Question of Day: How do the differences in structure between the oxygen transport proteins myoglobin (Mb) and hemoglobin (Hb) make each more best suited for their biological roles?

Introduction

• Both Hemoglobin (Hb) and Myoglobin (Mb) are oxygen-binding proteins.
  • Hb is used in mammals to transport oxygen from the lungs to the tissues.
  • Mb is used in the tissues to store the oxygen, once it gets there.

Introduction

• Together, Hb and Mb provide an excellent example of structure-function relationships in proteins.
  • They illustrate the substrate binding portion of an enzyme catalyzed reaction.
  • They illustrate allosteric regulation.
Introduction

- Hb also provided one of the first examples for the molecular basis of genetic diseases.
  - Sickle-cell anemia.

Genomics

- Identifying cause of genetic mutation, e.g. Sickle cell anemia
  - Due to a 1 base pair change in the gene for the $\beta$ subunit of hemoglobin

Hb-A: $\ldots$ATG GTG CAC CTO ACT CCT GAG GAG AAG TCT GCC GTT ACT...  
Hb-S: $\ldots$ATG GTG CAC CTO ACT GTG GAG GAG AAG TCT GCC GTT ACT...
**Introduction**

- Mb and Hb were also the first proteins to have their 3-dimensional structures determined.

- The crystal structure of Mb was determined by John Kendrew's lab in 1957 using X-ray diffraction.
- This was closely followed by the crystal structure for Hb, which was determined by Max Perutz's in 1958.

**The Medical Research Council (MRC) at Cambridge University**

- Kendrew
- Perutz
- Sanger
- Watson
- Crick

**Nobel Prizes in 1962**
Functions of Hb and Mb

- Hb and Mb provide an excellent example of how proteins have evolved to most efficiently carry out a particular function.

  - Hb binds oxygen in the lung, where the $O_2$ concentration is high, and delivers it to the tissues, where the $O_2$ concentration is low.
  - Mb then accepts the $O_2$ from the Hb in the tissues, where the $O_2$ concentrations are low.

What does this description of the roles of Hb and Mb tell you about the relative $O_2$ binding affinities for Hb and Mb?

- Hb binds oxygen in the lung, where the $O_2$ concentration is high, and delivers it to the tissues, where the $O_2$ concentration is low.
- Mb then accepts the $O_2$ from the Hb in the tissues, where the $O_2$ concentrations are low.

Structures of Hb and Mb

- Structures of Mb and the $\alpha$ and $\beta$ subunits of Hb are very similar.
Structures of Hb and Mb

What structural feature does Hb have that Hb lacks?

Mb
Hb

Structures of Hb and Mb

• Structures of Mb and the \( \alpha \) and \( \beta \) subunits of Hb are very similar

Mb
Hb

Structures of Hb and Mb

• The amino acid sequences for Mb and the \( \alpha \) and \( \beta \) chains of Hb are homologous (Chapter 6.2-6.4)

Structures of Hb and Mb

• The amino acid sequences for Mb is also homologous to the sequence for the plant protein leghemoglobin

25%
23%
Structures of Hb and Mb

- The amino acid sequences for Mb is also homologous to the sequence for the plant protein leghemoglobin.

Leghemoglobin is found in legumes. What metabolic feat are legumes known for?

These three proteins also have very similar 3-dimensional structures. The tertiary structure appear to be more highly conserved than the primary structure.

The amino acid sequences can be used to create an evolutionary tree.
The O$_2$ binding site for Hb & Mb

* Both Hb and Mb contain a heme group.
  * The heme group is an example of a protein cofactor.

Kendrew’s X-ray crystal structure for Mb showed the heme group inserted into a pocket produced in the tertiary fold of the protein.

The heme Fe$^{2+}$ ligated by the heme nitrogens and the nitrogen on the proximal histidine.
The $O_2$ binding site for Hb & Mb

* When bound, $O_2$ provides the sixth ligand for the heme Fe$^{2+}$

* When $O_2$ binds, the heme Fe$^{2+}$ gets smaller and moves into the plane of the heme.

* The heme Fe$^{2+}$ reduces the bound $O_2$ to a superoxide ion, $O_2^-$.

* Superoxide, like other reactive oxygen species (ROS's), is very damaging.

* It is the distal histidine that helps to prevent the release of the superoxide.
The O$_2$ binding site for Hb & Mb

- The heme Fe$^{2+}$ reduces the bound O$_2$ to a superoxide ion, O$_2^-$.
- Superoxide, like other reactive oxygen species (ROS's), is very damaging.
- The distal histidine helps to prevent the release of superoxide.

