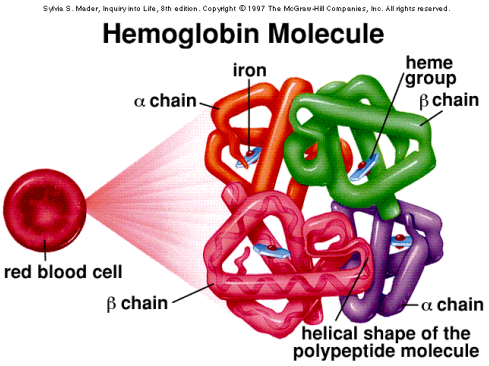
Myoglobin/Hemoglobin O_2 Binding and Allosteric Properties of Hemoglobin

•Hemoglobin binds and transports H^+, O_2 and CO_2 in an allosteric manner

•Allosteric interaction - a regulatory mechanism where a small molecule (effector) binds and alters an enzymes activity



'globin Function

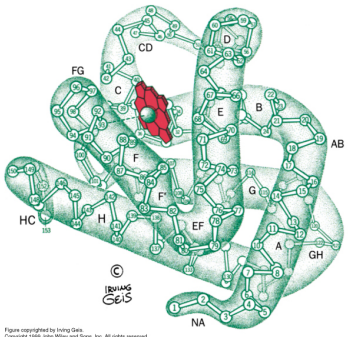
O_2 does not easily diffuse in muscle and O_2 is toxic to biological systems, so living systems have developed a way around this.

Physiological roles of:

- **Myoglobin**
  - Transports O_2 in rapidly respiring muscle
  - Monomer - single unit
  - Store of O_2 in muscle high affinity for O_2
  - Diving animals have large concentration of myoglobin to keep O_2 supplied to muscles
- **Hemoglobin**
  - Found in red blood cells
  - Carries O_2 from lungs to tissues and removes CO_2 and H^+ from blood to lungs
  - Lower affinity for O_2 than myoglobin
  - Tetrameter - two sets of similar units ($\alpha_2\beta_2$)

Myo/Hemo-globin

- Hemoglobin and myoglobin are oxygen- transport and oxygen-storage proteins, respectively
- Myoglobin is monomeric; hemoglobin is tetrameric
  - Mb: 153 aa, 17,200 MW
  - Hb: two α chains of 141 residues, 2 β chains of 146 residues



X-ray crystallography of myoglobin

- mostly α helix (proline near end of each helix *WHY?*)
- very small due to the folding
- hydrophobic residues oriented towards the interior of the protein
- only polar aas inside are 2 histidines

Structure of heme prosthetic group

Protoporphyrin ring w/ iron = heme

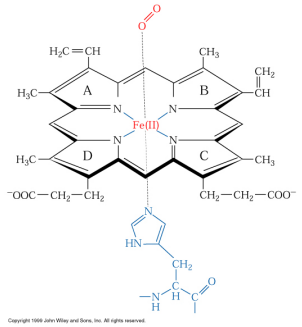
Oxygenation changes state of Fe

- Purple to red color of blood, Fe^{+3} - brown

Oxidation of Fe^{+2} destroys biological activity of myoglobin

Physical barrier of protein is to maintain oxidation state of Fe^{+2}

Propionate chain orients heme



Mb and Hb use heme to bind Fe^{2+} / Fe^{2+} is coordinated by His F8

- Iron interacts with six ligands in Hb and Mb
- Four of these are the N atoms of the porphyrin
- A fifth ligand is donated by the imidazole side chain of amino acid residue His F8
- When Mb or Hb bind oxygen, the O_2 molecule adds to the heme iron as the sixth ligand
- The O_2 molecule is tilted relative to a perpendicular to the heme plane *(IMPORTANT FOR LATER!)*

Structure of heme prosthetic group

- heme wedged between hydrophobic pocket of helix E & F
- Iron is out of plane due to his 8 bond
- distal vs. proximal histidines
- 3 states of 6th coordinate site

free vs. bound heme – role of apoprotein

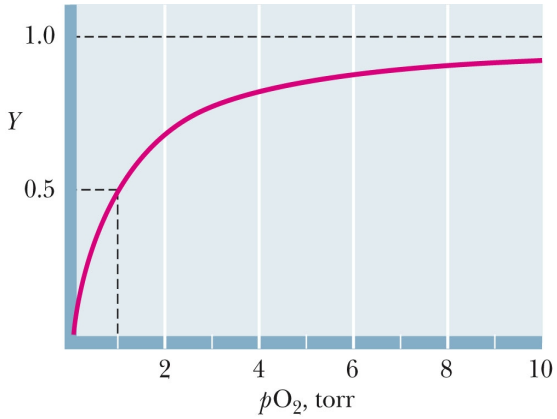
- Globin binding restricts heme dimers from forming
- Helps keep iron reduced
- Stabilizes transition state (O_2 binding)

