Lecture 17: Myoglobin & Hemoglobin

Margaret A. Daugherty
Fall 2003

Myoglobin

- Mb
  - Muscle protein;
  - Single polypeptide, 153 residues (18 kd protein);
  - All $\alpha$-helical;
  - Binds heme;
  - Functional role to bind and store $O_2$ in muscle;

Hemoglobin

- Multisubunit, plasma protein
  - $\alpha_2\beta_2$ (adult)
  - other globin subunits found in the fetus and newborns (zeta, epsilon, gamma, etc).
  - $\alpha$ chain = 141 AAs;
  - $\beta$ chain = 146 AAs;
  - Binds 4 hemes;
  - All $\alpha$-helical protein;
- Body contains 750g of Hb & it is replaced every 120 days;
- Hb also transports $H^+$ and $CO_2$ in addition to $O_2$;

Comparison of structures

The "globin" fold

Myoglobin ~ Hb alpha ~ Hb beta

Myoglobin ~ Hb beta

Quaternary structure!
**Myoglobin: hyperbolic curve**

Question: What if Mb was a transport protein, not a storage protein?

![Graph showing hyperbolic curve for myoglobin](image)

**Hemoglobin & Oxygen**

What Sigmoidal (S-shaped) binding curves tells us!!!

![Graph showing sigmoidal curve for hemoglobin](image)

**Hemoglobin Structure:**

Low resolution

2 \[\alpha\] + 2 \[\beta\] → 2 \[\alpha\beta\]

"dimer of dimers"

The "\(\alpha_1\beta_2\)" interface
Denote intersubunit interactions
Alpha1-Alpha2
Alpha1-Beta2
Alpha2-Beta1

**Protein-protein interactions:**

Play a major role in hemoglobin functions

*Homo-interactions* vs. *Hetero-interactions*

Hb as an example of both

Denote intersubunit interactions
Alpha-alpha
Alphabeta2
Alpha1-beta2
Alpha2-beta1

Hetero: \(\alpha\beta\) dimer
Homo: 2 dimers form tetramer

Stretching the truth: \(\alpha = \beta\); so 4 interacting monomers
Hb function: cooperative binding & release of oxygen

noncooperative

\[
\begin{array}{c}
2 \\
\alpha \\
+ \\
\beta \\
2
\end{array}
\]

cooperative

\[
\begin{array}{c}
\alpha_1 \\
\beta_1 \\
\alpha_2 \\
\beta_2
\end{array}
\]

\[\text{Mb, } \alpha, \beta \text{ or } \alpha\beta\]

\[\text{Mb} = \text{Myoglobin}\]

Ligand Binding Curves: Shape

\[\Delta G_{\text{binding}} \times = O_2\]

-5.4 kcal/mol

-5.7 kcal/mol

-6.7 kcal/mol

-9.3 kcal/mol

Oxygen binding site

Heme site: Protoporphyrin IX + Fe^{2+}

Heme is responsible for red color of blood!

Resonance delocalization: All bonds are equivalent in the heme and to the Fe

Heme binds Fe(II); Fe(II) has octahedral coordination => 6 ligands
Hemoglobin structure: Oxygen binding site

**Oxygen-induced heme-site changes are transmitted to the \( \alpha_1\beta_2 \) interface**

Deoxy form (blue): iron is 0.6 Å above center of domed porphyrin ring.

Oxy form (red): iron moves into plane of porphyrin ring & binds more tightly to \( O_2 \); pulls His 8 along with it; this causes an adjustment of Helix F

Result of adjustment of Helix F:
Rearrangement of salt bridges at \( \alpha_1\beta_2 \) interface

**Subunit Interactions Change when Oxygen Binds**

- The deoxy structure is stabilized by a network of salt bridges (hydrogen bond pairs)
- When \( O_2 \) binds:
  - the \( \alpha_1\beta_1 \) and \( \alpha_2\beta_2 \) contacts (30 AAs) change little;
  - the \( \alpha_1\beta_2 \) and \( \alpha_2\beta_1 \) contacts (19 AAs) undergo a large change (several ion pairs broken);

**Changes in the heme upon oxygen binding**

In deoxy state: heme is domed; Fe interacts with \( O_2 \) weakly; His F8 is tilted from the perpendicular by 8°

In oxy state: \( O_2 \) pulls Fe into plane of heme; His F8 becomes perpendicular. Steric strain builds up on Val FG5 which causes structural rearrangement in Hb.
Hb quaternary structural changes

Oxygen binding disrupts salt bridges in the $\alpha_1\beta_2$ interface thus structural rearrangement

Other Heme Ligands

- Other small molecules can also bind to the heme in Mb and Hb
- These include: CO & NO;
- CO has a 250 fold greater affinity for Hb than $O_2$ does

Allosteric regulation of oxygen affinity

Regulation by small ligands binding at sites other (allo-) than the oxygen binding site (-steric)
**The Bohr Effect:** allosteric regulation by $H^+$

Negatively charged BPG binds in positively charged central cavity
Net result = stabilization of deoxy hemoglobin

- Increased concentration in blood at high altitudes
- Lowers affinity of hemoglobin for oxygen - offsets decrease in $O_2$ uptake

---

**Carbon Dioxide Transport**

Bicarbonate reaction:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

Bicarbonate in blood serum reacts with N-terminal groups of Hb

"carbamate"

$$\text{-NH}_3^+ + HCO_3^- \leftrightarrow \text{-N-COO}^- + H_2O + H^+$$

Three effects:
- Transport $CO_2$ to lungs!
- Release of protons contributes to Bohr Effect.
- Stabilizes the deoxy (T) quaternary structure.

---

**Additive Effects of BPG and $CO_2$**
Hb Defects: 1 in 2000 people have a mutation in Hb

2 classes:

Thalassemias: decreased rate of synthesis of one or more peptide chains (different chains expressed at different times during development).

Hemoglobinopathies: alterations in function or stability of the molecule arising from amino acid changes in the chains

Hemoglobin changes during development

Expression of hemoglobins during development

Embryonic:
- Gower I: $\zeta_2\varepsilon_2$
- Gower II: $\alpha_2\varepsilon_2$
- Portland: $\zeta_2\gamma_2$

Fetal:
- Hb F: $\alpha_2\gamma_2$
- $G\gamma = \text{gly } 136$
- $A\gamma = \text{ala } 136$

Adult:
- Hb A: $\alpha_2\beta_2$ ($A_\beta$: 95%)
- Hb A2: $\alpha_2\delta_2$ (< 3.5%)
Fetal hemoglobin has higher affinity for $O_2$ than Hb A

Hb F
\[ \beta H143S \]

Loss of the 2 positive charges that stabilize 2,3- BPG binding.

SUMMARY

Myoglobin has evolved to function as a storage molecule. Hemoglobin has evolved to function as a transport molecule. Their oxygen binding curves reflect the significance of their functions.

The sigmoidicity of the hemoglobins' oxygen binding curve reflects the cooperativity in oxygen binding. The benefit of cooperativity comes in “dumping” off the oxygen to the body, not picking up the oxygen.

Changes in the heme site upon oxygenation are transmitted to the dimer-dimer interface, producing the T --> R quaternary transition.

The deoxy structure is most sensitive to mutations in the dimer-dimer interface. Additionally, it is the most stabilized by the allosteric effectors.

Keywords: “dimer-dimer” interface, cooperativity, hyperbolic binding curve, sigmoidal binding curve, allosteric regulation, allosteric effectors, T and R quaternary structures.