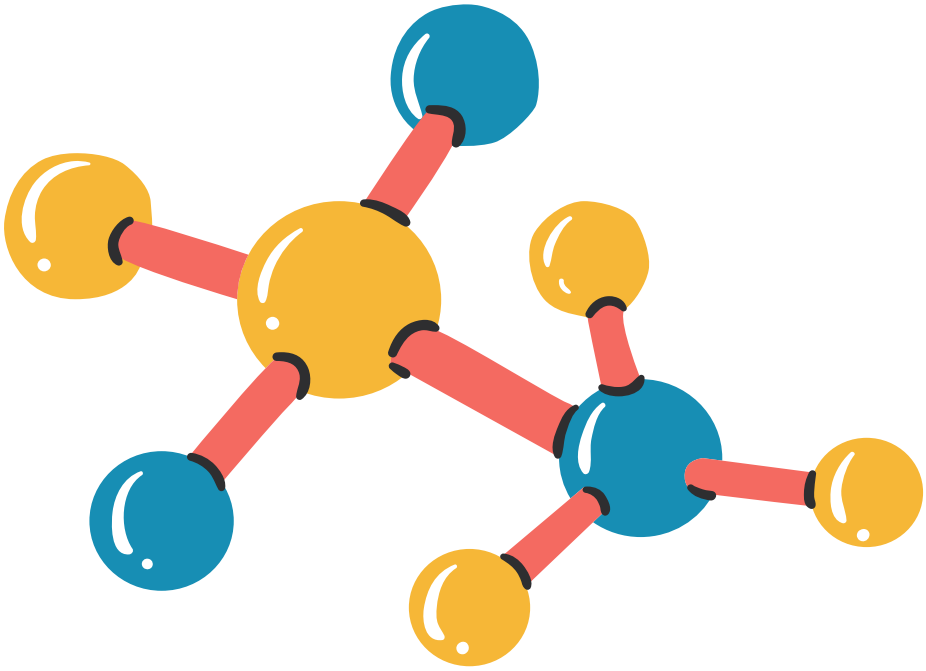
Sheet no.20

Biochemistry



Summer 2022

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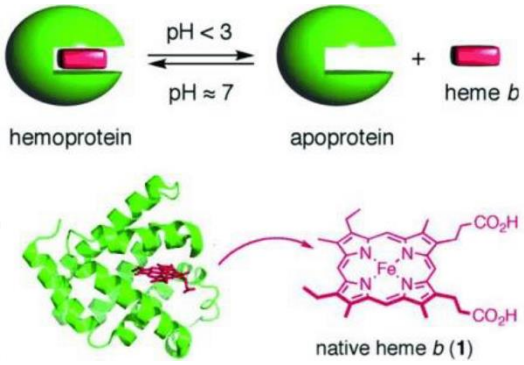
Globular proteins

Globular proteins have spherical shapes , such as hemoglobin and myoglobin

Hemoproteins : proteins which have prosthetic group called heme which is a non-protein group attach covalently to the protein (complex proteins with conjugated group which is heme) , Myoglobin and hemoglobin can be classified as hemoproteins because both of them contain heme group



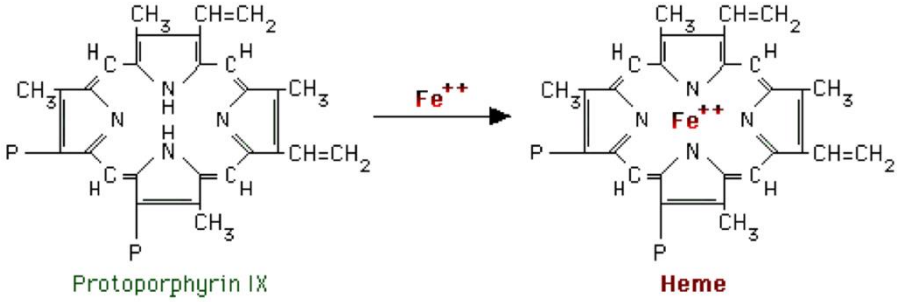
The protein environment dictates the function of the heme , because environment can change the affinity of the heme group and ph can change the bonds then the attraction between subunits will change and so on .

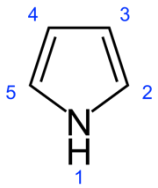
A prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.

