BIOCH 755: Biochemistry I Fall 2015

7a. Oxygen-Binding Proteins

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7.1 Oxygen Binding to Hemoglobin & Myoglobin

- **Key Concepts 7.1**
  - Myoglobin, with its single heme prosthetic group, exhibits a hyperbolic O_2-binding curve.
  - Hemoglobin can adopt the deoxy (T) or oxy (R) conformation, which differ in O_2-binding affinity (classical allosteric model).
  - Oxygen binding triggers conformational changes in hemoglobin so that oxygen binds to the protein cooperatively, yielding a sigmoidal binding curve.
  - The Bohr effect and BPG alter hemoglobin's O_2-binding affinity.
  - Mutations can change hemoglobin's O_2-binding properties and cause disease.

7.1A: Myoglobin

- First structure of proteins (1959 by John Kendrew)
- Vertebrate muscle
- 153 residues, 8 helices (A-H), ~44 x 44 x 25 Å
- Heme group: coordinate Fe(II), where O2 binds

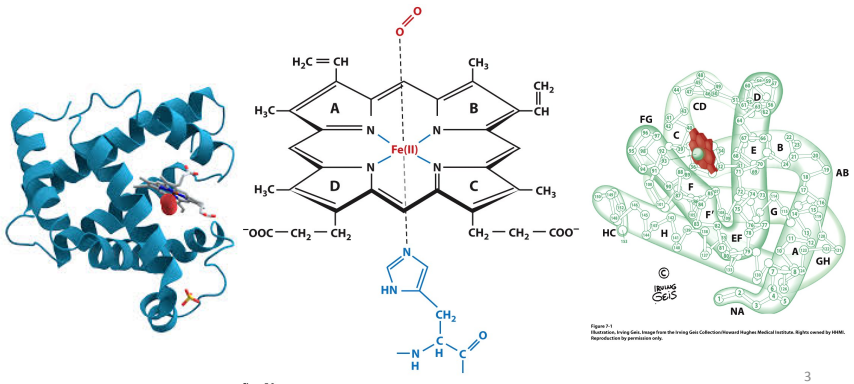

Figure 7-2
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Myoglobin

Roles of the protein

- Several residues bind and stabilize Heme
  - Val E11/Phe CD1/His E7/His F8
- Fe(II) in free heme is oxidized when exposed to O2 and Fe(III) does not bind O2
- Protein coordination shift the electronic states and prevent Fe(II) oxidization

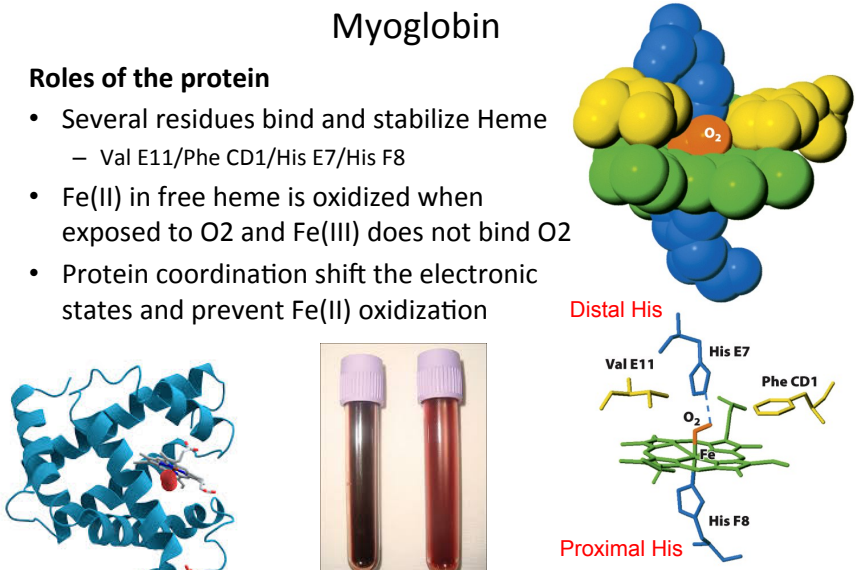

Figure 7-3
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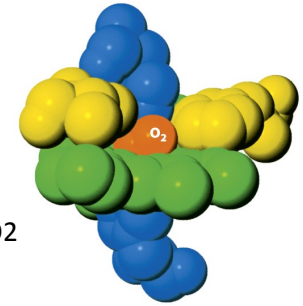
Myoglobin

