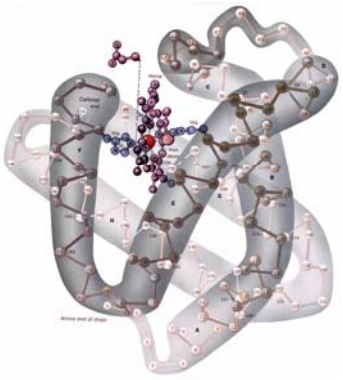
Globins

Lecture 10/01/2009

The Backbone structure of Myoglobin



Myoglobin: 44 x 44 x 25 Å single subunit 153 amino acid residues

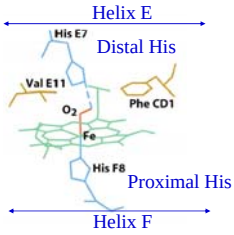
121 residues are in an a helix. Helices are named A, B, C, …F. The heme pocket is surrounded by E and F but not B, C, G, also H is near the heme.

Amino acids are identified by the helix and position in the helix or by the absolute numbering of the residue.

Role of the Globin

- **Modulate oxygen binding affinity**
- **Make reversible oxygen binding possible**

By introducing steric hindrance on one side of the heme plane interaction can be prevented and oxygen binding can occur.

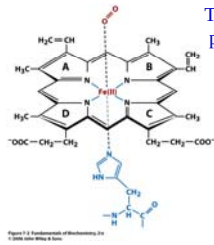


Fe^{3+} - O = O - Fe^{3+}

A heme dimer is formed which leads to the formation of Fe(III)

The Heme group

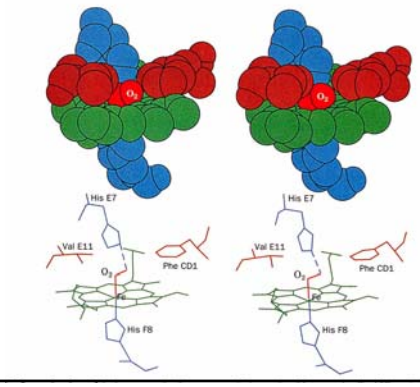
Each subunit of hemoglobin or myoglobin contains a heme.
- Binds one molecule of oxygen
- Heterocyclic porphyrin derivative
- Specifically protoporphyrin IX

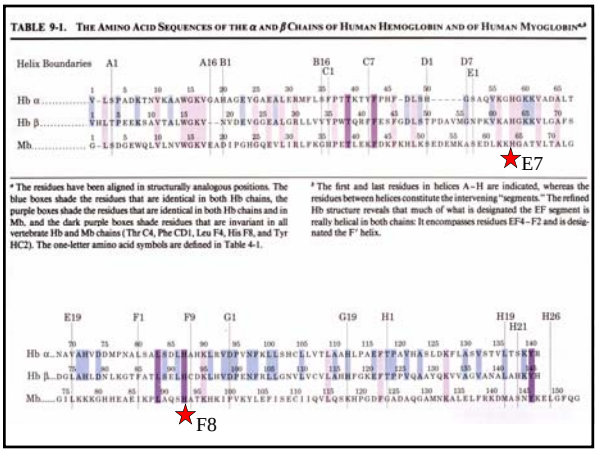


The heme **prosthetic group** in Mb ad Hb: protoporphyrin IX + Fe(II)

The iron must be in the **Fe(II)** form or **reduced** form (ferrous oxidation) state.

The Heme complex in myoglobin





Hemoglobin

Spherical 64 x 55 x 50 Å two fold rotation of symmetry α and β subunits are similar and are placed on the vertices of a tetrahedron. There is no D helix in the α chain of hemoglobin. **Extensive interactions between unlike subunits** α2-β2 or α1-β1 interface has 35 residues while α1-β2 and α2-β1 have 19 residue contact.