Heme structure

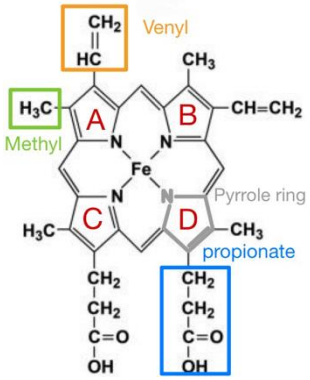
It is a complex of protoporphyrin IX (IX means 9) + Iron (Fe+2)(Fe II) , be careful about the name of each cation Fe+2 is called ferrous but Fe+3 is called ferric , this change in charge will change the function of the heme group

The porphyrin is planar and consists of four rings (designated A-D) called pyrrole rings





Pyrrole ring



Two rings have a propionate group each

Each pyrrole can bind two substituents (methyl and venyl - methyl and propionate and so on)

the molecule is hydrophobic

Fe has six coordinates of binding (4 with the nitrogens of the rings)

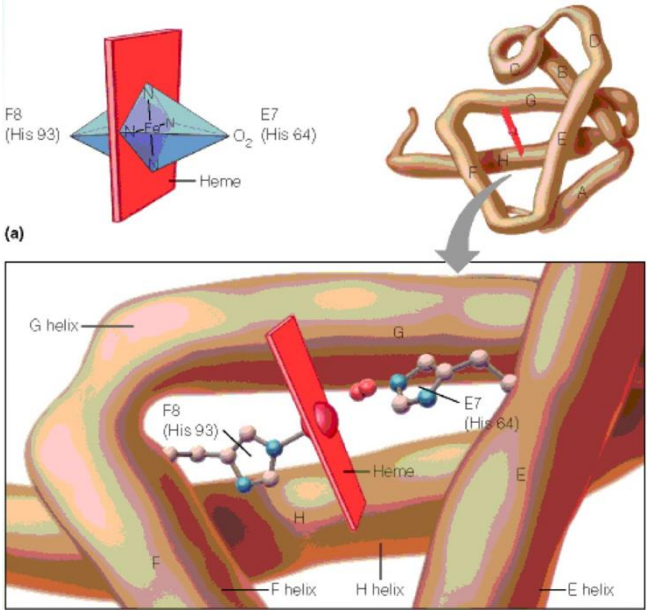
Structure of myoglobin (Mb)

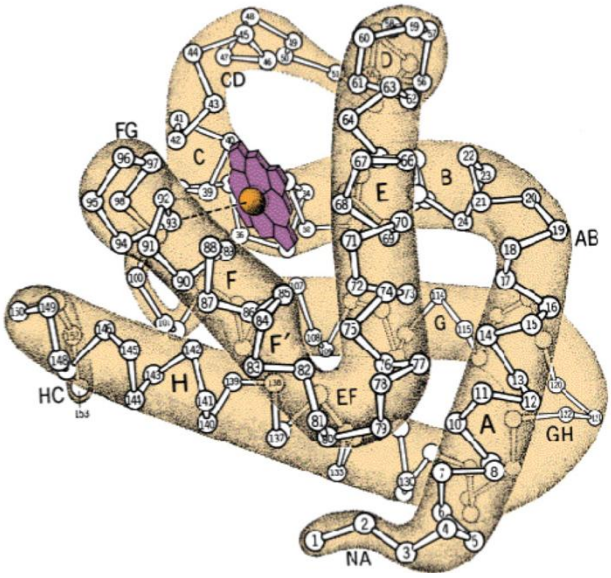
Myoglobin is a monomeric protein (monomeric protein means that the protein consists of one type of monomers which is alpha helices in the myoglobin) that is mainly found in in muscle tissue

It includes a prosthetic group which is heme group , then myoglobin is hemoprotein

It can be present in two forms 1.oxymyoglobin (oxygen-bound) 2.deoxymyoglobin (oxygen-free)

The tertiary structure of myoglobin 8 α-helices, designated A through H, that are connected by short non- helical regions , myoglobin doesn't have beta sheets





Myoglobin functions : storing O2 in muscles During periods of oxygen deprivation(lack or absence) , oxymyoglobin releases its bound oxygen.

Myoglobin is very sensitive to the O2 at any pressure , it's important because it's very sensitive at low pressure of O2 then it's very important to exist in the muscles , myoglobin collect O2 and store it to use in aerobic exercises (muscles have enough oxygen to produce energy) , The major source of energy in our muscles comes from aerobic but this doesn't mean that our muscles doesn't make any anaerobic exercises (with out O2) , anaerobic exercises causes muscle fatigue (because of lactic acid accumulation)

Iron can bind in the center of the four rings (4 pyrrole rings)

Like other globular protein, amino acid R-groups exposed on the surface of the molecule are generally hydrophilic, while those in the interior are predominantly hydrophobic Except for two histidine residues in helices E and F (known as E7 and F8) , as we say before that myoglobin consist of 8 alpha helices (A,B,C,D,E,F,G,H) now the 7 amino acid on the E (5 helice) is connected with Fe+2 and 8 amino acid in the F (6 helice) is connected to the Fe+2 the connection is between the Fe+2 and the nitrogen of the histidine imidazole , now Fe+2 has 6 bonds 4 of them with nitrogens of pyrrole rings and two bonds with two histidines now F8 histidine is called proximal histidine and E7 histidine is called distal histidine

The oxygen is bound at the sixth coordination site of the iron , residue E7 lies on the same side of the heme group as the bound oxygen then this histidine is not bound to the iron , or to any part of the heme group, but it acts as a gate that opens and closes as oxygen enters the hydrophobic pocket to bind to the heme.

