Lecture : 7

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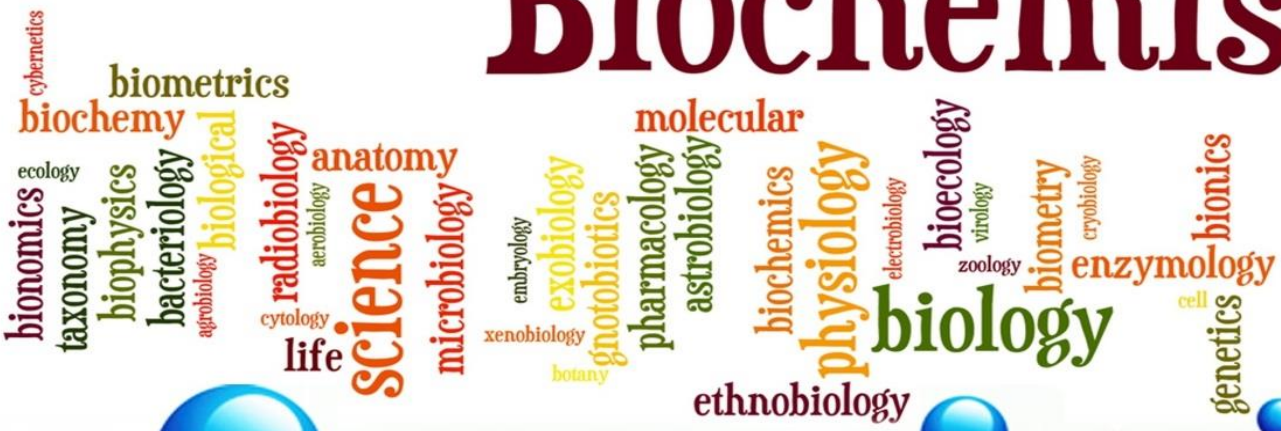
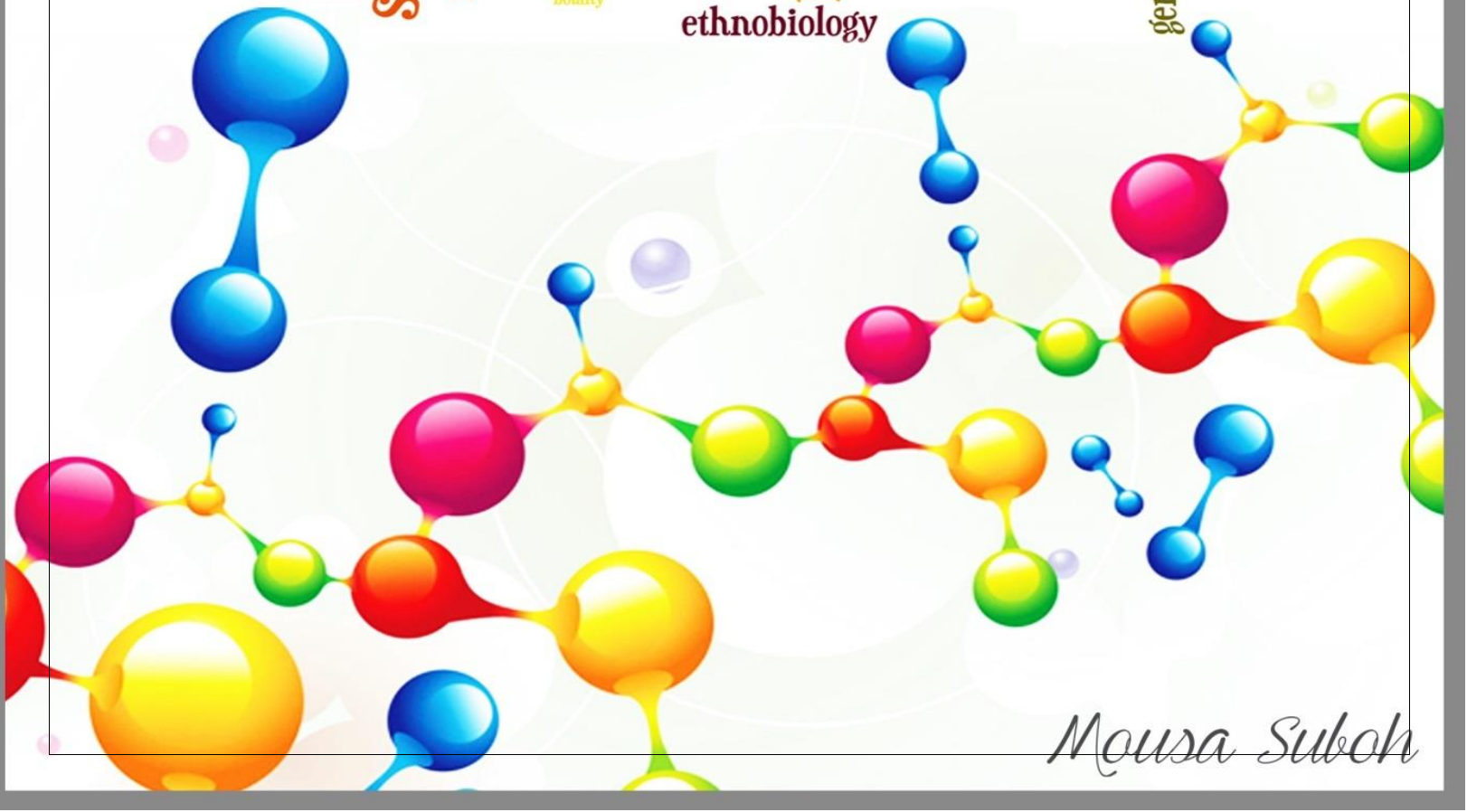
Medical Committee
The University of Jordan

