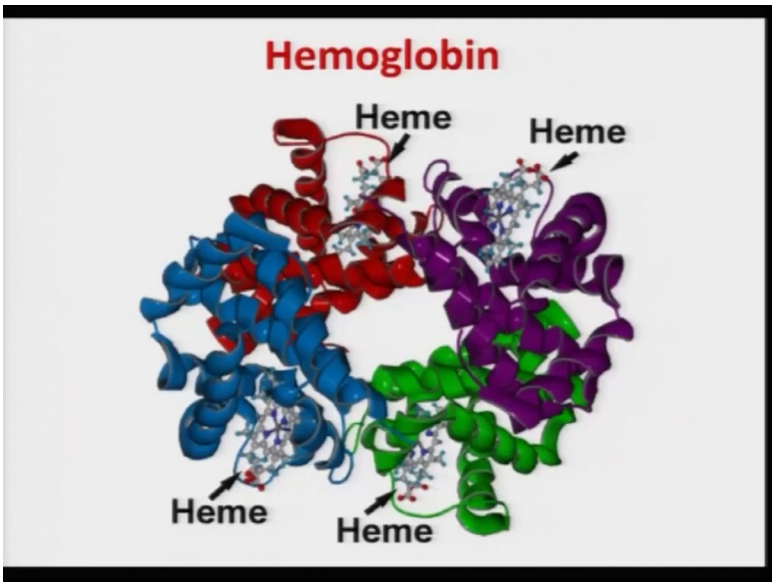
Bioinorganic Chemistry
Prof. S. P. Rath
Department of Chemistry
Indian Institute of Technology, Kanpur

Lecture - 17
Life with Oxygen: Reversible O_2 - Binding & Transport

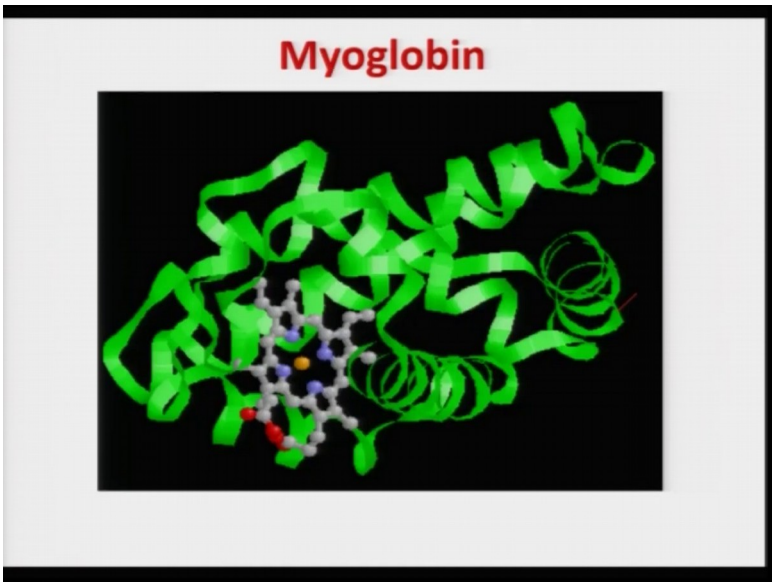
Hello everybody, and welcome back to this short course of "Bioinorganic Chemistry". Most organism require molecular oxygen in order to survive. We have been discussing various dioxygen carrying proteins in our life. In my last lecture, I was talking about hemoglobin and myoglobin in our body.

(Refer Slide Time: 00:49)



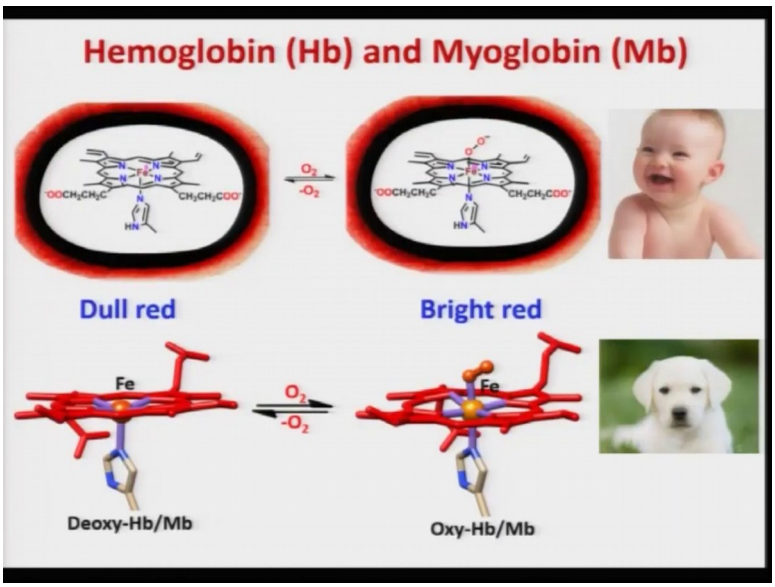
As I have discussed that hemoglobin is tetramer, as you can see that this is one heme, two heme, third, fourth. There are four heme centers in hemoglobin which is wrapped by huge protein chains around it. While in myoglobin this is the mono heme center and here also huge protein chains are surrounding these heme centers.

(Refer Slide Time: 01:05)



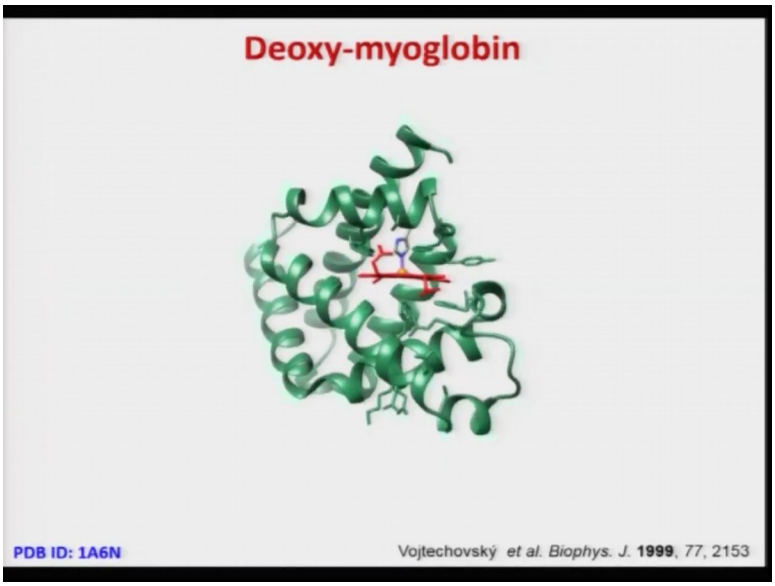
I had discussed that how the heme centers binds dioxygen in case of hemoglobin and myoglobin.

