Dioxygen: Uptake, Transport & Storage: Hemocyanin/Hemerythrin 
Hemoglobin/Myoglobin

References:


外籍王, I. Geis Hemoglobin 1983
D. Voet & J. G. Voet Biochemistry 2nd ed. 1995

Dioxygen: Uptake, Transport & Storage

Despite the huge body of synthetic work, NO model system comes even close to the biological parent!

It’s not only about reversible dioxygen binding!

1. The transport molecule must have a high affinity for O₂ in the presence of plentiful supply at the lungs and a lowered affinity in the O₂—poorer environment of the muscles…where it is needed!
2. The storage molecule must have a higher affinity for dioxygen than the carrier has at low dioxygen concentrations.
3. The carrier should not only bind to O₂, but to carbon dioxide as well to transport CO₂ back to the lungs where it can be ejected as a waste product.
4. The carrier should release its dioxygen more readily to working muscles than to resting tissue.

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The Dioxygen-Carrying Team: Hemoglobin/Myoglobin

Why do we have to bind O₂ in first place? Why not molecular diffusion?
At 100-mm O₂ pressure found in the lungs, 0.3 mL of O₂ can dissolve per 100 mL plasma…far too little to keep you going.
One would have to breath pure O₂ at 3 atm pressure for the solubility to rise to an acceptable 7 mL O₂/100 mL plasma…and that’s just enough to enable you to read this. In contrast, 15g of Hemoglobin (found in 100 mL of blood plasma) are capable of binding 20 mL of gases O₂…and that’s also why giant man-eating killer ants will only exist in Hollywood!

What it needs to do “the job”:
Every mL of blood has approx. 5 billion red blood cells (erythrocytes) …each erythrocyte is packed with 280 million molecules of hemoglobin.

Dickerson & Geis: “If an erythrocyte were enlarged 300 million times, it would be the size, and roughly the shape, of the Rose Bowl, piled with 280 million large grapefruits.”
Myoglobin/Hemoglobin Comparison:

Myoglobin:
- Only 1 heme and 1 polypeptide chain of 153 amino acids.
- MW = 17,800

Hemoglobin:
- 4 heme units and 4 polypeptide chains:
  - 2 α chains with 141 amino acids, and
  - 2 β chains with 146 amino acids.
- MW = 64,500

Size:

The richest source of myoglobin are the muscles of aquatic diving mammals: seals, whales, and porpoises. It is no surprise that the first x-ray crystal structure of myoglobin was obtained from the protein isolated from sperm whale.

A Three-Dimensional Fourier Synthesis at 2 Å Resolution.

...requires an awful lot of imagination!
**Myoglobin: Shapes & Perspectives:**
- an oblate spheroid: $44 \times 44 \times 25 \text{Å}$
- Front view:
- Side view:
  - a box for the heme group,
  - built up from 8 connected,
  - right-handed pieces of $\alpha$ helix.
- helices range in length from 7 (C,D) to 26 (H) residues.
- hydrophilic groups outside.
- hydrophobic groups inside.

**Dioxygen: Uptake, Transport & Storage**

"Little can be said as yet about the relation between structure and function. The haem groups are much too far apart for the combination with oxygen of any one of them to affect the oxygen affinity of its neighbours directly. Whatever interaction between the haem groups exists must be of subtle and indirect kind that we cannot yet guess." M. F. Perutz, *Nature* (1960), 185, 416.

**Hemoglobin: Structures of Deoxy- (left) and Oxyhemoglobin (right)**

2 $\alpha$ and 2 $\beta$ polypeptide chains. Each of the four chains is folded in much the same way as seen for Myoglobin. The $\alpha_1\beta_1$ and $\alpha_2\beta_2$ interactions (35 residues) are hydrophobic and stronger than $\alpha_1\beta_2$ and $\beta_1\beta_2$. They are also stronger than the $\alpha_1\beta_2$ (19 residues).
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Hemoglobin: Symmetry & Perspective:

Nearly 2,2,2 symmetry (pseudo $D_3$) roughly spherical: 64 x 55 x 50 Å

The quaternary structural changes preserve Hb's two-fold symmetry and takes place entirely across the $\alpha_1\beta_2$ and $\alpha_2\beta_1$ interface:

Oxygenation rotates the $\alpha_1\beta_1$ dimer ~ 15° with respect to the $\alpha_2\beta_2$ dimer (page 33), so that some atoms at the $\alpha_1\beta_2$ interface shift by as much as 6 Å relative to each other.