Biochemistry





Mousa Suboh

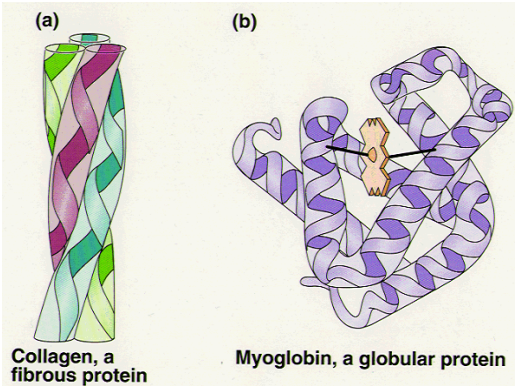
Globular Proteins

Recap:

- There are two types of proteins according to their structure:

  a. Fibrous proteins.

  b. Globular proteins: are globe-like (spherical) with three-dimensional compact structures (refer to the figure on the right).
  Examples: Myoglobin ,Hemoglobin and immunoglobulin **. The first two examples will be discussed thoroughly in this lecture.**



I. Functions of myoglobin and hemoglobin:

- **Myoglobin:** mainly present in muscles (that's why it's named "myo" , which indicates muscles). Its main function is to store oxygen to be released in cases of oxygen deprivation (in cases of emergency) , when the pressure of oxygen in tissues is very low (hypoxia) , because O_2 is very impotant to the function of muscles so muscles must have O_2 backup. Therefore, myoglobin binds oxygen at very high affinity (i.e.: it releases oxygen only when its pressure in the tissue is very low).

- **Hemoglobin**: its main function is to transport O_2 and CO_2 and buffer blood. Its affinity to bind oxygen will be discussed later in the lecture.

- **How Hemoglobin works as buffer ?**

- By protonation and deprotonation of the C-terminus carboxyl group, the N-terminus amino group and the R groups , depending on the PH.

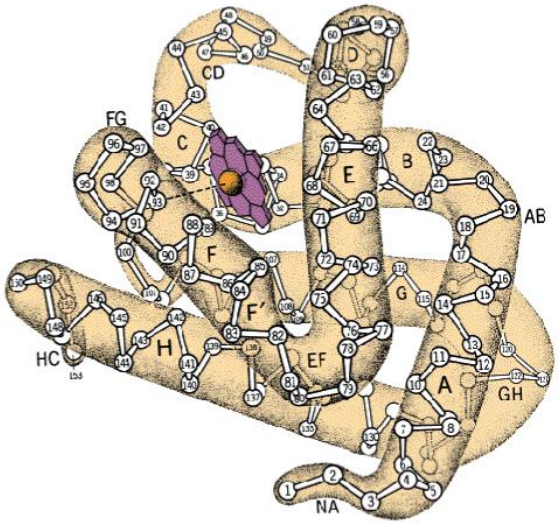
- ***Recall :***

- The buffering systems in your blood are :
- 1- bicarbonate 2- Hemoglobin 3- Albumin

II. Myoglobin:

1. The structure of myoglobin :

- Myoglobin is composed of one polypeptide chain (it is monomeric).