Roles of the protein

- Several residues bind and stabilize Heme
  - Val E11/Phe CD1/His E7/His F8
- Fe(II) in free heme is oxidized when exposed to O2 and Fe(III) does not bind O2
- Protein coordination shift the electronic states and prevent Fe(II) oxidization
- Other small molecules such as CO, NO, H2S can also bind to heme, and with higher affinities!
  - CO binds 200X stronger than O2 and is thus particularly toxic!
  - Incomplete combustion, car exhaust etc



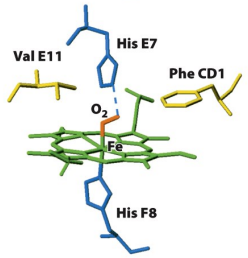


Figure 7-3

Myoglobin

- First structure of proteins (1959 by John Kendrew)
- 153 residues, 8 helices (A-H), ~44 x 44 x 25 Å
- Heme group: coordinate Fe(II), where O2 binds

- Function: mainly to facilitates O2 diffusion in muscle
  - O2 has low solubility in blood (~10^{-4} M), which limits diffusion from capillaries to tissues
  - Myoglobin increases the effective O2 concentration
- Also as O2 storage protein for aquatic mammals
  - 10X more myoglobin (first X-ray structure determined from sperm whale myoglobin)
- Other globins: prevent O2 radical damage prevention
  - Neuroglobin: brain, retina and endocrine tissues
  - Cytoglobin: most tissues

Binding Properties

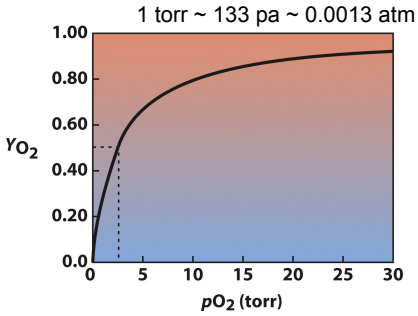
- Simple binary binding

  $Mb + O_2 <-> MbO_2$

  $K_D = [Mb][O_2]/[MbO_2]$
- Fractional saturation:

  $Y_{O2} = [MbO_2] / ([Mb] + [MbO_2])$

  $= [O_2] / (K_D + [O_2])$

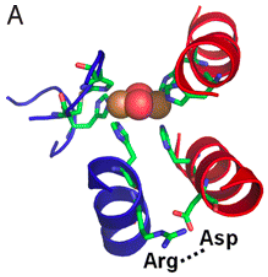


- Hyperbolic curve
- K_D = *p*50 (pO2 when 50% saturated)
  - 2.8 torr for myoglobin
  - pO2 ~100 torr in arterial blood and ~30 torr in venous blood
  - Y_{O2} ~ 0.97 and 0.91 respectively
- Hyperbolic binding curve ubiquitous for all simple binary binding

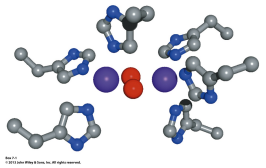
Other Oxygen Transport Proteins

- Myoglobin: vertebrate muscle
- O2 diffusion frequently a bottleneck and thus need for oxygen transport mechanism in many organisms
- Many use heme but some do not

A



(Two Cu atoms)

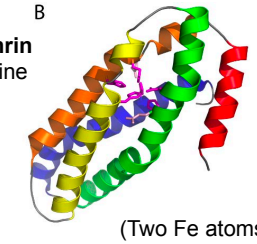


Hemocyanin (mollusks and arthropods)

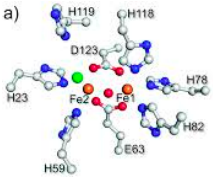
PDB: 1OXY

B

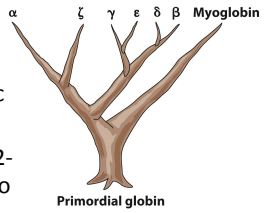
Hemerythrin (from marine worms)



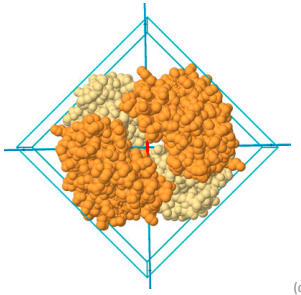
(Two Fe atoms)

