Globins & Enzyme Catalysis

10/06/2009

The Bohr Effect

Higher pH i.e. lower [H⁺] promotes tighter binding of oxygen to hemoglobin

and

Lower pH i.e. higher [H+] permits the easier release of oxygen from hemoglobin

$$Hb(O_2)_n H_x + O_2 \Leftrightarrow Hb(O_2)_{n+1} + xH^+$$

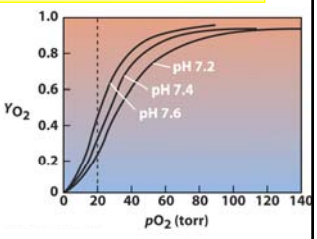
Where n = 0, 1, 2, 3 and x ≅ 0.6 A shift in the equilibrium will influence the amount of oxygen binding. Bohr protons

CO_2 Transport and The Bohr Effect

Higher pH i.e. lower [H⁺] (more basic) promotes tighter binding of oxygen to hemoglobin

and

Lower pH i.e. higher [H+] (more acidic) permits the easier release of oxygen from hemoglobin



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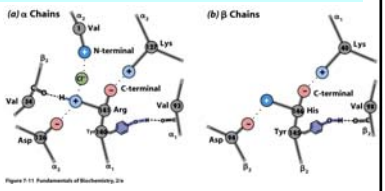
Origin of the Bohr Effect

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



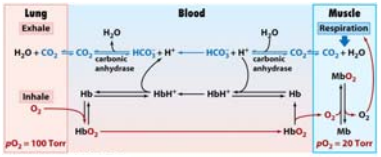
- The T-state is shown above.
- T→R transition causes breakage of terminal interactions and changes in ionization states of His146β and Val1α (part of Bohr effect)

Look at the relation between pH and the p_{50} values for oxygen binding. As the pH increases the p_{50} value decreases, indicating the oxygen binding increases (opposite effect,when the pH decreases).

At 20 torr 10% more oxygen is released when the pH drops from 7.4 to 7.2!!

The Bohr effect: Importance in transporting O_2 and CO_2

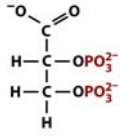
$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$



As oxygen is consumed CO_2 is released. Carbonic Anhydrase catalyzes this reaction in red blood cells.

- 0.6H+ released for each O_2 binding
- $CO_2 + H_2O \rightarrow H+ + HCO_3$-, catalyzed by carbonic anhydrase – main mode of elimination of CO_2

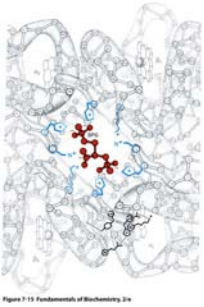
D-2,3-bisphosphoglycerate (BPG)



D-2,3-Bisphoglycerate (BPG)

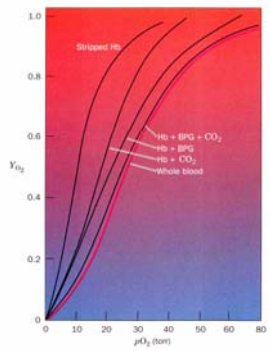
BPG binds 1:1 with a K=1x10^{-5} M to the deoxy form but weakly to the oxy form

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



Fetal Hb (α2γ2) has low BPG affinity β-His143 to Ser in γ chain

The P50 value of stripped hemoglobin increases from 12 to 22 torr by 4.7 mM BPG

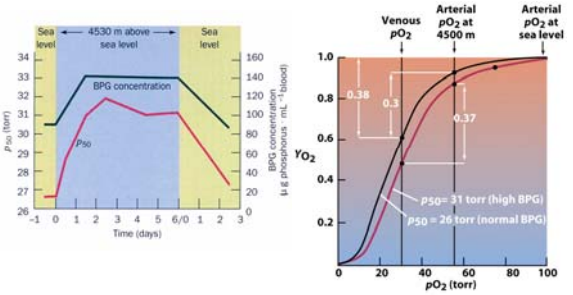


At 100 torr or arterial blood, hemoglobin is 95% saturated

At 30 torr or venous blood, hemoglobin is 55% saturated

Hemoglobin releases 40% of its oxygen. In the absence of BPG, little oxygen is released. Between BPG, CO_2, H^+, and Cl^- all O_2 binding is accounted for.

