Bioinorganic Chemistry
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Lecture – 16
Life with Oxygen: O_2-Carrying Proteins - Hemoglobin & Myoglobin

Hi everybody, welcome back to the short course of Bioinorganic Chemistry. Most organisms require molecular oxygen in order to survive and we have been discussing various dioxygen carrying proteins in biology.

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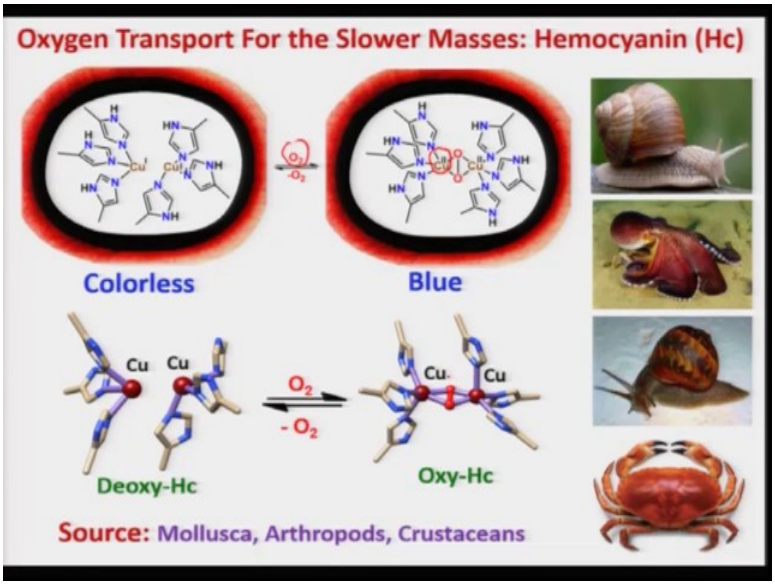

In my previous lecture, I discuss about oxygen transport for slower masse, I talk about hemocyanin and hemerythrim. In hemocyanin as one can see that this is dicopper center which is in the deoxy form. And once dioxygen binds between two copper unit then what is happening? first is there is a huge color change from colorless to blue, and then the Cu(I) center getting oxidized to Cu(II) and dioxygen getting reduced by two electron forming peroxide.

I discuss this in details in my last lecture, X ray structure of deoxy-hemocyanin and oxy-hemocyanin is also displayed over here.

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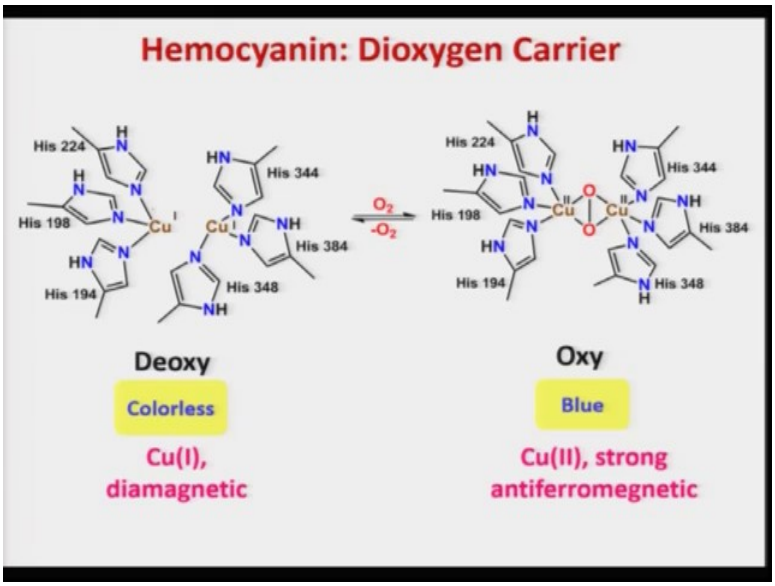

As you can see that two copper unit comes closer and bridged by the superoxide which is responsible for huge structural and magnetic change between deoxy and oxy-hemocyanin. What is happening in deoxy-hemocyanin? In deoxy-hemocyanincopper center is Cu(I) and it is a d^{10} system and thus it is a colorless and diamagnetic.