Oxygenation causes a considerable structural conformational change

Oxygenation rotates the α1β1 dimer in relation to α2β2 dimer about 15°

The conformation of the deoxy state is called the T state

The conformation of the oxy state is called the R state

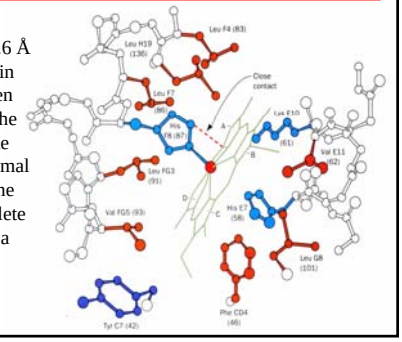
individual subunits have a **t** or **r** if in the deoxy or oxy state.

What causes the differences in the conformation 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.

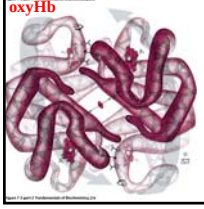
The Fe iron is about 0.6 Å out of the heme plane in the deoxy state. When oxygen binds it pulls the iron back into the heme plane. Since the proximal His F8 is attached to the Fe this pulls the complete F helix like a lever on a fulcrum.



Hemoglobin structure

DeoxyHb

oxyHb



β-monomers are related by 2-fold symmetry (same is true for α)

Note changes in structure:

between β-monomers – see big double-headed arrows

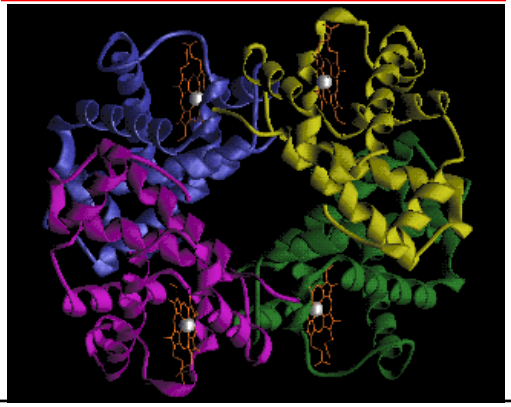
at points of contact – see small arrows

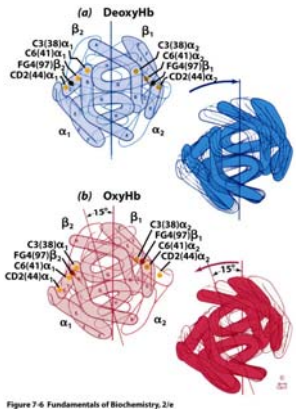
Binding of the O_2 on one heme is more difficult but its binding causes a shift in the α1-β2 (& α2-β1) contacts and moves the distal His E7 and Val E11 out of the oxygen's path to the Fe on the other subunit. This process increases the affinity of the heme toward oxygen.

The α1-β2 contacts have two stable positions .

These contacts, which are joined by different but equivalent sets of hydrogen-bonds that act as a binary switch between the T (deoxy) and the R (oxy) states

Hemoglobin switch T to R states





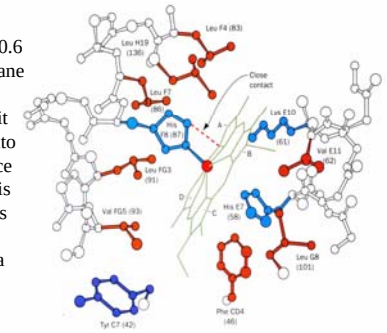
The major structural difference between the quaternary conformations of (a) deoxyHb and (b) oxyHb

Note: This view is from the right side relative to the previous slide.

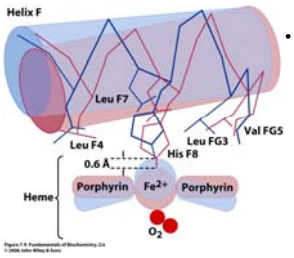
The positive cooperativity of O_2 binding to Hb

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

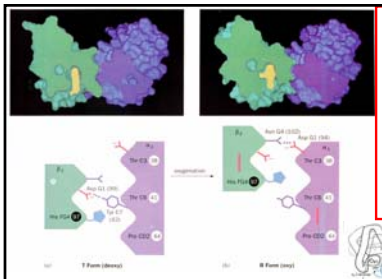
The Fe iron is about 0.6 Å out of the heme plane in the deoxy state. When oxygen binds it pulls the iron back into the heme plane. Since the proximal His F8 is attached to the Fe this pulls the complete F helix like a lever on a fulcrum.



