Chem 452 - Lecture 3
Hemoglobin & Myoglobin
Part 1

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

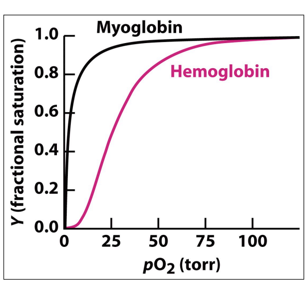
- Both Hemoglobin (Hb) and Myoglobin (Hb) are oxygen-binding proteins.
  - Hb is used in mammals to transport oxygen from the the lungs to the tissues.
  - Mb is used in the tissues to store the oxygen, once it gets there.

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 and Mb bind oxygen differently.



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 β subunit of hemoglobin

Hb-A:	...ATG GTG CAC CTG ACT CCT **GAG** GAG AAG TCT GCC GTT ACT...
Hb-S:	... ATG GTG CAC CTG ACT CCT **GTG** GAG AAG TCT GCC GTT ACT...

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	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

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	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

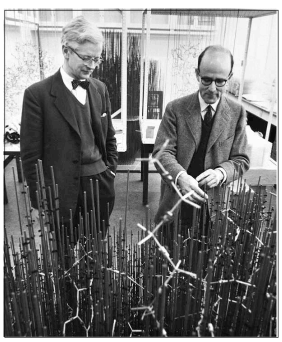
Introduction

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

Introduction

- 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.

Introduction



John Kendrew Max Perutz

The Medical Research Council (MRC) at Cambridge University

- Kendrew
- Perutz
- Sanger
- Watson
- Crick

Nobel Prizes in 1962

Introduction

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.

Functions of Hb and Mb

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

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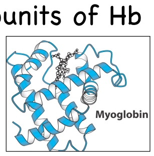
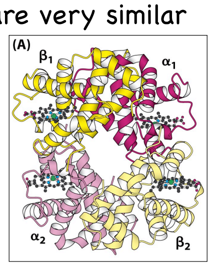
Structures of Hb and Mb

- Structures of Mb and the α and β subunits of Hb are very similar

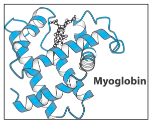


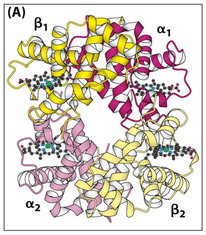


Mb Hb

Structures of Hb and Mb

What structural feature does Hb have that Hb lacks?





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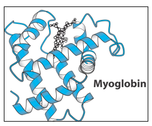
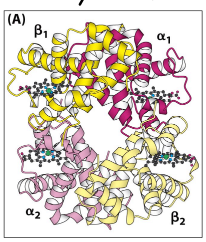
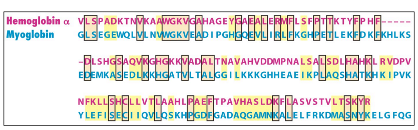




Mb Hb

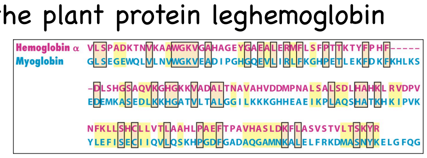
Structures of Hb and Mb

- The amino acid sequences for Mb and the α and β 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



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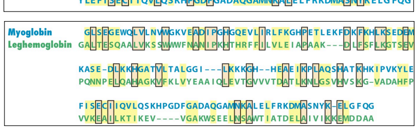

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Structures of Hb and Mb

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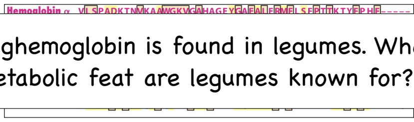

Leghemoglobin is found in legumes. What metabolic feat are legumes known for?

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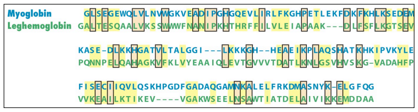

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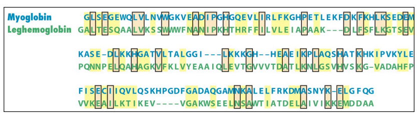

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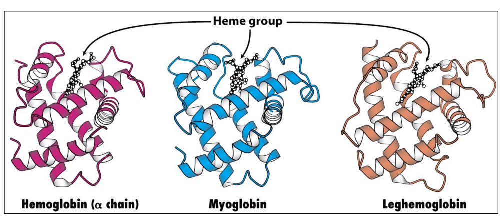
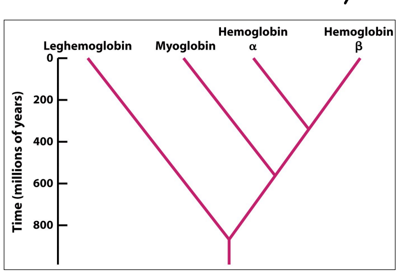
Structures of Hb and Mb

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



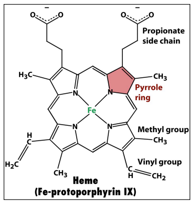
Structures of Hb and Mb

- 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**.



