Chem 352 - Lecture 5, Part III
Proteins: Function and Evolution

Question of the Day: *"How to the differences in the O_2-binding curves for Hb and Mb make each best suited for delivering the O_2 needed for respiration from he lungs to the tissues?"*

1

Outline

Myoglobin and Hemoglobin

7.8 Oxygen Transport from Lungs to Tissues: Protein Conformational Change Enhances Function

7.9 The Oxygen-Binding Sites in Myoglobin and Hemoglobin

7.10 The Role of Conformational Change in Oxygen Transport

7.11 Allosteric Effectors of Hemoglobin Promote Efficient Oxygen Delivery to Tissues

2

7.8 Oxygen Transport from Lungs to Tissues: Protein Conformational Change Enhances Function

3

Oxygen Transport and Storage

Aerobic organisms, such as ourselves, derive most of the energy they require by carrying out the complete oxidation of the foods they eat.

- For example, we will later see that the complete oxidation of the monosaccharide glucose ($C_6H_{12}O_6$) is accomplished by combining three metabolic pathways, including glycolysis, the citric acid cycle, and the electron transport chain.

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O + \text{energy}$$

- The energy released from this "reaction" is then coupled to the phosphorylation of ADP to make ATP

$$ADP + P_i + \text{energy} \longrightarrow ATP + H_2O$$

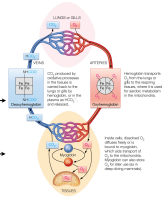
- For this to work, however, the tissues need an abundant supply of molecular oxygen.

4

Oxygen Transport and Storage

Hemoglobin (Hb) and Myoglobin (Mb) and are the two proteins that have evolved to transport molecular O_2 from the lungs to the tissues (Hb), and then to store it there until needed (Mb).

- Hb is a tetrameric heme protein that transports O_2 from lungs or gills to peripheral tissues and returns CO_2 to the gills or lungs for exhalation
- Myoglobin (Mb): a monomeric heme protein that binds and releases O_2 in tissues.



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Oxygen Transport and Storage

Mb comprises about 2 mg/g of human muscle tissue, and is used for efficient delivery of O_2 to the mitochondria, the cellular organelle where O_2 is reduced to H_2O by the electron transport chain during cellular respiration.

- The Mb proteinis what gives muscles their red color.
- Deep-diving mammals, such as whales, have 10-30 times more Mb per gram of muscle tissue than humans, and it is this capacity for storing oxygen that permits them to go for long periods underwater between breaths.

6

Oxygen Transport and Storage

Hb, on the other hand, is is found in the blood of humans within cells called erythrocytes (red blood cells).

- The protein Hb is what gives blood and erythrocytes their red colors.
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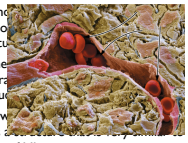

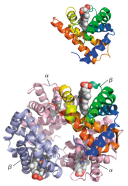

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7.9 The Oxygen-Binding Sites in Myoglobin and Hemoglobin

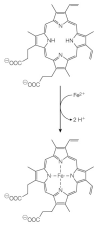
8

Oxygen Binding Sites in Mb and Hb

The oxygen binding site in both Mb and Hb provide an example of a protein ***prosthetic group***.

- A prosthetic group is a non-peptide component of some proteins, which usually facilitate the protein's ability carry out its function.
- The prosthetic group in both Mb and Hb is called a heme group and comprises two components
  - an Fe^{2+} ion
  - and a porphyrin ring
- A porphyrin ring is a conjugated tetrapyrrole ring system
  - The specific one used in the heme group is the protoporphyrin IX

Mb or Hb without heme is called an ***apoprotein***, while with heme it is called a ***holoprotein***.



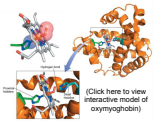
9

Oxygen Binding Sites in Mb and Hb

Fe^{2+} has six, octahedrally arranged, coordination sites

- four of these are provided by the nitrogens in the porphyrin ring
- another is provided by the bound O_2
- and the sixth is provided by a histidine side chain from the protein.
  - This histidine is called the ***proximal histidine*** residue (green)

There is also a second histidine that is called the ***distal histidine*** (cyan), which stabilizes the bound O_2 through H-bonding.