Oxidation of iron to the Fe3+, ferric, state makes the molecule incapable of normal O2 binding

Upon absorption of light, heme gives a deep red color

Structure-function relationship

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

The heme group stabilizes the tertiary structure of myoglobin.

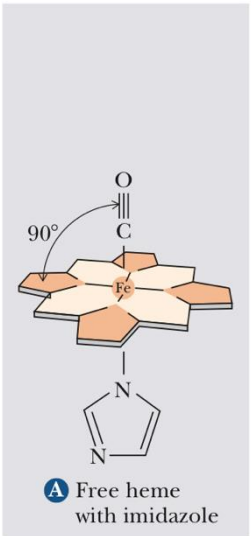
The distal histidine acts as a gate that opens and closes as O2 enters the hydrophobic pocket to bind to the heme.

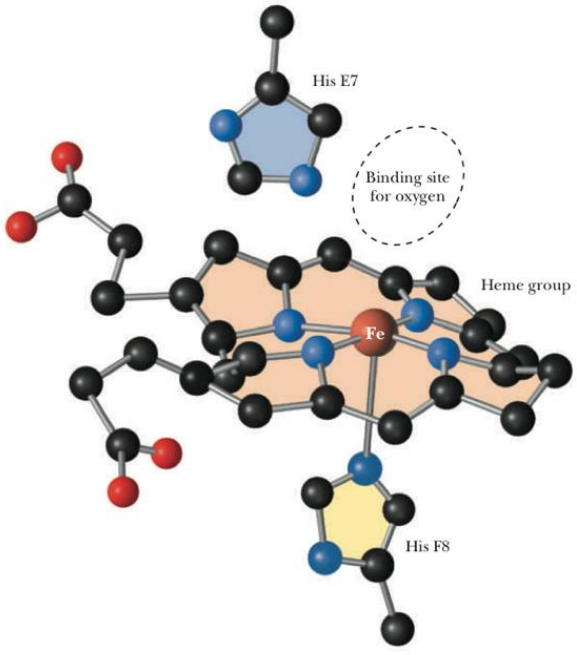
The hydrophobic interior of myoglobin (or hemoglobin) prevents the oxidation of iron, and so when O2 is released, the iron remains in the Fe(II) state and can bind another O2.

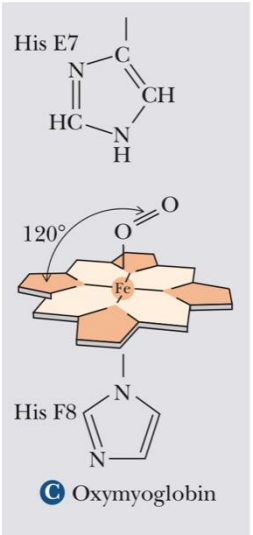
The surface of the protein contain two types of molecules : hydrophobic and hydrophilic

The function of the E7 histidine

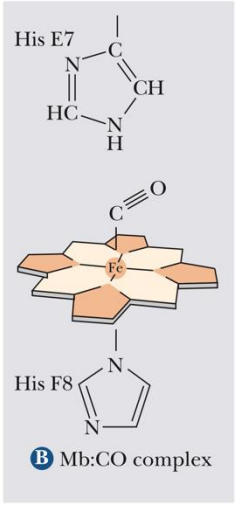
As we mention before residue E7 lies on the same side of the heme group as the bound oxygen then this histidine is not bound to the iron and act as a gate of O2 now the presence of E7 and it's steric strain make the angle between heme plane and O bonding is 120 degree







In complete combustion CO2 is produced but when it's incomplete combustion then CO is produced now the body can't handle with CO and the CO bonding is irreversible , we mean that our body can't separate CO molecule then the exchange of O2 will be affected , CO is produced when you smoke or when you inhale car smokes , heaters , ….



CO prefers straight bonding, but O2 prefer bent bonding

CO binds to free heme many orders of magnitude compared to O2 .

CO binding to myoglobin- bound heme only 250 times more than O2 .

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

Oxygen binding to myoglobin

Myoglobin binds O2 with high affinity , high affinity means that we have low p50% .