- It is composed of 8 α-helices, named A through H (refer to the figure to the right) , and these helices are connected by turns . (i.e. : A α-helix – turn - B α-helix –turn ... and so on).

- Myoglobin contains a non-protein group known as the **heme group**. This group is covalently bonded to myoglobin, and is called a prosthetic group .

- prosthetic groups : they are non-protein groups that bind covalently to a protein.

- Myoglobin can be found in two forms:
  a. Oxymyoglobin : when myoglobin is bound to oxygen (through the heme group).
  b. Deoxymyoglobin: when myoglobin is free of oxygen.

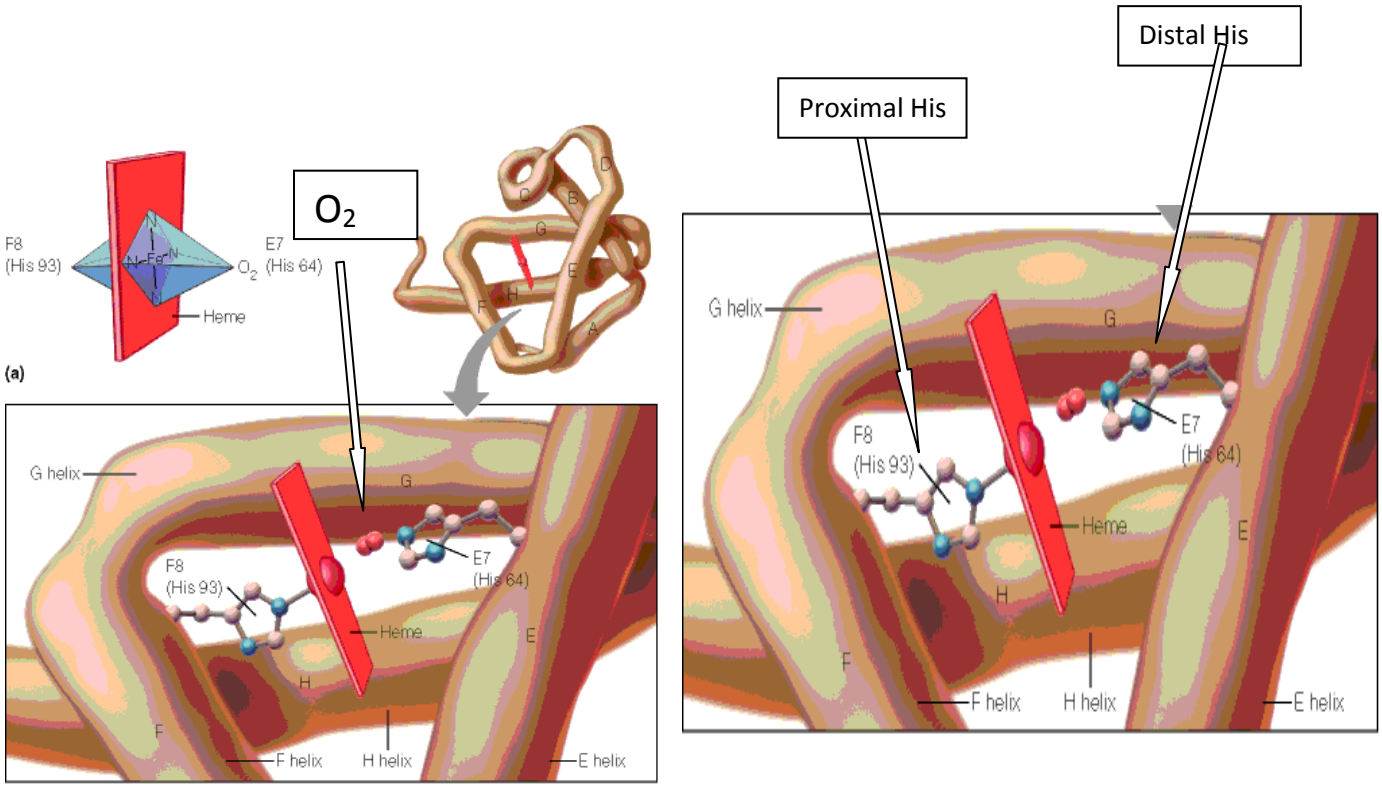
2. Arrangement of amino acids:

- Like any other globular protein, the hydrophobic R-groups of the amino acids of myoglobin are found at the interior of the structure, while hydrophilic R-groups are exposed on the surface of the molecule.

- The previous generalization has two exceptions to it. There are two histidines (remember that histidine is a polar/hydrophilic +ve charged amino acid) found

in the **interior** of the protein. One of them is present in helix F, covalently bound to heme, and is called F8 .

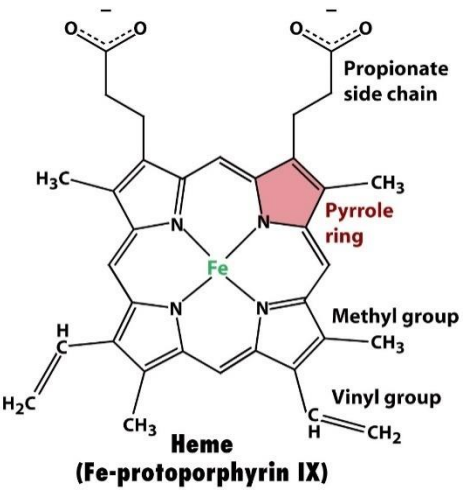
The other one is present in helix E, is **not** bound to heme and is known as E7 - (refer to the figures on the following page). F8 is known as **proximal His** (because it is closer to heme), while E7 is known as **distal His** (because it is further away from the heme group).



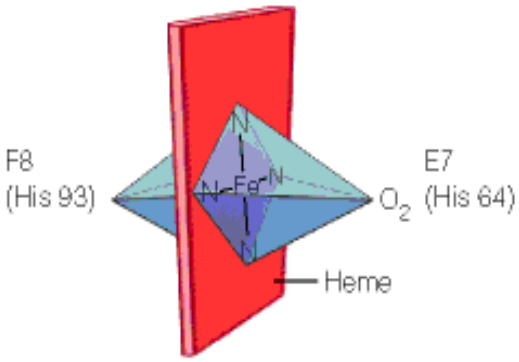
3. More on the heme group and iron:

- Heme is a non-protein molecule that consists of four rings/cyclic groups known as pyrrole rings (refer to the figure on the right).

- It is known as a prosthetic group, a non-protein group that is covalently attached to a protein.



- In the middle of the molecule there is an iron atom (Fe^{+2}) that can form six covalent bonds (refer to the figure at the bottom):
  - Four of these bonds are with the heme group itself, through the nitrogen atoms of its pyrrole rings.
  - One of the other two bonds (known as the fifth coordinate) is attached nitrogen of the imidazole ring of the proximal histidine.
  - The other one is free and binds oxygen. Therefore, oxygen doesn't bind to myoglobin directly but rather to the iron atom that is part of heme, which is linked to myoglobin.



- Iron can only bind oxygen when it is in the *ferrous state (Fe^{+2}).* In other words it cannot bind oxygen when it is in the *ferric/oxidized state (Fe^{+3}).*
- Our blood is red because of heme .

4. The structure-function relationship of myoglobin:

- The heme group fits into a hydrophobic pocket of the protein. Surrounding the heme are the hydrophobic amino acids (except for proximal and distal His).

- This hydrophobic pocket stabilizes the interaction between myoglobin and heme , if you have charged pocket ... the interaction is not stabilized .

- Heme also stabilizes the tertiary structure of myoglobin.