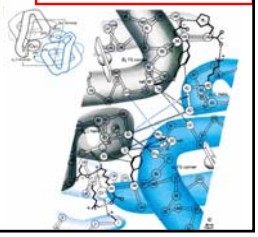
Mechanism of Cooperativity in Hemoglobin



- T-state (deoxyhemoglobin)
  - Fe is 0.6 Å out of heme plane
- R-state (oxyhemoglobin)
  - Fe is in the heme plane
  - Helix containing F8 shifts
  - Change in quaternary structure
  - C-terminal residues (Arg141α, His146β, and Val1α) change interactions and/or ionization state (Bohr effect)

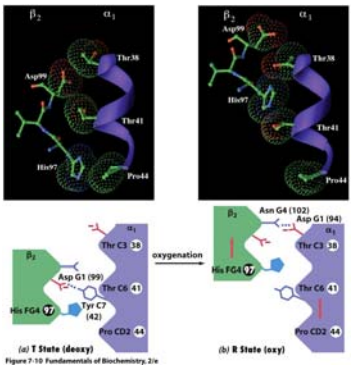


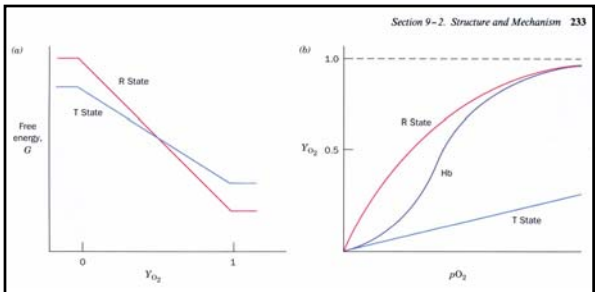
Binding causes a shift in the α1-β2 contacts and moves the distal His E7 and Val E11 out of the oxygen's path to the Fe on the other subunit. This process increases the affinity of the heme toward oxygen.



The α1-β2 contacts have two stable positions with different but equivalent sets of hydrogen bonds to act as binary switch between the T and the R states

Changes at the α1-β2 interface during T→R transition in Hb





a. Free energy changes with fractional saturation

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

Association kinetics of O_2 binding to myoglobin

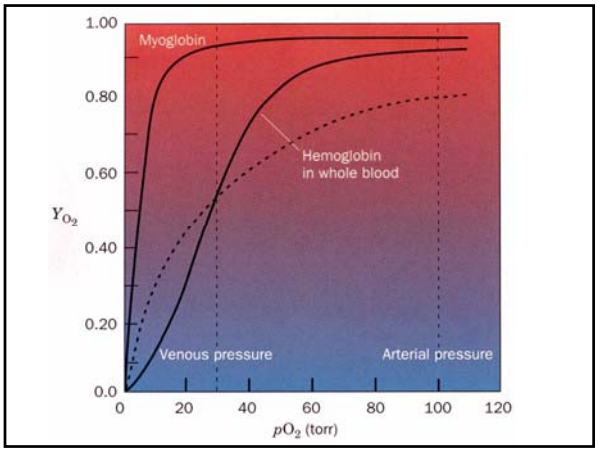
$$Mb + O_2 \leftrightarrow MbO_2$$

Written backwards we can get the dissociation constant

$$K_d = \frac{[Mb][O_2]}{[MbO_2]}$$

Fractional Saturation solve for $[MbO_2]$ and plug in

$$Y_{O_2} = \frac{[MbO_2]}{[Mb]+[MbO_2]} = \frac{[O_2]}{Kd+[O_2]}$$



The Hill Equation

E = enzyme, S = ligand, n= small number

$$E + nS \leftrightarrow ESn$$

This is for binding of 1 or more ligands

O_2 is considered a ligand

1. $$K = \frac{[E][S]^n}{[ESn]}$$

2. $$Ys = \frac{n[ESn]}{n([E]+[ESn])}$$

Fractional Saturation = bound/total

Hill Plot

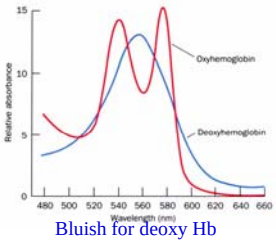
Rearrange equation 4.

$$\text{Log}\left(\frac{Ys}{1-Ys}\right) = n\text{Log}[S] - \log K$$

y = mx + b

n = slope and x intercept of -b/m

The visible absorption spectra for hemoglobin



The red color arises from the differences between the energy levels of the *d* orbitals around the ferrous atom.