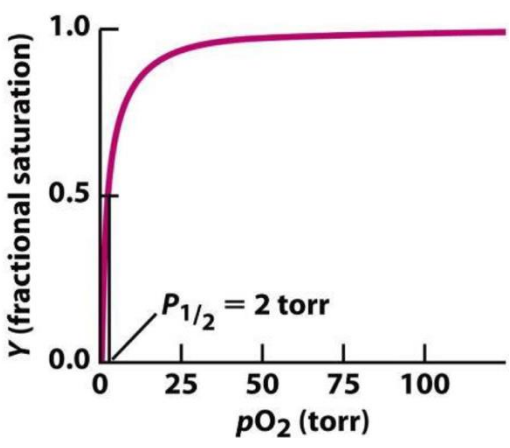
The P50 (oxygen partial pressure required where 50% of myoglobin molecules linked to oxygen) for myoglobin 2.8 torrs or mm Hg .

Given that O2 pressure in tissues is normally 20 mm Hg, it is almost fully saturated with oxygen at normal conditions , The binding of O2 to myoglobin follows a hyperbolic saturation curve.

Myoglobin has the function of oxygen storage in muscle. It must bind strongly to oxygen at very low pressures, and it is 50% saturated at 1 torr partial pressure of oxygen .

(The torr is a widely used unit of pressure, but it is not an SI unit) .

Myoglobin has low capacity of O2 if we compare it with hemoglobin (hemoglobin has 4 heme groups not 1) .

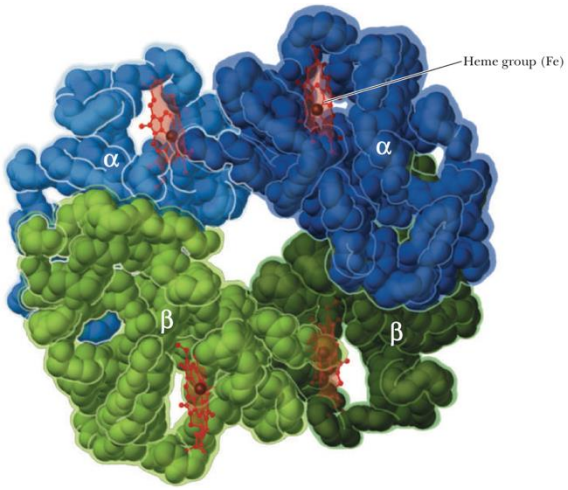


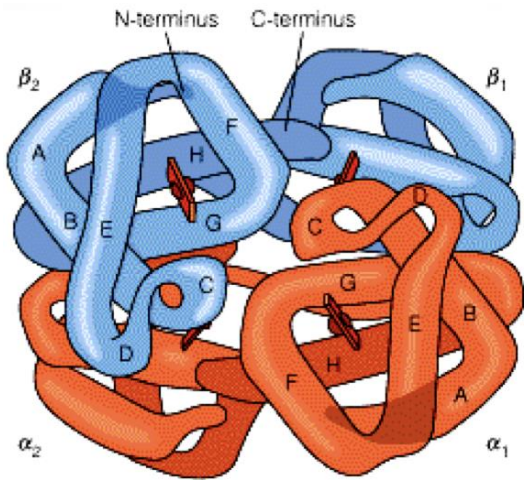
Hemoglobin (Hb) structure

Hemoglobin is tetrameric hemeprotein (four protein chains known as globins with each bound to heme , Hemoglobin (α2β2) is a tetramer consisting of four polypeptide chains (two α-chains and two β -chains) .

In adults, the four globin proteins are of two different types known as α and β, so a hemoglobin protein is an α2β2 globin protein .

The α and β chains contain multiple α-helices where α contains 7 α-helices and β contains 8 α-helices (similar to myoglobin) .





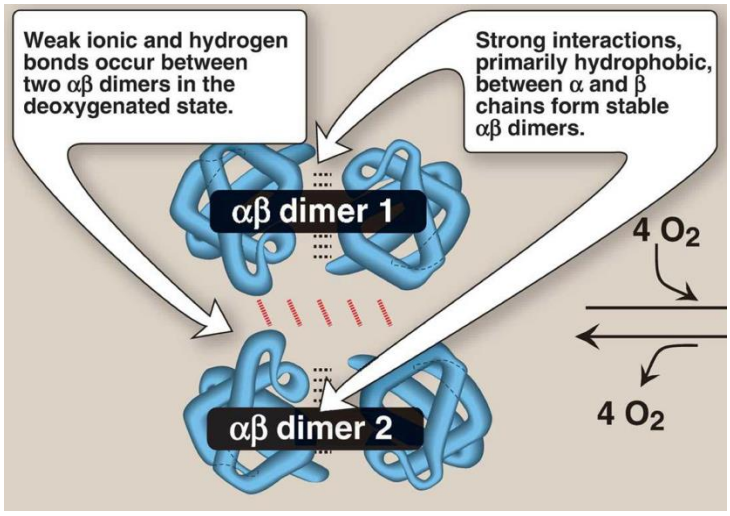
Hemoglobin functions : transport of O2 and CO2 , blood buffering .