- The hydrophobic interior/pocket of myoglobin prevents the oxidation of iron, so when O_2 is released, the iron remains in the (Fe^{+2}) state and can bind another O_2. If there was no hydrophobic pocket ,the iron atom would become oxidized (Fe^{+3}).

- How do they maintain the iron in the ferrous form?

- As the electrons of the non-polar R groups are always moving ,so they give the iron the electron it needs to stay as (Fe^{+2}).

-What is the function of proximal and distal histidine ?

- **The proximal histidine** connects the Heme group to myoglobin
- **The distal histidine:**
  - Acts as a gate that opens up to allow oxygen either to bind heme or to be released, and then closes back again.

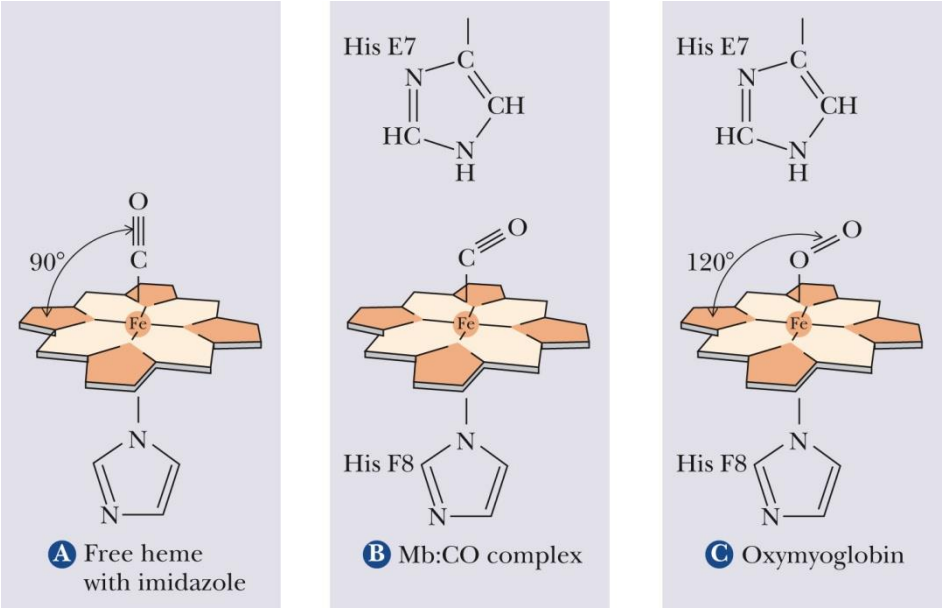
  - Another important function of the distal histidine is to decrease the affinity of heme to carbon monoxide (CO) relative to oxygen. CO has a much higher affinity towards heme than oxygen (about 25000 times the affinity of oxygen) , *only when Heme is free* . This is dangerous, since carbon monoxide is toxic and can be fatal if inhaled excessively. However, because heme is part of a protein and because of the presence of a distal histidine, the interaction of heme and CO becomes weaker than that of oxygen and heme. Why?

  - This can be explained through the figure on the next page.

- In picture (a) the distal histidine is absent and the binding is very strong between heme and CO because the bond is (90^0)perpendicular to the plane of heme (straight).

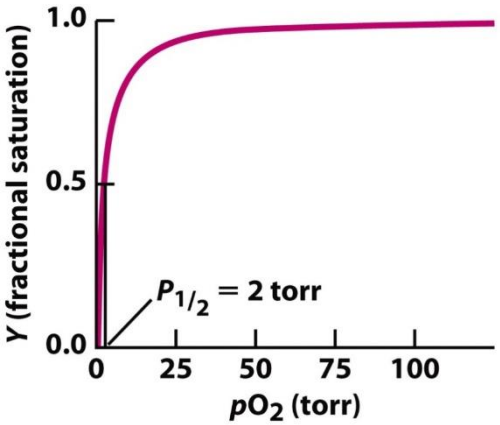
- In (b) we see the interaction between heme and CO in the presence of the distal histidine. Here , there is an angle between CO and heme and this causes the interaction to become weaker than that of oxygen and heme (c).

  - ❖ Notice that the action of distal His in lowering the affinity to CO is present in Hemoglobin (as its β chain has a similar structure of myoglobin) and Myoglobin.