(Refer Slide Time: 01:19)



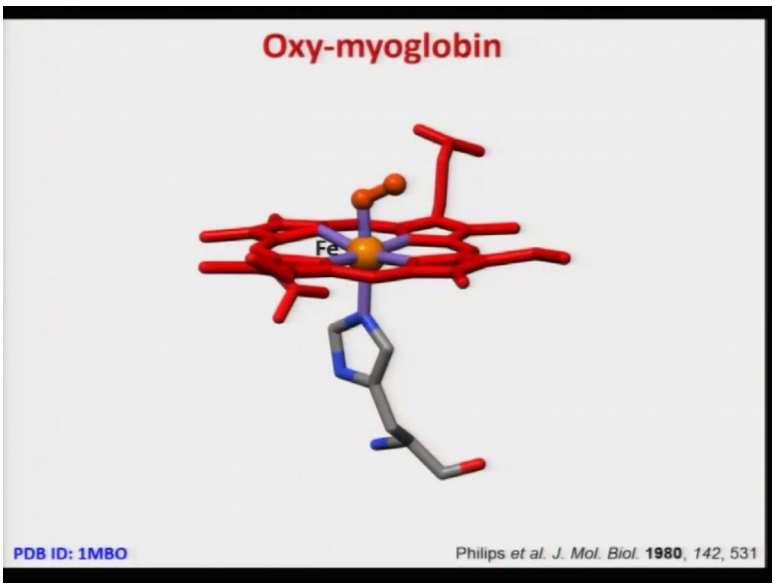
As ones can see this while Fe(II) heme center converts to Fe(III) in oxy-hemoglobin or myoglobin then dioxygen actually converts to O_2^- which is superoxides. During this process there is a color change happening, in deoxy form it was a dull red while it becomes bright red after dioxygen binding. The X-ray structure of monomeric unit is shown here, you can see that dioxygen binds to iron center in an angular fashion.

(Refer Slide Time: 02:15)



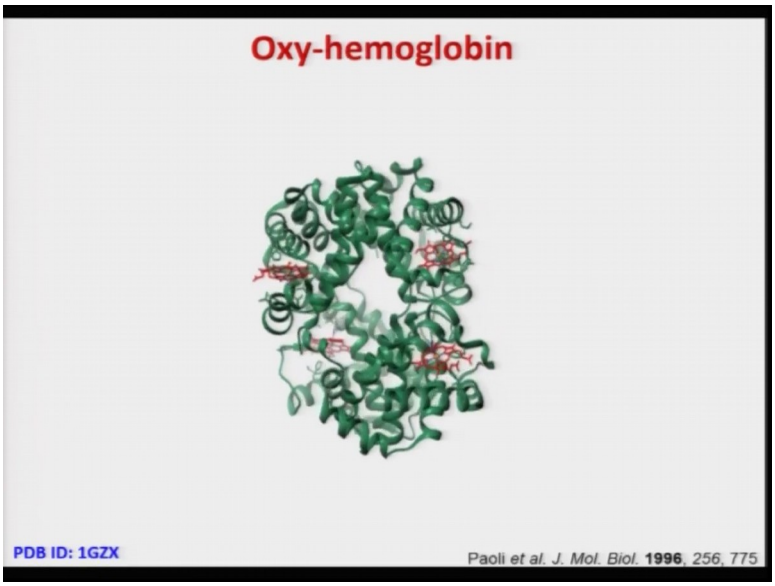
So, deoxy-myoglobin is shown over here and as one can see that the heme center which is sitting inside the huge protein chains which can easily be visualized just by removing proteins and as one can see this is the iron center which indeed binds dioxygen. The oxy-myoglobin structure is also shown, I have discussed in my previous lecture in details you see that the dioxygen binds to the iron center in an angular fashion.

(Refer Slide Time: 02:57)



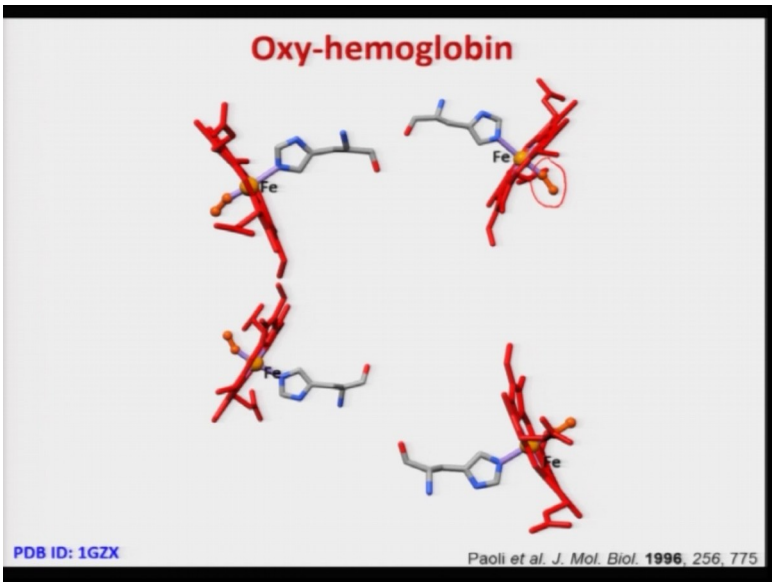
And distal histidine actually plays a role of stabilizing this dioxygen binding on iron.

(Refer Slide Time: 03:07)



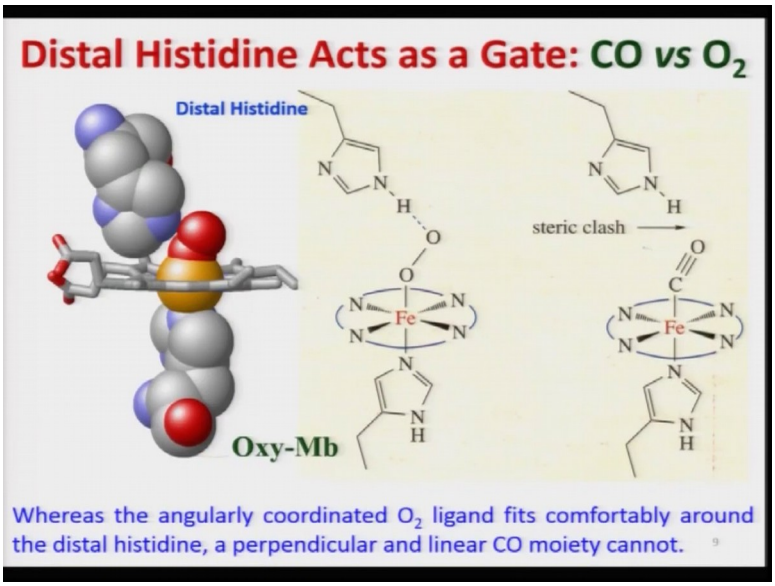
And oxy-hemoglobin as also you can see that there are four heme centers and huge protein chains which are actually covering and which are also separating these two iron centers.

(Refer Slide Time: 03:25)



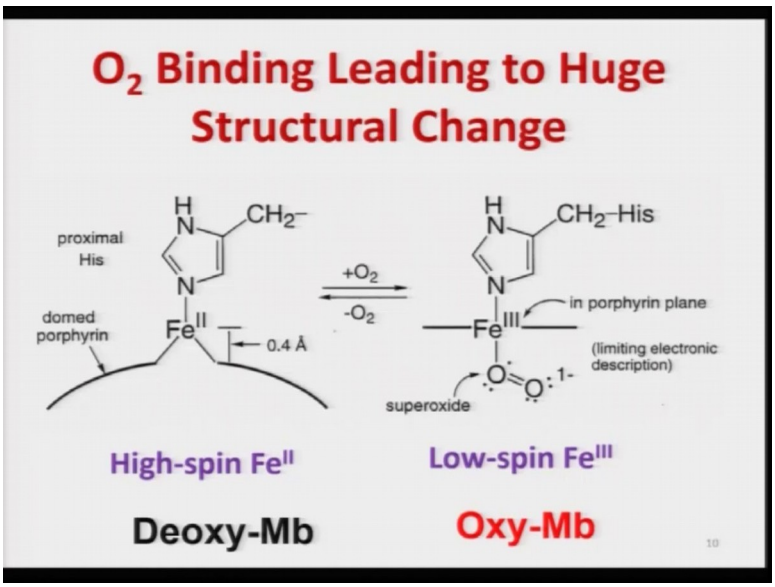
And once you remove these protein chains you see that these are four units, four heme centers and all these four coordinated dioxygen and dioxygen is in angular fashion.

