Doctor 022

Biochemistry

Sheet no.18

Globular Proteins

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Globular proteins

Myoglobin and hemoglobin

Functions of myoglobin and hemoglobin:

Myoglobin: functions in storing O_2 in muscles. During periods of oxygen deprivation, oxymyoglobin releases its bound oxygen.

Reminder: Myo means muscles, so it only makes sense that myoglobin is found in muscles and its purpose is to bind oxygen and release it only in cases of hypoxia (hypoxia: deficiency in oxygen), so when oxygen percentages are low in muscles, myoglobin tends to release oxygen.

Hemoglobin:

-transport of O_2 and CO_2

-blood buffering

you may wonder why we named hemoglobin in this way? this is simply because it contains the organic molecule known as heme (to be discussed later).

Now when we talk about the function of hemoglobin, we say that it plays a major role in binding oxygen in lungs and then transports and releases it in peripheral tissues. After that, hemoglobin goes back to the lungs where it repeats the previously mentioned steps. Hemoglobin possesses other functions as well, one of them is that when hemoglobin enters the tissues, it binds to carbon dioxide (CO_2) which is a product of metabolism (back to biology 101: in cellular respiration: glucose ⟹ pyruvate ⟹ Kreps cycle ⟹ CO_2, now this CO_2 that was produced must be released from cells so it simply binds to hemoglobin which releases this CO_2 in lungs), note that CO_2-hemoglobin binding mechanism is different from O_2-hemoglobin binding mechanism. In addition to all of that, hemoglobin is capable of binding to protons as well, so this allows it to act as a buffer system for the blood.

Hemoproteins

-many proteins have heme as a prosthetic group called hemoproteins.

Hemoproteins: is a class of proteins that contain the heme molecule which is considered to be a prosthetic group (remember the meaning pf prosthetic group: it's a non-proteinaceous organic (or inorganic) group that binds covalently to protein). we have many kinds of hemoproteins like:

★**Myoglobin and hemoglobin: that transfer and store oxygen**

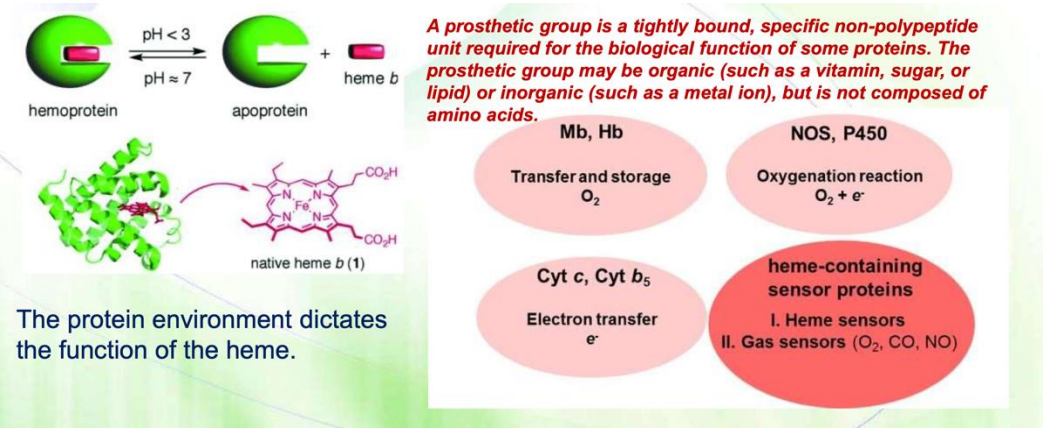
★ **NOS (Nitric oxide synthase) and cytochrome P450: enzymes that need heme in order to be functional (used in oxygenation reactions).(NOS: reduces arginine and produces NO which is a signaling molecule)(P450: cytochrome, very important for detoxification toxins or any other foreign molecules in the liver)**

★**Cyt c and Cyt b_s: used to transport electrons in the electron transport chain.**

★**Sensor proteins: bind to carbon monoxide (CO) and this way inform tissues about the presence or absence of CO.**

★**catalase is also a hemoprotein (peroxisomal enzyme) which removes ROS (H_2O_2)**

Refer to the picture below for more functions!



Since we have prosthetic groups in hemoproteins, that's means that hemoproteins are holoproteins (also referred to as conjugated proteins) (which are proteins that contain a non-protein group). Upon the removal of heme from hemoglobin, we obtain globin (the protein portion of hemoglobin), so now we have an apoprotein (a protein composed of amino acids only and no prosthetic groups).

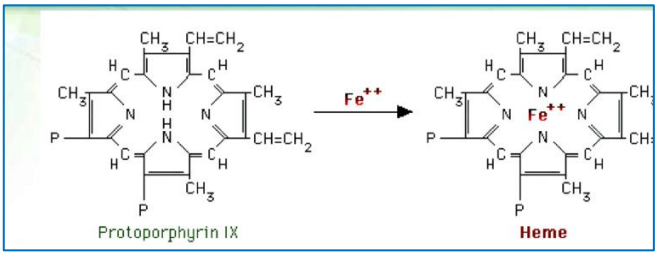
Now let's discuss the heme structure!!

Heme structure:

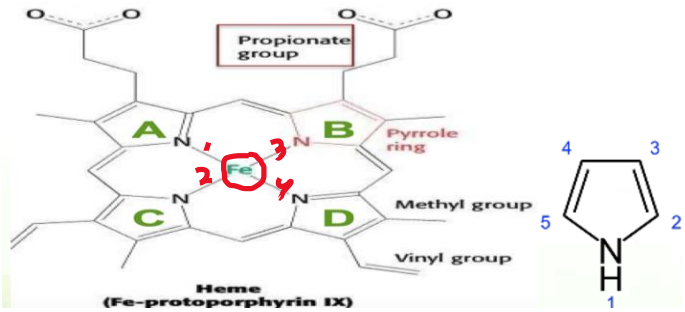
Heme is an organic molecule that can bind iron, and iron must be in the ferrous state Fe^{+2}

(Extra info: in an old system of naming ions, we used to add -ous and -ic to stem name of the element to indicate the ions of lower and higher charge, for example: iron contains +2 and +3 charges, so Fe^{+2} is ferrous and Fe^{+3} is ferric).