The O_2 binding site for Hb & Mb

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

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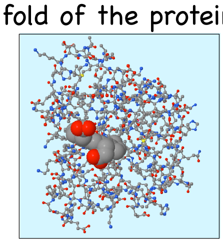
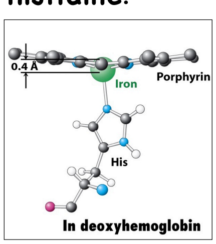


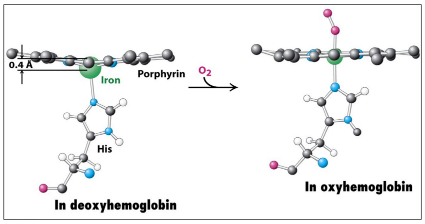
The O_2 binding site for Hb & Mb

- The heme Fe^{2+} ligated by the heme nitrogens and the nitrogen on the **proximal histidine**.



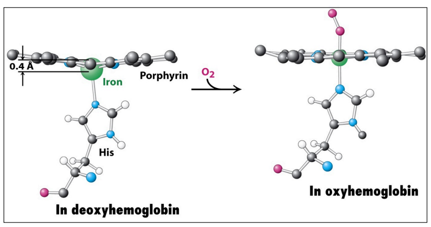
The O_2 binding site for Hb & Mb

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



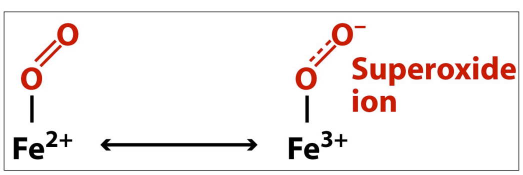
The O_2 binding site for Hb & Mb

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



The O_2 binding site for Hb & Mb

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

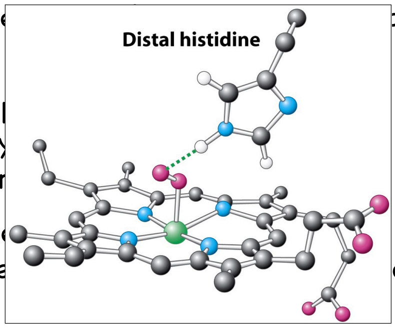


The O_2 binding site for Hb & Mb

- 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.
- It is the **distal histidine** that helps to prevent the release of the superoxide.

The O_2 binding site for Hb & Mb

- The ... d O_2 to
- Su... oxy... da...
- The ... pre... e.



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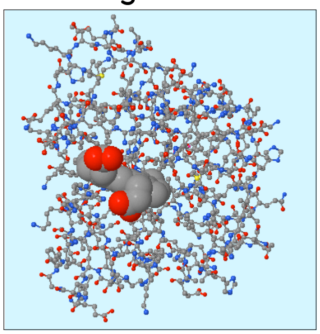
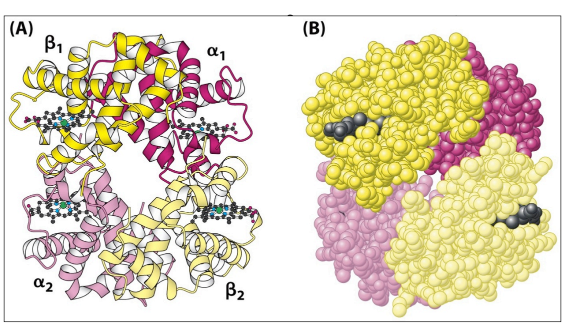