(Refer Slide Time: 03:43)



I also have discussed how the distal histidine acts as a gate and discriminates oxygen from carbon monoxides.

(Refer Slide Time: 03:49)

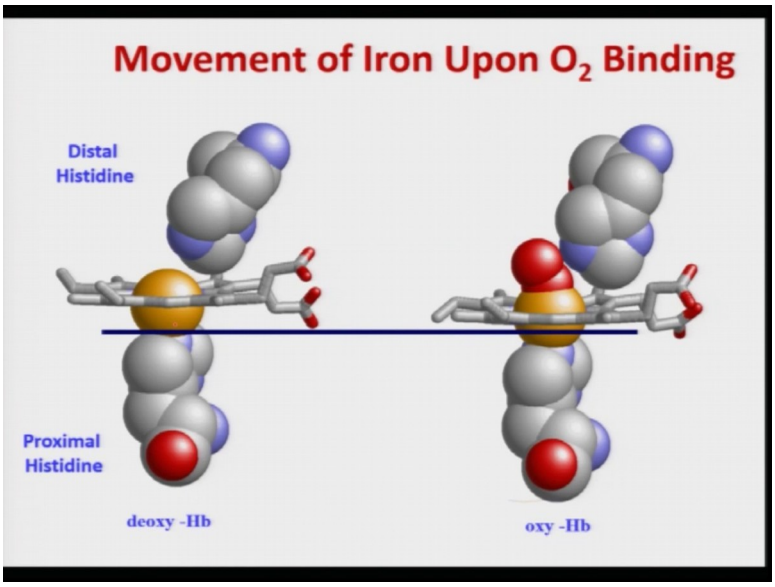


However, we also have seen that once dioxygen binds to the iron center there is a huge structural changes happening around this iron. So, what is exactly happening? This iron two which is in high spin state and which is also displaced towards this proximal histidine upon oxygenation, this deoxy converts to oxy form and iron center comes at the

plane of this porphyrin ring. Whereas, dioxygen becomes superoxides O_2^- and due to such displacement there is a huge change around the periphery of this heme center.

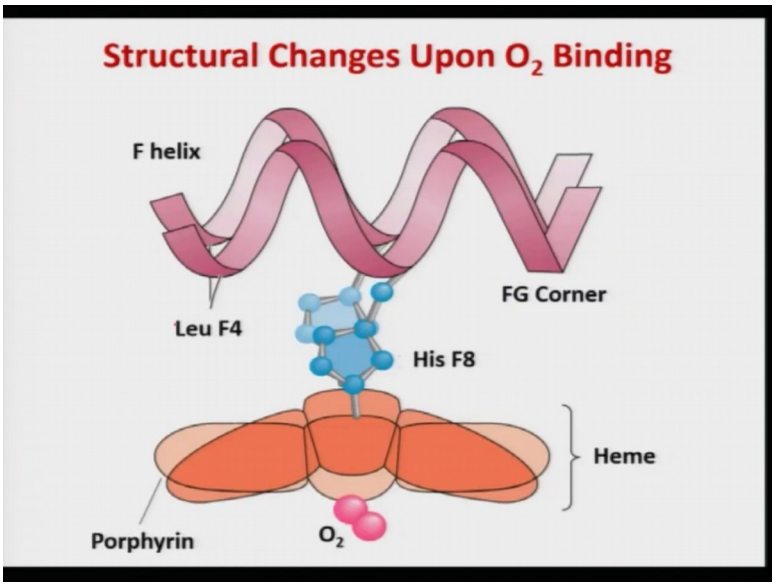
Here I have shown how dioxygen binding leading to huge structural change. In deoxy form which is five coordinated iron center and iron is in high spin state and when dioxygen comes and binds to the iron center. So, Fe(II) converts to Fe(III), while dioxygen converts to superoxide O_2^-, also iron two which was displaced towards this proximal histidine site and sitting about the mean porphyrin plane now, coming back to the porphyrin plane and thereby there is a huge structural and conformational change at the heme center and around the protein chains.

(Refer Slide Time: 05:43)



As one can see this movement of iron upon dioxygen binding that in deoxy to oxy, the distal histidine forcing dioxygen to bind in angular fashion. And also, another thing is happening as I have already discussed that iron center in deoxy which was it is a five coordinated species which was above then the mean porphyrin plane. Now, comes inside the porphyrin plane. So, it fits perfectly on the porphyrin plane. So, there is a movement of iron from deoxy to oxy is happening. This is a huge consequence and in next slides we will be talking mostly on that.

(Refer Slide Time: 06:35)

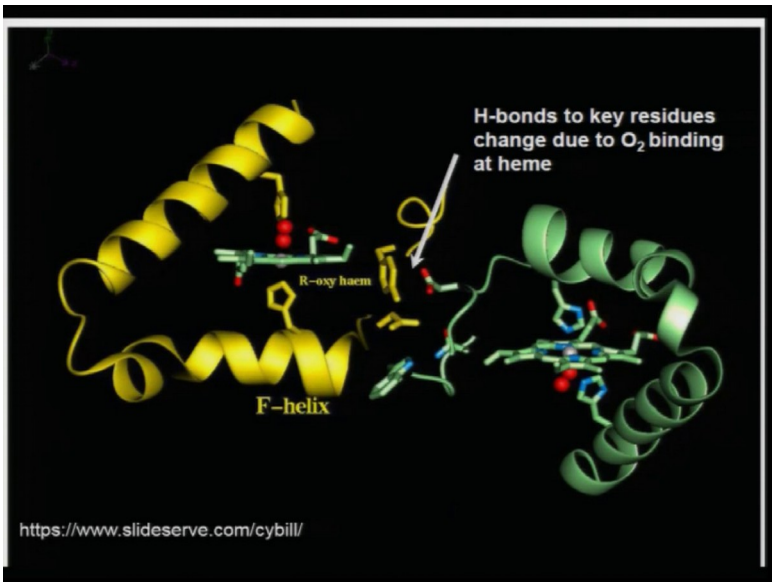


So, there is a huge structural change upon dioxygen binding. Here you can see that the proximal histidine which is ligated at the fifth position and it moves a lot before and after dioxygen binding which is clearly reflected in this diagram. Heme center also which were highly domed (Refer Time: 07:03), now, becomes planar after dioxygen binding.

So, there is a change in the heme porphyrin ring, there is a huge change on iron displacement, there is a huge change on proximal histidine. And as proximal histidine is also linked with this protein chains, so it makes a huge change in the entire protein around this molecule and which indeed generates cooperativity particularly in case of hemoglobin. Because all this is a hemoglobin is a tetrameric unit and they are all linked the protein chains are wrapping. All these four units in such a way that there is an effect between all these heme centers upon subsequent dioxygen binding which we will be discussing in next few slides.

So, this is the origin of Cooperativity that we often see in case of hemoglobin.