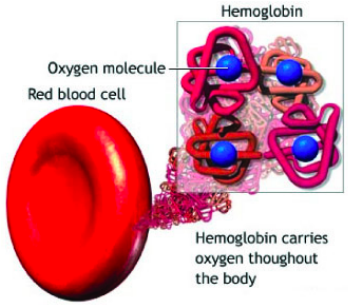


7.1B: Hemoglobin



- One of the first proteins to be associated with specific function (oxygen transport)
- Tetramer (α2β2): dimer of αβ protomers (related by 2-fold rotation, i.e., D2 symmetry); α and β subunits also have pseudo 2-fold symmetry
- Evolutionarily related to myoglobin with only ~18% identity (yet with very similar structures)





Deoxy- and Oxyhemoglobin Structures

- O2 binding induces (quaternary) structure changes
  - ~15o rotation of one αβ w.r.t. the other

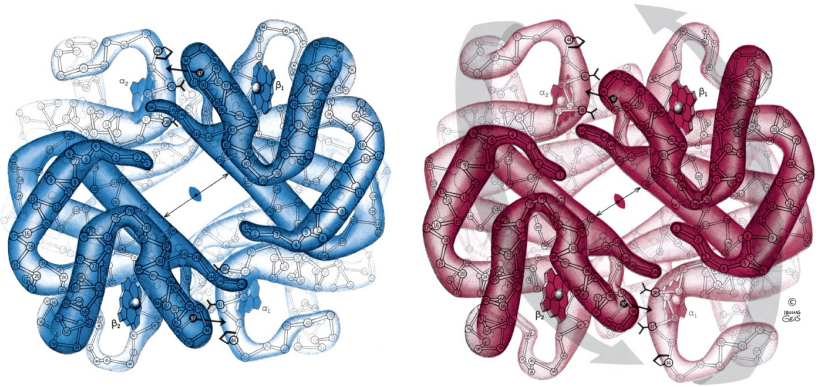


Figure 7-5
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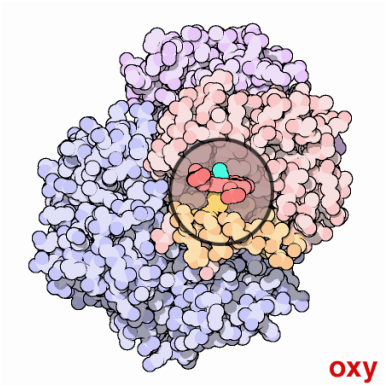
Deoxy- and Oxyhemoglobin Structures

- O2 binding induces (mostly quaternary) structure changes

(show VMD)

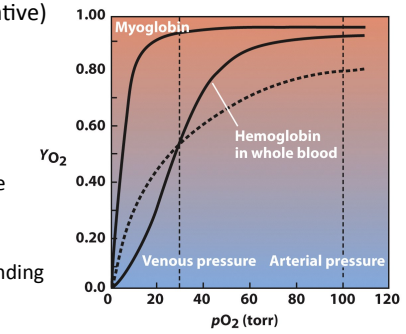


http://www.rcsb.org/pdb/101/motm.do?momID=41

Cooperative binding of O2: Importance for O2 delivery

- Binding of O2 to Hb is (quite) cooperative (i.e., tend to be all or none)
  - Arise from conformational changes upon O2 binding
- Hb + n O_2 <-> $Hb(O_2)_n$ (when fully cooperative)

  $Y_{O2} = [pO_2]^n / (p_{50}^n + [pO_2]^n)$
- n also known as Hill's coefficient
- Cooperative binding: sigmoidal (S-shape) curve instead hyperbolic curve
- Provide a mean for empirical fitting to infer binding mechanism
  - n > 1: positive cooperativity
  - n < 1: negative cooperativity



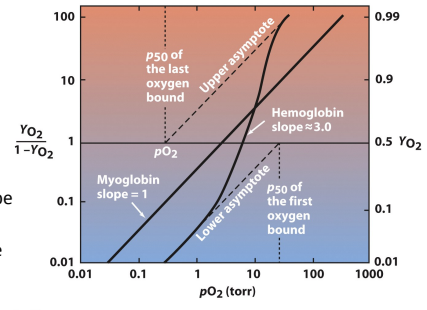
Hill's Plot

- $Y_{O2} = [pO_2]^n / (p_{50}^n + [pO_2]^n)$

  $Y_{O2}/(1-Y_{O2}) = [pO_2]^n / p_{50}^n$

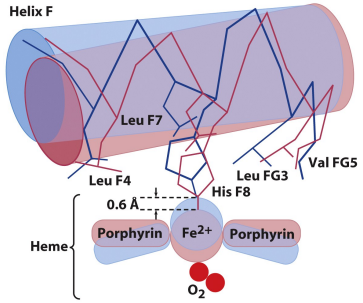
$\log(Y_{O2}/(1-Y_{O2})) = n \log[pO_2] - n \log p_{50}$