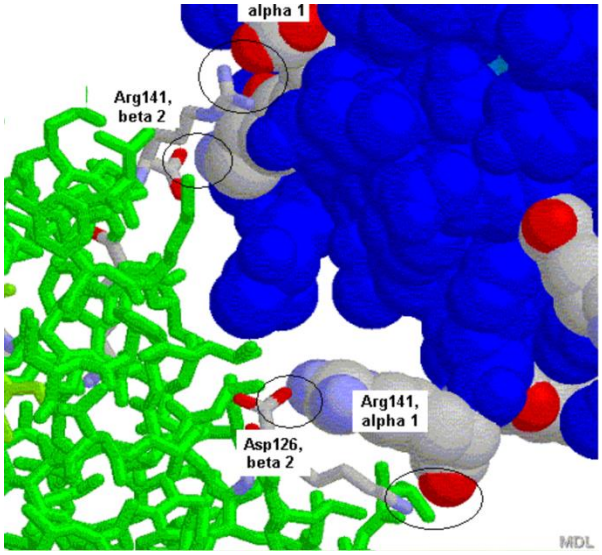
Chain interaction

The chains interact with each other via hydrophobic interactions .

Therefore, hydrophobic amino acids are not only present in the interior of the protein chains, but also on the surface.

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 the high oxygen pressure found in lungs (approximately 100 mm Hg) .

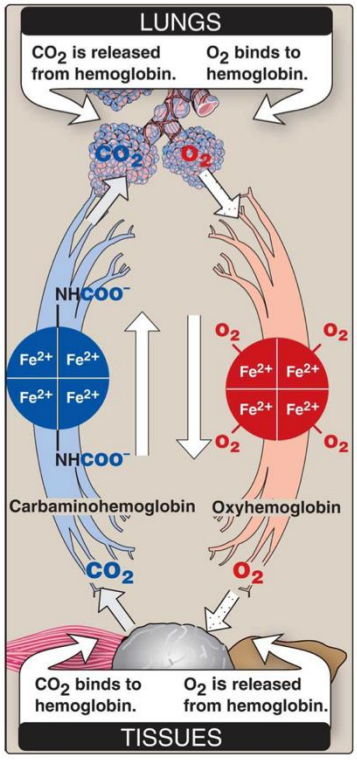
Then, it releases oxygen and become unsaturated in tissues where the oxygen pressure is low (about 20 mm Hg) .

Myoglobin located in muscles and store O2 but how the O2 arrives to the muscles ?

Hemoglobin main function is transporting O2 and CO2 now hemoglobin will be saturated until reaching the capillaries , at this point hemoglobin exchange the O2 with CO2 and go back to the lungs

now why hemoglobin act as a buffer ?

We know that we have H2CO3 buffer in our body now hemoglobin transport CO2 then this CO2 can be be used in the H2CO3 buffer , the excess of CO2 can be exhalated .



The saturation curve

The saturation curve of hemoglobin binding to O2 has a sigmoidal shape .

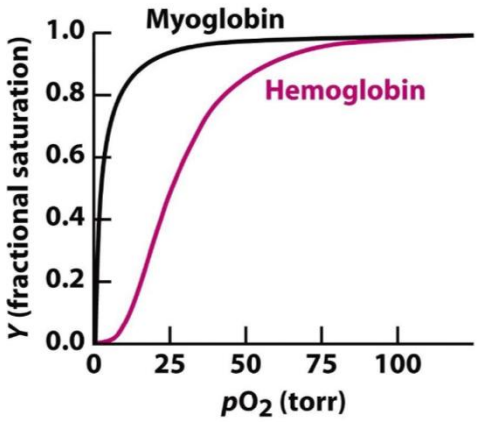
At 100 mm Hg, hemoglobinis 95-98% saturated (oxyhemoglobin) .

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

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

Myoglobin has the function of oxygen storage in muscle. It must bind strongly to oxygen at very low pressures, and it is 50% saturated at 1 torr partial pressure of oxygen .

The function of hemoglobin is oxygen transport, and it must be able both to bind strongly to oxygen and to release oxygen easily, depending on conditions. In the alveoli of lung .



Hemoglobin is allosteric

Hemoglobin is an allosteric protein (from Greek "allos"= "other",and"stereos"="shape").

