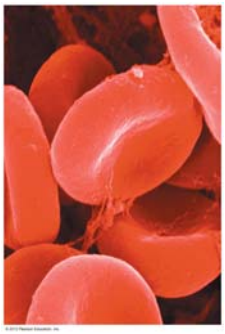
Hemoglobin, an Allosteric Protein

Stryer Short Course

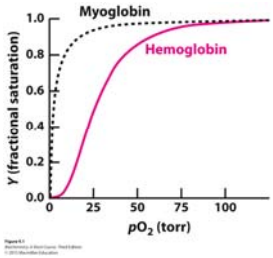
Case Study: Hemoglobin

- Structure: Quaternary, heme group
- Function: Oxygen binding
- Physiology: oxygen delivery from lungs to tissue
- Myoglobin: no quaternary structure; stores oxygen in muscle tissue



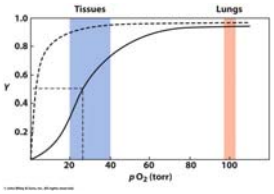
Oxygen Binding Curves

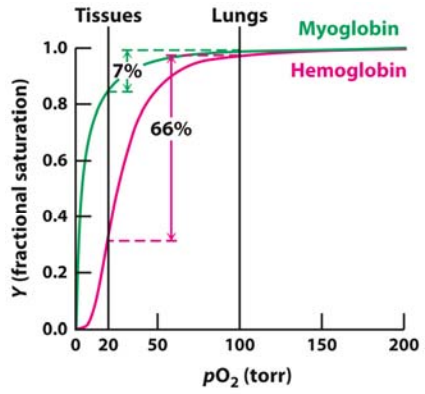
- Fractional saturation
- Partial pressure of oxygen
  - 176 torr, 100 torr in lungs with water vappr
- Rectangular hyperbola vs. sigmoidal
  - cooperativity



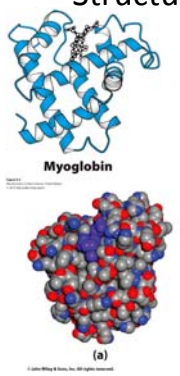
Physiological impact

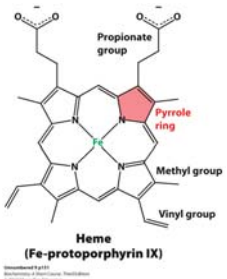
- Myoglobin is half-saturated at 2 torr
- Hemoglobin is half-saturated at 26 torr O_2
- Hemoglobin has less affinity for oxygen
- Hb saturated in lungs
- When it reaches tissues, oxygen is released
- Steep in important region





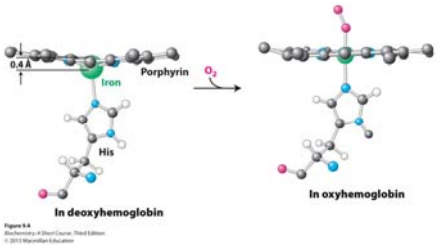
Structure of Myoglobin





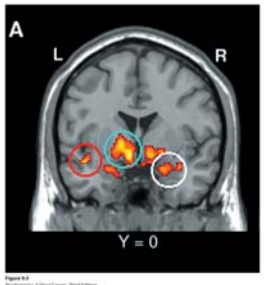
Structure of Myoglobin

- Noncovalent binding in hydrophobic pocket
- His F8 (proximal); His E7 (distal)
- With oxygen bound, iron fits in porphyrin ring

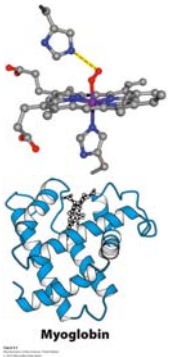


Functional MRI

- MRI = NMR: detects water protons
- Oxygenated hemoglobin has different magnetic qualities
- Can detect "active" sites of brain, where oxygenated blood is being used



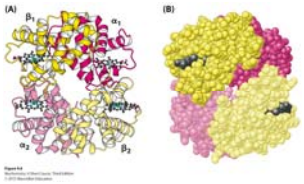
Reversible Oxygen Binding



- Tight hydrophobic pocket
- Fe^{+1} easily oxidized outside of globin
- Distal histidine and hydrophobic pocket limits Fe^{+3} formation

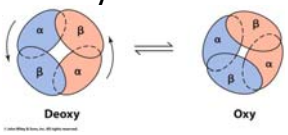
Structure of Hemoglobin

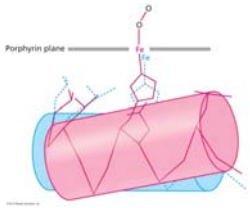
- Oligomer of four units resembling Mb
- $\alpha_2\beta_2$ tetramer
- Treated as dimer of $\alpha\beta$ units
- Hollow core in center



Cooperativity

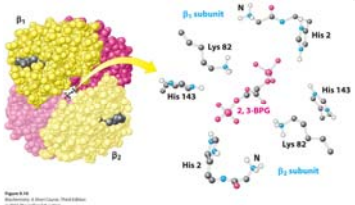
- Binding of Oxygen changes shape of unit
- Shape of subunit affects shapes of other subunits
  - Oxygen-bound unit causes other subunits to become **relaxed**
  - The rich become richer
  - Cooperative binding





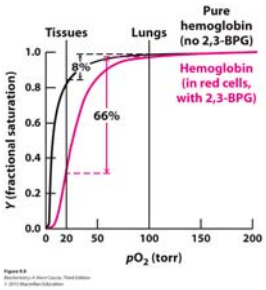
Allosteric Regulation

- 2,3-BPG binds in central cavity, but only to T
- Holds all subunits toward the "Tight" conformation
- Disfavors oxygen binding