- maximal slope ~ $Y_{O2} = 0.5$
- Hemoglobin not infinitely cooperative
- Three linear regimes
  - Low pO2: first O2 binding, myoglobin like
  - Intermediate O2: cooperative, maximal slope reached near $Y_{O2} = 0.5$
  - High pO2: last O2 occupancy, no observable cooperativity either
  - Note that p50 of last O2 bound is much lower than p50 of first O2 bound



Hemoglobin Cooperativity Mechanism

- Heme binding sites distal on four subunits of Hb
- Cooperativity arises from conformational changes driven by O2 binding
- The classical Perutz mechanism of allostery
  - Two stable state: T (deoxy-) and R (oxy-) states
  - R-state driven by binding of first O2 and has high O2 affinity

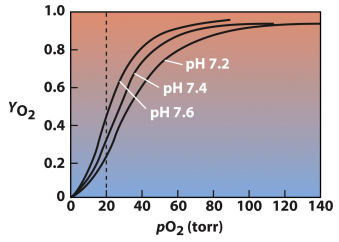


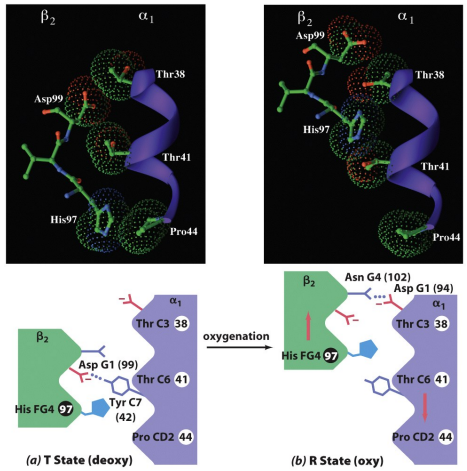
T (blue) to R (red) transformation:

Fe(II) is ~0.6 Å above in T-state and does not coordinate O2 as well and leads to structural strains

Bohr Effect

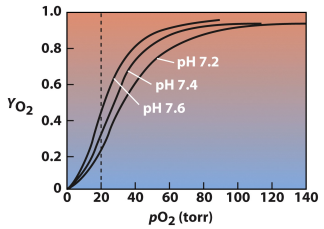
- The conformation changes associated with O2 binding (T->R) also lead to disruption of several salt-bridges and pKa decrease
- ~0.6 proton release per O2
- pH can thus modulate O2 binding to Hb
  - Increasing pH stimulates O2 binding and vice versa!

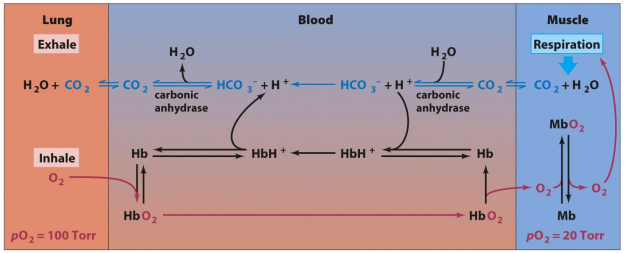




Bohr Effect

- The conformation changes associated with O2 binding (T->R) also lead to disruption of several salt-bridges and pKa decrease
- ~0.6 proton release per O2
- pH can thus modulate O2 binding to Hb
  - Increasing pH stimulates O2 binding and vice versa!
- In tissues: CO_2 accumulation lowers pH and reduces Hb-O_2 binding and releases O_2!
  - Robust built-in feed back loop!