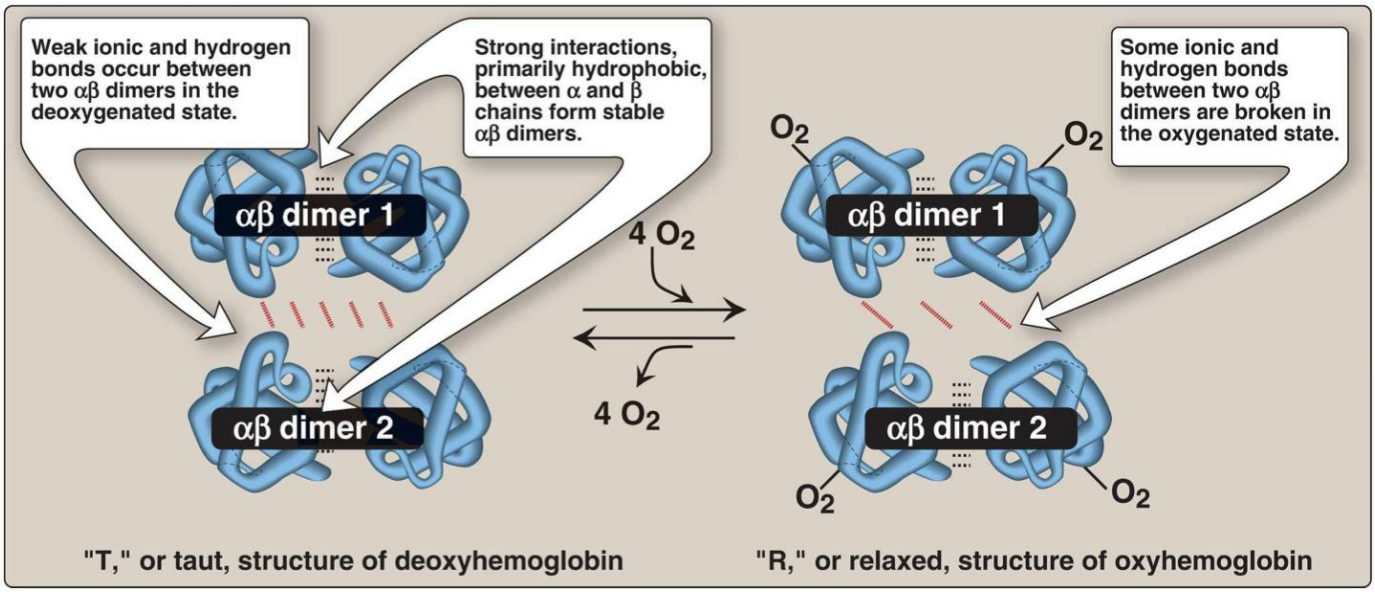
An allosteric protein: a protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or a different ligand to another part of the protein.

Hemoglobin exists in two forms : T-state and R-state .

The T-state is also 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 500 times higher affinity to oxygen than as the T conformation.

Binding of O2 causes conformational changes in hemoglobin, converting it from the low affinity T-state to the high affinity R- state .



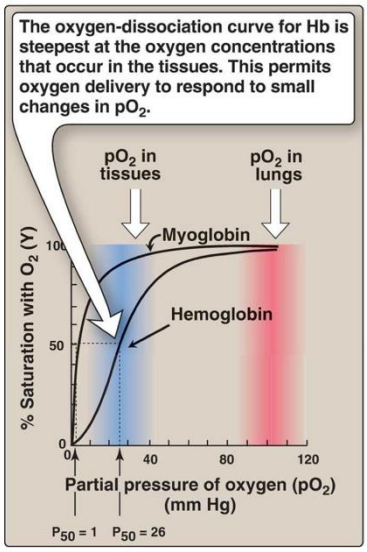
T form: The deoxy form of hemoglobin is called the “T,” or taut (tense) form. In the T form, the two αβ dimers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains. The T conformation is the low-oxygen-affinity form of hemoglobin .

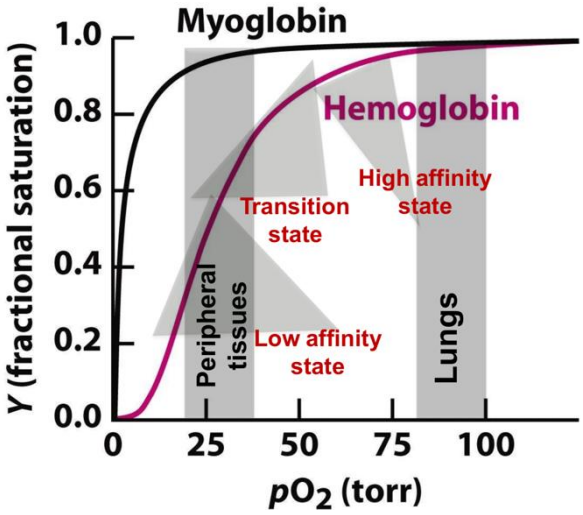
R form: The binding of O2 to hemoglobin causes the rupture of some of the polar bonds between the two αβ dimers, allowing movement. Specifically, the binding of O2 to the heme Fe2+ pulls the iron into the plane of the heme Because the iron is also linked to the proximal histidine (F8), the resulting movement of the globin chains alters the interface between the αβ dimers. This leads to a structure called the “R,” or relaxed form The R conformation is the high-oxygen-affinity form of hemoglobin .