Heme's name before the addition of Fe^{+2} is protoporphyrin IX, but once iron is added it becomes heme.



we can see that iron binds right in the middle of the heme molecule, now iron could form 6 covalent bonds, four of them will be with the heme molecule.



If we take a closer look to the heme structure, we can see that it is composed of four rings designated as A, B, C and D, each one of these four ring structures is known as pyrrole ring (thus heme is called tetrapyrrole).

Overall, we consider heme to be hydrophobic, it contains rings, methyl and vinyl (attached to C, D rings) but the top rings (A and B) contain propionate groups (-CH_2-CH_2-COO^-) which can be charged, so the overall heme molecule is hydrophobic with an exception of the propionate groups that are on top (it contains 4 methyl groups, 2 vinyl groups and 2 propionate groups).

-it's a complex of protoporphyrin IX + iron (Fe^{+2})
-the porphyrin is a planar and consists of four rings (designated A-D) called pyrrole rings.

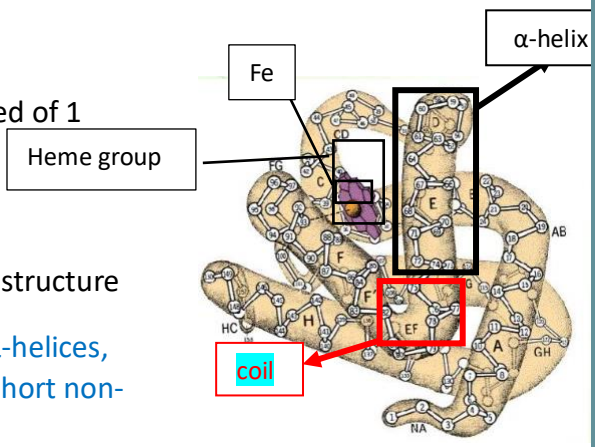
- each pyrrole ring can bind two substituents

-two rings have a propionate group each

-Note: the molecule is mainly hydrophobic

-Fe has six coordinates of binding

Structure of myoglobin



- Myoglobin is a monomeric protein (it's composed of 1 polypeptide chain) that is

  mainly found in (skeletal) muscle tissue.

REMEMBER: 1 polypeptide → doesn't have quaternary structure

- The tertiary structure of myoglobin contains 8 α-helices, designated from A to H, that are connected by short non-helical regions.
- The eight α-helices are connected by short coils, a structure that is known as the **globin fold**, which is a hydrophobic O_2-binding pocket.
- It contains heme as a prosthetic group internally.
- Myoglobin can be present in two forms (states):

  oxymyoglobin (oxygen-bound)

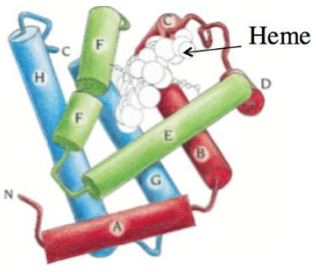
  deoxymyoglobin (oxygen-free)

GLOBIN FOLD:

It's full of helices

8 (A → H)

Extra image:



Arrangement of amino acids

- Like other globular proteins, the hydrophilic amino acids are generally on the surface, while hydrophobic amino acids are predominantly internal.

→ Except for two histidine residues in helices E and F (known as E7 and F8):

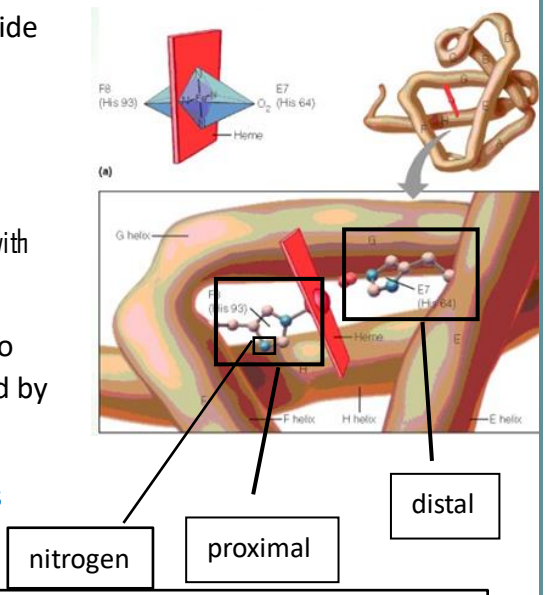
Although they are polar AAs (charged), they are present inside the protein for different functions 😉:

1- **proximal** His (in F helix)→ binds covalently to iron → Stabilizing interactions between heme and myoglobin.

that's why we call heme "prosthetic group" (because it's bound covalently with protein)

2- **distal** His (in E helix) → it's a GATE بوابة(It allows only O_2 to enter and bind with iron) → strength of binding is facilitated by distal His (stabilization of interaction between O_2 and iron).

- F8 His is designated as **proximal His**, whereas E7 His is known as **distal His.**



How distal histidine is hydrogen bonded with O_2?

The partial positive charge in imidazole group will bind unshared pair of electrons that oxygen contains.

Iron

- Iron can bind in the center of the four rings.
- Fe is in the **ferrous state (Fe^{2+})** and can form 6 (covalent) bonds (that's how it is stabilized in its position):
  - 4 bonds with the nitrogen of the rings (heme)
  - One bond (known as the fifth coordinate) with the nitrogen of the proximal His.
  - A last one with O_2 (the sixth coordinate) when O2 is there.
- Oxidation of iron to the Fe^{3+}, ferric state, makes the molecule incapable of normal O_2 binding.
  When O_2 is released from iron, iron becomes oxidized → from ferrous (+2) to ferric (+3)
  But this is prevented (doesn't happen) why?? We will know in the next page 😉
- Upon absorption of light, heme gives a deep red color.
  When heme is bound to O_2 it gives the reddish color (that's why our blood is red)
  - Why the arteries are red while veins are blue?
  Because in arteries the hemoglobin is bound with O_2 → red color
  While in veins, hemoglobin is unbound (actually it's bound but in little percentage) → blue color

Structure-function relationship

Three things stabilize the interactions of heme with myoglobin:

1. Covalent bond between proximal His with iron.
2. Hydrophobic Interactions between heme and the hydrophobic AAs(inside the protein).
3. Electrostatic interactions between the two propionate groups with polar AAs in the surface of myoglobin.