Oxymyoglobin

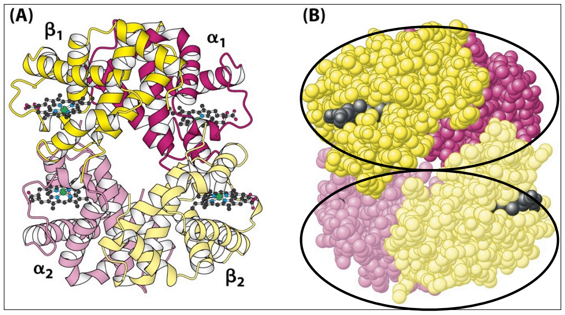
Hb is a Tetramer

- Hb's quaternary structure causes it to bind O_2 differently than Mb
- Hb is a tetramer of myoglobin-like subunits
  - Two α subunits
  - Two β subunits
- Combine as two $\alpha\beta$ dimers
  - $\alpha_1\beta_1$ and $\alpha_2\beta_2$

Hb is a Tetramer

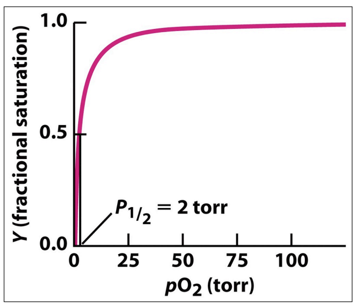


Hb is a Tetramer



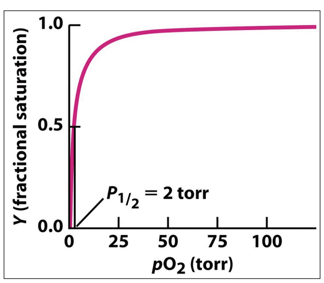
Hb Binds O_2 Cooperatively

- Mb has a P_{50} of 2 Torr



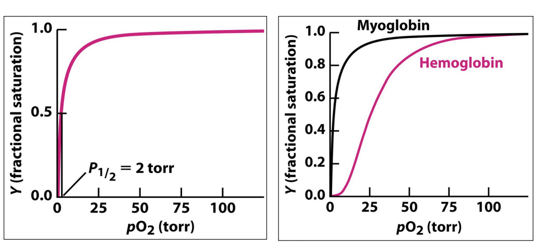
Questions

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



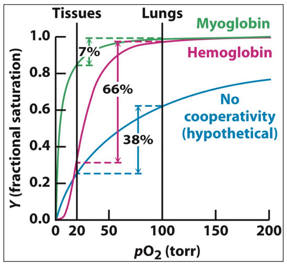
Hb Binds O_2 Cooperatively

- Hb binds O_2 more weakly than Mb



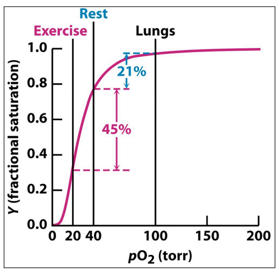
Hb Binds O_2 Cooperatively

- Cooperative binding makes Hb a more efficient transporter of O_2 than Mb.



Hb Binds O_2 Cooperatively

- Hb is efficiently delivers O_2 to the tissues during stress or exercise.

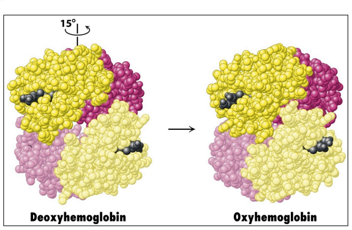


Problem 7.12 & 7.14

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

Hb Binds O_2 Cooperatively

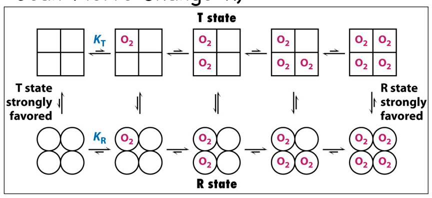
- Cooperativity is associated with changes in the quaternary structure of Hb



Tense (T) State Relaxed (R) State

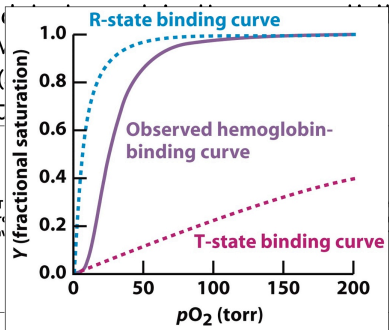
Hb Binds O_2 Cooperatively

- Models to explain the cooperativity:
  - MWC Model
    - (Jacques **M**onod, Jeffries **W**yman & Jean-Pierre **C**hangeux)



Concerted Model

Hb Binds O_2 Cooperatively



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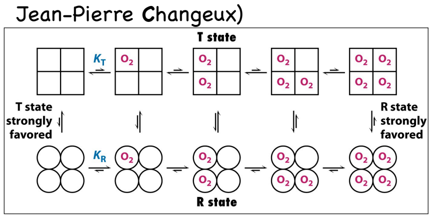
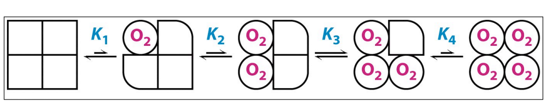


Concerted Model

Hb Binds O_2 Cooperatively

- Models to explain the cooperativity:
    - Sequential Model



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.

