Chapter 10

Oxygen Transporting Proteins
Oxygen-transport proteins

- **Vertebrates**
  - Myoglobin (Muscle)
  - Hemoglobin (Blood)
- **Invertebrates**
  - Hemerythrin
  - Hemocyanin
Myoglobin’s oxygen-binding curve is hyperbolic

\[ \text{Mb} + \text{O}_2 \rightleftharpoons \text{MbO}_2 \]

\[ K = \frac{[\text{Mb}][\text{O}_2]}{[\text{MbO}_2]} \]

Fractional saturation (\( Y_{O_2} \))

\[ Y_{O_2} = \frac{[\text{MbO}_2]}{[\text{Mb}]+[\text{MbO}_2]} \]

Because \([\text{MbO}_2] = \frac{[\text{Mb}][\text{O}_2]}{K}\)

\[ Y_{O_2} = \frac{\frac{[\text{Mb}][\text{O}_2]}{K}}{[\text{Mb}]+\frac{[\text{Mb}][\text{O}_2]}{K}} = \frac{[\text{O}_2]}{K + [\text{O}_2]} \]

\[ Y_{O_2} = \frac{p\text{O}_2}{K + p\text{O}_2} \]
Hemoglobin

A tetrameric protein with two alpha and two beta chains

Both chains fold into a Mb-like structure

An oxygen carrier protein

Provides oxygen to different types of tissues

Hb has two different conformations; oxygen-bound and unbound
Deoxyhemoglobin
Oxyhemoglobin
Differences between deoxy- and oxy-Hb
Why do we need blood to transport $O_2$?

Why do we need hemoglobin?

Why does hemoglobin have 4 subunits?
In organisms larger than 2 mm, diffusion is too slow to supply the tissues with $O_2$.

We need hemoglobin to increase $O_2$ solubility of blood.
Hemoglobin transports $O_2$ from the lungs to the tissues

Take up as much $O_2$ in the lungs as possible
Leave as much $O_2$ behind as possible in the tissues

How is the affinity to $O_2$ controlled?
Hemoglobin’s oxygen-binding curve is sigmoidal
Sigmoidal binding curve suggests cooperativity

- Hb contains 4 $O_2$ binding sites
- Binding at one site affects the binding at other sites
- Binding of $O_2$ to one site increases the affinity of the remaining sites (lungs)
- Release of $O_2$ from one site reduces the affinity of the remaining sites (tissues)
Mathematical model (Hill equation)

Assuming that hemoglobin (Hb) bound $n$ molecules of $O_2$ in a single step with infinite cooperativity,

$$Hb + nO_2 \leftrightarrow Hb(O_2)_n$$

$$Y_{O_2} = \frac{(pO_2)^n}{(p50)^n + (pO_2)^n}$$

$n$ (Hill coefficient): Degree of cooperativity

- $n = 1$: noncooperative (myoglobin)
- $n > 1$: positively cooperative
- $n < 1$: negatively cooperative
Hill plot

\[
\log \left( \frac{Y_{O_2}}{1 - Y_{O_2}} \right) = n \log pO_2 - n \log p_{50}
\]

Slope = \( n \)

Intercept = \( p_{50} \)

The last (4th) \( O_2 \) to bind to hemoglobin does so with 100-fold greater affinity than the first
Mechanism of cooperativity
(Perutz Mechanism)

Helix F

Leu F4

Leu F7

Leu FG3

Val FG5

0.6 Å

Heme

Porphyrin

Fe^{2+}

Porphyrin

O_2

R State (oxyHb)

T State (deoxyHb)
Switch between T and R state
Stabilization of T state without Fe-O$_2$ bonds
Hemoglobin is the perfect $O_2$ transporter

- Sigmoidal $O_2$ binding curve
- Decrease in pH reduces the affinity for $O_2$
- $CO_2$ reduces the affinity for $O_2$

Interaction between 4 subunits
Bohr effect

Decrease in pH reduces the affinity for $O_2$

- Protonation of Hb at low pH
- N-amino group ($\alpha$ subunits)
- C-terminal His ($\beta$ subunits)