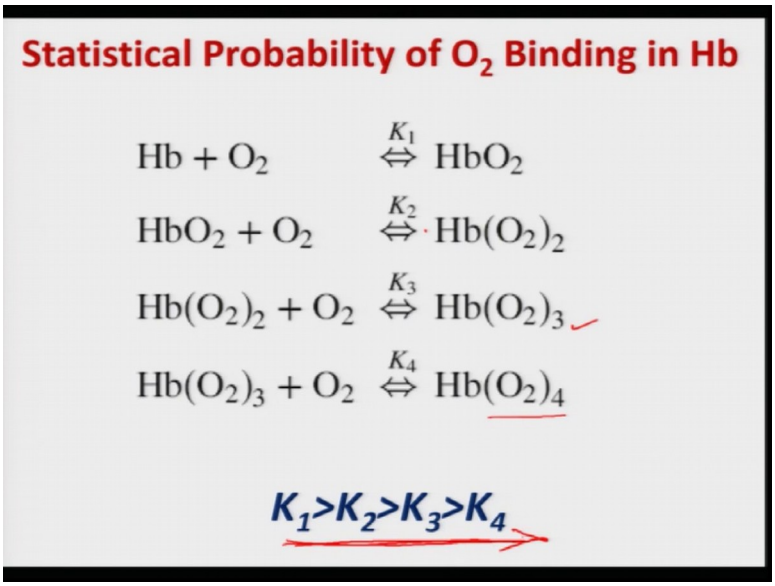
(Refer Slide Time: 08:17)



As I have said that this is the deoxy and oxy, there is a huge structural change and this protein chains they also are linked within hydrogen bonding, salt bridge interaction. And as you can see that there are two heme centers over here, one heme over here one heme over here.

Now, once a dioxygen binds you see that what would happen that there is a huge structural change and some of these hydrogen bonding actually breaks and there is a huge conformational change in the protein also. We have seen the structural change around iron centers, so there is a huge conformational change within the proteins after dioxygen binding which indeed generates that cooperativity.

(Refer Slide Time: 09:15)



Now, let us look at the statistical probability of oxygen binding in hemoglobin. As I have said that hemoglobin is a tetrameric unit. There are four heme centers which is wrapping a huge protein chains is wrapping, and there is a one center there is a change a small conformational change it effects all four, ok.

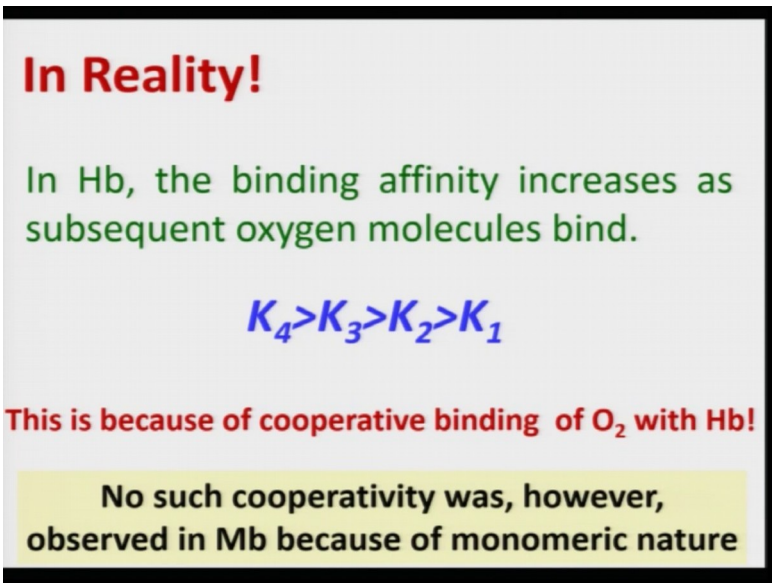
What would happen if dioxygen comes and bind stepwise? So, stepwise binding constant like if Hb + O_2 HbO_2, another HbO_2 as Hb is a tetramer. As I said that there are four heme units (Refer Time: 10:06) first one unit binds one dioxygen, then another unit binds the second dioxygen giving rise to $Hb(O_2)_2$ to then the three dioxygen, then the four dioxygen because four heme units can binds four dioxygen, ok.

Now, if you consider a simple system where these two heme center are not interacting or not linked, not influencing each other, what you can see? You can see that statistical probability of binding dioxygen will decreases in this fashion. Like what? That K_1, this is K_1, this is K_2, two dioxygen, this is third one it is K_3 and the fourth one is K_4. So, $K_1 > K_2 > K_3 > K_4$. So, the binding constant decreases in this fashion. So, that is one good aspect statistically.

(Refer Slide Time: 11:17)

So, this is just like musical chair. Many of you have seen or performed in the musical chair and as you remove one after another chairs the probability of getting or that chairs is reduced, ok. So, just I have shown you the statistical expectation is $K_1 > K_2 > K_3 > K_4$, and that you can see in the musical chair performance as well. When one chair is removed your probability get reduce, second chair removed your probability again further reduced and when you have only one chair left so probability of getting that chair is reduced, ok.

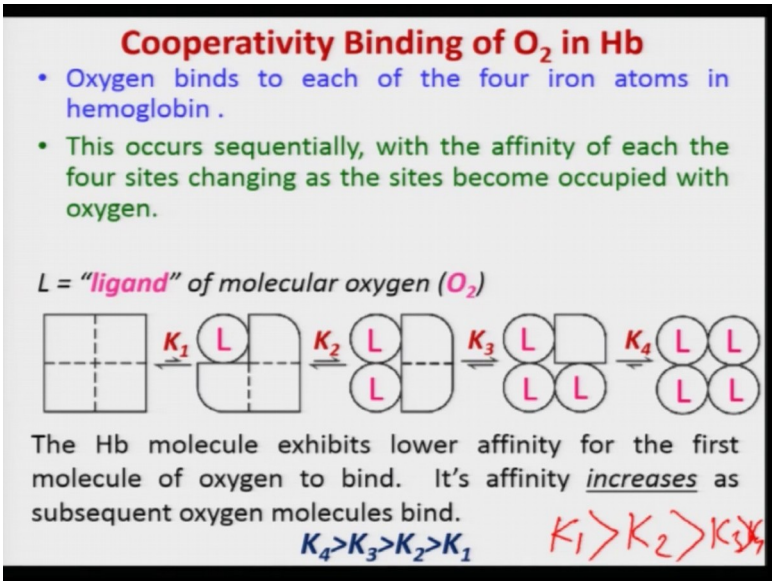
(Refer Slide Time: 12:09)



Now, what is happening in reality in case of hemoglobin? In hemoglobin the binding affinity increases as subsequent oxygen molecule bounds, it is just an opposite i.e., $K_4 > K_3 > K_2 > K_1$. This is a remarkable sequence because the statistical probability which we expected out of four heme centers are completely inverted in reality, this is what is happening in hemoglobin. Now, this is because of cooperative binding of dioxygen with hemoglobin.

Please note that no such cooperativity, however, observed in case of myoglobin because of its monomeric nature.

(Refer Slide Time: 13:15)



So, let us look at a little bit more on cooperative binding of dioxygen in hemoglobin. So, oxygen binds to each of the four iron atoms in hemoglobin. This occurs sequentially with the affinity of each four sites changing as the sites becomes occupied with oxygen. Let us think that L as a ligand of molecular oxygen. So, this is the tetrameric unit in our deoxy-hemoglobin.