In contrast after dioxygen binding this deoxy-hemocyanin what is happening? this copper is getting oxidized to Cu(II) it is a d^9 system has one unpaired electron and both the copper center since they have one unpaired electron each. .. They undergo strong antiferromagnetic coupling through this superoxides bridge.

So, magnetic property is completely different from deoxy to oxy, as I also have discussed in details in my last lecture.

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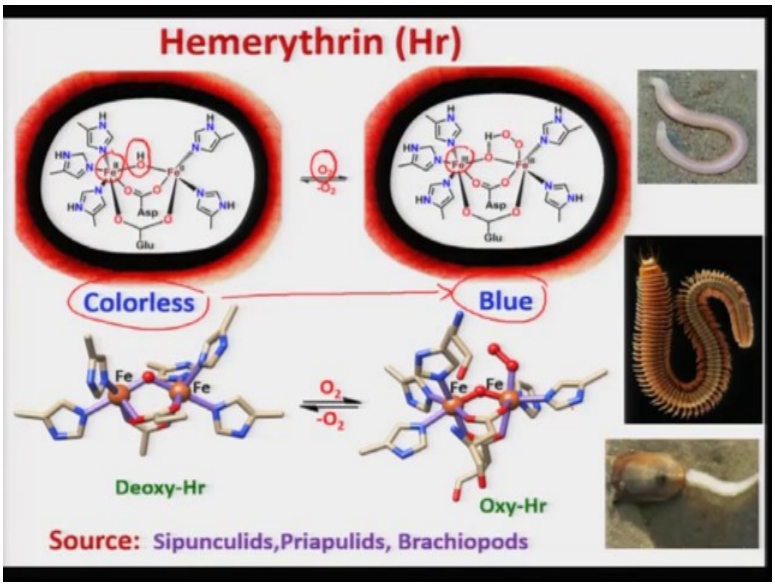

In the hemocyanin as well as one see this is a non-heme protein where two iron centers are present in the deoxy form. As one can see that two iron centers are in equivalent, one center is a six coordination, you know three histidine residue is legated to iron center. And these two iron center is bridged by aspartate and glutamate and also the two iron center is bridged by a hydroxyl group. However, interestingly one iron center is six coordinated while the other iron center is five coordinated, where indeed dioxygen comes in binds.

So, what is exactly happening? In deoxy form this Fe(II) converts to Fe(III), it's oxidize by one unit. However, the dioxygen is getting reduced by two electrons forming peroxides. Also you can see that this colorless which is in the color of the deoxy form, converted to the blue which is the color of the oxy-hemerythrin.

The X-ray structure of deoxy and oxy-hemerythrin is shown over here.

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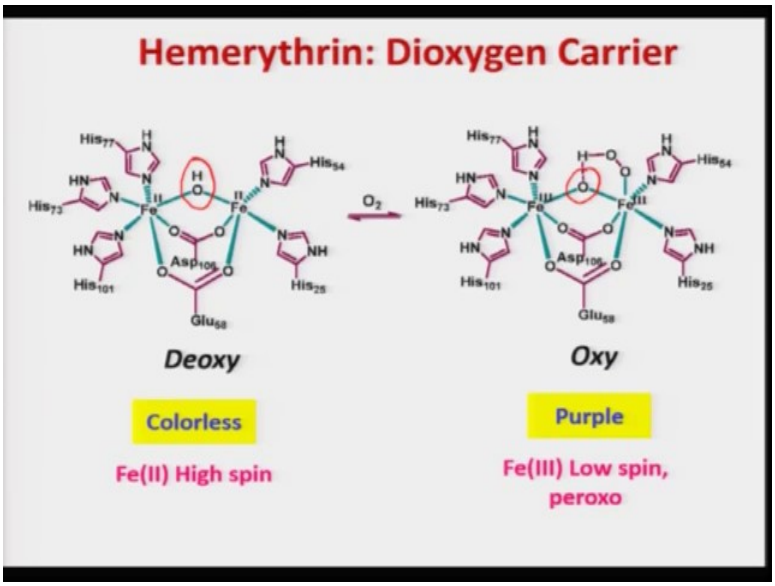