When protonated these groups are involved in ion-pairs that stabilize the T form (deoxy-Hb)

$$\text{HbH}^+ + O_2 \leftrightarrow \text{HbO}_2 + H^+$$
Stabilization of T state without Fe-O$_2$ bonds
The $O_2$ affinity increase with increasing pH
Hemoglobin and CO$_2$

Lung

Exhale

$\text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{CO}_2 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$

carbonic anhydrase

Blood

$\text{HCO}_3^- + \text{H}^+ \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O}$

$\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$

Carbonic anhydrase

Muscle

Respiration

$\text{MbO}_2 \rightleftharpoons \text{Mb} + \text{O}_2$

$p\text{O}_2 = 20 \text{Torr}$

$p\text{O}_2 = 100 \text{Torr}$

Inhale

$\text{O}_2$

$\text{Hb} \rightleftharpoons \text{HbH}^+ \rightleftharpoons \text{HbH}^+ \rightleftharpoons \text{Hb}$

$\text{HbO}_2 \rightleftharpoons \text{Hb}$

$\text{Mb} \rightleftharpoons \text{MbO}_2$

$\text{O}_2$
CO$_2$ can also bind directly to Hb

N-terminal NH$_2$ groups react with CO$_2$ to form a carbamate

$$R-\text{NH}_2 + \text{CO}_2 \rightleftharpoons R-\text{NH-COO}^- + \text{H}^+$$

- The T form binds more CO$_2$ as carbamate
- The carbamate formation stabilizes the T form (O$_2$ release)
H⁺ production in tissues

- CO₂ + H₂O ⇄ H⁺ + HCO₃⁻
- Lactic acid ⇄ H⁺ + Lactate⁻
$O_2$ → $H^+$ → $CO_2$
Lung

- High pH
- Low pCO$_2$
- High pO$_2$

O$_2$ binding

Tissue

- Low pH
- High pCO$_2$
- Low pO$_2$

O$_2$ release
BPG decreases the affinity for $O_2$

- Synthesized in red blood cells
- Lowers the affinity for $O_2$
- In the absence of BPG, the affinity for $O_2$ would be too high
- Binds to the central cavity of T-state Hb

`d-2,3-Bisphosphoglycerate (BPG)`
Figure 7-14. The effects of BPG and CO$_2$ on hemoglobin's O$_2$ dissociation curve. [After Kilmarlin, J.Y. and Rossii-Bernard, L., Physiol. Rev. 53, 884 (1973).]
BPG plays a role in altitude adaptation and delivery of $O_2$ to the fetus

- At high altitude the $pO_2$ is lower
- Increase in BPG concentration
- Small decrease in $O_2$-binding (lungs)
- Much larger increase in $O_2$ release (tissues)
Allosteric effect

Binding of a molecule to one site affects the activity of the protein at a different site

- Positive effector (modulator)
- Negative effector (modulator)

- $O_2$ binding at one site increases the affinity of the other sites for $O_2$
- $H^+$ decreases the affinity of Hb for $O_2$
- $CO_2$ decreases the affinity of Hb for $O_2$
- DPG decreases the affinity of Hb for $O_2$
Allosteric models

Symmetry model  Sequential model (induced-fit model)
Symmetry model of allosterism
Sequential model of allosterism

1. Substrate
2. Transition from T to T'
3. Reaction from R' to R

Diagram depicts the sequential model of allosterism with substrate binding, transition states, and reaction products.
# Abnormal hemoglobins

## 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)α → Phe</td>
<td>Disrupts hydrogen bonding at the α₁–β₁ interface</td>
</tr>
<tr>
<td>Boston</td>
<td>His E7(58)α → Tyr</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Milwaukee</td>
<td>Val E11(67)β → Glu</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Iwate</td>
<td>His F8(87)α → Tyr</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Yakima</td>
<td>Asp G1(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).*

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*Table 7-1* Fundamentals of Biochemistry, 2/e
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Normal vs Sickled blood cells
HbS Fiber
HbS fiber
Malaria and HbS

The sickle cell gene confers resistance to malaria

Malaria parasite increases the acidity of the infected erythrocytes
→ Promote formation of deoxyHb
→ Promote sickling of the infected erythrocytes
→ Removal of the infected sickle cells by the spleen (early stage)
→ Disruption of the parasite by sicking (late stage)