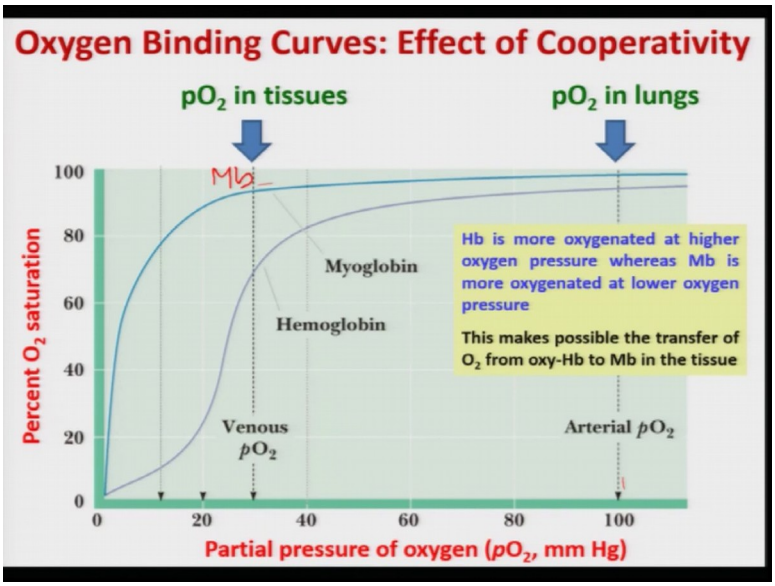
Now, one oxygen comes and occupy one heme center and binding constant is K_1, the next dioxygen comes and binds the second position, second heme center, third oxygen comes and binds the third heme center and the fourth oxygen comes and binds the fourth center. So, all these four unit is now saturated with dioxygen. So, the hemoglobin molecule exhibits lower affinity at the first molecule of oxygen to bind because of all

these salt bridge interaction which indeed are actually blocking the six position it was difficult for dioxygen to goes and bind on that iron center.

Now, more and more dioxygen binds on the iron, it becoming easier to binds dioxygen to the subsequent heme centers and that is what is happening. So, this binding affinity rather increases as subsequent oxygen molecule binds and that is the reason why we see a completely reversal of what is being expected from the statistical point of view.

So, $K_4 > K_3 > K_2 > K_1$ whereas, statistical order is $K_1 > K_2 > K_3 > K_4$ thinking that if there is no interactions through protein chains between two iron centers or two heme center, if they are isolated. So, this says that hemo in hemoglobin the heme centers are not isolated, they are actually linked through the protein chains and protein chains which actually controlling this reactivity.

(Refer Slide Time: 16:09)



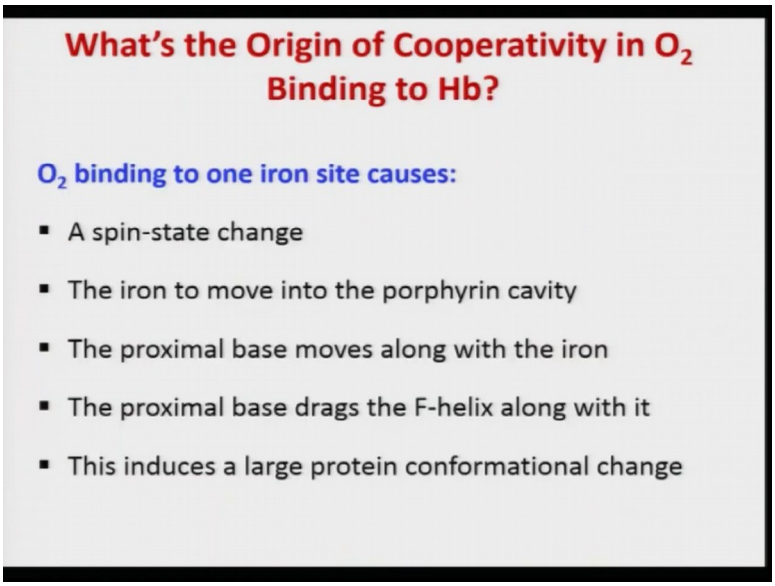
So, this is actually percent of oxygen saturation. So, the dioxygen binding and cooperativity can be clearly visualized if one see this curve. What is this plot? Here this percent of oxygen saturation is being plotted with partial pressure of dioxygen and one can see that the venous partial pressure of oxygen is very low, they are in case of tissue, in tissue the partial pressure of oxygen is low whereas, in lungs partial pressure of oxygen is very high, which is clearly reflected here.

Now, both hemoglobin and myoglobin behavior is plotted here. As you can see that this is what is the behavior of myoglobin which is basically a hyperbolic saturation curve and as you can see that myoglobin Mb is saturated at very low oxygen pressure which is present in the tissue. In the tissue, the whatever low oxygen pressure good enough for myoglobin to get fully saturated out of that.

In contrast, hemoglobin the saturation is much less, hemoglobin requires large oxygen concentration to get fully saturated which is present in lungs. So, this curve is sigmoidal for hemoglobin and which is a clear reflection of cooperative binding of dioxygen, otherwise if there is no cooperativity you could expect the hyperbolic saturation curve as one can see in case of myoglobin. This is very important.

Hemoglobin is more oxygenated at higher oxygen pressure which is present in the lungs whereas, myoglobin it more oxygenated at lower oxygen pressure such as in tissues. This makes possible the transfer of dioxygen from oxy-hemoglobin to myoglobin in the tissue. So, this is a clear reflection of cooperative binding of dioxygen in case of hemoglobin. However, this cooperativity is completely absent in case of myoglobin as we have discussed in previous slides.

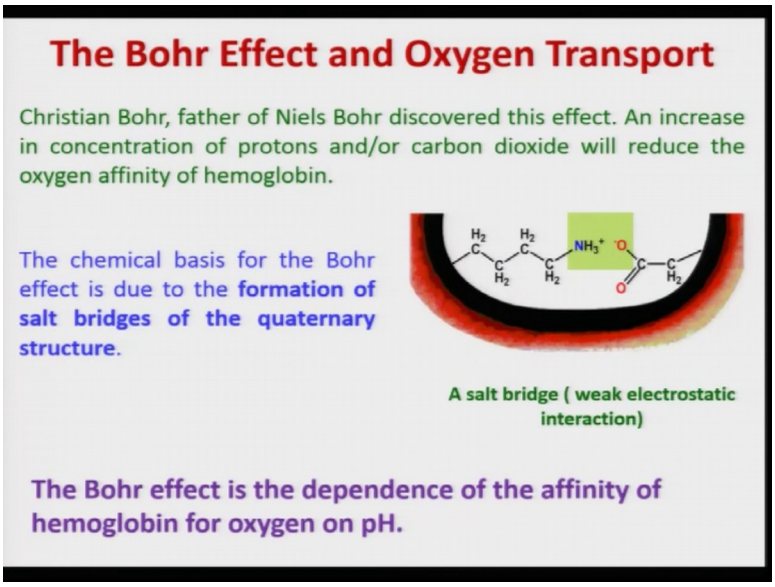
(Refer Slide Time: 18:57)



Question is that, what is the origin of the cooperativity in dioxygen binding to hemoglobin? Now, dioxygen binding to one iron site causes, first is that a spin state change converting high spin to low spin. The iron move into the porphyrin cavity in oxy-

hemoglobin, the proximal base moves along with the iron, the proximal base drags the fhelix along with it and this induces a large protein conformational change which is reflected in the cooperativity. And that is the reason why nature design two proteins, one is hemoglobin, one is myoglobin, one is having cooperativity in myoglobin there is no cooperativity. And we have seen that how this cooperativity helps to transfer dioxygen from hemoglobin to myoglobin.

