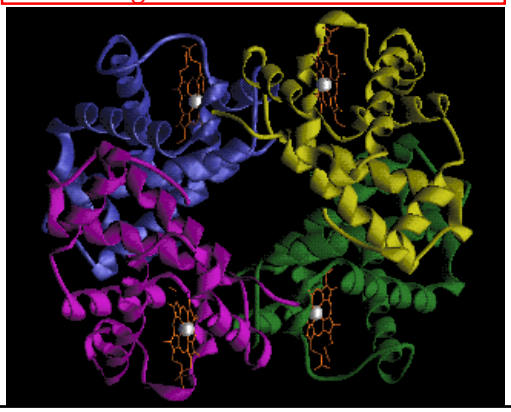
Left-handed 3.0 residues/turn pitch 9.4° extended conformation the prolines avoid each other.

3 left handed helices combine in a triple right handed coil.



Rope twist or metal cable longitudinal force (pulling) is supported by lateral compression opposite twisted strands prevents twists from pulling out.

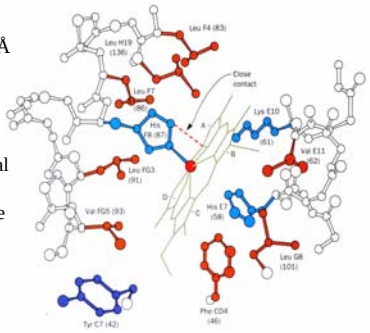
Hemoglobin switch T to R states

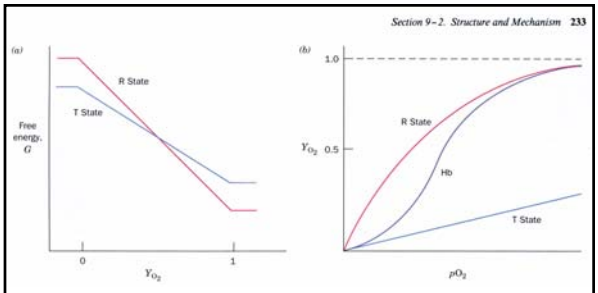


The positive cooperativity of O_2 binding to Hb

The effect of the ligand-binding state of one heme on the ligand-binding affinity of another.

The Fe iron is about 0.6 Å out of the heme plane in the deoxy state. When oxygen binds it pulls the iron back into the heme plane. Since the proximal His F8 is attached to the Fe this pulls the complete F helix like a lever on a fulcrum.





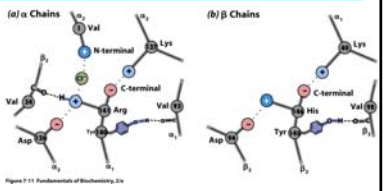
a. Free energy changes with fractional saturation

b. Sigmoidal binding curve as a composite of the R state binding and the T state binding.

Origin of the Bohr Effect

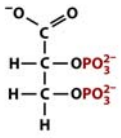
The T → R transition causes the changes in the pK's of several groups. The N-terminal amino groups are responsible for 20-30% of the Bohr effect. His146β accounts for about 40% of the Bohr effect salt bridged with Asp 94β. This interaction is lost in the R state.

Networks of H-bonds & ion pairs in T-state



- The T-state is shown above.
- T→R transition causes breakage of terminal interactions and changes in ionization states of His146β and Val1α (part of Bohr effect)

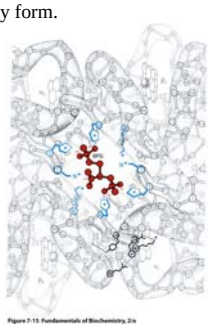
D-2,3-bisphosphoglycerate (BPG)



D-2,3-Bisphosphoglycerate (BPG)

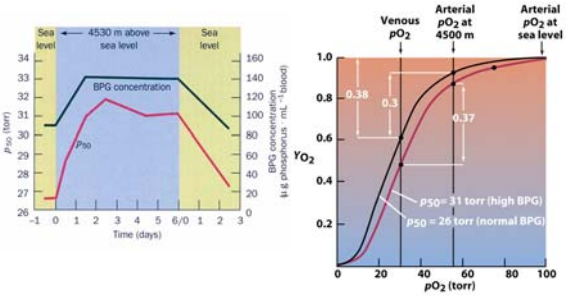
BPG binds 1:1 with a $K=1x10^{-5}$ M to the deoxy form but weakly to the oxy form

BPG binds to Hb (deoxy state) and decreases the O_2 affinity and keeps it in the deoxy form.



