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

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
Myoglobin

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- Protein coordination shift the electronic states and prevent Fe(II) oxidation
- 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

**Binding Properties**
- Simple binary binding
  \[ \text{Mb} + \text{O}_2 \leftrightarrow \text{MbO}_2 \]
  \[ K_D = [\text{Mb}][\text{O}_2]/[\text{MbO}_2] \]
- Fractional saturation:
  \[ Y_{O2} = [\text{MbO}_2] / ([\text{Mb}] + [\text{MbO}_2]) = [O_2] / (K_D + [O_2]) \]
- Hyperbolic curve
  \[ K_D = p50 \text{ (pO2 when 50% saturated)} \]
  - 2.8 torr for myoglobin
  - pO2 ~100 torr in arterial blood and ~30 torr in venous blood
  - \( Y_{O2} \sim 0.97 \) 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

**Hemerythrin** (from marine worms)
- (Two Cu atoms)

**Hemocyanin** (mollusks and arthropods)
- PDB: 1OXY

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7.1B: Hemoglobin

- One of the first proteins to be associated with specific function (oxygen transport)
- Tetramer ($\alpha_{2} \beta_{2}$): dimer of $\alpha \beta$ protomers (related by 2-fold rotation, i.e., D2 symmetry); $\alpha$ and $\beta$ 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
  - ~15° rotation of one $\alpha \beta$ w.r.t. the other

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
- $\text{Hb} + n\text{O}_2 \leftrightarrow \text{Hb(O}_2)_n$ (when fully cooperative)
  \[ Y_{O2} = \frac{[\text{pO}_2]^n}{(p_{50})^n + [\text{pO}_2]^n} \]
- $n$, 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

Deoxy- and Oxyhemoglobin Structures
- O2 binding induces (mostly quaternary) structure changes

http://www.rcsb.org/pdb/101/molm.do?momID=41
Hill’s Plot

- $Y_{O2} = \frac{[pO_2]^n}{(p_50^n + [pO_2]^n)}$
- $Y_{O2}/(1-Y_{O2}) = \frac{[pO_2]^n}{p_50^n}$
- $\log(Y_{O2}/(1-Y_{O2})) = n \log[pO_2] - n \log p_50$

- maximal slope $\sim Y_{O2} = 0.5$
- 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 pS50 of last O2 bound is much lower than pS50 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

Bohr Effect

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

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- 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

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

**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**

<table>
<thead>
<tr>
<th>Name*</th>
<th>Mutation</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hammersmith</td>
<td>Phe CD1(42)β → Ser</td>
<td>Weakens heme binding</td>
</tr>
<tr>
<td>Bristol</td>
<td>Val E11(67)β → Asp</td>
<td>Weakens heme binding</td>
</tr>
<tr>
<td>Bibba</td>
<td>Leu H19(136)α → Pro</td>
<td>Disrupts the H helix</td>
</tr>
<tr>
<td>Savannah</td>
<td>Gly B6(24)β → Val</td>
<td>Disrupts the B-E helix interface</td>
</tr>
<tr>
<td>Philly</td>
<td>Tyr C1(35)β → Phe</td>
<td>Disrupts hydrogen bonding at the α1-β1 Interface</td>
</tr>
<tr>
<td>Boston</td>
<td>His E7(28)α → Tyr</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Milwaukee</td>
<td>Val E11(67)β → Gln</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Iwate</td>
<td>His F8(17)α → Tyr</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Yakima</td>
<td>Asp G5(99)β → His</td>
<td>Disrupts a hydrogen bond that stabilizes the T conformation</td>
</tr>
<tr>
<td>Kansas</td>
<td>Asn G4(102)β → Thr</td>
<td>Disrupts a hydrogen bond that stabilizes the R conformation</td>
</tr>
</tbody>
</table>

*Hemoglobin variants are usually named after the place where they were discovered (e.g., hemoglobin Boston).

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:

Summary

• Describe the O₂-binding behavior of myoglobin in terms of pO₂ 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 O₂-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 O₂ 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?