5. *The saturation curve of myoglobin with oxygen* :

- The X axis represent the pressure of oxygen in tissues (as an indicator of its availability), while the Y axis represents the fraction of myoglobin that is saturated with oxygen (i.e.: 1.0 on the Y axis means that all myoglobin molecules are saturated and 0.5 means that half of the myoglobin molecules are saturated and 0.0 means all molecules are free of O_2).



- As mentioned earlier, myoglobin binds oxygen with high affinity.

- The shape of the curve is *hyperbolic* (قطع زائد).

- In the lungs, the pressure of oxygen is 100 torr. As we can see from the curve, this means that if any myoglobin is present in the lungs will be saturated (i.e.: all myoglobin molecules are bound to oxygen). In peripheral tissue, where the pressure of oxygen is around 25-30 torrs, myoglobin will also be *saturated*. In tissues where oxygen is not available abundantly (i.e. when oxygen pressure is very low and drops from 25 torr to 4--5 torr for example), the saturation of myoglobin will be low, because myoglobin is providing the tissue with oxygen to substitute for its unavailability and in cases of emergency .

- An important value on the curve is P50, which is the value of oxygen pressure when 50% of myoglobin molecules are saturated with oxygen. This value is equal to 2.8 torrs (P50 = 2.8 torrs).

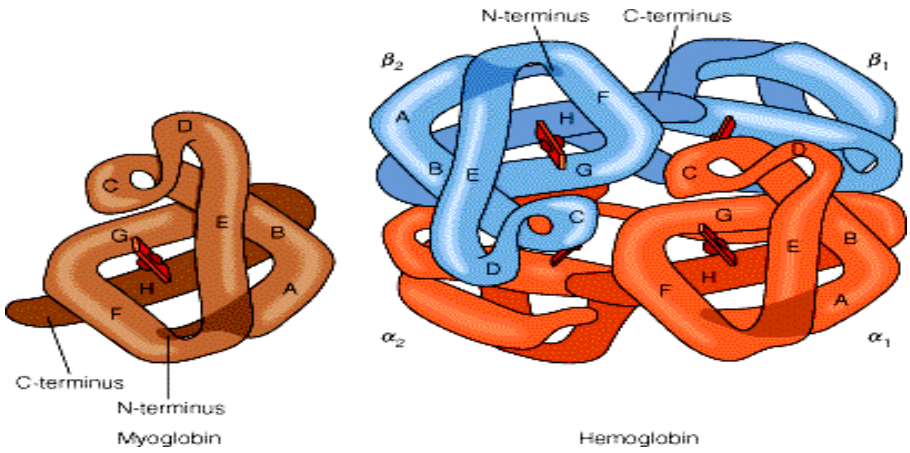
III. Hemoglobin:

- Hemoglobin is a protein that binds O_2 from the lungs and delivers it to the tissues of the body and then binds CO_2 and delivers it to the lungs.

1. Hemoglobin structure and chain interactions:

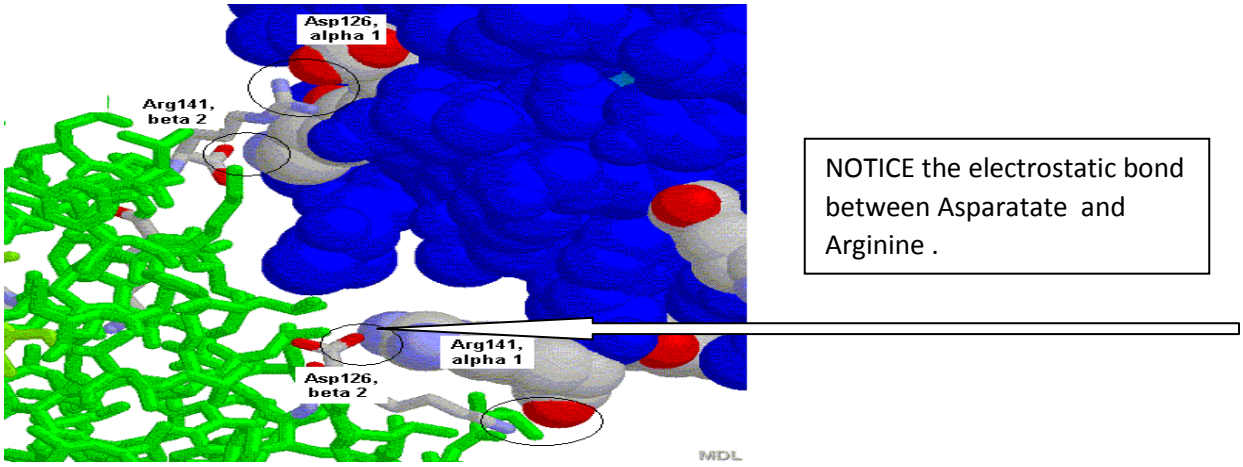
- Hemoglobin is a tetrameric hemeprotein (composed of four polypeptide chains , known as globins with each bound to a heme group).