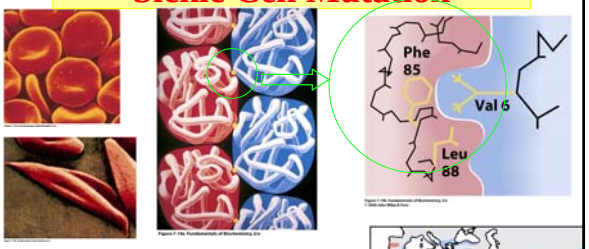
Fetal Hb ($\alpha_2\gamma_2$) has low BPG affinity β-His143 to Ser in γ chain

BPG levels are partially responsible for High-Altitude adaptation



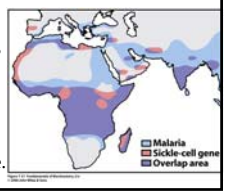
BPG restores the 37% release of O_2 at higher elevations between arterial and venous blood

Sickle Cell Mutation



Glu 6 ---> Val 6 mutation on the hemoglobin B chain

Heterozygotes carrying only one copy of the sickle-cell gene are more resistant to malaria than those homozygous for for the normal gene.

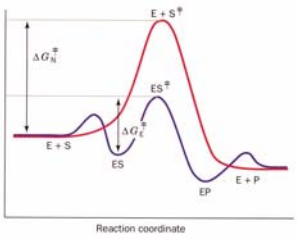


Enzymes: **The more tightly an enzyme binds its reaction's transition state (K_T) relative to the substrate (K_R) , the greater the rate of the catalyzed reaction (k_E) relative to the uncatalyzed reaction (k_N)**

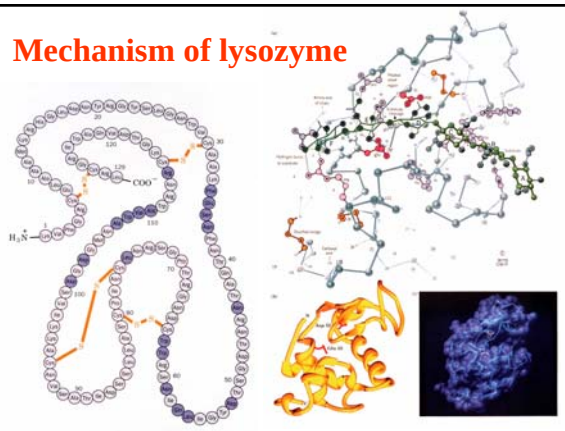
10^6 rate enhancement requires a 10^6 higher affinity which is 34.2 kJ/mol

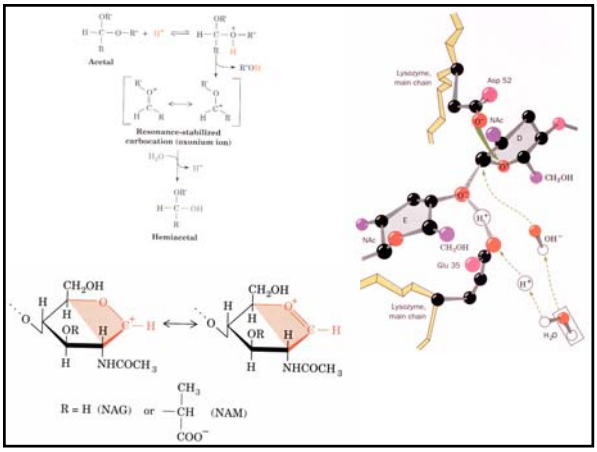
Catalysis results from the preferred binding and therefore the stabilization of the transition state ($S^{\ddagger}$) relative to that of the substrate (S).

$$\frac{k_E}{k_N} = \exp\left[\left(\Delta G^{\ddagger}_N - \Delta G^{\ddagger}_E\right)/RT\right]$$