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Myoglobin/Hemoglobin: A comparison

The similarity of their conformations is evident... yet unexpected, because their amino acid sequences are rather different!

Different amino acid sequences can specify very similar 3-D structures.

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Myoglobin/Hemoglobin: A comparison of the amino acid sequences.

In the three chains are identical at only 24 of 141 position.

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Highly conserved amino acid residues in hemoglobins:

<table>
<thead>
<tr>
<th>Position</th>
<th>Amino Acid</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8</td>
<td>Histidine</td>
<td>Proximal heme-linked His</td>
</tr>
<tr>
<td>E7</td>
<td>Histidine</td>
<td>Distal His near the heme</td>
</tr>
<tr>
<td>CD1</td>
<td>Phenylalanine</td>
<td>Home contact</td>
</tr>
<tr>
<td>F4</td>
<td>Leucine</td>
<td>Home contact</td>
</tr>
<tr>
<td>B6</td>
<td>Glycine</td>
<td>Allows close approach of the B and E helices</td>
</tr>
<tr>
<td>C2</td>
<td>Proline</td>
<td>Helix termination</td>
</tr>
<tr>
<td>HC2</td>
<td>Tyrosine</td>
<td>Cross-links the H and F helices</td>
</tr>
<tr>
<td>C4</td>
<td>Threonine</td>
<td>?</td>
</tr>
<tr>
<td>H10</td>
<td>Lysine</td>
<td>?</td>
</tr>
</tbody>
</table>

Additionally, highly non-polar interior and polar character of the exterior of the molecule is conserved.

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The interior consists almost entirely of non-polar residues such as leucine (Leu), valine (Val), phenylalanine (Phe), and methionene.

The only polar residues are two histidines with their critical function at the binding site.
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The Role of the Proximal (F8) & Distal (E7) Histidine:

Discriminate in favor of O₂ and against CO. How?

Linearly coordinated CO binds ~ 25,000 more tightly to Fe than dioxygen does.

A bent Fe—CO geometry weakens the ligand interaction significantly. CO binds only 200x stronger.

The dioxygen ligand binds to the iron-heme with an Fe-O-O angle of ~ 115° – 159°.

The imidazole ring of the distal His E7 (pKₐ = 5.5) acts as a proton trap, thereby protecting the iron from H⁺.

Heme iron oxidation is catalyzed by H⁺ that are reduced by the heme Fe and that in turn reduce O₂ to O₂⁻.

The status of the oxy-form is diamagnetic spin ground state! Solution: Antiferromagnetic spin-spin coupling.

Status Quo

Two strikingly similar proteins, hemoglobin (Hb) & myoglobin (Mb), that immobilize and protect the active site, a heme—iron unit, which reversibly bind dioxygen.

But how does the system address the following important issues:

- Hb binds dioxygen and transports O₂ to the tissue. How does Mb manages to get O₂ transferred from Hb—O₂ + Mb to Mb—O₂ + Hb?
- Active tissue produces CO₂ and H⁺. Who takes care of the waste products?
- Maternal blood needs to transfer O₂ to fetal blood. How do fetal red blood cells manage to receive O₂?

Hemoglobin is a much more intricate and sentient molecule than Myoglobin is!

Hb transports H⁺ and CO₂ in addition to O₂.

O₂ binding properties in Hb are regulated by interactions between separate, non-adjacent sites.