- In adults, the four globin polypeptides are of two different types known as α and β, two globins are α and two are β, so a hemoglobin protein is an α2β2 globin protein (refer to the figure).



- The α and β chains contain multiple α-helices , where α subunit contains 7 α-helices and β subunit contains 8 α-helices (similar to myoglobin).
- Each polypeptide chain binds to heme, so hemoglobin has a total of four heme groups , so it can bind four O_2 molecules .
-
- The chains interact with each other Primarily via hydrophobic interactions. Therefore, hydrophobic amino acids are *not only present in the interior of the protein chains, but also on the surface ,* which are responsible for the interactions.

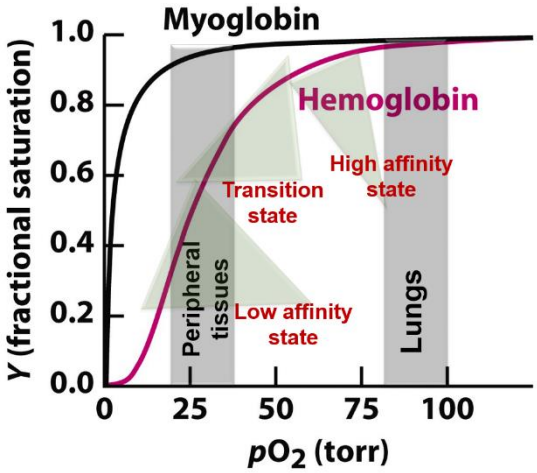
- Electrostatic interactions (salt bridges) and hydrogen bonds also exist between the two different chains (α and β). These interactions give hemoglobin its Function (refer to the figure on the right).



NOTICE the electrostatic bond between Asparatate and Arginine .

2. Oxygen binding and the saturation curve of hemoglobin:

- Hemoglobin must bind oxygen efficiently and become saturated at the high oxygen pressure found in lungs (approximately 100 mm Hg). Then, it releases oxygen and become unsaturated in tissues where the oxygen pressure is low (about 30 mm Hg).
- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape (looks like an S) (refer to the figure on the right).



- At 100 torr (in the lungs), hemoglobin is fully saturated (oxyhemoglobin) and that's what we want. As the oxygen pressure falls, oxygen is released to the cells, and the saturation/ affinity decreases in order to give tissues the O_2 they want.

- In contrast to a low p50 for myoglobin, the p50 of hemoglobin is approximately 26 torr.

- Hemoglobin exists in two states:
  a. A high affinity state/the R-state: when oxygen pressure is high (i.e. in the lungs, in order to bind to 4 oxygens). The R-state is known as the "relaxed" state and it has 500 times higher affinity to oxygen than the T conformation.
  b. A low affinity state/the T-state: when oxygen pressure is low (i.e. in peripheral tissues , in order to release oxygen). The T-state is also known as the "taut" or "tense" state and it has a low-binding affinity to oxygen.

- Hemoglobin is therefore an allosteric protein (from Greek "allos" = "other", and "stereos" = "shape"), that is, it has *two shapes/conformations*.