BPG Regulation of Hb-O2 binding

- Bisphosphoglycerate (BPG) binds to deoxy-Hb much tighter than oxy-Hb
- Presence of BPG in red blood cells stabilize deoxy-Hb (w.r.t oxy-Hb) and thus facilitates O2 release

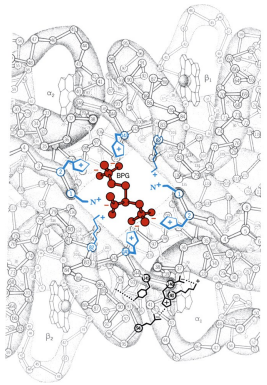


Figure 7-14
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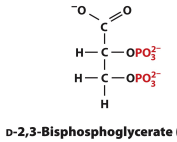
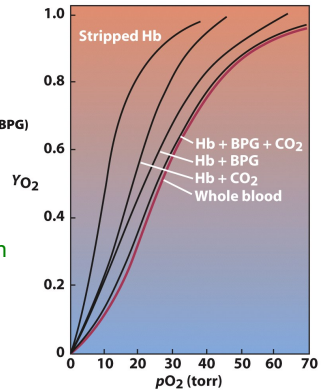


T->R transformation reduce the central cavity where BPG binds (and not favorable)



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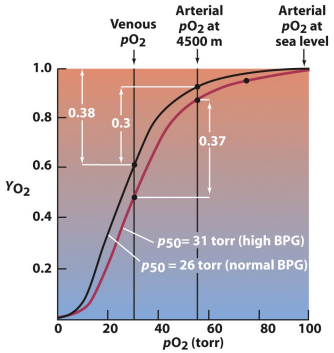
BPG Regulation of Hb-O2 binding

- Bisphosphoglycerate (BPG) binds to deoxy-Hb much tighter than oxy-Hb
- Presence of BPG in red blood cells stabilize deoxy-Hb (w.r.t oxy-Hb) and thus facilitates O2 release
- BPG level regulates p50 values and plays a role in high-altitude adaptation



Box 7-3
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- 1-day stay could increase BPG from ~4 mM to 8 mM
- p50 of Hb increases from 26 to 31 torr
- @ sea level: 38% O2 released with arterial to venous pO2 change
- If not BPG modulation, only 30% O2 release due to lower arterial pO2
- w/ BPG increase: ~37% O2 release
- Thus, a rapid response before more Hb can be made

Hemoglobin Mutations

- ~300,000 people born every year with serious Hb disorders!
- Consequences of mutations ultimately considered in the ability to transport (and properly release) O2!

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)β → 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	Disrupts 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
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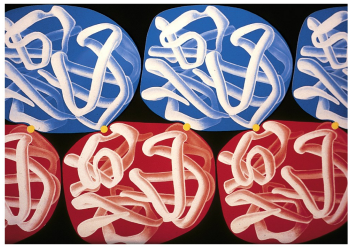
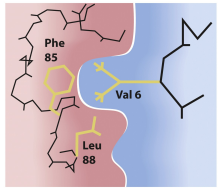
Sickle-Cell Anemia: Single Amino Acid Change!

- Glu6 -> Val
- ~10% African American and ~25% black African carries a single copy of the sickle-cell Hb gene (Hemoglobin S)
- Deformed red blood cells (and thus the name)
- Speculated and later demonstrated by Linus Pauling in 1950's
- Structural data shows that Val forms hydrophobic pockets and promote linear polymers of Hb, and could lead to blood flow blockage





Hemoglobin S and Malaria

- Sickle-cell genes confers resistance to malaria
- Bohr effect:

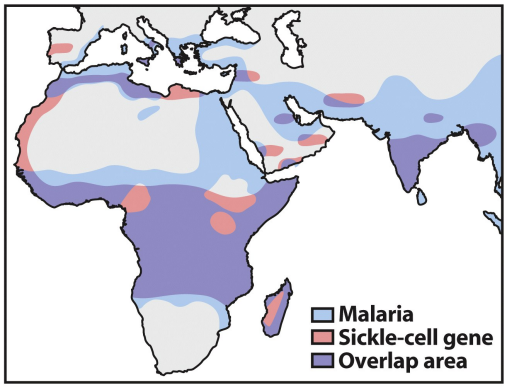


Figure 7-20

Summary

- Describe the O_2-binding behavior of myoglobin in terms of pO_2 and K. How is K defined?
- Explain the structural basis for cooperative oxygen binding to hemoglobin.
- Sketch a binding curve (% bound ligand versus ligand concentration) for cooperative and noncooperative binding.
- Explain why the O_2-binding behavior of myoglobin and hemoglobin can be summed up by a single number (the p50).
- Could a binding protein have a Hill constant of zero?
- Describe how myoglobin and hemoglobin function in delivering O_2 from the lungs to respiring tissues.
- What is the physiological relevance of the Bohr effect and BPG?
- Mutations can increase or decrease the oxygen affinity and cooperativity of hemoglobin. How can the body compensate for these changes?