CO, NO and H_2S binding - poison of O_2 binding bind with greater affinity than O_2

His E 7 decreases affinity of ligands (CO and O_2) for Fe^{+2}

Form	Oxidation	Fe in plane of heme	6th Coordinate State	Distal His

Myoglobin O_2 affinity

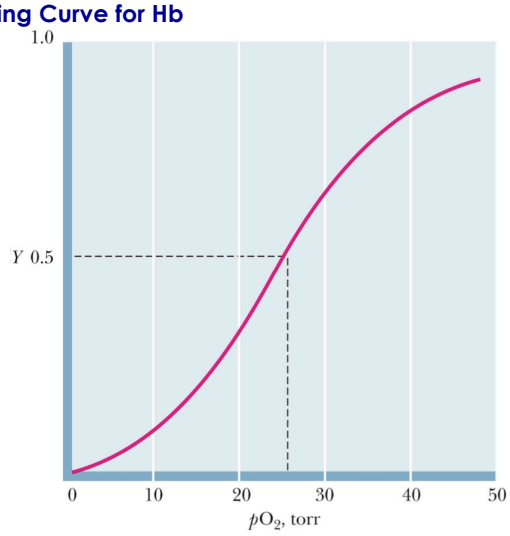


O_2-Binding Curve for Hb

Oxygen saturation curve for Hb in the form of Y versus pO_2 assuming n=4 and P_{50} =26 torr.

Y is the fractional saturation of Hb:

$$Y = \frac{[pO_2]^4}{[pO_2]^4 + K}$$



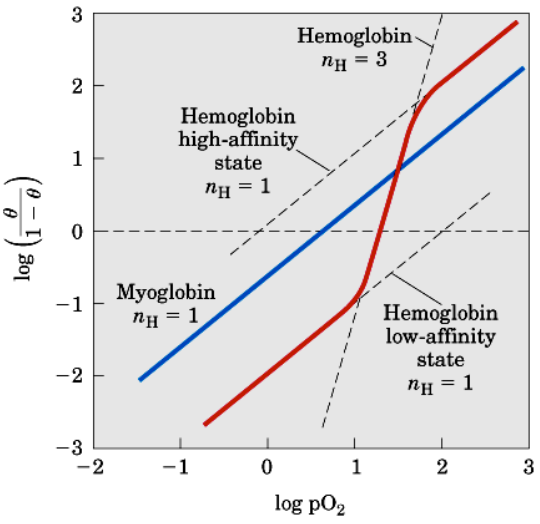
Hemoglobin -sigmoidal dissociation

$Hb(O_2)_n = Hb + n\ O_2$ $\quad X_n$ = number of Hb subunits

$$\log \frac{Y}{1-Y} = n \log pO_2 - n \log P_{50}$$

Y = fraction of globin bound to O_2

n = Hill constant - determined graphically by the - hill plot
n is the slope at midpoint of binding of log (Y/1-Y) vs log of pO_2
if n = 1 then non cooperativity
if n < 1 then negative cooperativity
if n >1 then positive cooperativity

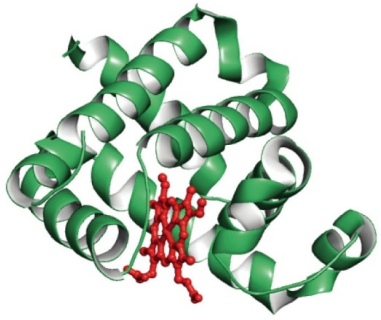


The experimentally determined slope does not reflect the number of binding sites however. It reflects, instead, the degree of cooperativity.