- The planar heme group fits into a hydrophobic pocket of the protein and the myoglobin-heme interaction is stabilized by hydrophobic attractions.

Interactions between the hydrophobic heme and the surrounding hydrophobic AA

- The heme group stabilizes the **tertiary** structure of myoglobin.

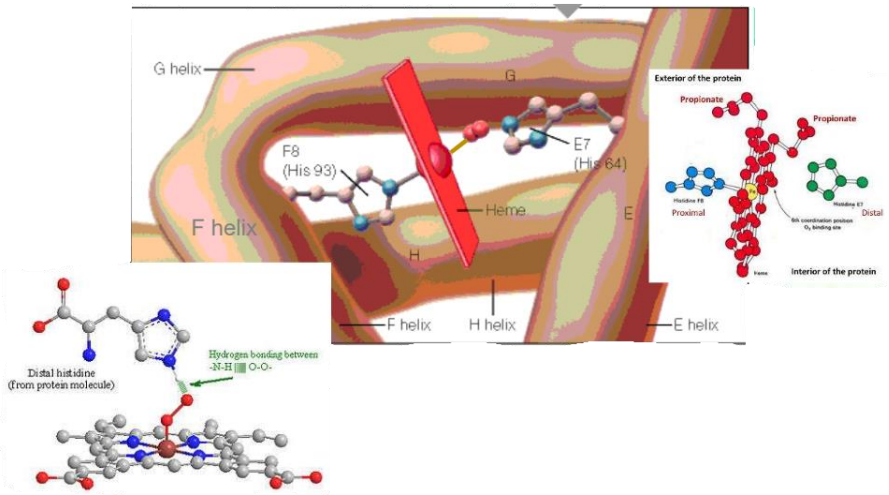
REMEMBER: Iron should be Fe^{+2} (ferrous ion).

When O_2 is released → Fe^{+2} become oxidized to be Fe^{+3} (ferric ion)

But that does NOT happen, Why?

-because the surrounding hydrophobic AAs prevent iron from being oxidized by electrons rearrangement with it, allowing for another O_2 molecule to bind with myoglobin.

- The hydrophobic interior of myoglobin (or hemoglobin) prevents the oxidation of iron, and so when O_2 is released, the iron remains in the Fe (II) state and can bind to another O_2.



Repeated information:

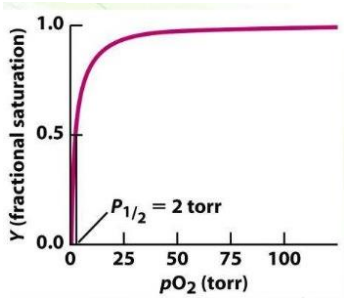
- The distal histidine acts as a gate that opens and closes as O2 enters the hydrophobic pocket to bind to the heme.
- It also stabilizes the interaction with oxygen.

Oxygen binding to myoglobin

Myoglobin releases O_2 only in case of hypoxia (medical emergency)

→ the interaction between O_2 and heme has to be tight (very strong), so that releasing of O_2 ONLY can happen in case of Hypoxia.

O_2 saturation curve.



The binding of O2 to myoglobin follows a **hyperbolic** saturation curve.

- Myoglobin binds O_2 with high affinity.
- The $P_{1/2}$ (oxygen partial pressure that is required for 50% of all myoglobin molecules to be bound with O_2) for myoglobin ~2.8 torrs (or mm Hg).
- Given that O_2 pressure in tissues is normally 20-40 mmHg, it is almost fully saturated with oxygen at normal conditions.

in lungs: pO_2 = 100 torr → myoglobin almost saturated 100%

in tissues at normal metabolism pO2 =40 torr → almost saturated 100%

in tissues (if you're playing soccer) pO2 =25 torr → almost saturated 100%

but, in HYPOXIA there will be a sudden drop of O_2 saturation (quick release of O_2)

NOTE:

Saturation = myoglobin is bound to O_2

Hemoglobin structure

Hemoglobin is a tetrameric hemoprotein (four globin protein chains with each bound to heme).

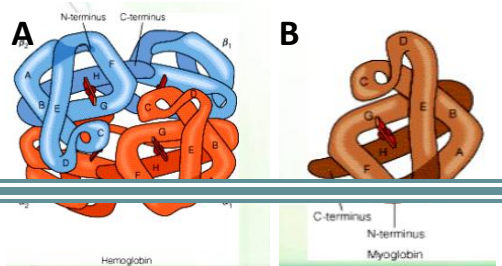
Tetrameric= it is composed of 4 polypeptide chains (so it can bind at most 4 O_2)

2 alpha chains + 2 beta chains (alpha2beta2 protein).

In adults, the four globin proteins are of two different types known as alpha and beta, so a hemoglobin protein is termed a2b2 globin protein.

This hemoglobin (a2b2) is the adult hemoglobin that exists in our bodies, there are many types of hemoglobin molecules that we will discuss later.

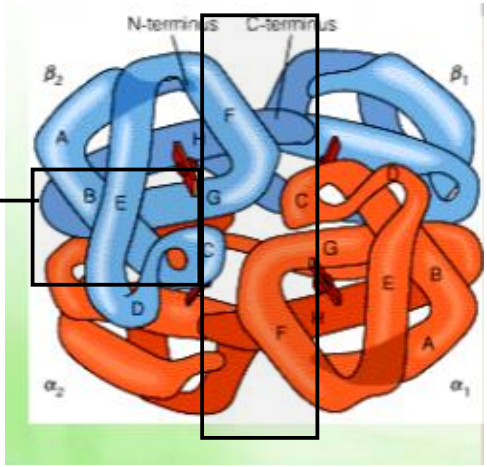
B resembles the myoglobin protein, there are some differences but as a structure A and B are similar (globular proteins, contain α helices).



A=Hemoglobin

Blue chains are Beta, Orange chains are Alpha

B=Myoglobin



The alpha and beta chains contain multiple alpha helices:

Alpha subunit contains 7 alpha-helices (141 Amino acid residues).

Like myoglobin, Beta subunit contains 8 alpha-helices (146 Amino acid residues).

How are these subunits bound?

We said before in quaternary structure that we have proteins that are composed of multiple polypeptides that can be connected either via non-covalent interactions or covalent interactions (according to the protein we have, we can determine the type of interactions).

