Protein structure-function relationship: Allostery and cooperativity illustrated by hemoglobin (Hb) and myoglobin (Mb)

Devlin, section 9.4

1. Physiological role of Hb
2. Structure of Hb and comparison with Mb
3. Cooperativity of binding O$_2$
4. Regulation of O$_2$ binding
Hemoglobin: physiological role = a transport protein

- Concentration in erythrocytes = $3 \times 10^8$ molecules/cell. High concentration!
- Function: Transport $O_2$ and $CO_2$ between lung and tissues.
Oxygen transport system

Glucose or Fatty Acids + O$_2$ → CO$_2$ + H$_2$O + HCO$_3^-$ + H$^+$ → Energy

Lung

CO$_2$ + H$_2$O

Tissue Capillaries

Heart
Structure of Hemoglobin

- All-α protein:
  - 7-8 helices labelled A to H
- tetrameric: 2 x αβ dimer
- intersubunit interactions are critical for function
Hemoglobin, a heme protein

- **Prosthetic group**
  - Organic molecule needed for activity
  - Apoprotein = no prosthetic group
  - Holoprotein = + prosthetic grp

- **Heme**
  - Binds Fe
  - $O_2$ coordinates Fe of heme
  - Causes red color
  - Noncovalent association to Hb

*figure 9.21, Devlin*
Tetrameric Hb

\[ \alpha_1 \beta_1 \alpha_2 \beta_2 \]
Comparison with myoglobin (Mb)

- **Structure**: single chain, also with heme.
  - A monomeric “version” of hemoglobin
- **Function**: to store $O_2$ in muscle tissue.

Myoglobin

β chain of Hb
## Comparison of Hemoglobin And Myoglobin

<table>
<thead>
<tr>
<th>Features</th>
<th>Hemoglobin</th>
<th>Myoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Polypeptide</td>
<td>4 (2α, 2β)</td>
<td>1</td>
</tr>
<tr>
<td>No. of Oxygen Bound</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>141 x 2, 146 x 2</td>
<td>153 residues</td>
</tr>
<tr>
<td>Sites for transport</td>
<td>All cells</td>
<td>Skeletal muscle cells</td>
</tr>
<tr>
<td>Transport molecules</td>
<td>O₂, CO₂, NO</td>
<td>O₂</td>
</tr>
</tbody>
</table>
O₂ Binding Curves

Fractional saturation of heme plotted against partial pressure of oxygen, \( pO_2 \).

- Mb binding \( O_2 \): single binding site; hyperbolic saturation curve
- Hb binding \( O_2 \): 4 binding sites; sigmoidal binding curve indicates cooperativity, i.e. binding to one site alters affinity of subsequent binding

\( P_{50} \) indicates \( O_2 \) affinity (see eqn 9.3, Devlin). Why??

\[ Y = \frac{[XO_2]}{[XO_2]+[X]}, \ X = \text{Mb or Hb} \]

50% of heme sites have \( O_2 \) bound
O₂ Binding Curves

Cooperativity for ligand binding

• Binding at one site (i.e. heme for Hb) facilitates binding to the second site.

• Positive cooperativity is an increase in binding affinity for each O₂ bound.

• Hill equation & coefficient: $n_H$
  
  ➔ determined from the slope of a log-log plot
  ➔ measures the degree of cooperativity

\[
\log \left( \frac{Y}{1-Y} \right) = \text{const} + n \log(pO_2)
\]

Fig 9.25, Devlin 7e

n=1 no cooperativity
n>1 positive cooperativity
O₂ Binding Curves

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  - determined from the slope of a log-log plot
  - measures the degree of cooperativity

Slope at 50% saturation is equal to the Hill coefficient
Crystallography shows oxyhemoglobin and deoxyhemoglobin differ in quaternary structure.

- One $\alpha \beta$ dimer is rotated relative to the second dimer.
- Changes interactions at dimer interface between $\alpha_1 \beta_1$ and $\alpha_2 \beta_2$

oxy-Hb is R state; deoxy-Hb is T state
$O_2$ binding changes the quarternary structure of Hb

Allosteric contact region

15 deg
O_2 binding changes the quaternary structure of Hb

Different view from ~90° rotation about an axis in the plane of the slide
O$_2$ binding changes the quaternary structure of Hb

Different view from ~90° rotation about an axis in the plane of the slide
Quaternary Change: how does $O_2$ binding induces it

deyoxy Hb: Fe has only 5 ligands and is out-of-plane

oxy Hb: Fe has only 6 ligands and is in-plane
Quarternary Change
triggered by oxygen binding that allows Fe to move into heme plane