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

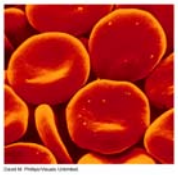
Fetal Hemoglobin

- Fetal hemoglobin has a different β subunit called a γ subunit or $\alpha_2\gamma_2$.
- In Fetal hemoglobin, BPG does not affect this variant and the baby's blood will get its oxygen from the mothers hemoglobin.
- The transfer of oxygen is from the mother (less tightly bond) to the baby (more tightly bond).

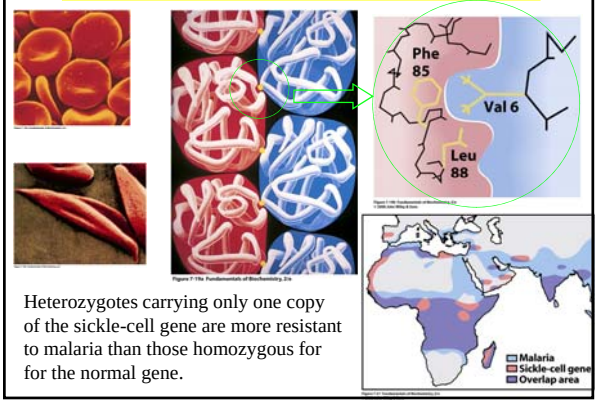
Sickle Cell Mutation

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

- Decreases surface charge
- More hydrophobic
- Frequency 10% USA versus 25% in africa.
- Forms linear polymers



Normal and sickled erythrocytes



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

Hemoglobin mutants

There are about 500 variants of hemoglobin 95% are single amino acid substitutions.

5% of the world's population carries a different sequence from the normal.

- **Changes in surface charge**
- **Changes in internally located residues**
- **Changes stabilizing Methemoglobin (oxidized Fe(III))**
- **Changes in the α1-β2 contact**

Changes in surface rarely change the function of hemoglobin with the exception of the sickle cell mutation.

Internal residues cause the hemoglobin to contort to different shapes and alter its binding properties. Heinz bodies are precipitated aggregates of hemoglobin. Usually cause hemolytic anemia characteristic by cell lysis.

Table 7-1 Some Hemoglobin Variants

Name[a]	Mutation	Effect
Hammersmith	Phe CD1(42)β → Ser	Weakens heme binding
Bristol	Val E11(67)β → Asp	Weakens heme binding
Bibba	Leu H19(136)α → Pro	Disrupts the H helix
Savannah	Gly B6(24)β → Val	Disrupts the B–E helix interface
Philly	Tyr C1(35)α → Phe	Disrupts hydrogen bonding at the α_1–β_1 interface
Boston	His E7(58)α → Tyr	Promotes methemoglobin formation
Milwaukee	Val E11(67)β → Glu	Promotes methemoglobin formation
Iwate	His F8(87)α → Tyr	Promotes methemoglobin formation
Yakima	Asp G1(99)β → His	Disrupts a hydrogen bond that stabilizes the T conformation
Kansas	Asn G4(102)β → Thr	Distrupts a hydrogen bond that stabilizes the R conformation

[a]Hemoglobin variants are usually named after the place where they were discovered (e.g., hemoglobin Boston).

Table 7-1 Fundamentals of Biochemistry, 2/e

General Properties of Enzymes

- **Increased reaction rates sometimes 10^6 to 10^{12} increase**

  Enzymes do not change ΔG between the reactants and products.

  They increase reaction rates (catalysts).

- **Milder reaction conditions**
- **Great reaction specificity**
- **Can be regulated**

Preferential transition state binding

Binding to the transition state with greater affinity to either the product or reactants.

RACK MECHANISM

Strain promotes faster rates

The strained reaction more closely resembles the transition state and interactions that preferentially bind to the transition state will have faster rates

$S \xrightarrow{k_N} P$

$ES \xrightarrow{k_E} EP$

k_N for uncatalyzed reaction

and

k_E for catalyzed reaction

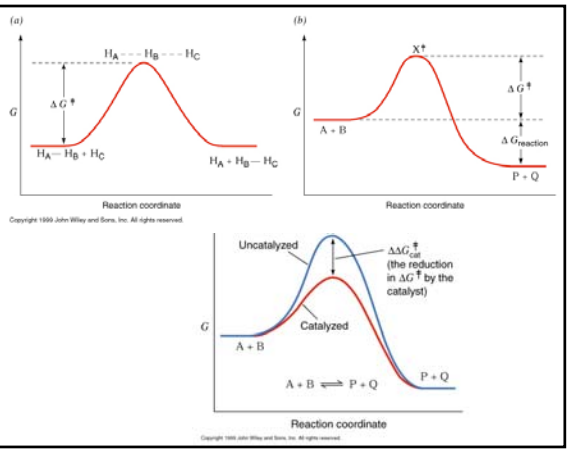
$$E + S \underset{}{\overset{K_N^{\ddagger}}{\longleftrightarrow}} S^{\ddagger} + E \rightarrow P + E$$