The two structures of hemoglobin

In the previous pages we mention what happens to the hemoglobin in the T&R states , changing the environment of the hemolgobin changes the affinity .

Changes can be related with Ph or PO2-PCO2.

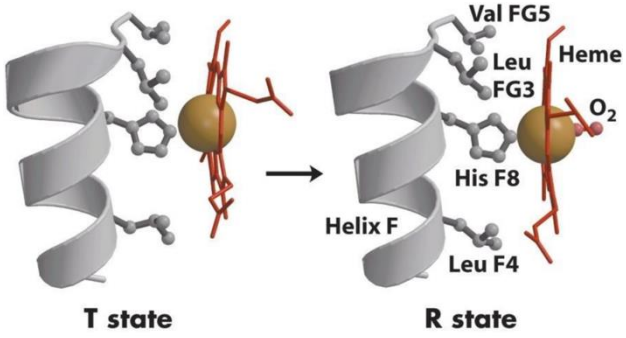


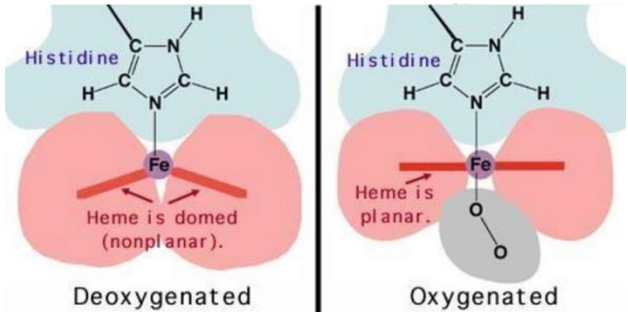


How does the structure change?

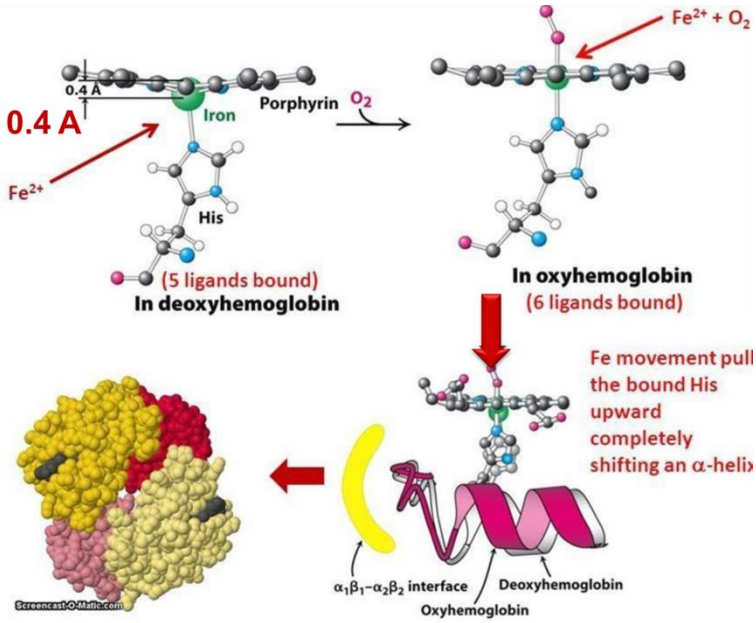
When heme is free of oxygen, it has a domed structure and iron is outside the plane of the heme group.

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) along with it.



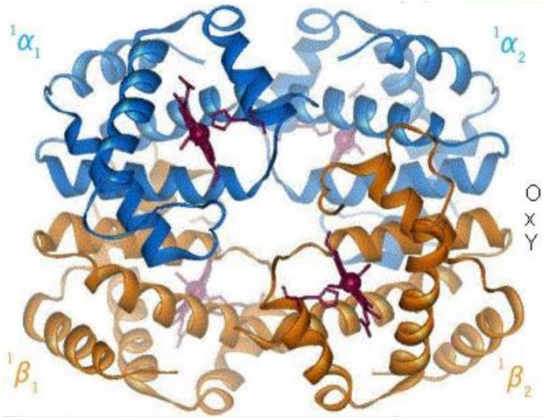


When we add O2 then the O2 will change the distance between Ferrous and distal histidine then the iron will be at the same level of heme plane (more planner) .
now we change the conformation of the molecule then the covalent interactions will decrease .



This movement triggers changes in tertiary structure of individual hemoglobin subunits breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.

In myoglobin, movement of the helix does not affect the function of the protein.



The saturation curve is sigmoidal because

Conformational changes lead to cooperativity among binding sites .