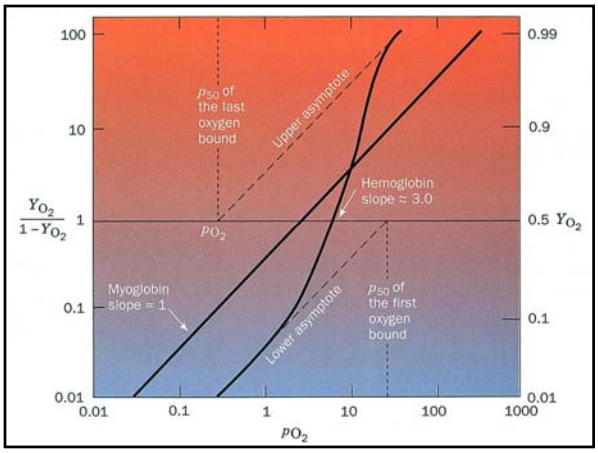
Fe(II) = *d6* electron configuration low spin state ↓↑ ↓↑ ↓↑

Binding of oxygen rearranges the electronic distribution and alters the *d* orbital energy.

This causes a difference in the absorption spectra.

Bluish for deoxy Hb
Redish for Oxy Hb

Measuring the absorption at 578 nm allows an easy method to determine the percent of O_2 bound to Hb



Things to remember

Hb subunits independently compete for O_2 for the first oxygen molecule to bind

When the Y_{O2} is close to 1 i.e. 3 subunits are occupied by O_2 , O_2 binding to the last site is independent of the other sites

However by extrapolating slopes: the 4th O_2 binds to hemoglobin 100 fold greater than the first O_2

A ΔΔG of 11.4 kJ•mol $^{-1}$ in the binding affinity for oxygen

When one molecule binds, the rest bind and when one is released, the rest are released.

Contrast Mb O_2 binding to Hemoglobin

Y_{O2} = 0.95 at 100 torr

but

0.55 at 30 torr

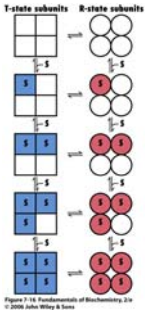
a ΔYO_2 of 0.40

Understand Fig 9-3

Hb gives up O_2 easier than Mb and the binding is Cooperative!!

Allosteric Proteins

Symmetry model (Monod-Wyman-Changeux)



Sequential model (Koshland)

Chapter 12 Enzymes!!

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

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

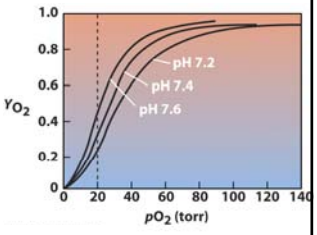
Where n = 0, 1, 2, 3 and x ≅ 0.6 A shift in the equilibrium will influence the amount of oxygen binding. Bohr protons

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



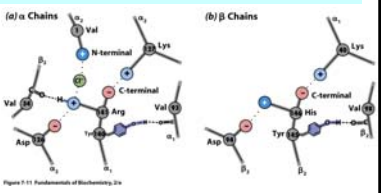
$$Hb(O_2)_n H_x + O_2 \Leftrightarrow Hb(O_2)_{n+1} + xH^+$$

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

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



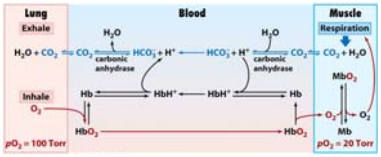
- The T-state is shown above.
- T→R transition causes breakage of terminal interactions and changes in ionization states of His146β and Val1α (part of Bohr effect)