$$\Updownarrow K_R \qquad \Updownarrow K_T \qquad \Updownarrow$$

$$ES \overset{K_E^{\ddagger}}{\longleftrightarrow} ES^{\ddagger} \rightarrow EP$$

$$K_R = \frac{[ES]}{[E][S]} \qquad K_T = \frac{[ES]^{\ddagger}}{[E][S]^{\ddagger}} \qquad K_N^{\ddagger} = \frac{[E][S]^{\ddagger}}{[E][S]}$$

$$K_E^{\ddagger} = \frac{[ES^{\ddagger}]}{[ES]} \qquad \frac{K_T}{K_R} = \frac{[S][ES^{\ddagger}]}{[S^{\ddagger}][ES]} = \frac{K_E^{\ddagger}}{K_N^{\ddagger}}$$

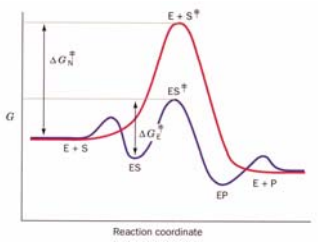


$$\frac{k_E}{k_N} = \exp\left[\left(\Delta G^{\ddagger}_N - \Delta G^{\ddagger}_E\right)/RT\right]$$

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

Enzymes:

The enzyme binding of a transition state ($ES^{\ddagger}$) by two hydrogen bonds that cannot form in the Michaelis Complex (ES) should result in a rate enhancement of 10^6 based on this effect alone

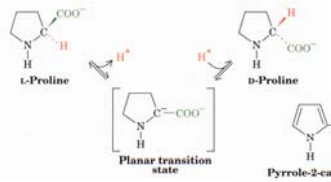


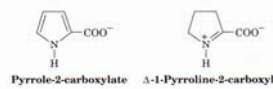
Enzymes: Preferential transition state binding

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)

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

Transition state analogues are competitive inhibitors





both of which bind to the enzyme with 160-fold greater affinity than does proline. These compounds are therefore thought to be analogs of the transition state in the proline racemase reaction. In contrast, **tetrahydrofuran-2-carboxylate,**

Tetrahydrofuran-2-carboxylate

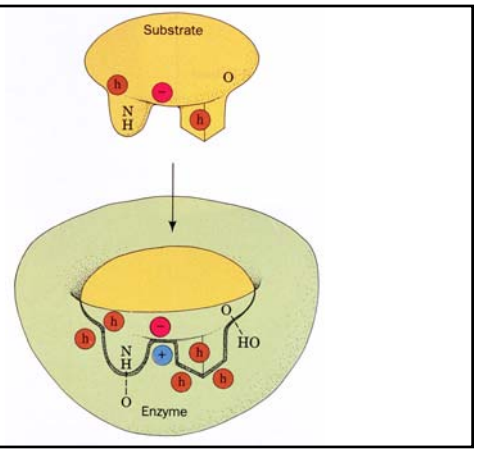
Substrate specificity

The non-covalent bonds and forces are maximized to bind substrates with considerable specificity

- Van der Waals forces
- electrostatic bonds (ionic interactions)
- Hydrogen bonding
- Hydrophobic interaction

