Bohr Effect Continued

The Bohr effect is the reversible shift in Hb affinity for O2 with changes in pH.

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

- deoxyform (T form) brings Asp 94 close to His 146 (fig 7-11 (b))
- 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 O2
- 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

CO_2 - In the red blood cells the picture is even more complicated. CO_2 is removed by converting CO_2 to bicarbonate

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

- bicarbonate (HCO_3^-) formed by the enzyme carbonic anhydrase
- H+ produced by this enzyme is removed by the Hb as described above
- This allows more CO_2 to be removed in the form of bicarbonate
- CO2 binding aids in reducing O2 affinity by changing conformation by the production of more H+ (R to T change)
- In the lungs the O_2 binds Hb and forces the R conformation

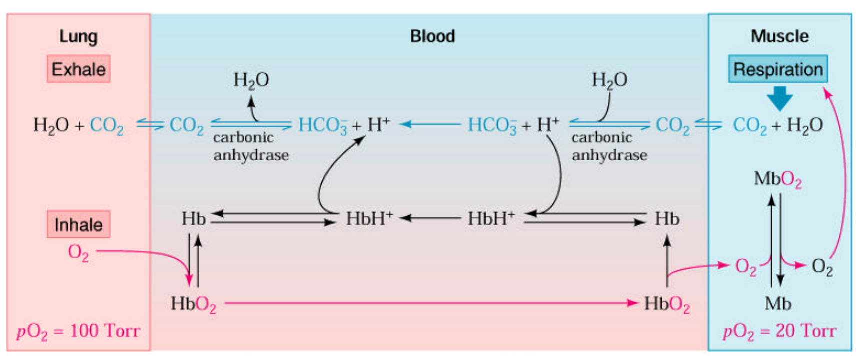

Figure 7-13 Key to Function. The roles of hemoglobin and myoglobin in transporting O_2 from the lungs to respiring tissues and CO_2 (as HCO_3^-) from the tissues to the lungs.

This all aid the function of Hb. In active tissues respiration, (glycolysis) results in lactic acid formation. These tissues need more O2. Without the H+ effect Hb would hold on to more of the O2. The H+ induces Hb to dump 10% more of it's O_2.

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

  $R - NH_2 + CO_2 <-> R - NH - COO^- + H^+$

  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.

2,3 bisphosphoglycerate (BPG) Effects

- 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?

Cooperative interactions between subunits

- Both models do not fully account for the effects of allosteric effectors
- sequential model (D Koshland)
  - binding of one O_2 induces T-R conformation change
    - 1st change is most difficult due to influence by 3 other subunits
  - binding of next three subunits happens sequentially, with higher affinity (easier T-R changes)
  - kinetics increase to the fully oxy Hb state as more O_2 is bound
- concerted model (J Monod)
  - All R or all T no in between as in the Koshland model
  - concerted model means as more O_2 binds, the R conformation is favored until all units are in the R conformation regardless of the total units bound to O_2
  - Affinities do NOT change until conformation changes
  - 1 O_2 - all T; 2 O_2 - nearly even equilibrium; 3 O_2 mostly R; 4 O_2 - mostly R form
  - energy from O2 binding causes the change in equilibrium
  - this model best fits O2 dissociation curve but with limits.

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

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

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

The disease is environmentally selective

- 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

Molecular Diseases

Methemoglobinemia - instead of aggregation, mutation leads to changes in O_2 affinity

Hb boston form: distal His replaced with tyrosine - stabilizes Fe^{+3} state

- Heme cannot bind O_2, T form favored

Hb Milwaukee Val near distal His site is mutated to a glutamate

- This allows tight association with O_2. Causes oxidation of iron
- Blood is brown (Fe^{+3} state)
  - Only heterozygous individuals survive

Thalassemias -

α thalassimia, missing the α chain - usually due to a mutation that leads to a lack of gene expression

- Heterozygous people are usually asymptotic (show no signs)
- Homozygous need blood transfusions to live
- Delta chain are very important