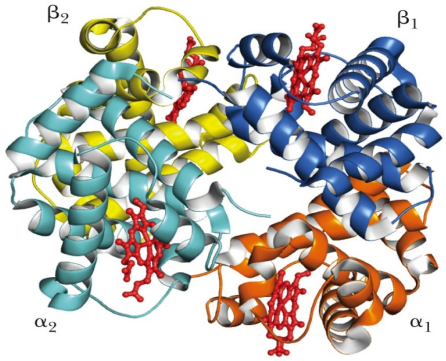
So how does this relate the biological effect of O_2 affinity of Hb?
- *look at pO2 of alveoli vs. metabolically active tissue*
- *What if oxygen dissociation were hyperbolic rather than sigmoidal?*

Myoglobin and Hemoglobin

- Hemoglobin is structurally related to myoglobin
- very different primary sequence about an 18% homology in the primary sequence
- 2 alpha subunits and 2 beta subunits
- in adults there are very small amount of alpha $_2$delta $_2$ hemoglobin



Myoglobin (Mb)

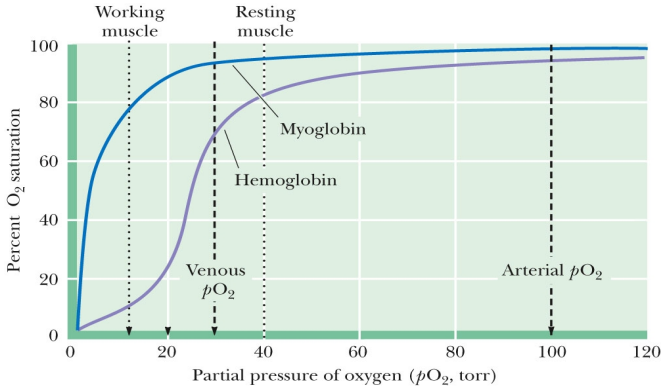


Hemoglobin (Hb)

Cooperative Binding of Oxygen Influences Hemoglobin Function

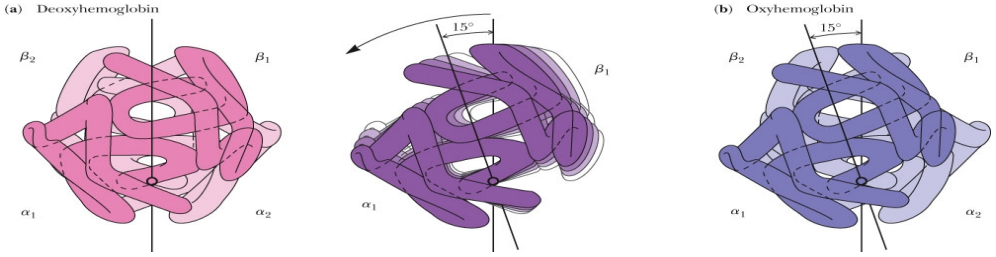
- Mb, an oxygen-storage protein, has a greater affinity for oxygen at all oxygen pressures
- Hb is different – it must bind oxygen in lungs and release it in capillaries
- Hb becomes saturated with O_2 in the lungs, where the partial pressure of O_2 is about 100 torr
- In capillaries, pO_2 is about 40 torr, and oxygen is released from Hb
- The binding of O_2 to Hb is cooperative – binding of oxygen to the first subunit makes binding to the other subunits more favorable

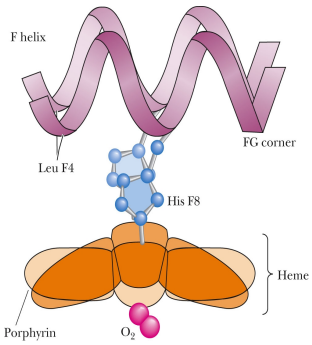
Impact of differences of O_2 binding affinity – allosterism



Oxygen Binding by Hb Induces a Quaternary Structure Change

- When deoxy-Hb crystals are exposed to oxygen, they shatter. Evidence of a large-scale structural change
- One alpha-beta pair moves relative to the other by 15 degrees upon oxygen binding
- This massive change is induced by movement of Fe by 0.039 nm when oxygen binds





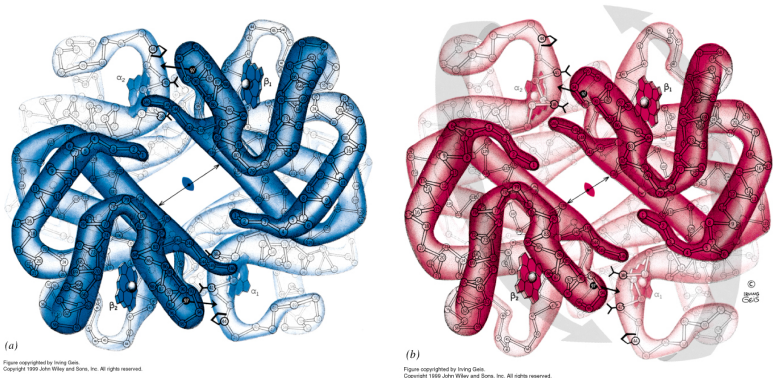
Fe^{2+} Movement by Less Than 0.04 nm Induces the Conformation Change in Hb