7.12.a 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

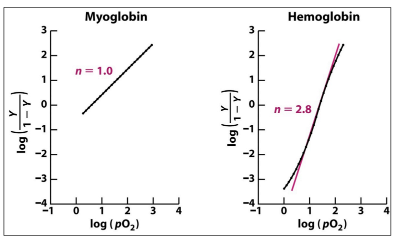
A) Plot data and determine P50

B) Make Hill plot and determine n

C) Propose model to explain cooperativity

Hb Binds O_2 Cooperatively

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



Hb Binds O_2 Cooperatively

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

$$X + nS \Leftrightarrow X(S)_n$$

$$Y = \frac{[S]^n}{[S]^n + [S_{50}]^n}$$

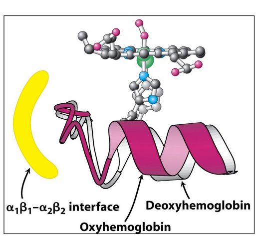
$$Y = \frac{pO_2^n}{pO_2 + P_{50}^n}$$

$$\frac{Y}{1-Y} = \frac{pO_2^n}{P_{50}^n}$$

$$\log\left(\frac{Y}{1-Y}\right) = n\log(pO_2) - n\log(P_{50})$$

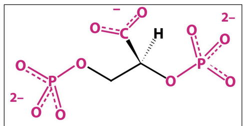
Hb Binds O_2 Cooperatively

- At the molecular level.
  - Conformational changes occurring upon O_2 bonding to one subunit are transmitted to other subunits



Allosteric Regulation

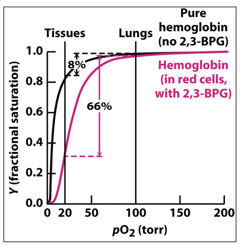
- Hb provides and example of **allosteric regulation**.
  - In red blood cells (RBC's), the metabolite 2,3-Bisphosphoglycerate (2,3-BPG) alters the O_2 binding behavior of Hb.



2,3-Bisphosphoglycerate (2,3-BPG)

Allosteric Regulation

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



Hb Binds O_2 Cooperatively

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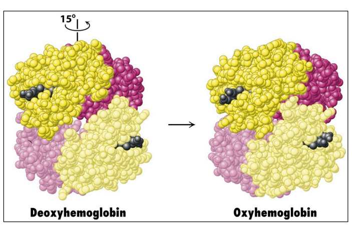
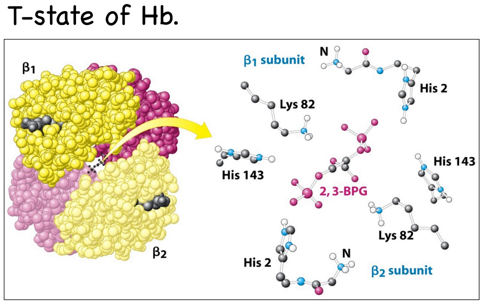


Tense (T) State Relaxed (R) State

Allosteric Regulation

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

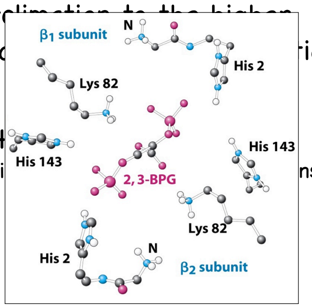


Allosteric Regulation

- 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)

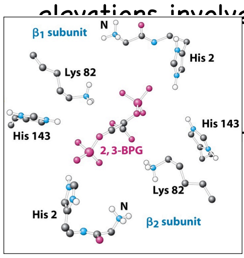
Allosteric Regulation

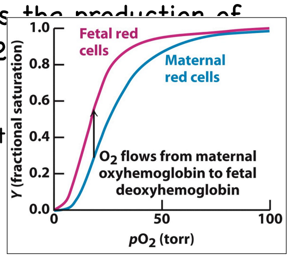
- The ac
  elevati ion of
  higher
- Fetal H
  - γ chai ns (H143S)



Allosteric Regulation

- The acclimation to the higher





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)