And you can see there is a huge structural and magnetic changes happening between deoxy and oxy. For example, in case of oxy-hemerythrin iron center is in Fe(II) high spin state in contrast in case of oxy-hemerythrin, this is the low spin and this is dioxygen is in peroxo form. And this hydroxo which was bridging between two Fe(II) centers in deoxy form converted to new oxo species. There is a huge magnetic changes happening between deoxy to oxy. The coupling constant in the deoxy form was less negative is around to -10 cm^{-1} and in oxy it becomes around -77 cm^{-1}.

So, I will be now talking about oxygen transport and storage in our own body. As one can see that hemoglobin and myoglobin have been designed by our mother nature and as you already know hemoglobin is responsible for transporting dioxygen, while myoglobin stores dioxygen.

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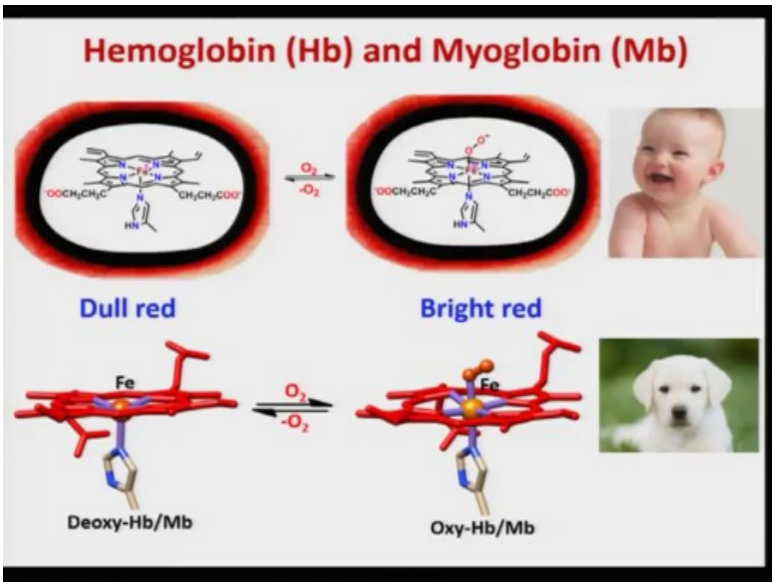

Now, this is a heme unit, a mono heme unit indeed. This is as you can see that this macro cycle is porphyrin and a iron is sitting inside the cavity of this porphyrin and the fifth position is a histidine group. Now this is five coordinated iron center which binds dioxygen reversibly, converted to a six coordinated situation, and as you can see that during this process the color also getting changed from dull red to bright red.

The X-ray structure of both the deoxy and oxy forms are shown over here. As you can see that the five coordinated iron center converted to six coordinate and vice versa upon oxygen binding and oxygen releasing. So, this is what is happening all the time in our body.

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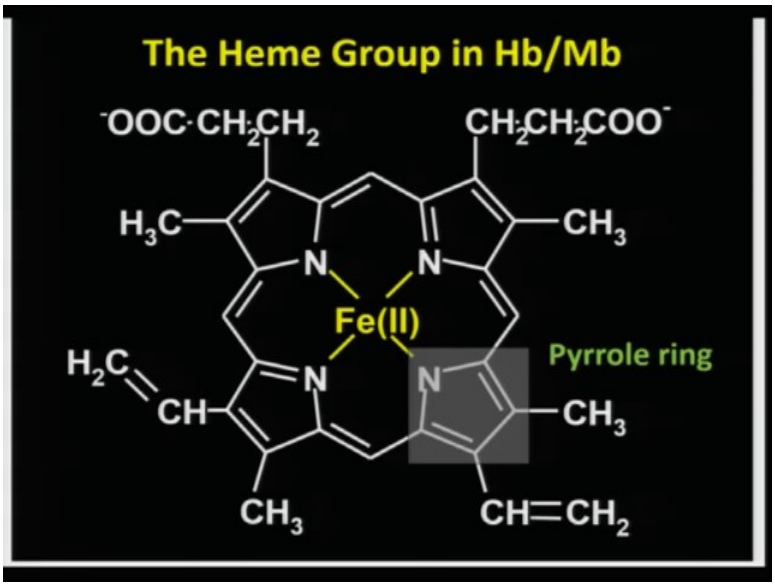