$$A + B \xleftrightarrow{enz} P + Q$$

Substrates **Products**

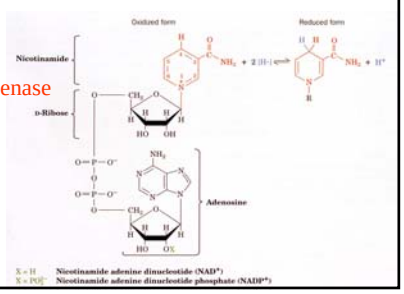


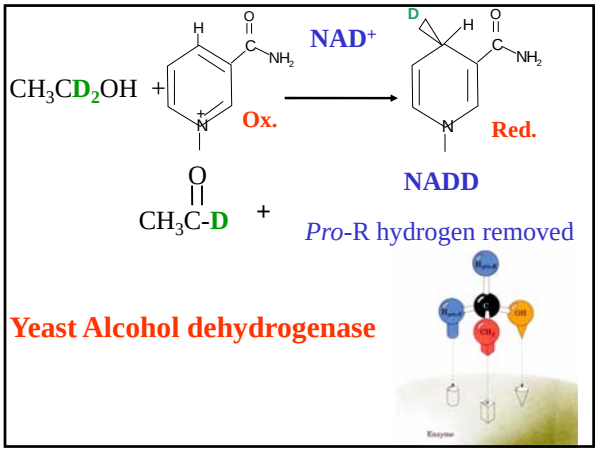
Enzymes are Stereospecific

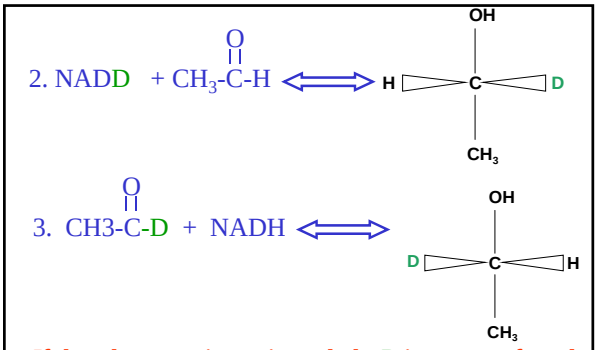
$$CH_3CH_2OH + NAD^+ \Leftrightarrow CH_3\overset{O}{\overset{\|}{C}}H + NADH + H^+$$

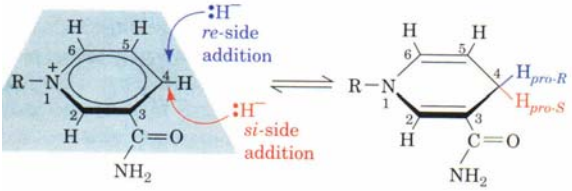
Yeast

Alcohol dehydrogenase









Both the Re and Si faced transfers yield identical products. However, most reactions that have an Keq for reduction >10^{-12} use the *pro*-R hydrogen while those reactions with a Keq <10^{-10} use the *pro*-S hydrogen. The reasons for this are still unclear

Specific residues help maintain stereospecificity

Liver alcohol dehydrogenase makes a mistake 1 in 7 billion turnovers. Mutating Leu 182 to Ala increases the mistake rate to 1 in 850,000. This is a 8000 fold increase in the mistake rate, This suggests that the stereospecificity is helped by amino acid side chains.

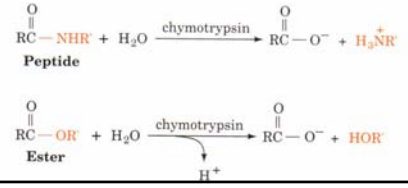
Geometric specificity

Selective about identities of chemical groups

but

Enzymes are generally not molecule specific

There is a small range of related compounds that will undergo binding or catalysis.



Coenzymes

Coenzymes: smaller molecules that aid in enzyme chemistry.
Enzymes can:

a. Carry out acid-base reactions
b. Transient covalent bonds
c. Charge-charge interactions

Enzymes can not do:

d. Oxidation -Reduction reactions
e. Carbon group transfers

Prosthetic group - permanently associated with an enzyme or transiently associated.
Holoenzyme: catalytically active enzyme with cofactor. ★
Apoenzyme: Enzyme without its cofactor ★

Common Coenzymes	
Coenzyme	Reaction mediated
Biotin	Carboxylation
Cobalamin (B12)	Alkylation transfers
Coenzyme A	Acyl transfers
Flavin	Oxidation-Reduction
Lipoic acid	Acyl transfers
Nicotinamide	Oxidation-Reduction
Pyridoxal Phosphate	Amino group transfers
Tetrahydrofolate	One-carbon group transfers
Thiamine pyrophosphate	Aldehyde transfer

Vitamins are Coenzyme precursors

Vitamin	Coenzyme	Deficiency Disease
Biotin	Biocytin	not observed
Cobalamin (B_{12})	Cobalamin	Pernicious anemia
Folic acid	tetrahydrofolate	Neural tube defects Megaloblastic anemia
Nicotinamide	Nicotinamide	Pellagra
Pantothenate	Coenzyme A	Not observed
Pyridoxine (B_6)	Pyridoxal phosphate	Not observed
Riboflavin (B2)	Flavin	Not observed
Thiamine (B_1)	Thiamine pyrophosphate	Beriberi

Lecture 14
Thursday 10/08/09
Enzymes II