In terms of the hemoglobin molecule, the interactions that connect the subunits are hydrophobic interactions, hydrophobic amino acids exist on the surface and they facilitate the interactions between the alpha and beta subunits.

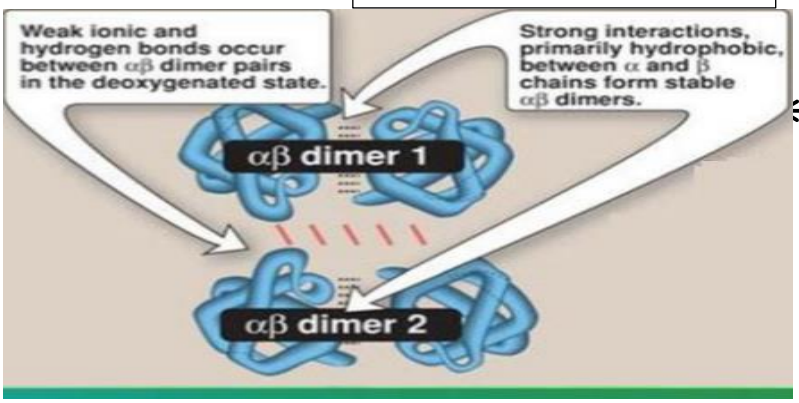
A dimer of dimers (I made up this term) or 2αβ-protomers

***(a-b)2**

We have two heterodimers (alpha and beta) and they are connected via hydrophobic interactions (α and β).The (alpha and beta 1) are connected with (alpha and beta 2) via electrostatic interactions.

Hydrophobic interactions

Electrostatic interactions



electrostatic interactions (salt bridges) and hydrogen bonds also exist between the two DIFFERENT chains .

Oxygen binding to hemoglobin

Hemoglobin must bind oxygen efficiently and become saturated at high oxygen pressure found in lungs (approximately 100 mmHg).

Then it must release oxygen and become unsaturated in tissues where the oxygen pressure is low (about 30 mmHg).

RBCs contain high number of hemoglobin molecules, hemoglobin molecules are routed to the lungs and bound to oxygen. After that, they move to the tissues and release oxygen and the cycle repeats.

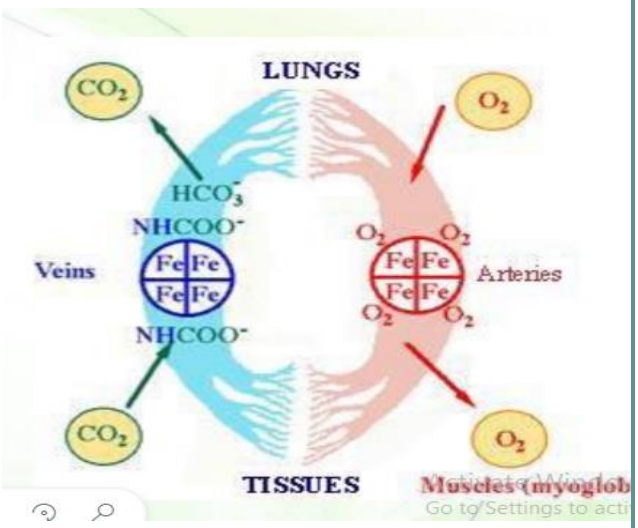
Dr asked, would you expect hemoglobin to have high affinity (strength of interaction) or low affinity to oxygen?

Answer and explanation: If it has a low affinity to oxygen, it wouldn't bind to oxygen in lungs, and if it has a high affinity to oxygen, it wouldn't release oxygen in tissues.

So we want hemoglobin to have both affinities (high affinity in lungs and low affinity in tissues).

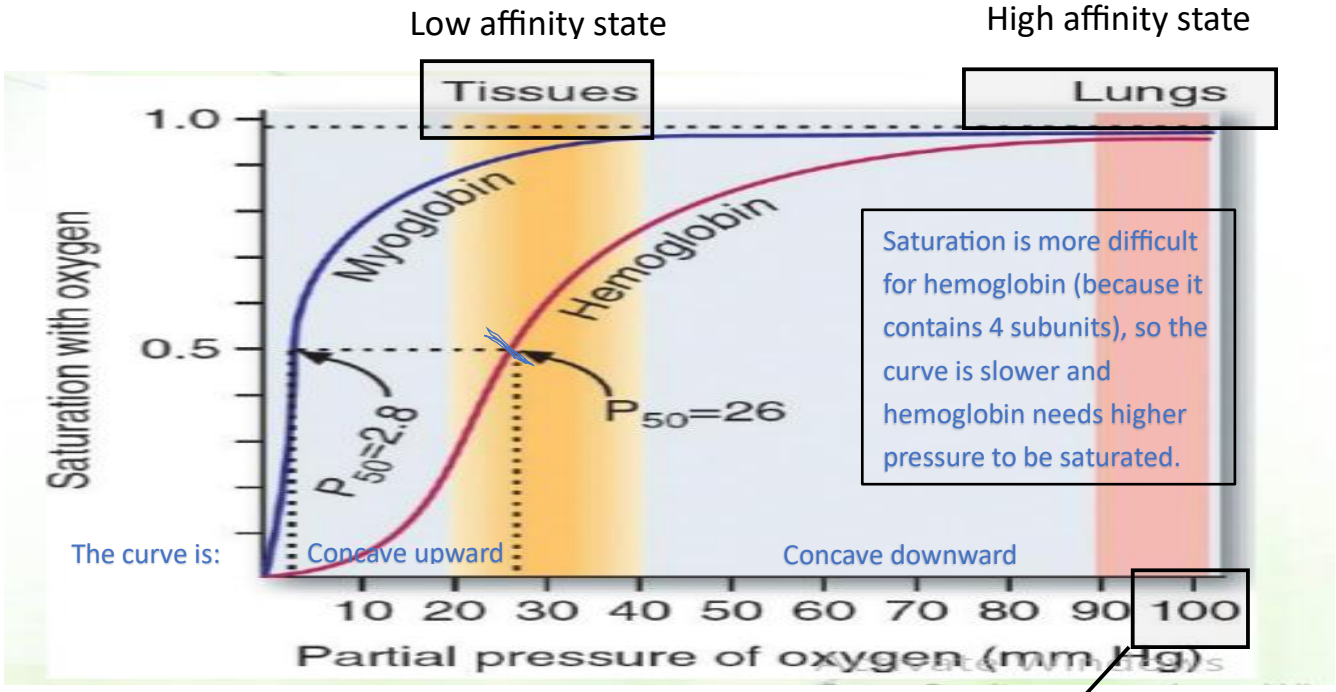
High affinity in lungs => becomes saturated with oxygen from lungs.