(Click here to view interactive model of oxymyoglobin)

10

Oxygen Binding Sites in Mb and Hb

When oxygen is bound, these proteins are called

- Oxymyoglobin
- Oxyhemoglobin

And when they are oxygen-free, the proteins are called

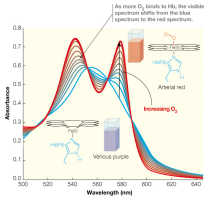
- Deoxymyoglobin
- Deoxyhemoglobin

11

Oxygen Binding Sites in Mb and Hb

The binding of O_2 to Hb and Mb can be monitored by observing changes in visible light absorption.

- Oxyhemoglobin that is present in the arteries is a bright red color,
- Whereas, deoxyhemoglobin that is present in the veins has a darker, purple color.



12

A COVID-19 Connection

Patients with severe COVID-19 infections are hospitalized because they have difficulty breathing.

- A consequence is they are unable to deliver an adequate supply of O_2 to their tissues.
- If not reversed, this can lead to massive organ failure and death.

13

A COVID-19 Connection

The extent to which a patient's Hb is saturated with O_2 can be monitored with a device called a ***pulse oximeter.***

- The device can be clipped onto one of the patient's fingers to determines what percentage of the patient's Hb is saturated with O_2.
- If the patient's O_2-saturation level falls below about 80%, concern arises that the patient will need to be put on a ventilator to force sufficient O_2 into their lungs.

click on the image

14

A COVID-19 Connection

A pulse oximeter works by monitoring light absorbance at two different wavelengths

- 660 nm, which is at the red end of the visible spectrum
- 940 nm, which is in the near IR range.

Because Hb and HbO_2 have different absorptions spectra, the relative absorption at these two wavelengths can be used to calculate the percent saturation.

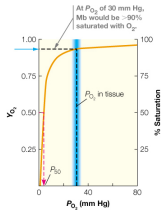
click on the image

15

The Oxygen Binding Curve

We will first look at the oxygen binding curve for myoglobin, because it is simpler to interpret and understand.

- In a binding curve, the fraction of myoglobin molecules bound with oxygen, Y_{O_2}, is plotted as a function of the O_2 concentration.
- When working with gases that are dissolved in a solution, it is easier to measure the partial pressure of the gas above that solution, P_{O_2}, than to measure the concentration of the gas in a solution, $[O_2]$, directly.
  - While the P_{O_2} is not equal to $[O_2]$, it is directly proportional to it, and so it provides the same binding information.



16

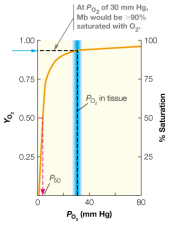
The Oxygen Binding Curve

This kind of curve, which rises quickly and then plateaus, is mathematically described as a ***binding isotherm*** or ***hyperbolic-binding curve***.

- The equation that describes this curve is,

$$Y_{O_2} = \frac{P_{O_2}}{P_{50} + P_{O_2}}$$

- Where P_{50} is a constant and represents the P_{O_2} required to bind O_2 to 50% of the Mb molecules.



17

The Oxygen Binding Curve

We can derive this equation by considering what goes on in the binding process.

- The dissociation of O_2 from Mb can be described by the following reaction equation, where k_{off} is the dissociation rate constant.

$$MbO_2 \xrightarrow{k_{off}} Mb + O_2$$

- This reaction is reversible, so the association reaction can be described by the reverse reaction equation

$$MbO_2 \xleftarrow{k_{on}} Mb + O_2$$

- When the two rates become equal an equilibrium is reached,

$$MbO_2 \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} Mb + O_2$$

18

The Oxygen Binding Curve

This equilibrium can be characterized by the equilibrium constant, K_D,

$$K_D = \frac{[Mb][O_2]}{[MbO_2]} \text{ at equilibrium}$$

- The subscript D for K_D indicates this is the equilibrium constant for a dissociation reaction.