Hemoglobin is an allosteric protein; whereas Myoglobin is not!
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- Dioxygen binds cooperatively to hemoglobin!
  - The binding of O₂ to hemoglobin enhances the binding of additional O₂ to the same hemoglobin (take advantage of high concentrations of O₂ in the lungs; sigmoidal curve). Binding of O₂ to Myoglobin is not cooperative; hyperbolic curve).
- Affinity of hemoglobin for O₂ is pH dependent!
  - H⁺ and CO₂ promote the release of bound dioxygen (for instance in active tissues such as in muscles). Reciprocally, higher concentrations of O₂ promote the release of CO₂ (e.g. in the lungs).
- Dioxygen affinity of the tetrameric hemoglobin is regulated by 2,3-BPG (lowered by the presence of BPG).

H⁺ and CO₂ Promote the Release of O₂: The Bohr Effect (1904)

- Most of the CO₂ is transported as bicarbonate, which is formed within red blood cells by the action of carbonic anhydrase:
  \[
  \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+ 
  \]
- The major portion of the Bohr Effect is due to the fact that increasing \( p(\text{CO}_2) \) causes a decreased red cell pH (acidosis).
- A secondary part of the Bohr Effect is due to the fact that CO₂ reacts covalently with hemoglobin to form carbamino-hemoglobin which has a reduced O₂ affinity.
  \[
  \text{R—NH}_2 + \text{CO}_2 \rightleftharpoons \text{R—NH—COO}^- + \text{H}^+ 
  \]
  - The bound carbamates form salt bridges that stabilize the T-form!
  - (The Tense-form of hemoglobin possesses a lower O₂ affinity).

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Molecular Structure in the Region of the Heme:

<table>
<thead>
<tr>
<th>Controlling ligand binding:</th>
<th>Distances in Å</th>
<th>Dioxyhemoglobin</th>
<th>Oxhemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out-of-plane position of the iron ion is not exclusively due larger ionic radius of the Fe(II). “It takes” less than 0.5 kcal to place the Fe(II) ion in the mean plane of the porphyrin.</td>
<td>Fe—N(8)</td>
<td>0.16(6)</td>
<td>0.16(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(Heme)</td>
<td>0.16(6)</td>
<td>0.16(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(5)</td>
<td>0.12(6)</td>
<td>0.12(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—S</td>
<td>2.1(6)</td>
<td>2.1(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(Fe)</td>
<td>2.0(6)</td>
<td>2.0(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(NH₃)</td>
<td>3.2(6)</td>
<td>3.2(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(H₂O)</td>
<td>3.7(6)</td>
<td>3.7(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(amide)</td>
<td>3.7(6)</td>
<td>3.7(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(H₂O)</td>
<td>3.2(6)</td>
<td>3.2(6)</td>
</tr>
<tr>
<td></td>
<td>Fe—N(H₂O)</td>
<td>3.1(3)</td>
<td>3.1(3)</td>
</tr>
<tr>
<td>Angle between Fe—N(amide)</td>
<td>5(2)</td>
<td>5(2)</td>
<td>3(2)</td>
</tr>
</tbody>
</table>

Dioxygen: Uptake, Transport & Storage

Hb/Mb is a very interrelated and complex system.

Methanol (methyl alcohol) is highly poisonous because it is converted to a toxic product (formaldehyde) in a reaction catalyzed by the enzyme alcohol dehydrogenase. Part of the medical treatment for methanol poisoning is to administer ethanol (ethyl alcohol) in large amounts. WHY???
The Movement of Fe(II) into the Heme Plane triggers the T → R Conformation Shift in the Quaternary Structure:

The binding of BPG to deoxy-Hb as viewed down the molecule’s exact 2-fold axes (central cavity).

In the R-state, the central cavity is too narrow to contain BPG.

Expression of Hemoglobin Genes in Human Development:

- Adult hemoglobin: α₂β₂, Hb A
- Fetal hemoglobin: α₂γ₂, Hb F

Why does BPG bind more weakly to fetal than to adult Hb? (or: why is O₂ transferred from maternal to fetal blood?)

Residue 143 in hemoglobin F is an uncharged serine instead of the positively charged histidine in hemoglobin A.