In deoxy-Hb, the iron atom lies out of the heme plane by about 0.06 nm
Upon O_2 binding, the Fe^{2+} atom moves about 0.039 nm closer to the plane of the heme
As Fe^{2+} moves, it drags His F8 and the F helix with it
This change is transmitted to the subunit interfaces, where conformation changes lead to the rupture of salt bridges

3D structure of hemoglobin and myoglobin

- a_1- b_1 units have 35 interactions
- a_1- b_2 units have 19 interaction sites
- similar units have few polar contacts
- the two a and two b subunits face each other through aqueous channels

- Binding of oxygen dramatically alters the interactions and brings about a twisting of the two halves (alpha beta pairs)
- Much of the quaternary changes takes place in the salt bonds between the C terminals of all four chains



Salt bridges that stabilize deoxy-Hb are broken in oxy-Hb

- There are two general structural states - the deoxy or T form and the oxy or R form.

One type of interactions shift is the polar bonds between the alpha 1 and the beta 2 subunits.

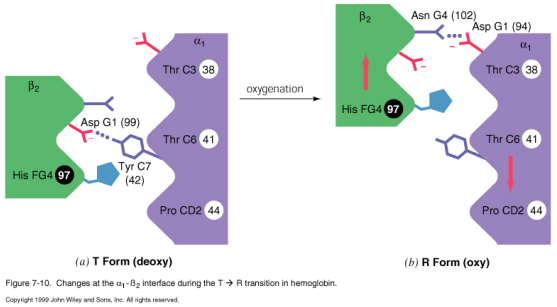


Figure 7-10. Changes at the α_1-β_2 interface during the T → R transition in hemoglobin.

So… to recap!

Oxygen binding shifts quaternary structure at long distances

- binding of O_2 ligand at 6th coordinate position pulls Fe into heme
- moves proximal histadine (F8) and the alpha helix it is attached to.
- shift in the helix is transmitted throughout of molecule
- Impacts interactions between Hb subunits.
- Myoglobin? No interactions
- Thus one has allosteric potential while the other doesn't

The Physiological Significance of the Hb:O_2 Interaction

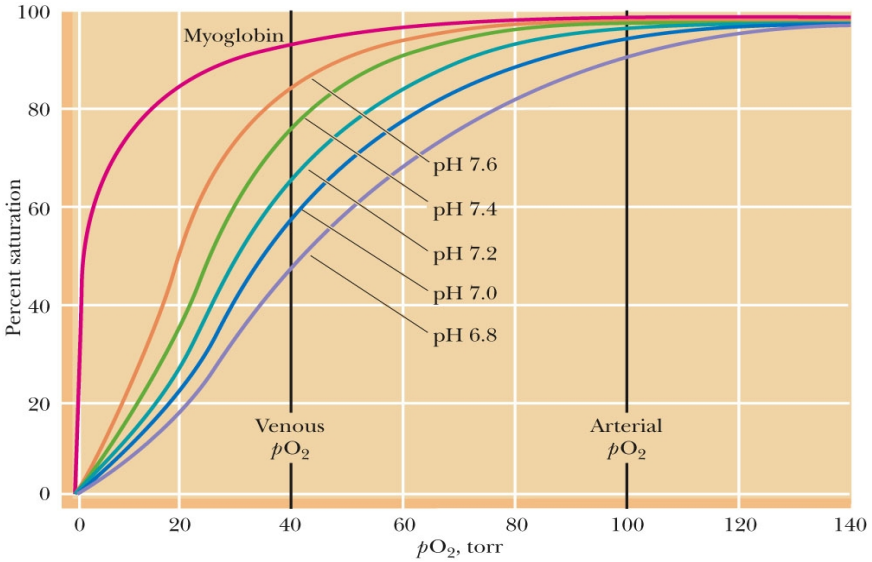
Hb must be able to bind oxygen in the lungs
Hb must be able to release oxygen in capillaries