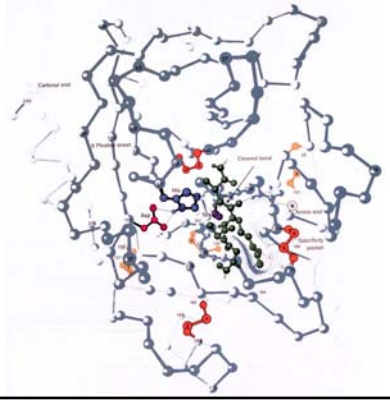


Mechanism of lysozyme

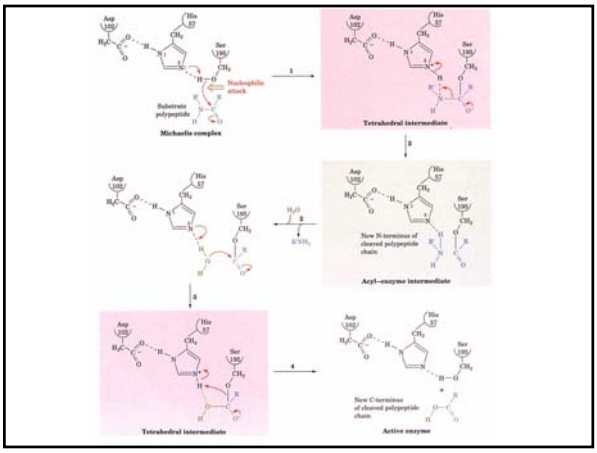


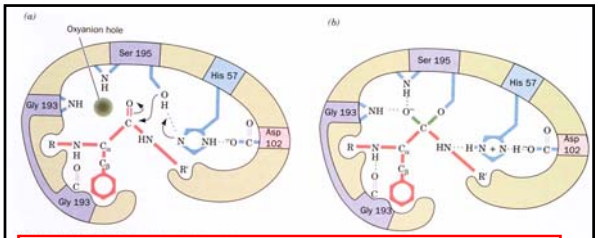


Serine proteases



Bovine Trypsin





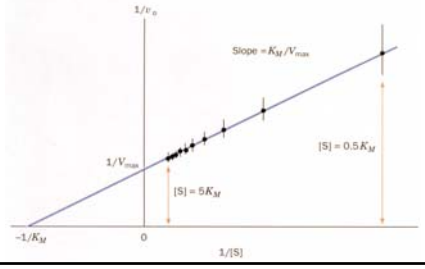
1. Conformational distortion forms the tetrahedral intermediate and causes the carboxyl to move close to the oxyanion hole

2. Now it forms two hydrogen bonds with the enzyme that cannot form when the carbonyl is in its normal conformation.

3. Distortion caused by the enzyme binding allows the hydrogen bonds to be maximal.

Enzyme Kinetics: The double reciprocal plot

$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



What is catalytic perfection?

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

When $k_2 >> k_{-1}$ or the ratio $\frac{k_1 k_2}{k_{-1} + k_2}$ is maximum

Then $\frac{k_{cat}}{K_M} = k_1$

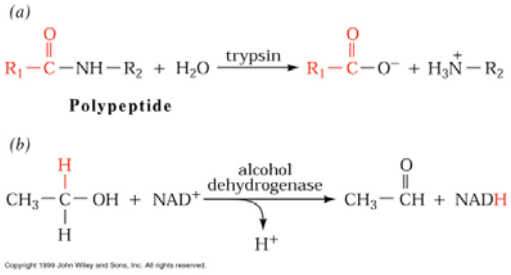
Or when every substrate that hits the enzyme causes a reaction to take place. This is catalytic perfection. Note that for Michaelis - Menton kinetics $k_2 = k_{cat}$

Diffusion-controlled limit- diffusion rate of a substrate is in the range of 10^8 to 10^9 $M^{-1}s^{-1}$. An enzyme lowers the transition state so there is no activation energy and the catalyzed rate is as fast as molecules collide.

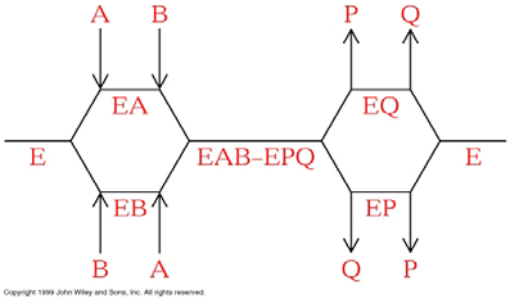
Reaction Mechanisms
A: Sequential Reactions