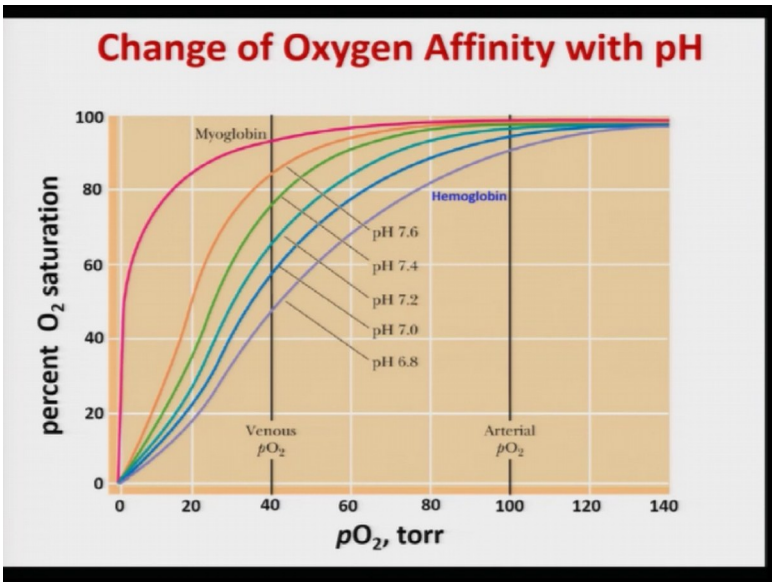
(Refer Slide Time: 20:09)



Now, there is another interesting effects happening once dioxygen binds. This is actually first observed by Christian Bohr which is the father of Niel's Bohr, all of you know about him. Actually first seen this effect and increased concentration of protons and or carbon dioxide will reduce the oxygen affinity of hemoglobin. The chemical basis for the Bohr Effect is due to the formation of the bridge of quaternary structure.

You see that this is the salt bridge interactions between two amino acid, this amine group and acid group, they have this interactions in terms of hydrogen bonding and Bohr effect is the dependence of the affinity of hemoglobin for oxygen on pH. Just by changing the pH you can also breaks some of this salt bridge interactions and this Bohr effect is very important for transporting oxygen from hemoglobin to myoglobin which we will see in the subsequent slides.

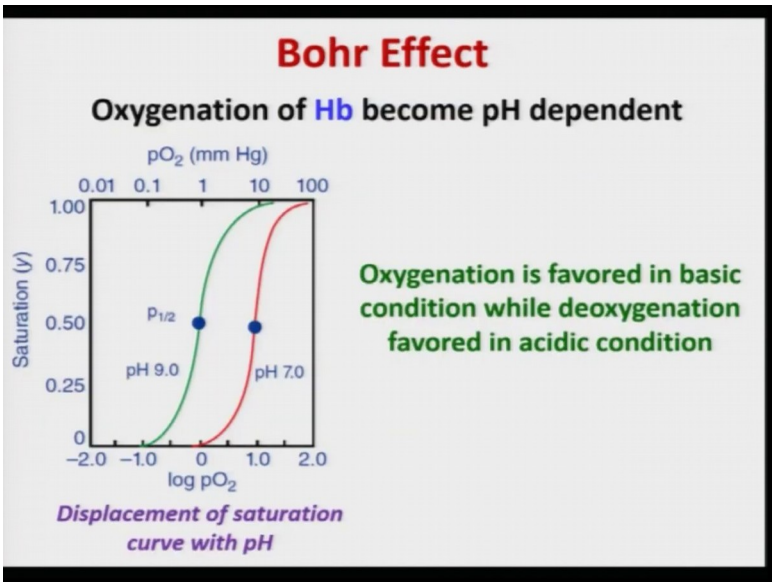
(Refer Slide Time: 21:35)



This is a curve which shows the change of oxygen affinity with pH. So, percent of O_2 saturation versus partial pressure of O_2 is plotted over here. And as one can see that myoglobin the curve is hyperbolic in nature and has greater affinity for oxygen at low partial pressure. In contrast, in hemoglobin the sigmoidal curve indicate that the binding of oxygen is cooperative in nature. Also the affinity towards dioxygen decreases as pH decreases.

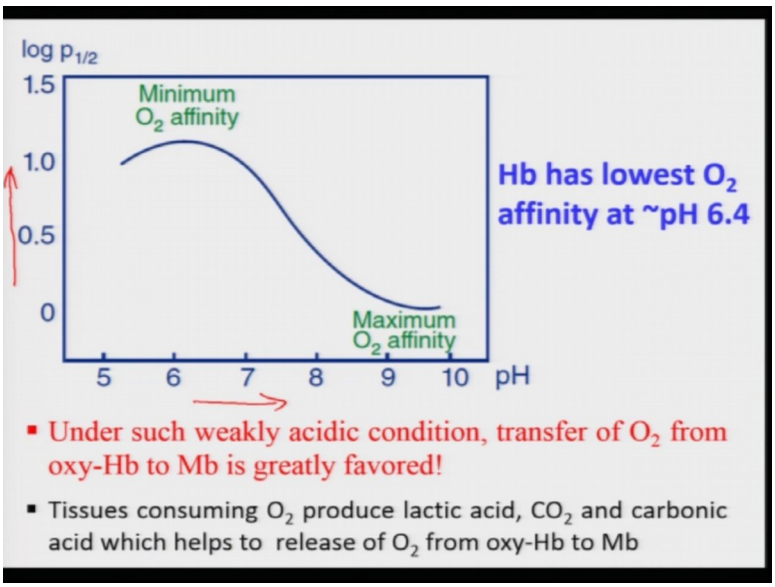
As one can see that oxygenation is favored in basic condition in hemoglobin due to the elimination of hydrogen bonding. whereas, in deoxy-hemoglobin is favored in acidic condition as shown in the curve. Due to the absence of cooperative interaction, as there are only one subunit myoglobin oxygenation does not show any Bohr effect. Oxygenation is favored in basic condition in hemoglobin, due to the elimination of hydrogen bonding whereas, deoxygenation is favored in acidic condition as shown in the curve. And what we observed?

(Refer Slide Time: 23:13)



We observe is that oxygenation favored in basic condition while deoxygenation favored in acidic condition. You can see that the saturation curve you know the dioxygen which is require to saturate for hemoglobin is more if you increase the pH in the basic medium, but in acidic medium the saturation that is require for hemoglobin its less. So, Bohr effect basically the oxygenation of hemoglobin becomes pH dependent. As one can see that oxygenation is favored in basic condition while deoxygenation favored in acidic condition.

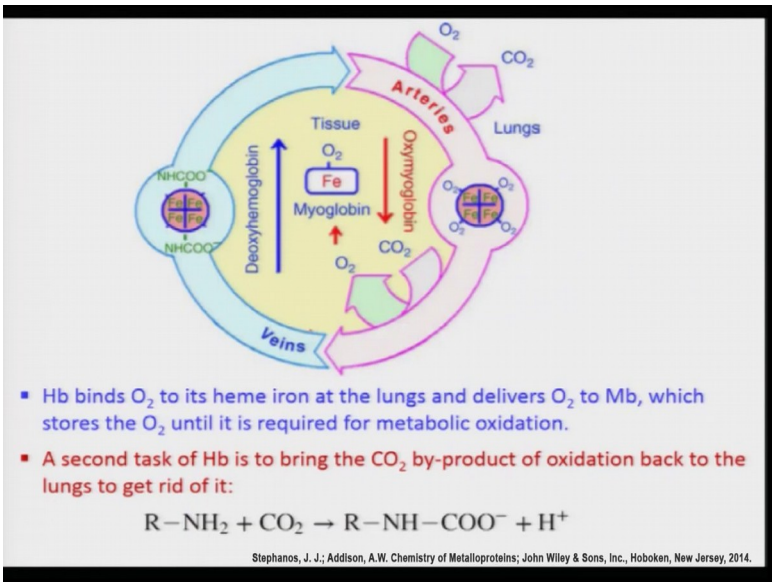
(Refer Slide Time: 24:05)



Now, this is what is being plotted, the partial pressure of dioxygen which is required for its saturation which is plotted in the y-axis, in the x-axis it is the pH is being plotted. Now, what you see in case of hemoglobin? The lowest affinity of dioxygen towards heme center is at around 6.4-6.5 pH, whereas, pH in basic medium the binding affinity is very large. So, Hb has lowest, oxygen affinity at pH 6.4. In contrast, myoglobin the affinity towards oxygen does not change much by changing pH.