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

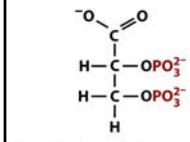
$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$



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

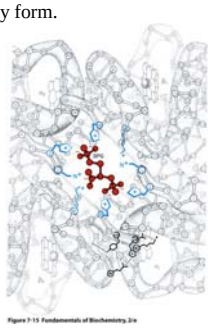
- 0.6H+ released for each O_2 binding
- $CO_2 + H_2O \rightarrow H+ + HCO_3$-, catalyzed by carbonic anhydrase – main mode of elimination of CO_2

D-2,3-bisphosphoglycerate (BPG)



D-2,3-Bisphosphoglycerate (BPG)

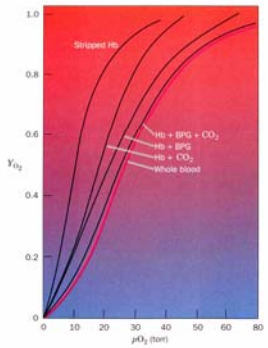
BPG binds to Hb (deoxy state) and decreases the O_2 affinity and keeps it in the deoxy form.



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

Fetal Hb ($\alpha 2\gamma 2$) 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

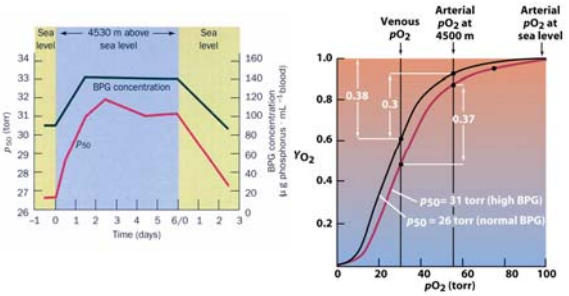


At 100 torr or arterial blood, hemoglobin is 95% saturated

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

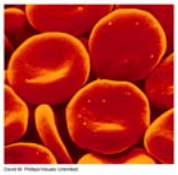
Fetal Hemoglobin

- 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).

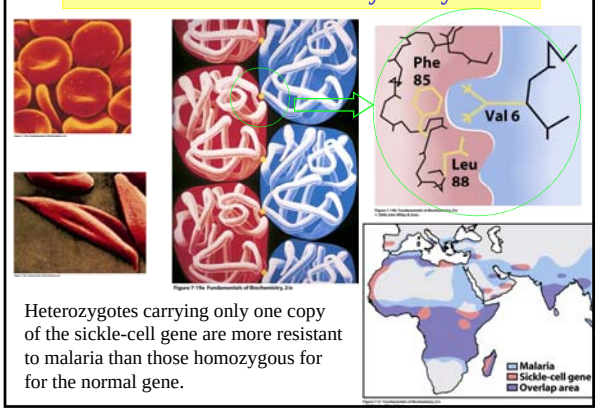
Sickle Cell Mutation

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)$\beta \rightarrow$ Ser	Weakens heme binding
Bristol	Val E11(67)$\beta \rightarrow$ Asp	Weakens heme binding
Bibba	Leu H19(136)$\alpha \rightarrow$ Pro	Disrupts the H helix
Savannah	Gly B6(24)$\beta \rightarrow$ Val	Disrupts the B–E helix interface
Philly	Tyr C1(35)$\alpha \rightarrow$ Phe	Disrupts hydrogen bonding at the α_1–β_1 interface
Boston	His E7(58)$\alpha \rightarrow$ Tyr	Promotes methemoglobin formation
Milwaukee	Val E11(67)$\beta \rightarrow$ Glu	Promotes methemoglobin formation
Iwate	His F8(87)$\alpha \rightarrow$ Tyr	Promotes methemoglobin formation
Yakima	Asp G1(99)$\beta \rightarrow$ His	Disrupts a hydrogen bond that stabilizes the T conformation
Kansas	Asn G4(102)$\beta \rightarrow$ 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

Lecture 13
Tuesday 10/06/09
Protein Function / Enzymes