Low affinity in tissues => releases oxygen in tissues.



And that what actually happens (I mean that hemoglobin has both high and low affinities).

Saturation curve



95-98% of hemoglobin is saturated

The saturation curve of hemoglobin binding to oxygen has a sigmoidal shape

Sigmoidal curve indicates that the protein has different structures

Myoglobin has a hyperbolic shape (very rapid increase then platue),

For hemoglobin curve (the red line) it has Sigmoidal shape (looks like S letter).

Sigmoidal plot indicates that the protein has two states: ** مهم تضلكم متذكرينه

1)High affinity state (R-state) **2)Low affinity state(T-state)**

As oxygen pressure falls, oxygen is released to the cells.

$P_{1/2}$ or P_{50} is 26mmHg for hemoglobin, but what we mean by P_{50}?

It indicates the pressure of oxygen when oxygen binding sites are 50% full of oxygen and 50% free of oxygen.

So when the pressure of oxygen is 26mmHg, 50% of binding sites are full of oxygen and 50% are free of oxygen, 50% are oxyhemoglobin and 50% are deoxyhemoglobin.

The P_{50} of myoglobin is 2.8torr, means that when the pressure of oxygen in the muscles is 2.8mmHg, we will have 50% of myoglobin in the oxygenated form and 50% of it in the deoxygenated form.

In contrast to a low P_{50} for myoglobin, the P_{50} of hemoglobin is approximately = 26mmHg.

Relate the value of P_{50} to affinity.

Note: the more pressure of the gas, the more concentration of it.

Does myoglobin have a higher affinity to bind oxygen than hemoglobin or hemoglobin has the higher affinity? Dr asked.

ANS: myoglobin! because it needs a less concentration of oxygen to be 50% saturated.

REMEMBER THE P_{50}.

Hemoglobin? owww we need a much higher concentration of oxygen to fill 50% of the binding sites (50% oxygenated).

Note: since we need a higher concentration of oxygen to get 50% of the protein oxygenated, that indicates that we have a lower affinity to oxygen.

As P_{50} increases the affinity decreases.

Extra information from Dr, when you are playing football the release of oxygen is faster, why? Because affinity will become lower for many reasons that will be discussed later on.

Hemoglobin is allosteric

Hemoglobin is an allosteric protein (different structures)

(from Greek "allos" = "other" and "stereos" = "shape")

An allosteric protein: a multi-subunit protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or different ligand to another part of the protein by changing its structure slightly.

Proteins that are allosteric are proteins that have different structures, not totally different structures (a minor change in protein's structure).

Hemoglobin exists in two allosteric forms (T and R States).

The T-state is known as the "taut" or "tense" state and it has a low binding affinity to oxygen.

The R-state is known as the "relaxed" state, and it has a 500 times higher affinity to oxygen than the T conformation.

These structures are high and low affinity structures they called them

R-state and T-state. R=Relaxed, T=Tight (or taut)

So hemoglobin while being in the R state it will be relaxed so it will bind to oxygen instead of not binding to oxygen like in the T-state.

Summary: T-state = low affinity to oxygen (like in tissues)

R-state = high affinity to oxygen (like in lungs).

Note that myoglobin isn't an allosteric protein, it's a monomeric protein.

While hemoglobin is an allosteric protein that has a quaternary structure (reason of being allosteric "quaternary structure").

Binding oxygen causes conformational changes in hemoglobin, converting it from the low affinity T-state to the high affinity R-state

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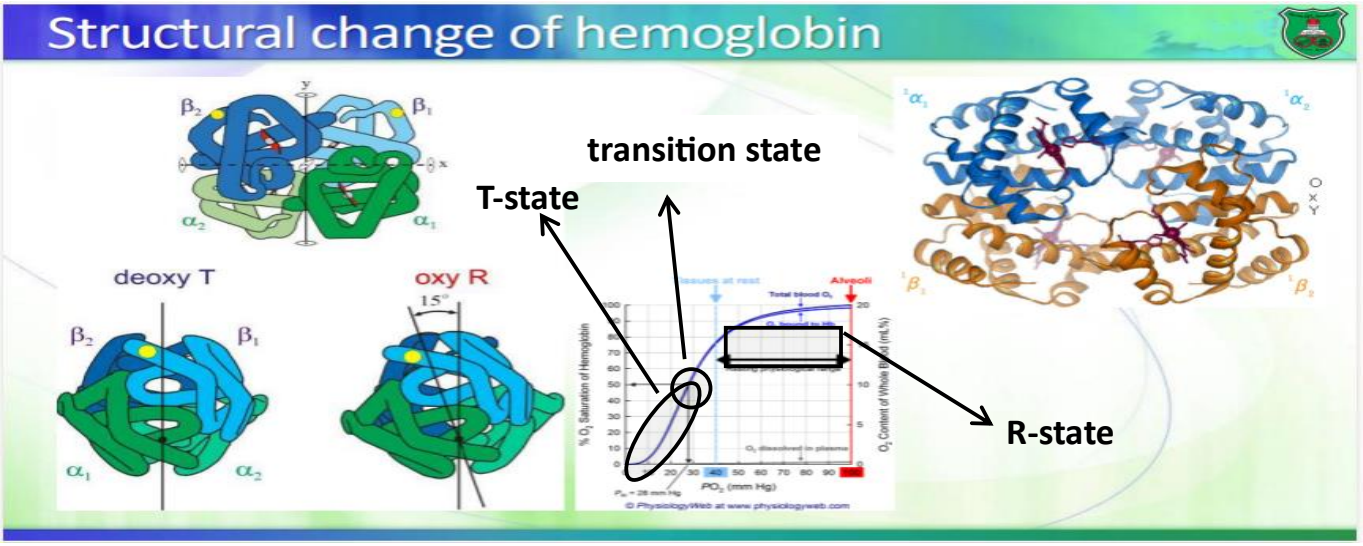


The two states are found in equilibrium, we can find hemoglobin in both states even if it is not bound with oxygen

NOW WE WILL SEE THE CHANGE BETWEEN THE TWO STATES (A MINOR CHANGE AS WE SAID IN THIS LECTURE).