- All substrates must combine with enzyme before reaction can occur

Bisubstrate reactions

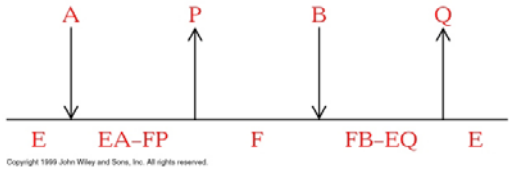


Random Bisubstrate Reactions

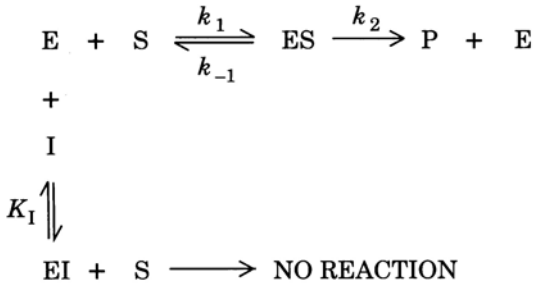


Ping-Pong Reactions

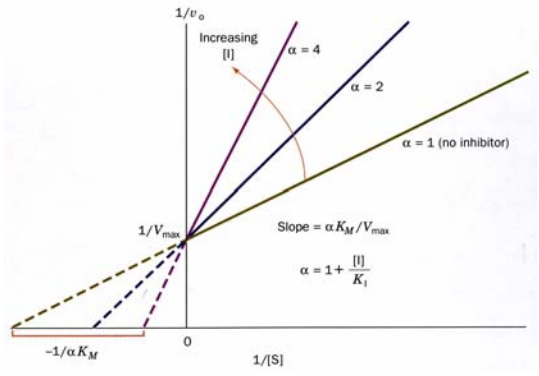
- Group transfer reactions
- One or more products released before all substrates added



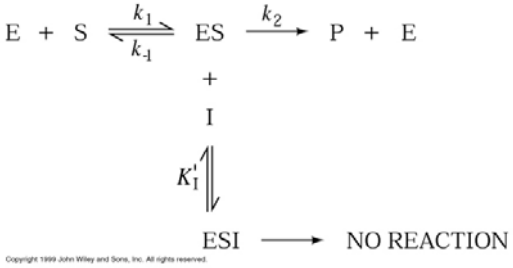
Competitive Inhibition



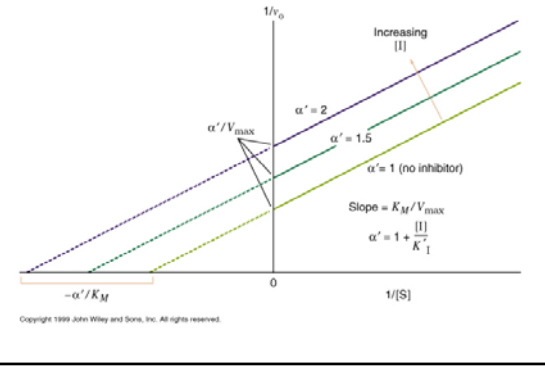
Competitive Inhibition: Lineweaver-Burke Plot



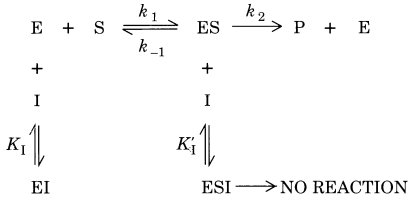
Uncompetitive Inhibition

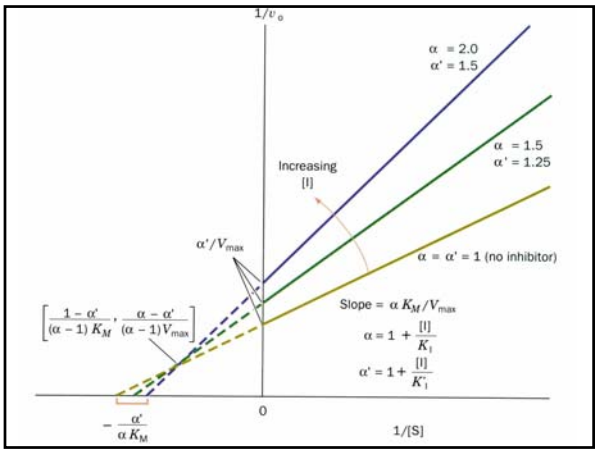


Uncompetitive Inhibition: Lineweaver-Burke Plot



Mixed inhibition





Mixed inhibition is when the inhibitor binds to the enzyme at a location distinct from the substrate binding site. The binding of the inhibitor will either alter the K_M or V_{max} or both.

$$K_I = \frac{[E][I]}{[EI]} \qquad K'_I = \frac{[ES][I]}{[ESI]}$$

$$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} \qquad \alpha' = \left(1 + \frac{[I]}{K'_I}\right)$$

TABLE 13-2. THE EFFECTS OF INHIBITORS ON THE PARAMETERS OF THE MICHAELIS–MENTEN EQUATION[a]

Type of Inhibition	V_{max}^{app}	K_M^{app}
None	V_{max}	K_M
Competitive	V_{max}	αK_M
Uncompetitive	V_{max}/α'	K_M/α'
Mixed	V_{max}/α'	$\alpha K_M/\alpha'$

[a] $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K'_I}$

Next Lecture 18
Exam II (10/23/08)

Lecture 19 10/28/08
Metabolism