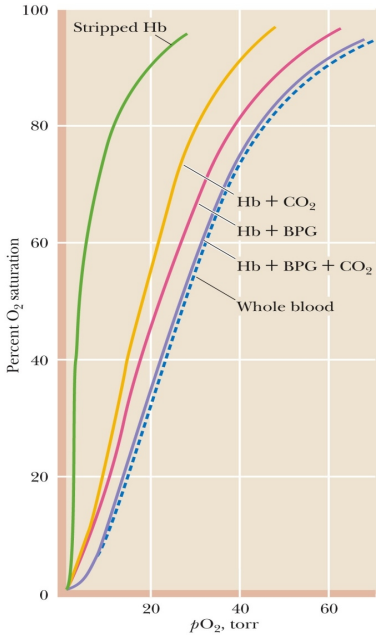
- If Hb behaved like Mb, very little oxygen would be released in capillaries
- The sigmoid, cooperative oxygen-binding curve of Hb makes its physiological actions possible!

Bohr Effect

H^+ Promotes Dissociation of Oxygen from Hemoglobin
The effect of H^+ is particularly important
Deoxy-Hb has a higher affinity for H^+ than oxy-Hb

Thus, as pH decreases, dissociation of O_2 from hemoglobin is enhanced





The Antagonism of O_2 Binding by H^+ is Termed the Bohr Effect

- The effect of H^+ on O_2 binding was discovered by Christian Bohr (the father of Neils Bohr, the atomic physicist)
- Binding of protons diminishes oxygen binding
- Binding of oxygen diminishes proton binding
- Important physiological significance

CO_2 Also Promotes the Dissociation of O_2 from Hemoglobin

Carbon dioxide diminishes oxygen binding

Hydration of CO_2 in tissues and extremities leads to proton production:

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

These protons are taken up by Hb as oxygen dissociates
The reverse occurs in the lungs

pH and CO_2 impact

1) In active tissues respiration, (glycolysis) results in lactic acid formation. These tissues need more O_2. Without the H+ effect Hb would hold on to more of the O_2. The increase [H+] induces Hb to dump 10% more of it's O_2.

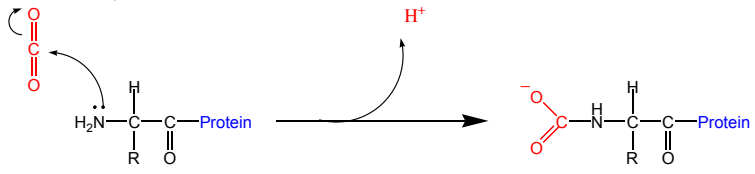
2) CO_2 reversibly binds to N term (carbamate) to remove remaining CO_2

$$R - NH_2 + CO_2 \text{ <-> } R - NH - COO^- + H^+$$

R is the Hb N term amide

The carbamide increases the T formation - deoxy form.

The reverse occurs in the lungs. This results in 1/2 of CO2 removal from tissues.



Amino Terminus Carbamate on Amino Terminus

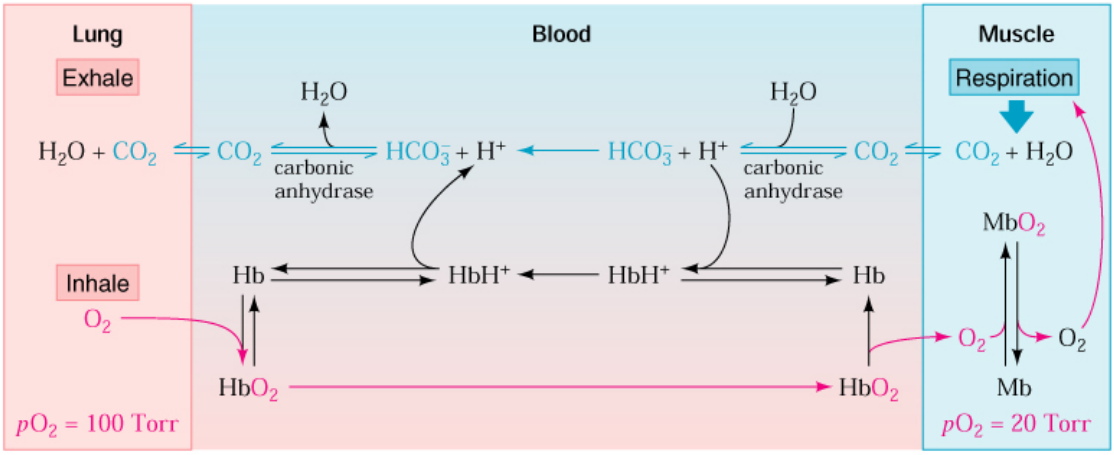
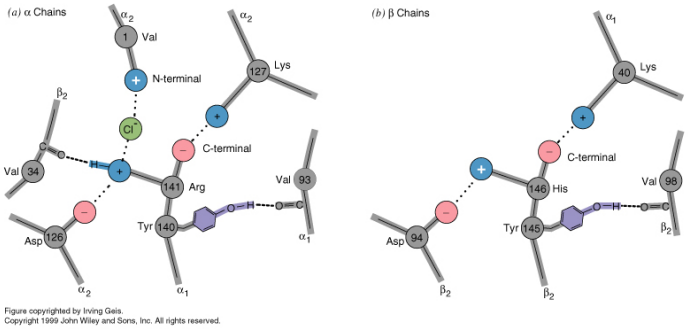