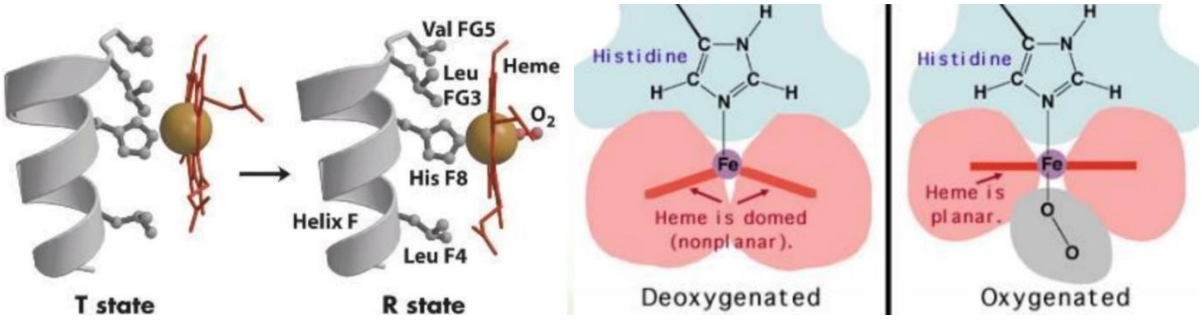
We have a little change in structure which is a movement around 15 degrees around the axis of hemoglobin molecule (make it open able to receive oxygen or close not able to bind oxygen).

How does the structure of hemoglobin changes

- **When heme is free of oxygen, it has a domed structure زي شكل القُبّة and iron is outside the plane of the heme group.**

Why? Because the hydrophobic heme is repelled by the proximal His.

- **When oxygen binds to an iron atom, heme adopts a planar structure and the iron moves into the plane of the heme pulling proximal histidine (F8).**



***Heme molecule is not really totally flat. it looks like a dome, so it's bent (because there is repulsion between proximal histidine and heme, because**

heme are hydrophobic and histidine is hydrophilic we will have repulsion between the two groups so the heme will be bent).

***Iron exists outside the plane يعني طالع لبرا شوي**

***Explanation of the pictures above:**

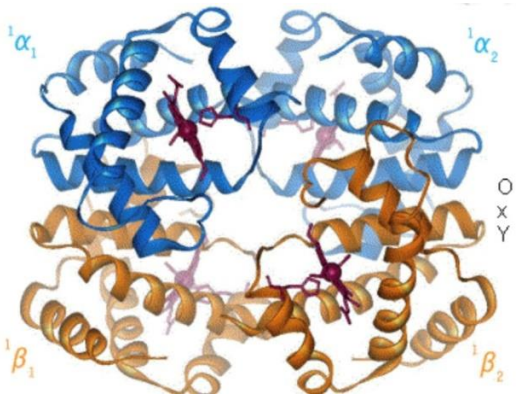
When oxygen comes in and binds to iron, the structure of heme will change, it'll become flat. The iron atom will be pulled inside رح تنسحب الى الداخل because the oxygen will make interactions with the distal Histidine (the charge or proton in distal Histidine makes a hydrogen bond with oxygen). So, the oxygen atom will get closer to distal Histidine, pulling the iron atom with it. Which means that the heme is now flat. The proximal Histidine also gets pulled because it's bonded to iron, which means that THE WHOLE alpha-helix is now pulled.

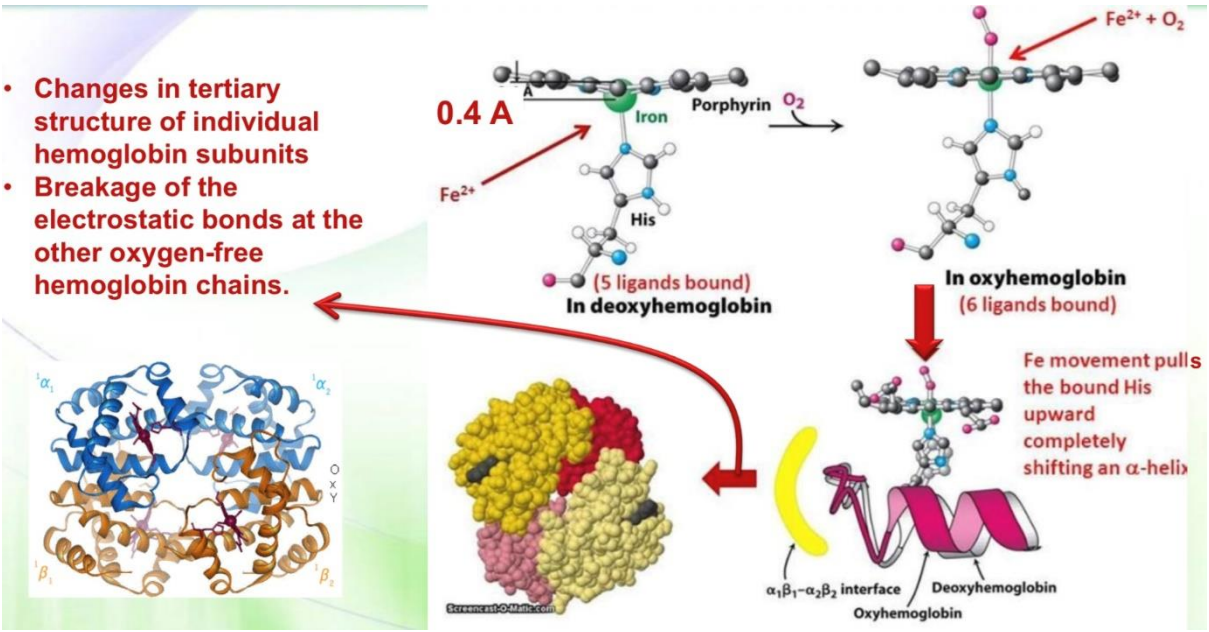
Now that we have changed the structure of the alpha-helix, the tertiary structure is changed, and so the quaternary structure is also changed.