So, under such weakly acidic condition so, 6.4 is basically a weak acidic condition transfer of oxygen from oxy-hemoglobin to myoglobin is indeed greatly favored. So, therefore, tissues which are consuming dioxygen produce lactic acid, carbon dioxides and carbonic acid which helps to release dioxygen from oxy-hemoglobin to myoglobin by lowering the pH of the medium.

(Refer Slide Time: 25:43)



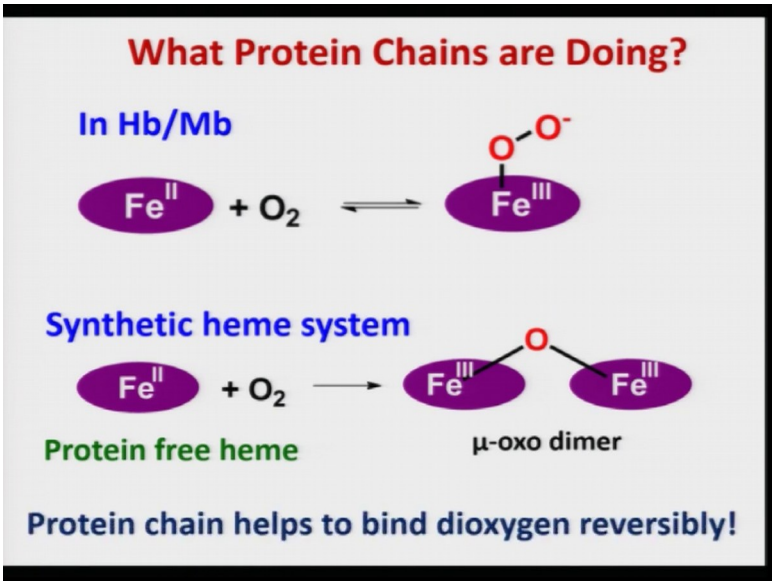
This is a carton diagram, as you know that hemoglobin takes oxygen from the environment convert to oxy-hemoglobin at the lungs, then this passes through the arteries and then it comes to the tissue where it transform dioxygen to myoglobin. And then this hemoglobin converted to deoxy-hemoglobin whereas, myoglobin converts to oxy-myoglobin which is stored in the tissue.

Now, then this deoxy-hemoglobin, it takes carbon dioxide which is released in tissue out of this respiration and converts to (Refer Time: 26:32), some of this amino acid as you can see that this amine plus carbon dioxide, this (Refer Time: 26:40) and releases a

proton. So, this is what is the cycle happening all the time in our body. So, Hb hemoglobin binds oxygen to heme iron at the lungs and delivers oxygen to myoglobin which stores oxygen until it is required for metabolic oxidation.

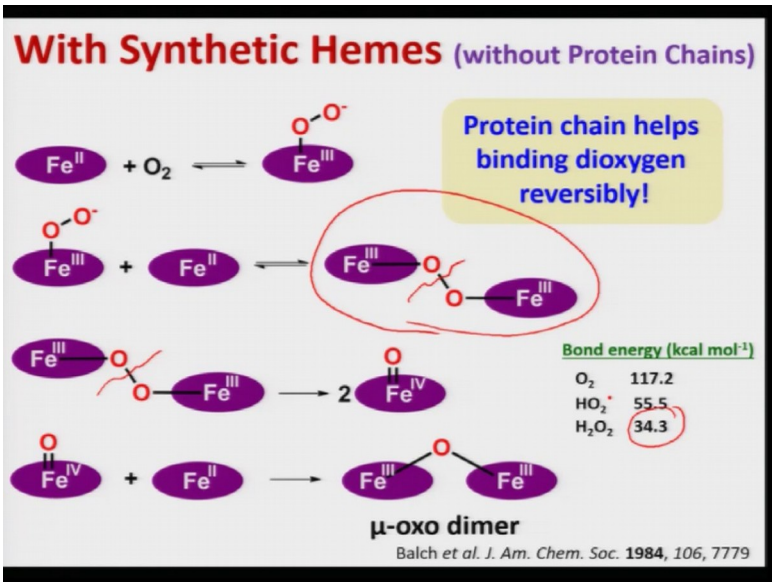
And then again this deoxy-hemoglobin is actually doing another job, its bring back the carbon dioxide which is the byproduct of oxidation backs to the lungs to get rid of it. So, actually hemoglobin does the both things together. We now try to understand what protein chains are doing. If you take hemoglobin and myoglobin what is exactly happening?

(Refer Slide Time: 27:43)



This Fe(II) heme binds to dioxygen reversibly to form Fe(III)-O_2^- and then once dioxygen leaves Fe(III) converted to Fe(II) again. So, dioxygen binding is indeed reversible in hemoglobin and myoglobin. In contrast, if you make such heme unit in the laboratory and without protein chains what would happen? This Fe(II) heme center bind dioxygen irreversibly immediately form this μ-oxo dimer which is a very stable unit. So, one can see that dioxygen binding cannot be reversible anymore because this Fe(III) no longer can convert it to Fe(II). So, this suggest that protein chain helps to bind dioxygen reversibly.

(Refer Slide Time: 28:49)



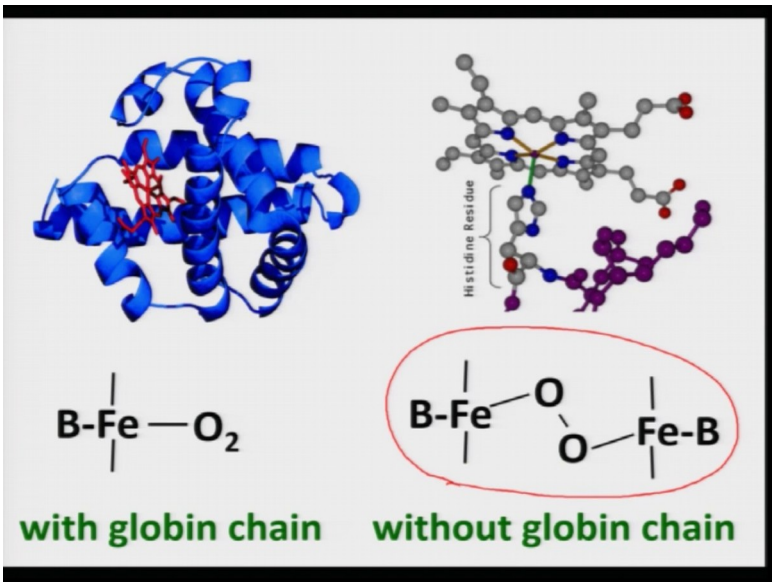
What exactly is happening with synthetic hemes? Now, Fe(II) heme binds dioxygen as observed in hemoglobin and myoglobin converts to Fe(III)-O_2^-, so metal is getting oxidized and dioxygen is getting reduced to superoxide first. Now, once it forms this O_2^- complex then this react with another Fe(II) heme and convert this μ-peroxo species iron.

As I have already informed you earlier that if you look at this bond energy of dioxygen this is 117.2 kcal/mol, if it is superoxide then this bond dissociation energy becomes 55.5 kcal/mol. However, if it is hydrogen peroxides the bond dissociation energy is very low 34.3 kcal/mol, indeed this is the state where that O-O bond get cleaved very easily in biology.

Now, this μ-peroxo species undergo O-O cleavage and converts to two (Refer Time: 30:11) ferryl heme centers, Fe(IV)=O (Refer Time: 30:13) and which is a highly reactive intermediate, which immediately binds to another Fe(II) heme center forms this Fe(III)-μ-oxo dimer which is very stable. So, this is what is being proposed for synthetic hemes which converts to μ-oxo dimer.