- drags proximal His in helix F and moves helix F
- this alters FG loop (of one chain) - C helix (another chain) interactions
- changes are propagated to neighboring heme and increase affinity
- T state (deoxy) and R state (oxy)

oxy Hb: Fe in plane alters proximal His

Analogous to Fig 9.38, Devlin
Bohr Effect

- Additional regulation of oxygen binding is from the Bohr Effect
  - Increase in [H+] (lower pH)
    - Decreases oxygen affinity, i.e. stabilizes deoxy Hb

\[ \text{Hb}^T + 4\text{O}_2 \rightleftharpoons \text{Hb}^R (\text{O}_2)^4 + n\text{H}^+ \]

Increase in [H+] drives equil to left by law of mass action

Important in metabolizing tissues: CO₂ produced; leads to lower pH; reduces Hb affinity for O₂.
Structural basis of Bohr effect

- In deoxy-Hb, H146 forms salt bridge with D94.
- In oxy-Hb, dimer rotation disrupts this interaction and $H^+$ is released:
  
  $pKa$ in deoxyHb $> pKa$ in oxyHb

\[
Hb^T + 4O_2 \rightleftharpoons Hb^R (O_2)_4 + nH^+ \]

Deoxy-Hb

Figure 9.30, Devlin
Structural basis of Bohr effect

pH changes are coupled to carbon dioxide levels: in erythrocytes, carbonic acid dissociates to bicarbonate and a proton:

\[
\text{CO}_2 + \text{H}_2\text{O} \xleftrightarrow{\text{carbonic anhydrase}} \text{H}_2\text{CO}_3 \xleftrightarrow{\text{spontaneous}} \text{HCO}_3^- + \text{H}^+
\]

CO\textsubscript{2} produced in tissues is converted to HCO\textsubscript{3} which increases \([H^+]\) (i.e. decreases pH) and promotes deoxy form/oxygen release.

HCO\textsubscript{3} is transported in plasma to lung, a process called isohydric transport, which accounts for \(~80\%\) of CO\textsubscript{2} transport to lungs.

Deoxy-Hb
Figure 9.30, Devlin

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Another Hb regulatory mechanism

2,3 bisphosphoglycerate (BPG)

- Allosteric effector: binds at a site different from $O_2$ and modulates Hb function. Check out fig in Devlin
- Effect is to change equilibrium for $O_2$ binding by lowering affinity, i.e. increasing $P_{50}$.
- Binds to one form of Hb (deoxy-HB, not oxy-HB)
- Purpose: regulate binding under oxygen deficiency conditions. (Changes in BPG concentration occur over hours or days.)

$$\text{Hb}^T (\text{BPG}) + 4O_2 \rightleftharpoons \text{Hb}^R (O_2)_4 + \text{BPG}$$

*Hb was one of first targets for allosteric drugs*
Hb Variants

• Over 800 mutant Hb have been characterized
• Most are single amino acid substitutions
  ➔ Surface residues - usually innocuous
  ➔ Internal residues - destabilize the folded structure; carriers suffer from hemolytic anemia
  ➔ Residues in heme binding pocket - eliminate binding of $O_2$
  ➔ Changes in the $\alpha1\beta2$ interface - changes in cooperativity. If oxy form is stabilized then release in tissues is less than normal. T form could also be stabilized.

• HbS, sickle-cell anemia
  ➔ Substitution of $\beta6$ Glu, surface residue, by Val
  ➔ Hb concentration is extremely high in red blood cells, nearly as dense as in a crystal
  ➔ E to V changes the surface and causes inter-deoxyHb binding, which leads to polymerization in RBCs
HbS, sickle-cell anemia

Glu 6 in the beta chain is mutated to valine. This change allows the deoxygenated form of the hemoglobin to stick to each other, as seen in PDB entry 2hbs

Summary of hemoglobin and cooperativity

1. Hb transport function meets the physiological need to bind O$_2$ in lungs but release O$_2$ in tissue through several processes that affect the equilibrium between deoxy Hb$^T$ and oxy Hb$^R$ (O$_2$)$_4$

2. Positive cooperativity of O$_2$ binding derives from conformational changes, which propagate from the heme to certain tetrameric interfaces, and increases O$_2$ affinity

3. O$_2$ binding Hb is linked to CO$_2$ generation in tissues through pH (Bohr effect). This linkage leads to CO$_2$ transport opposite in direction to that of O$_2$.

4. O$_2$ binding affinity depends also on BPG concentration; BPG binds deoxy Hb and thus promotes release of O$_2$. 

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