All of these causes the myoglobin to switch from T to R in lungs, and from R to T when it goes to tissues. (High affinity state to low affinity state and vice versa).

- **This movement triggers:**
  - **Changes in tertiary structure of individual hemoglobin subunits.**
  - **Breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains. The positions of amino acids changed a little due to the previous pulling, so the electrostatic interactions between each (alpha-beta) AND (alpha-beta) are broken or changed. Leading to the molecule simply switching:**
    from T to R (lungs, بيرجع زي قبل)
    and from R to T (reformation of electrostatic interactions).

In myoglobin, movement of the helix DOES NOT affect the function of the protein.

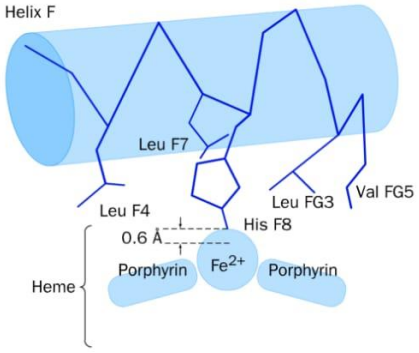




"This movement is only 0.4 Angstrom (Å) long, which is amazing!!"

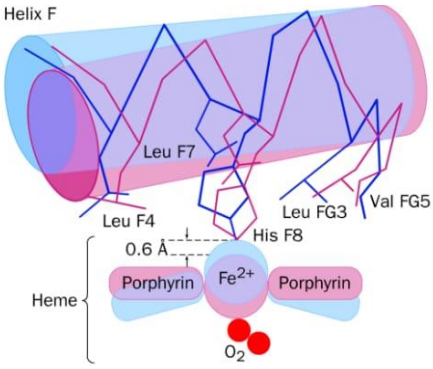
Look around, move, get some fresh air. no need to stress too much, you got this!! :D

-Notice that this little change will have a huge impact on the hemoglobin affinity to oxygen.



Stage 1 = T state

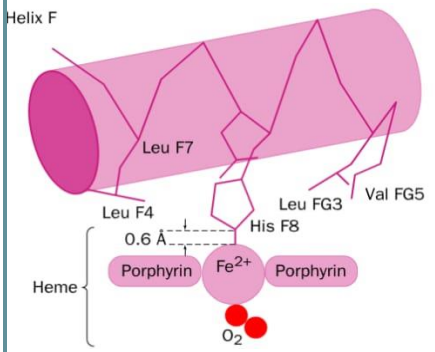
In the absence of bound O_2 ,the Fe(II) lacks a sixth ligand, and resides about 0.6 Angstrom out of the plane of the heme toward its His ligand (the proximal His).



Stage 2 = changing its state

Movements of the hemoglobin's heme and F helix during the T → R transition.

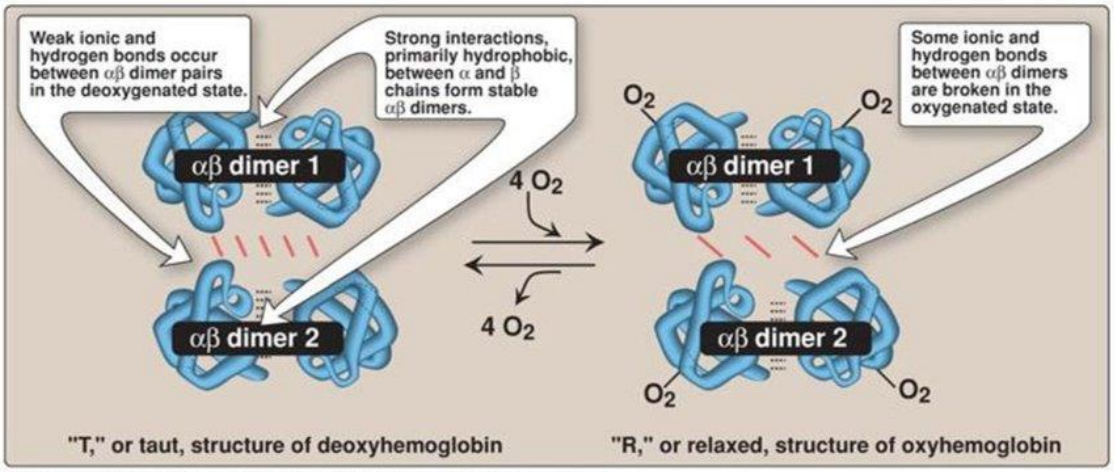
Fig. 7-9 diagrams how the binding of O_2 to one hemoglobin site induces conformation changes that influence the O_2 -binding affinity of the other sites.



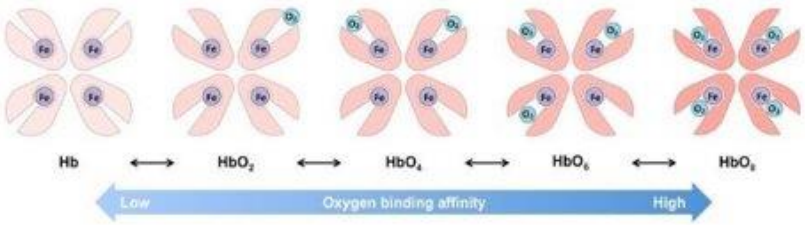
Stage 3 = R state

Upon binding O_2 ,the Fe(II) is pulled towards the O_2 into the plane of the heme. This also pulls the attached proximal His towards the heme. Since the proximal His is part of the F helix, this entire helix is also pulled toward the heme. These conformational changes induce a rearrangement of the alpha and beta subunits in the hemoglobin tetramer.

-Notice that some electrostatic interactions between αβ dimers are broken when hemoglobin is in the oxygenated state (oxyhemoglobin).



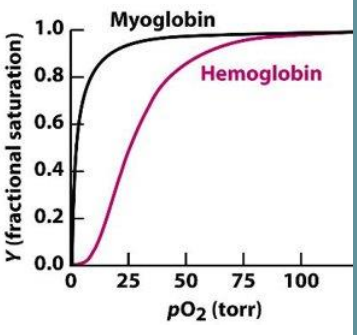
Binding is cooperative



-It basically means that binding of an oxygen to one heme makes it easier for the next oxygen to bind to the other heme, which makes it even easier for the third oxygen to bind to the third heme and so on.