- To relate this to the fraction bound, Y_{O_2}, we can describe the fraction bound as

$$Y_{O_2} = \frac{\text{sites occupied}}{\text{total sites available}}$$

- Since each Mb molecule has only one binding site we can let "sites occupied" = $[MbO_2]$, and the "total sites available" = $[Mb] + [MbO_2]$.

$$Y_{O_2} = \frac{[Mb]}{[Mb] + [MbO_2]}$$

19

The Oxygen Binding Curve

- By combining these two equations,

$$K_D = \frac{[Mb][O_2]}{[MbO_2]} \text{ and } Y_{O_2} = \frac{[Mb]}{[Mb] + [MbO_2]}$$

  - we can derive the following equation (see p.204 in your text for details).

$$Y_{O_2} = \frac{[O_2]}{K_D + [O_2]}$$

- If you set $Y_{O_2} = \frac{1}{2}$, you can show that K_D is equal to the O_2 concentration needed to saturate half of the available binding sites, $K_D = [O_2]_{50\%}$

- As discussed earlier, $[O_2]$ is proportional to P_{O_2}, so

$$Y_{O_2} = \frac{P_{O_2}}{P_{50} + P_{O_2}}$$

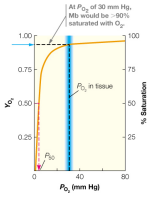
20

The Oxygen Binding Curve

We now have an equation to describe the oxygen binding curve for Mb,

$$Y_{O_2} = \frac{P_{O_2}}{P_{50} + P_{O_2}}$$

- A value for P_{50} can be determined by setting it equal to the P_{O_2} required to saturate half of the available sites.
  - Here that looks to be about 4 mmHg
- The partial pressure of O_2 in tissues fed by arterial capillaries is around 30 mmHg.
  - The binding curve shows that at this pressure, the Mb should be > 90% saturated with O_2.
- In cells that are actively metabolizing foods, the P_{O_2} will fall to 3-18 mmHg.
  - At these low pressures, Mb will release its load of O_2.



21

The Oxygen Binding Curve

Since P_{50} is proportional to the dissociation constant, K_D, it can be used to assess the relative binding affinity of Mb for O_2.

- The higher the P_{50}, the weaker the binding.
- This should make intuitive sense since the higher the P_{50}, the greater the P_{O_2} needs to be in order to saturate half of the Mb molecules.

22

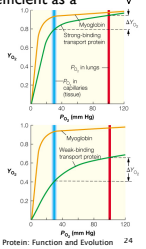
7.10 The Role of Conformational Change in Oxygen Transport

23

Binding and Unloading of Oxygen

The hyperbolic binding curve for Mb makes it efficient at binding O_2 at low P_{O_2}, but not very efficient as a transporter of O_2 to the tissues.

- This is because the P_{O_2} needs to go quite low to have the O_2 released from MbO_2
- Using a Mb-like transport protein with a higher P_{50} would help, but still would not be that efficient.
- Hb, with it four O_2 binding sites, provides for a more efficient option

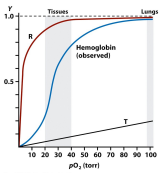


24

Binding and Unloading of Oxygen

Because Hb has four subunits, each with it own O_2 binding site, the four binding sites are able to communicate with one another.

- The four O_2 binding sites have two states
  - A low affinity state ***T-state*** (tense)
  - And a high affinity ***R-state*** (relaxed)
- At low O_2 concentrations the T-state is favored and at high O_2 concentrations the R-state is favored.
- This switching from T favored to R favored produces a ***sigmoidal*** (S-shaped) O_2 binding curve.



25

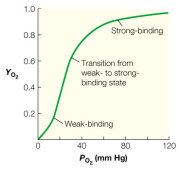
Binding and Unloading of Oxygen

When binding of a ligand at one site on a protein affects the affinity for the binding of a ligand at a second site, it is called an ***allosteric effect***.