Now, this iron porphyrin, we call it as a heme group. Now this iron porphyrin macro cycle is shown over here as one can see that iron is sitting at the center of this core and these are a ligated with four macro cyclic nitrogen and also there are substituents outside this macro cycle which are responsible for it is solubility in water. Now there are four pyrrole ring 1 2 3 4, four pyrrole ring which are also undergoing a conjugation gives rise to this heme centered.

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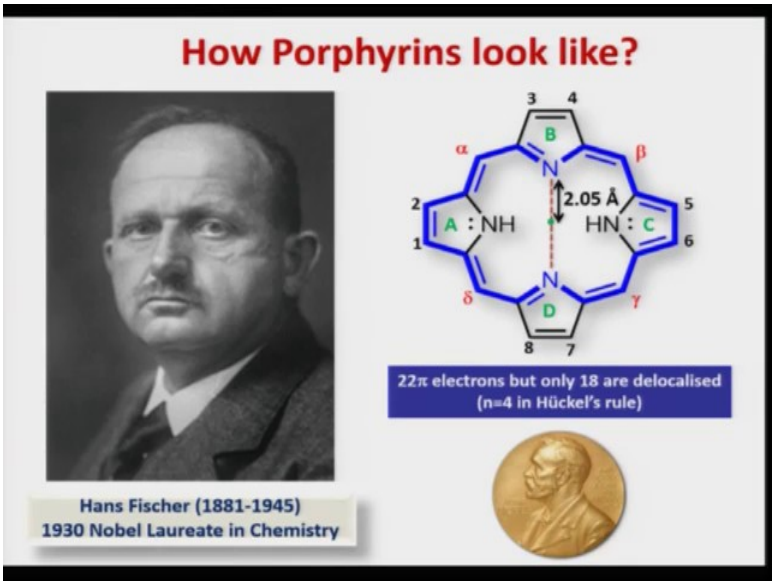

Now, this macro cycle which is porphyrin ring as I have just shown this is 22 pi electrons are there but only eighteen are delocalized. You know as for the Huckels rule N= 4 this is an aromatic ring and Hans Fischer who has synthesized this molecule in the laboratory and was awarded Nobel Prize in 1930 for doing that, it is so important in our biological system.

Now, let us look at the effect of conjugation in the porphyrin.

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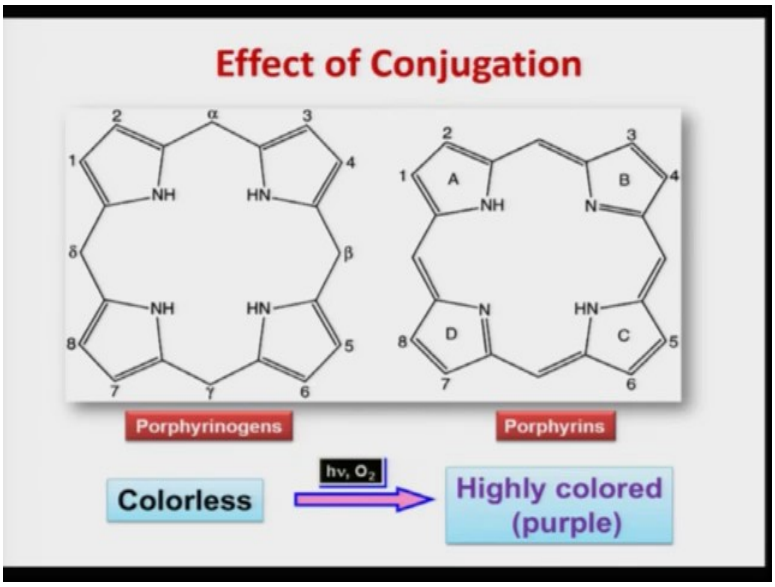