Hb is a Tetramer

- Hb’s quaternary structure causes it to bind O$_2$ differently than Mb
- Hb is a tetramer of myoglobin-like subunits
  - Two $\alpha$ subunits
  - Two $\beta$ subunits
- Combine as two $\alpha \beta$ dimers
  - $\alpha_1 \beta_1$ and $\alpha_2 \beta_2$
Hb is a Tetramer

Hb is a tetramer of myoglobin-like subunits

- Two \( \alpha \) subunits
- Two \( \beta \) subunits
  - Combine as two \( \alpha \beta \) dimers
  - \( \alpha_1 \beta_1 \) and \( \alpha_2 \beta_2 \)

Hb Binds \( O_2 \) Cooperatively

- Mb has a \( P_{50} \) of 2 Torr

When exposed to air at 1 atm pressure, what fraction of the myoglobin molecule will be bound with \( O_2 \)?

Hb Binds \( O_2 \) Cooperatively

- Hb binds \( O_2 \) more weakly than Mb
**Hb Binds O\(_2\) Cooperatively**

* Cooperative binding makes Hb a more efficient transporter of O\(_2\) than Mb.

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**Hb Binds O\(_2\) Cooperatively**

* Hb is efficiently delivers O\(_2\) to the tissues during stress or exercise.

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**Problem 7.12 & 7.14**

For Wednesday, work Problems 12 and 14 at the end of Chapter 7 and be ready to discuss them in class.

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**Hb Binds O\(_2\) Cooperatively**

* Cooperativity is associated with changes in the quaternary structure of Hb.
Hb Binds O₂ Cooperatively

• Models to explain the cooperativity:
  • MWC Model
    *(Jacques Monod, Jeffries Wyman & Jean-Pierre Changeux)*

![MWC Model Diagram]

Concerted Model

Hb Binds O₂ Cooperatively

• Models to explain the cooperativity:
  • MWC Model
    *(Jacques Monod, Jeffries Wyman & Jean-Pierre Changeux)*

![MWC Model Diagram]

Concerted Model

Hb Binds O₂ Cooperatively

• Models to explain the cooperativity:
  • MWC Model
    *(Jacques Monod, Jeffries Wyman & Jean-Pierre Changeux)*

![MWC Model Diagram]

Concerted Model

Hb Binds O₂ Cooperatively

• Models to explain the cooperativity:
  • Sequential Model

![Sequential Model Diagram]

Sequential Model
Problem 7.12a & 7.14

Problems 12a and 14 at the end of Chapter 7 and be ready to discuss it in class.

7.12a Using the Hill equation, plot an oxygen binding curve for a hypothetical two-subunit hemoglobin with $n = 1.8$ and $P_{50} = 10$ torr.

7.14 Oxygen binding for primitive Hb from a lamprey eel is given

A) Plot data and determine $P_{50}$
B) Make Hill plot and determine $n$
C) Propose model to explain cooperativity

---

Hb Binds O₂ Cooperatively

* Cooperativity can be assessed with a Hill plot.
  * $n$ is the Hill coefficient

\[
\begin{align*}
X + nS & \leftrightarrow X(S) \\
Y &= \frac{[S]^n}{[S]^n + [S_{50}]} \\
Y &= \frac{pO_2}{P_{50}} \\
Y &= \frac{pO_2}{P_{50}} \\
\log\left(\frac{Y}{1-Y}\right) &= n \log(pO_2) - n \log(P_{50})
\end{align*}
\]

---

Hb Binds O₂ Cooperatively

* Cooperativity can be assessed with a Hill plot.
  * $n$ is the Hill coefficient

At the molecular level.

* Conformational changes occurring upon O₂ bonding to one subunit are transmitted to other subunits
**Allosteric Regulation**

- Hb provides an example of **allosteric regulation**.
  
  - In red blood cells (RBCs), the metabolite 2,3-Bisphosphoglycerate (2,3-BPG) alters the $O_2$ binding behavior of Hb.

![2,3-Bisphosphoglycerate (2,3-BPG)](image)

**2,3-BPG lowers Hb’s affinity for $O_2$, allowing it to release $O_2$ more efficiently to the tissues.**

**Cooperativity** is associated with changes in the quaternary structure of Hb.

![Tense (T) State and Relaxed (R) State](image)

**2,3-BPG binds to, and stabilizes, the T-state of Hb.**

**Allosteric Regulation**

- 2,3-BPG lowers Hb’s affinity for $O_2$, allowing it to release $O_2$ more efficiently to the tissues.
Allosteric Regulation

- The acclimation to the higher elevations involves the production of higher levels of 2-BPG.
- Fetal Hb
  - γ chains are substituted for β chains (H143S)

To Summarize

- Question of Day: How do the differences in structure between the oxygen transport proteins myoglobin (Mb) and hemoglobin (Hb) make each more best suited for their biological roles?
Next up

• Hemoglobin and Myoglobin (con’d).
  • Bohr effect
  • Sickle-cell Hb

• Enzymes (Chapter 8)