- Later we will see how this is used to regulate enzyme activity.

When this leads to increased affinity for subsequent binding it is called ***cooperative binding***.

- A sigmoidal binding curve is a characteristic of cooperative binding



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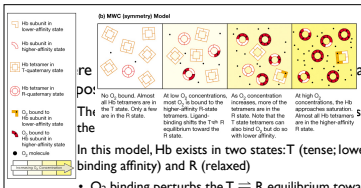

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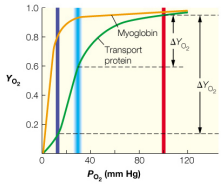
In this model, Hb exists in two states: T (tense; lower O_2 binding affinity) and R (relaxed)

- O_2 binding perturbs the T ⇌ R equilibrium toward the R state
- O_2 release favors the T state
- Mixed tetramers are not allowed in this model

27

Binding and Unloading of Oxygen

With its cooperative binding curve, Hb better is able to offload a greater percentage of its O_2 cargo out in the tissues to the awaiting Mb molecules.



28

Binding and Unloading of Oxygen

The sigmoidal Hb O_2 binding curve is described by the Hill equation.

$$Y_{O_2} = \frac{P_{O_2}^h}{P_{50}^h + P_{O_2}^h}$$

- where *h* is the Hill coefficient

Because Hb has 4 O_2 binding sites, *h* can range from 1 to 4.

- When n=1, there is no cooperative binding and the equation reduces to a hyperbolic equation that is equivalent to that for Mb.
- for $1 < h \leq 4$ the binding becomes increasingly cooperative.
- The observed value of *h* for Hb is around 2.8.

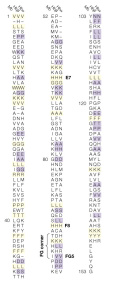
29

Binding and Unloading of Oxygen

While Hb contains four subunits, they are not all identical.

- There are α subunits and two β subunit, each with different primary structures.
- Even though their amino acid sequences are different, their tertiary structures are quite similar
- They also share a very similar tertiary structure to Mb.

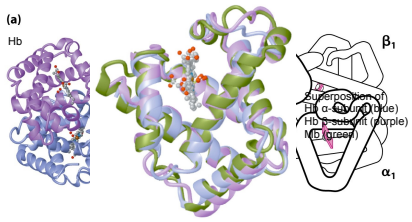
Hb is described as having an $\alpha_2\beta_2$ quaternary structure.



30

Myoglobin *vs* Hemoglobin Structures

Hb and Mb share similar I°, II° and III° structures

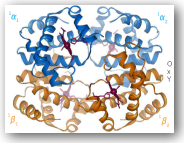


31

Structural Changes in Hemoglobin and Cooperative Binding

Max Perutz was the first to solve a 3-dimensional structure for Hb in 1960 using X-ray crystallography.

- He, and others, followed that by solving higher resolution structures for both oxyhemoglobin and deoxyhemoglobin
- Comparing these structures has revealed conformational changes that accompany the transition from the T to the R state.
- The α and β subunits pair up as two rigid αβ dimers, which move relative to one another in the transition

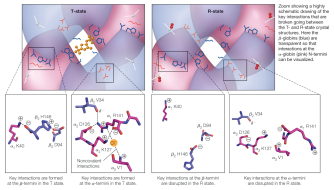


32

Structural Changes in Hemoglobin and Cooperative Binding

When the T → R occurs, a group of non-covalent interactions between the two dimers are disrupted

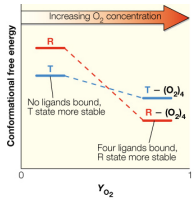
- These are primary charge-charge interactions.