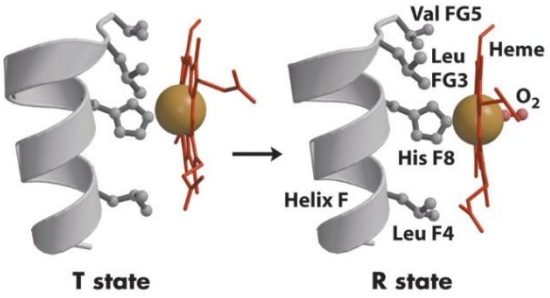
- An allosteric protein: a protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or a different ligand to another part of the protein.

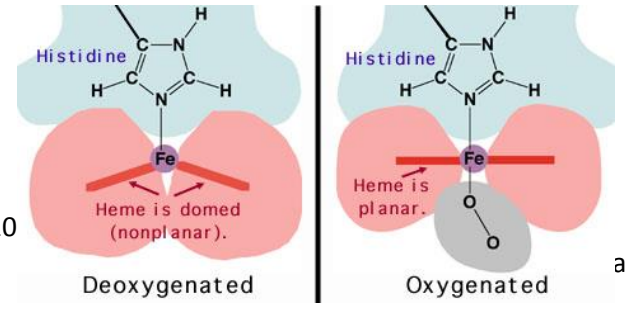
- Binding of O_2 causes conformational changes in hemoglobin gradually , converting it from the low affinity T-state to the high affinity R-state.

3. The transition between states:

- When heme is free of oxygen, it has a dome structure (not planar / flat) and iron is outside the plane of the heme group (refer to the figure below).

- When oxygen binds to an iron atom, heme adopts a planar/flat structure and the iron moves into the plane of the heme pulling proximal histidine (F8) along with it , also pulling the F - α helix ,changing the tertiary and then the quaternary structure , so change is gradual .





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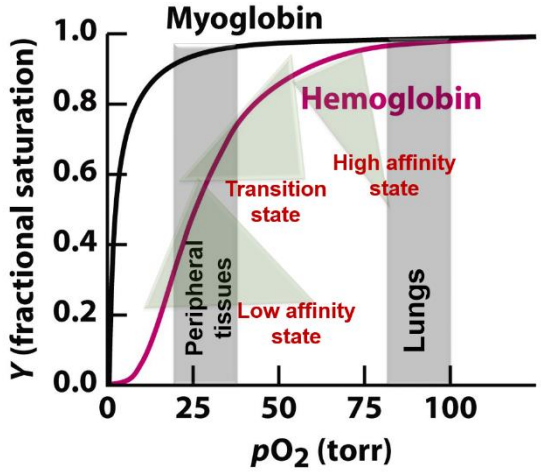
- This movement triggers :
    - changes in tertiary structure of individual hemoglobin subunits and
    - breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains, changing the shape of neighboring polypeptides and making them relaxed / opened and ready to accept oxygen, and that's why electrostatic bonds between subunits are important for the function of Hemoglobin.

- Binding is therefore considered to be cooperative, because binding of the first O_2 breaks some salt bridges with the other chains increasing the affinity of the binding of a second molecule. Binding of the second O_2 molecule breaks more salt bridges increasing the affinity towards binding of a third O_2 even more, and so on. This explains why the saturation curve of hemoglobin is **sigmoidal.**

- Unbinding of O_2 is also cooperative .. it means that releasing the last oxygen molecule is much easier than the first one .

- In myoglobin, binding of O_2 and movement of the helix does not affect the function of the protein and this transition is not seen. Why? Because in order for a protein to be allosteric or have two states it has to have more than one polypeptide (it has to be oligomeric), and myoglobin is monomeric (consists of one polypeptide chain)

Quick summary ☺

	Hemoglobin	Myoglobin
Function	Transporting O_2 and CO2 , buffer	Storage of O_2
location	In RBCs	In muscles
# of subunits	4 (heterotetramric)	1 (monomeric)
O_2 binding affinity	Depends on the pressure of O_2 : *low pressure.. low affinity *high pressure.. high affinity	High , except in case of emergency
O_2 binding capacity	4 molecules	1 molecule
O_2 saturation curve	Sigmoidal	hyperbolic
		
P 50	26 torr	2.8 torr

END OF LECTURE 12

Good luck to all ☺

Amer Al Musa