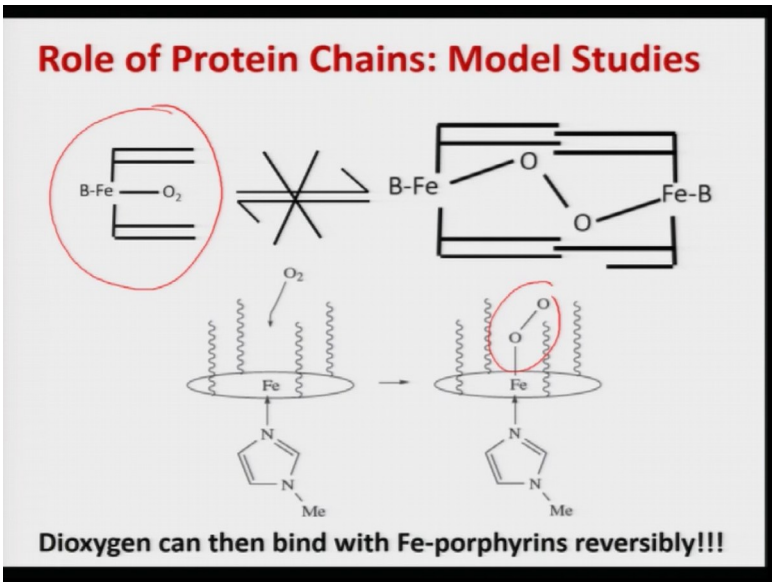
Now, in order to understand that the mechanism properly, so, what is happening? If we want to stop the formation of μ-oxo dimer, we need to stop the formation of μ-peroxo complex and actually protein chains help binding dioxygen reversibly otherwise without protein it converts to μ-oxo dimer.

(Refer Slide Time: 31:07)



Now, this is what is happening when dioxygen binds to a heme center. As you can see in case of myoglobin so, this heme center is wrapped with a huge protein chains. So, another heme center cannot come close, so that it can form μ-peroxo complex. In contrast, if you have synthetic heme center or without protein chain, what would happen? Then these species Fe(III)-O_2^- species convert to μ-peroxo species because it immediately reacts with another heme center and form this μ-peroxospecies as shown over here which eventually leads to Fe(IV)=O then (Refer Time: 32:02) μ-oxo dimer which is very stable.

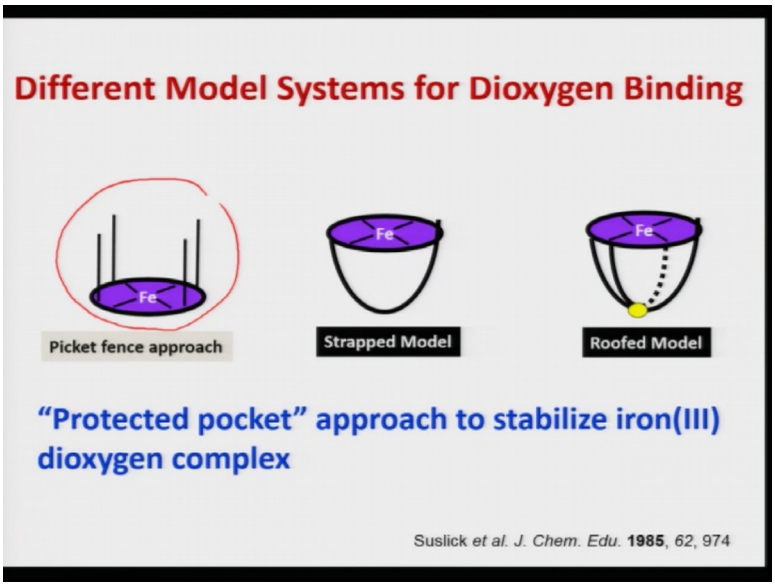
(Refer Slide Time: 32:07)



Now, how we know that protein chain is actually responsible for binding dioxygen reversibly? In order to prove that lot of synthetic chemist have made different molecules in the laboratory and one of this design I will be illustrating to you. Now, this is a heme centers having four long substituents at the porphyrin periphery which are directed towards the same site of the ring resembles the pickets of a fence. So, this if one can design such molecule then if it reacts with another heme center it is supposed to form μ-peroxo species.

However, as one can see because of the steric it will not form μ-peroxo species because there is a steric interaction between these substituents so, we can stop the formation of μ-peroxo and indeed this has happened. Say, once we make this artificial heme center with substituents at the periphery and because of the steric it will not convert to μ-peroxo rather it binds dioxygen in a reversible fashion. This proves that dioxygen can binds with even artificial iron porphyrins reversibly without protein chain if one design appropriate molecules for that.

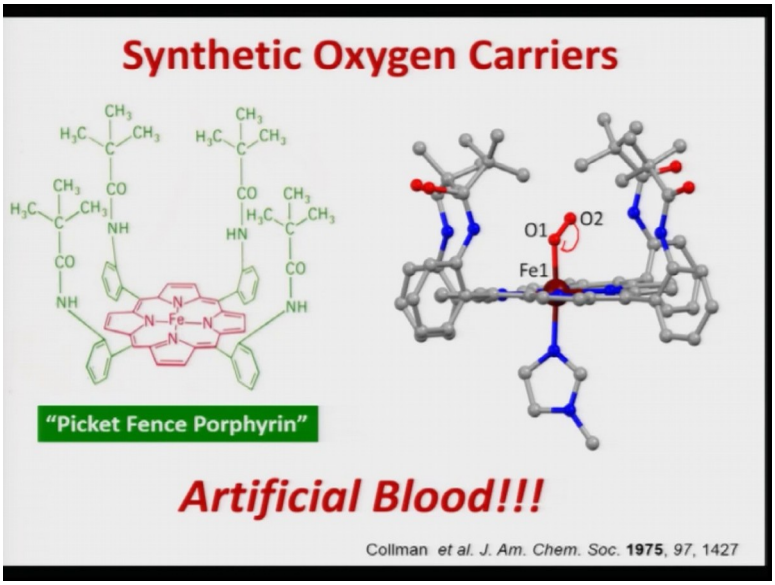
(Refer Slide Time: 34:01)



Now, there have been several such model system available in the literature for dioxygen binding and people have made protected pockets, such as picket fence approach as shown over here all the substituents are directed towards the same side of the ring. There is a strapped model also as you can see that this one side is completely blocked, so that another heme center cannot come close and there is also roofed model where one side is

completely blocked, it inhibits the formation of μ-peroxo species. However, all this molecule binds dioxygen reversibly.

(Refer Slide Time: 34:51)



I will be showing one such synthetic molecule picket fence porphyrin which is synthesized by Collman *et al.* long back and this molecule indeed able to bind dioxygen reversibly, although affinity is low. But it is possible to bind dioxygen and this molecule is also structurally characterized as you can see dioxygen binds in a angular fashion over here all these substituents are towards the same site and provide a hydrophobic environment so that dioxygen can bind in a reversible fashion.

So, there have been attempt to make artificial molecule which can carry dioxygen reversibly. If we can make this kind of artificial molecule which can indeed replace our blood, indeed one day we can make this artificial blood to fight against various blood related disease such as thalassemia, blood cancer all these problem could be solved if we can make artificial blood which can replace the real blood of our body.

In conclusion, I have discussed today how protein chains are actually responsible for reversible dioxygen binding in hemoglobin and myoglobin. I have also highlighted how the beautiful design principle makes smooth transfer of oxygen between hemoglobin and myoglobin and thereby responsible for our survival. In my next lecture, however, I will talk about heme oxygenase which is indeed responsible for the decomposition of the red blood cells.

Thank you.