So, this is a reduce species, we call it porphyrinogens and this is the conjugated one we call it porphyrin. And this conversion from porphyrinogen to prophyrins are a very simple, please note that the porphyrinogen is colorless whereas, the porphyrin ring is highly colored, purple in color and this is because of it is conjugation, highly conjugated. And this can be easily converted using light or dioxygen, spontaneously it becomes conjugated and produces this dark purple color which is actually that what you see in your blood.

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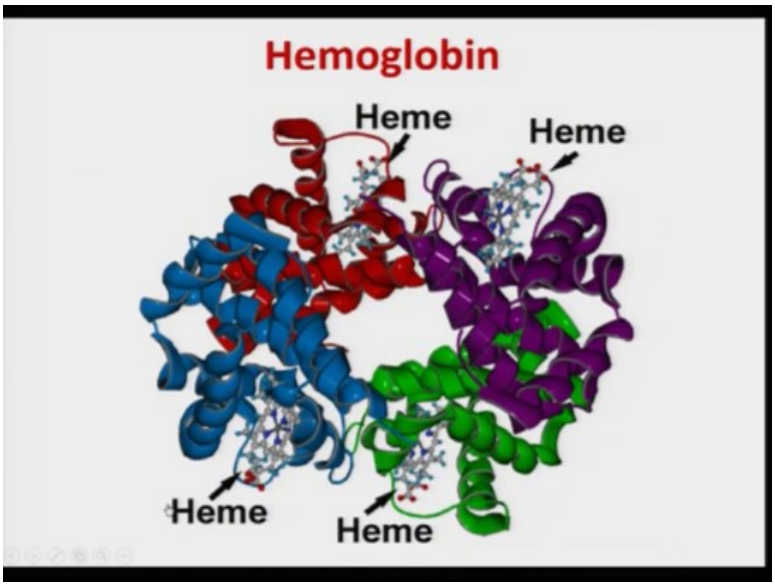

As you can see this is the structure of hemoglobin; there are four heme centers, heme 1 2 3 4 and iron is sitting at the center of this core and as one can see that there is a huge protein chains which are wrapping around this four heme unit, so we call it is a tetramer.

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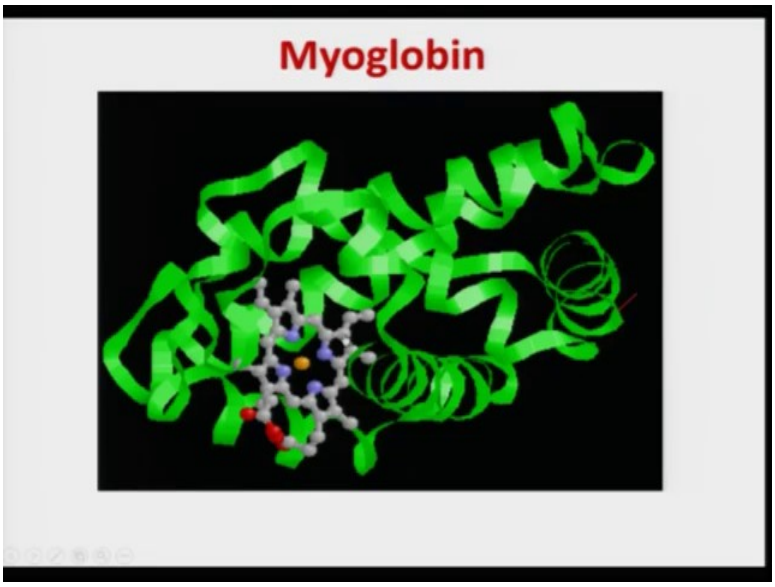

In contrast myoglobin is monomer, there is a one heme unit and as you can see that iron is sitting at the center of this porphyrin ring and it is in a monomer. And so, hemoglobin is tetrameric, myoglobin is monomeric in nature.