-This is a characteristic of allosteric proteins, as we will see later in enzymes (binding of a ligand makes it easier for the next ligand to bind).

-And also, the opposite is true; the release of one oxygen makes it easier for the next oxygen to be released.



Cooperativity results in sigmoidal (S-shaped) rather than hyperbolic binding curves

Conformational changes lead to cooperativity among binding sites.

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.

Oxygen is a homotropic effector (the allosteric modulator is the substrate itself).

-A homotropic effector refers to a substrate (e.g., oxygen in our case) that modulates its own binding or release. On the other hand, a heterotrophic effector describes a substance that, upon binding, influences the binding or release of a different substrate that is distinct from the effector itself.

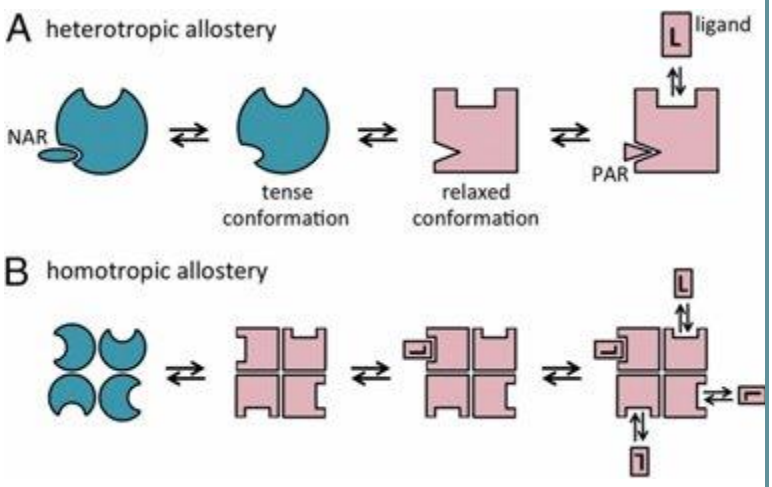
Remember: that all allosteric proteins have quaternary structures

Homotropic allosteric regulator/effector: effector and ligand regulated by the effector are the same molecules (e.g., O_2 binding affects subsequent O_2 binding).

Heterotropic allosteric regulator: effector and ligand are different molecules (e.g., H+ or BPG binding affects O_2 binding).

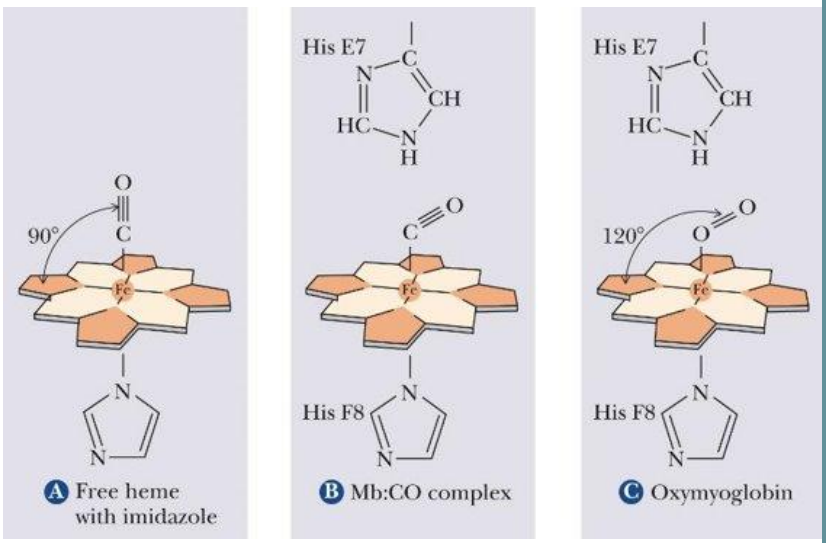
Positive allosteric interaction: effector binding increases affinity for ligand.

Negative allosteric interaction: effector binding decreases affinity for ligand.



Another significance of distal Histidine

-Theoretically speaking, if we took the heme group and placed it in an equal amount of O_2 and CO (carbon monoxide, which is lethal), the heme group would bind to the CO with a much greater affinity than O_2.-when the heme group is bonded to a protein (like hemoglobin or myoglobin) the affinity for CO will drop (from thousands-fold



more than O_2 binding affinity to only 250 times more than O_2).

-This is due to the presence of distal histidine (it causes the CO-Fe bond to be bent (to avoid steric hindrance with distal histidine) which is not favorable and causes the affinity of CO to heme to be lower).

CO prefers straight bonding, but O_2 prefers bent bonding.

CO binds to free heme with higher affinity (thousands folds more) than O_2.

The affinity of CO to myoglobin-bound heme is only 250 times more than O_2.

Yet, CO occupies 1% of hemoglobin, but 99% if distal His does not exist.

Test your knowledge:

Distal histidine has the significant role in hemoglobin:

a- It prevents the entry of CO into the heme binding core
b- It covalently links the heme group to hemoglobin
c- It reduces iron when oxygen is released and iron is oxidized
d- It stabilizes oxygen binding to heme via the formation of hydrogen bonding with it.
e- It makes affinity of hemoglobin to CO lower than that of O_2
Ans: D

The sigmoidal shape of the oxygen saturation curve of hemoglobin indicates:

a- Hemoglobin is a hetero-multimeric protein
b- Hemoglobin is a conjugated protein
c- Hemoglobin is a holoprotein
d- Hemoglobin is an allosteric protein
e- Hemoglobin has a prosthetic group
Ans: D

The correct statement about myoglobin is

a- It is made of beta sheets completely
b- Its P_{50} towards oxygen is constant with changing oxygen pressure in muscle tissues
c- It is an allosteric protein
d- It has high affinity towards oxygen
e- Oxygen is released during light exercise

Ans: B+D

If the O_2 pressure in a certain tissue is 3mmHg, what will happen?

a- The myoglobin will be partially saturated
b- The myoglobin will be fully saturated
c- The hemoglobin will be fully saturated
d- Can't be determined

Ans: A

V2

And the saturation curve

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