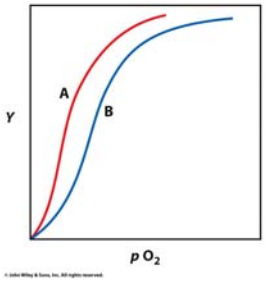
Physiological Role of 2,3-BPG

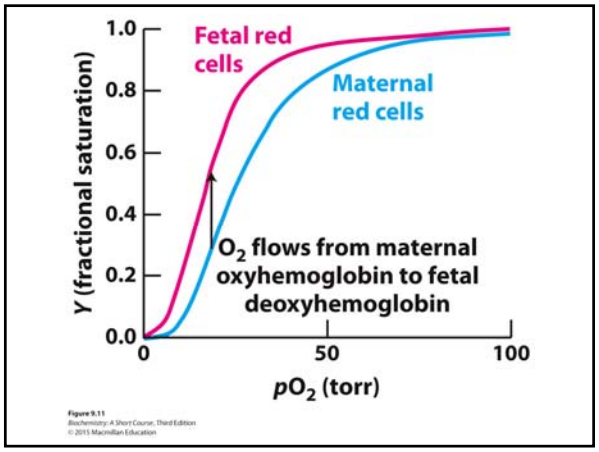
- Without 2,3-BPG, hemoglobin curve becomes hyperbolic
- No 2,3-BPG leads to too great affinity
- Hb would only deliver 8% of its oxygen
- Fetal Hb doesn't bind BPG; has greater oxygen affinity



Problem

- Fetal Hb is an $\alpha_2\gamma_2$ protein. At birth, adult Hb is produced so that by 6 months, 98% of the baby's Hb is adult. In the graph, which is the binding curve for fetal Hb? What is the physiological purpose?

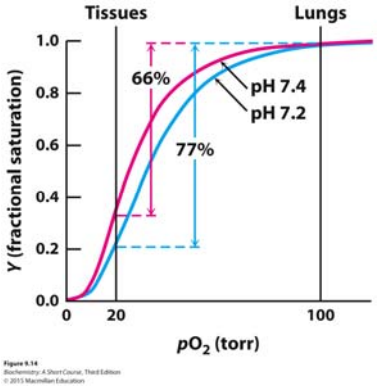




Bohr Effect

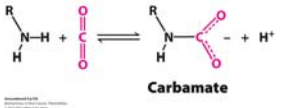
- pH affects oxygen binding
- Lower pH in tissue leads to protonation of Hb
- Ion pairs form in central cavity that stabilize the deoxy (tense) form
- 77% of O_2 released in acidic tissue

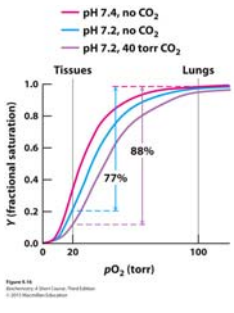
$Hb{\cdot}H^+ + O_2 \rightleftharpoons Hb{\cdot}O_2 + H^+$



Effect of Carbon Dioxide

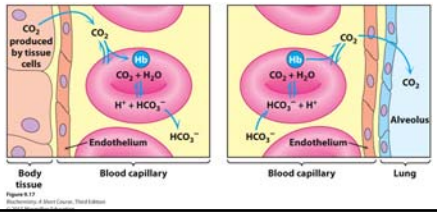
- CO2 produced in tissues also contributes to release of O2
- Reacts in cavity; makes salt bridges that stabilize deoxy form
- Now 88% released





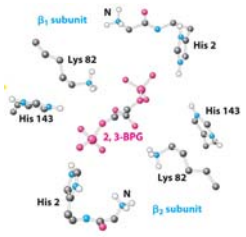
Physiology

- In tissue, CO_2 produced; H^+ concentration raised
- decrease oxygen affinity in tissue compared to lungs
- Protons, CO2 shuttled to lungs on Hb
  - minor process—mostly returns to lungs in blood buffer



Problem

- Propose a few explanations of how a K→N mutation of a residue in the central cavity could lead to a mutant Hb with greater oxygen binding affinity.



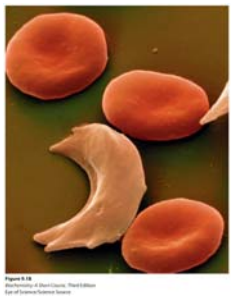
Problem

- Propose a few explanations of how a K→N mutation of a residue in the central cavity could lead to a mutant Hb with greater oxygen binding affinity.

- It might change the conformation of the F-helix such that His F8 binds oxygen better
- Since the central cavity is less +, BPG might bind worse, favoring R
- It might destabilize ion pairs that normally stabilize the T state

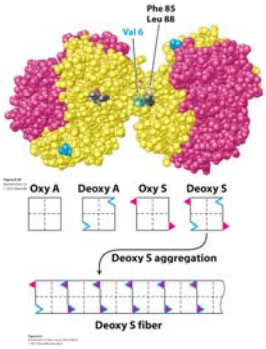
Pathologies

- Sickle-cell anemia
- Thalassemia



Sickle-cell Anemia

- D→V mutation
  - HbS
- Hydrophobic effect in deoxy state
- Leads to aggregate fibrils that distort the cell and block blood vessels



Thalassemia

- Loss of inadequate production of one chain
- α-thalassemia: tetramer is all β form
  - Called Hemoglobin H (HbH)
  - No cooperativity
  - oxygen affinity too high
  - Usually fatal
- β-thalassemia: tetramer is all α form
  - Precipitates, kills cell
  - Transfusions needed