Binding of the first O2 breaks some salt bridges with the other chains increasing the affinity of the binding of a second molecule .

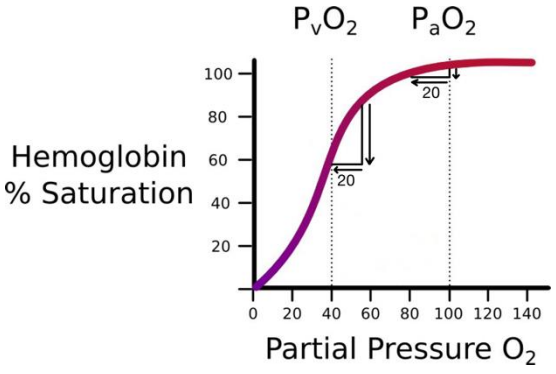
Binding of the second O2 molecule breaks more salt bridges increasing the affinity towards binding of a third O2 even more, and so on .

Binding is cooperative , notice that O2 is binding gradually and each O2 change the conformation will increase the affinity of binding with more O2 and this is called positive cooperativity .

It is a protective mechanism

A sudden drop in pulmonary capillary oxygen tension does not affect hemoglobin saturation.

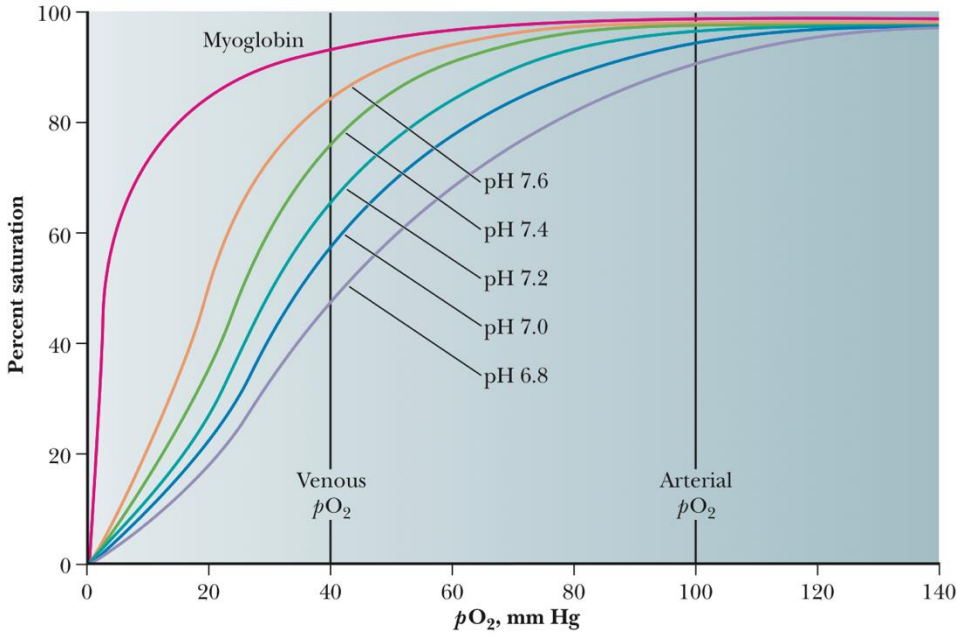
High altitudes .



In lungs we have PO2= 100, in tissues it's 20. now when someone climb a mountain then the PO2 relatively will decrease for example from 100 to 80 then the change of the pressure will be very small , but if the PO2 is very low such as 60 and then decreased to 40 then the change of the saturation of hemoglobin will be more than the change in the last one (from 100—80 drop) , this means that if we have very low PO2 then hemoglobin will release O2 with high efficiency and that's a protective mechanism .

When we add 4 O2 to hemoglobin the sensitivity of O2 will be less than the case when we add O2 for myoglobin .

Last note (I'm not sure that we need to know it , but I will write it just in case) : The oxygen-binding ability of myoglobin is not affected by the presence of H+ or of CO2 .



Quick quiz (T/F)

Myoglobin is composed of one polypeptide chain and hemoglobin is composed of four ( )

In comparison with hemoglobin , Myoglobin is more saturated with oxygen at a lower pressure ( )

After binding of oxygen to the heme group of hemoglobin , the iron atom of the heme group becomes at the level of the plane ( )

After binding of oxygen to the heme group of hemoglobin , the proximal histidine is pulled upward the polypeptide chain attached to it ( )

iron center in the heme coordinate 6 bonds ( )

The dissociation curve for myoglobin is hyperbolic ( )

The the T-form of hemoglobin has the least affinity for oxygen ( )

Circulating hemoglobin reach half saturation in the peripheral tissues ( )

Distal histidine holds oxygen in place when it binds heme in myoglobin & hemoglobin ( )

P1/2 of Hemoglobin equal 26 p1/2 of myoglobin ( )