Figure 7-13 Key to Function. The roles of hemoglobin and myoglobin in transporting O_2 from the lungs to respiring tissues and CO_2 (as HCO_3^-) from the tissues to the lungs.

- The T form finds the terminals in several important H bonds and salt bridges.
- In the T form the C terminus of each subunit are "locked" into position through several hydrogen and ionic bonds.
- Shifts into the R state break these and allow an increased movement throughout the molecule.



Bohr Effect Continued

The Bohr effect is the reversible shift in Hb affinity for O_2 with changes in pH.

H+ Transport (effect) - O_2 binding to Hb releases H^+ due to conformational changes in Hb

- deoxyform (T form) brings Asp 94 close to His 146
- -the proximity of an acidic amino acid increases the pK of histidine (pKa is now above the pH) and results in H^+ "binding" to deoxyHb
- in other words the His becomes protonated where it normally would be ionized
- increasing pH stimulates Hb to bind to O_2

- Bottom line - when O_2 binds Hb, H+ is released from several amino acid's functional groups.
- When O_2 is released, the amino acids become protonized and then "picks" up a H+.

So when the H+ is high (acidic conditions) the H+ is driven onto the terminal amino acids driving it into the T conformation

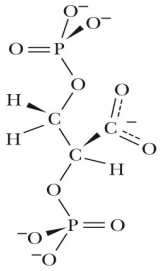
Summary of the Physiological Effects of H^+ and CO_2 on O_2 Binding by Hemoglobin

At the tissue-capillary interface, CO_2 hydration and glycolysis produce extra H^+, promoting additional dissociation of O_2 where it is needed most

$$CO_2 + H_2O \xrightleftharpoons{\text{carbonic anhydrase}} \underset{\text{carbonic acid}}{H_2CO_3} \rightleftharpoons H^+ + \underset{\text{bicarbonate}}{HCO_3^-}$$

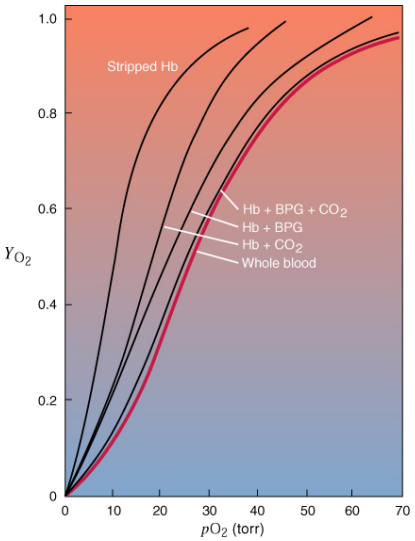
At the lung-artery interface, bicarbonate dehydration (required for CO_2 exhalation) consumes extra H^+, promoting CO_2 release and O_2 binding

–Purified Hb has a different O_2 affinity than in blood
–26 fold decrease change in affinity
–