33

Structural Changes in Hemoglobin and Cooperative Binding

Fe-O_2 bonds are strong and stabilize the R state, even though it has fewer inter-subunit interactions than in the T state



34

Structural Changes in Hemoglobin and Cooperative Binding

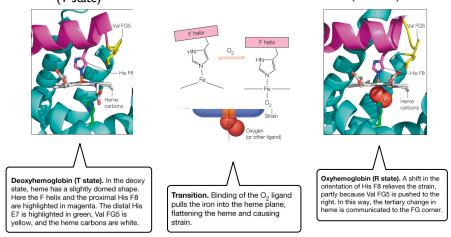
- When O_2 binds, it pulls the Fe^{2+} ion into the plane of the heme, causing steric strain between the flattened heme and the proximal His (F8) and Val FG5
- Val FG5 is at the corner between F and G helices. This strain is relieved by a change in the orientations of both His F8 and Val FG5
- These movements are what lead to the disruptions charge-charge interactions between the subunits and triggering the transition from the T to R state.

35

Structural Changes in Hemoglobin and Cooperative Binding

Deoxyhemoglobin (T state)

Oxyhemoglobin (R state)



Deoxyhemoglobin (T state). In the deoxy state, heme has a slightly domed shape. Here the F helix and the proximal His F8 are highlighted in magenta. The distal His E7 is highlighted in green, Val FG5 is yellow, and the heme carbons are white.

Transition. Binding of the O_2 ligand pulls the iron into the heme plane, flattening the heme and causing strain.

Oxyhemoglobin (R state). A shift in the orientation of His F8 relieves the strain, partly because Val FG5 is pushed to the right. In this way, the tertiary change in heme is communicated to the FG corner.

36

7.11 Allosteric Effectors of Hemoglobin Promote Efficient Oxygen Delivery to Tissues

37

Binding of Allosteric Effectors

Allosteric effectors are ligands that bind to a target protein and promote a conformational change that modulates the functional properties of these proteins

- We will see this with Hb, where the binding of certain substances can lead to either in increase or decrease in O_2 binding affinity.
- We will later see this later with enzymes, where their catalytic activities can be modulated in response to the binding of ligands.

Homotropic allosteric effectors bind at the active site, whereas ***heterotropic effectors*** bind at other sites on the protein

38

Binding of Allosteric Effectors

- O_2 is a ***positive homotropic effector*** for hemoglobin
  - Its binding to one heme increases the binding affinity of O_2 to other hemes in the tetramer
- H^+, CO_2, and 2,3-bisphosphoglycerate (2,3-BPG) are ***negative heterotropic effectors*** for hemoglobin
  - binding of one or more of these effectors decreases the binding affinity of O_2 to hemoglobin

Lungs:

Binding of O_2 favors R state; releases BPG, CO_2, and H^+

$4O_2$ + Hb-BPG-CO_2-H^+ ⇌ Hb-$(O_2)_4$ + BPG + CO_2 + H^+

Capillaries:

Binding of BPG, CO_2, and H^+ favors T state; releases O_2

BPG + CO_2 + H^+ + Hb-$(O_2)_4$ ⇌ Hb-BPG-CO_2-H^+ + $4O_2$

Respiring cells:

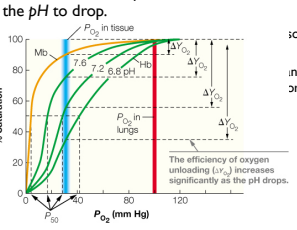
O_2 + Mb ⇌ Mb-O_2 ⇌ Mitochondrion(O_2) + Mb (CO_2)

39

Binding of Allosteric Effectors

When tissues are actively catabolizing food molecules, they produce acids as end-products, which in turn causes the *pH* to drop.

- H^+ mo…so
- Th… wh… sal… …ind …orms a
- Th…

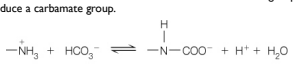


40

Binding of Allosteric Effectors

Another end-produce of catabolism is CO_2

- Most of this CO_2 is dissolved in the plasma and transported back to the lungs to be exhaled.
  - dissolved CO_2 reacts with H_2O to form carbonic acid, which dissociates to produce bicarbonate ions
- About 5–13% of the CO_2 reacts with the N-terminal amino groups to produce a carbamate group.



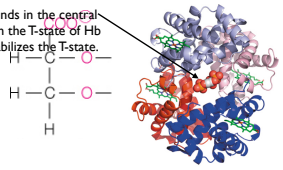
- This changes a positively charged amino group into a negatively charged carbamate group and leads to charge-charge interactions that favor the T-state.
- The reaction is reversible, so when the Hb reaches the lungs, it releases its cargo of CO_2

41

Binding of Allosteric Effectors

2,3-Bisphosphoglycerate (2,3-BPG) is a metabolite that allows organisms to adapt to environments with lower O_2 pressure

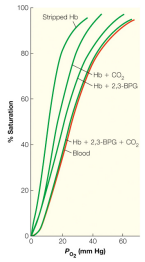
- For example, when individuals spend extended periods at high altitudes
- The 2,3-BPG binds in the central cavity formed in the T-state of Hb and thereby stabilizes the T-state.



42

Binding of Allosteric Effectors

The binding of H^+, CO_2, and 2,3-BPG are additive, allowing these heterotrophic negative effectors to work independently of one another in modulating the affinity of Hb for O_2.



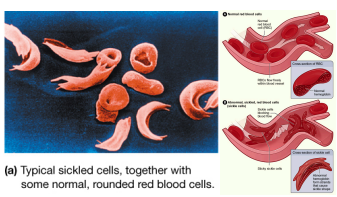
43

7.14 Hemoglobin Variants and Their Inheritance: Genetic Diseases

44

Sickle-Cell Disease

Abnormal (sickle-celled) erythrocytes block circulation in capillaries and lyse due to their fragility, causing anemia.



(a) Typical sickled cells, together with some normal, rounded red blood cells.

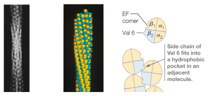
45

46

Sickle-Cell Disease

The sickling is due the the polymerization or assembly of Hb molecules into long, rod-like fibers.

- The sickle cell trait arises from a mutant Hb molecule (Hb-S), in which a glutamic acid (Glu) residue at position 6 on the β-subunit has been substituted with a valine (Val) residue



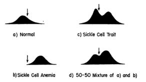
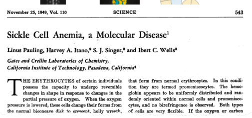


FIG. 3. Longsworth scanning diagrams of carbonmonoxyhemoglobins in phosphate buffer of 0.1 ionic strength and pH 6.90 taken after 20 hours' electrophoresis at a potential gradient of 4.73 volts/cm.



November 25, 1949, Vol. 110 SCIENCE 543

Sickle Cell Anemia, a Molecular Disease[1]

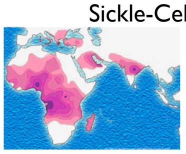
Linus Pauling, Harvey A. Itano,[2] S. J. Singer,[2] and Ibert C. Wells[3]

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California[4]

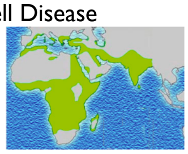
THE ERYTHROCYTES of certain individuals possess the capacity to undergo reversible changes in shape in response to changes in the partial pressure of oxygen. When the oxygen pressure is lowered, these cells change their forms from the normal biconcave disk to crescent, holly wreath, that form from normal erythrocytes. In this condition they are termed promeniscocytes. The hemoglobin appears to be uniformly distributed and randomly oriented within normal cells and promeniscocytes, and no birefringence is observed. Both types of cells are very flexible. If the oxygen or carbon

47

Sickle-Cell Disease



Incidence of sickle cell anaemia



Historic distribution of malaria

- This may explain why the sickle cell trait is so prevalent in populations living in Sub-Saharan Africa.

48

Summary

- Hemoglobin and myoglobin are heme-containing oxygen-binding proteins that are used by vertebrates for oxygen transport and storage
- Hemoglobin undergoes a transition between the T (tense) and R (relaxed) state, which is influenced by various allosteric effectors such O_2 (positive homotropic), H^+, CO_2, and 2,3-bisphosphoglycerate (negative heterotropic)