2,3-Bisphosphoglycerate

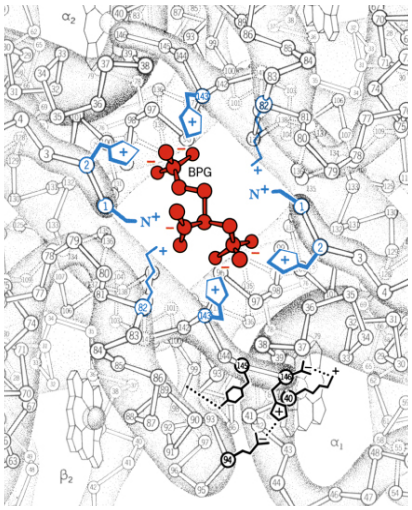
- In the absence of 2,3-BPG, oxygen binding to Hb follows a rectangular hyperbola!
- The sigmoid binding curve is only observed in the presence of 2,3-BPG
- Since 2,3-BPG binds at a site distant from the Fe where oxygen binds, it is called an allosteric effector



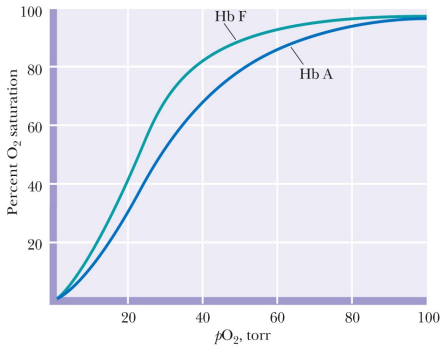
BPG Binding to Hb Has Important Physiological Significance

Where does 2,3-BPG bind?
- "Inside"
- in the central cavity

What is special about 2,3-BPG?
- Negative charges interact with 8 positive charges in the cavity: 2 Lys, 4 His, 2 N-termini



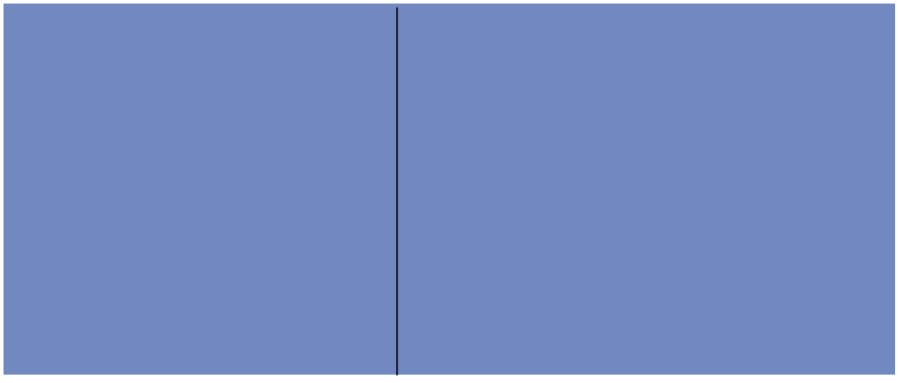
2,3 bisphosphoglycerate (BPG)
–Purified Hb has a different O_2 affinity than it does in blood
26 fold decrease change in affinity is due to 2,-3 diphosphoglycerate BPG
– (BPG replaced by nucleotides IHP and ATP in fish and birds)
– 1 BPG per Hb - binds in central cavity of Hb
– binds preferentially to deoxy Hb
– hydrophobic bonds with Lys and salt bridge with His
– O_2 binding changes conformation and "kicks out" BPG
– change in altitude increases concentration of BPG
Fetal F Hb has replaced His 143 with Ser - *What might the consequences be?*



Fetal and adult forms of hemoglobin

Expression of fetal Hb

z_2e_2 , a_2e_2 and a_2g_2 chains have a higher affinity for O_2 than a_2b_2 chains giving fetus ability to get O_2 from mother
Hemoglobin F, the hemoglobin of late fetal life is made of two a and two g subunits. The beta chain is not fully produced until a few weeks after birth



Fetal Hemoglobin Has a Higher Affinity for O_2 Because it has a Lower Affinity for BPG

- The fetus depends on its mother for O_2, but its circulatory system is entirely independent
- Gas exchange takes place across the placenta
- Fetal Hb differs from adult Hb – with γ-chains in place of β-chains – and thus a $\alpha_2\gamma_2$ structure
- As a result, fetal Hb has a higher affinity for O_2
- Why does fetal Hb bind O_2 more tightly?
- **Fetal γ-chains have Ser instead of His at aa 143 and thus lack two of the positive charges in the BPG- cavity**
- BPG binds less tightly and Hb F thus looks more like Mb in its O_2 binding behavior

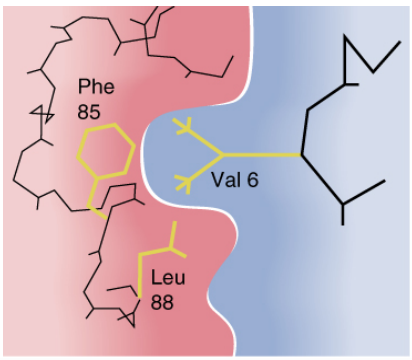
Sickle-Cell Anemia, a Molecular Disease

One of the first "molecular" diseases found - sickle cell anemia
- sickle cell - blood cell is elongated , mis-shaped (sickle)
  - occurs at low O_2 concentration
  - caused by hemoglobin aggregates
  - inflammation in capillaries and pain
  - red blood cells break down - anemia
- between 10% of American blacks and 25% of African blacks are heterozygous for sickle cell anemia
- homozygous usually do not survive into adult hood
- heterozygous individuals usually have no problem except when in severe oxygen deprivation

Sickle-Cell Anemia is a Molecular Disease

A single amino acid substitution in the β-chains of Hb causes sickle-cell anemia
- Glu at position 6 of the β-chains is replaced by Val

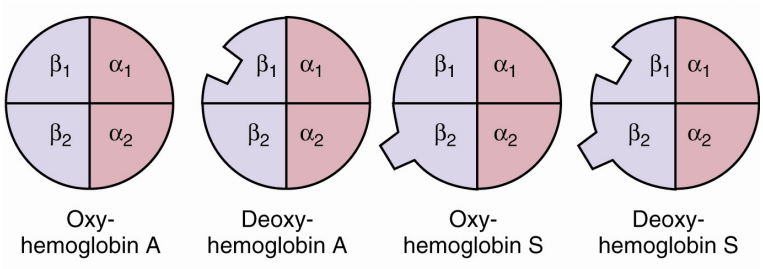
As a result, Hb S molecules aggregate into long, chainlike polymeric structures



Single amino acid (point mutation) HbS vs. HbA changes structure
- sickle cell b chains have a valine in place of glutamate
- leads to more Hb S (sickle cell) has 2 more + charges than normal hemoglobin
- Glu -Val occurs on exterior of protein - does not change O_2 dissociation/allosteric properties of protein

Sickle-Cell Anemia is a Molecular Disease

The polymerization of Hb S molecules arises because Val replaces His on the surface of β-chains. The "block" extending from Hb S below represents the Val side chains. These can insert into hydrophobic pockets in neighboring Hb S molecules.



Deoxy HbS precipitates
- oxyHb phenylalanine b85 and leucine b88 interior
- phe and leu shift to exterior
- create a sticky patch with valine (hydrophobic bonding)
- nucleation (cluster of aggregate) occurs logarithmically
- homozygous - 1000 times faster than heterozygous
- that means mixed genes can re-oxygenate faster than polymerization can occur

How can such a disease occur?
- highest concentration of gene mutation occurs where there is high incidence of malaria
- heterozygous individuals survive this disease better than those without
- malaria causing parasite lives in red blood cells during part of its life cycle
- partial sickling must interrupt life cycle of malaria parasite

Methemoglobinemia - instead of aggregation, mutation leads to changes in O2 affinity

Hb Boston form: distal his replaced with tyrosine - stabilizes Fe_3^+ state
- heme cannot bind O2, T form is favored

Hb Milwaukee Val near distal His site is mutated to a glutamate
- This allows tight association with O2. Causes oxidation of iron
- blood is brown (Fe+3 state)
- only heterozygous individuals survive

Thalassemias - alpha thalassemia, missing alpha chain - usually due to way the DNA mutation in promoter
- heterozygous are usually asymptotic (show no signs)
- homozygous need blood transfusion to live
- delta chains are very important here

Hemoglobin and Nitric Oxide

Nitric oxide (NO·) is a simple gaseous molecule that acts as a neurotransmitter and as a second messenger in signal transduction

NO· is a high-affinity ligand for Hb, binding to the heme iron 10,000 times more tightly than O_2

So why is NO· not bound instantaneously to Hb, preventing its physiological effects?

NO· reacts with the –SH of $Cys^{93}β$, forming an S-nitroso derivative:

The S-nitroso group is in equilibrium with other S-nitroso compounds formed by reaction of nitric oxide with small-molecule thiols such as free Cys or glutathione:

These small-molecule thiols transfer NO· from erythrocytes to endothelial receptors, where it exerts its physiological effects