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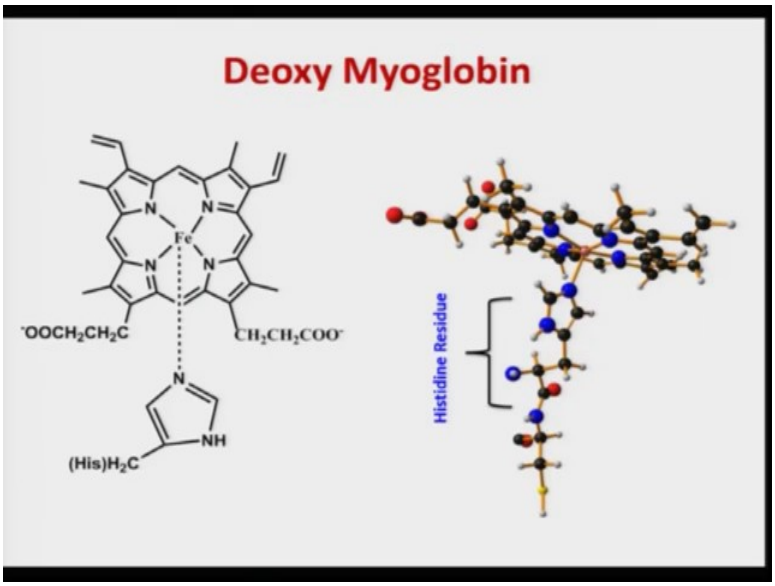

Now, deoxy myoglobin is shown over here, as I have shown that myoglobin is a monomeric unit whereas, hemoglobin is a tetramer. So, this monomer, what you see here that is a iron porphyrin which is called heme and the fifth position is ligated to a histidine group. You can see the structure X-ray structure over here that, iron porphyrin or heme, this is the iron center it is five coordinated in the deoxy myoglobin.

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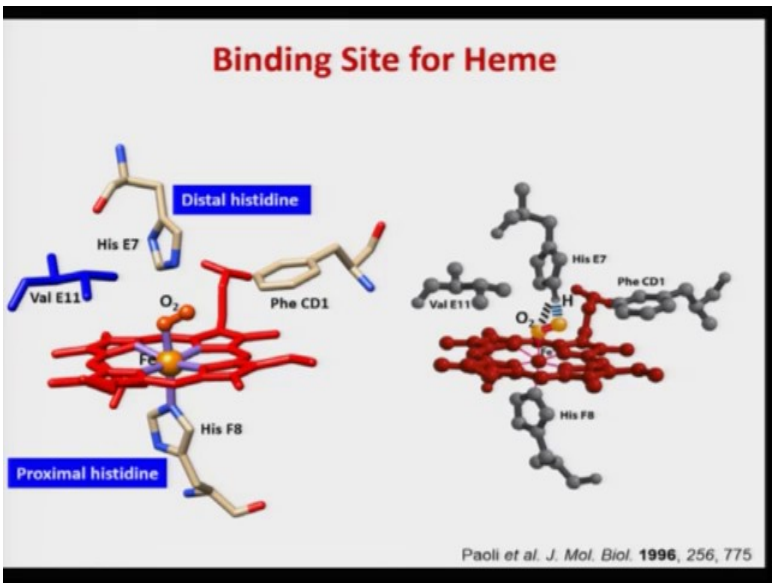

Now, upon oxygen binding what happens? this it forms the oxy-myoglobin and as you can see that the dioxygen binds at the sixth position. Now this dioxygen also hydrogen

bonded with the distal histidine which is actually placed at the top of iron heme, which is not ligated to iron. However, this is there just above this iron center and dioxygen can form in hydrogen bonding with this histidine N-H proton and thus stabilize this confirmations. Dioxygen binds angular fashion, you can see here.

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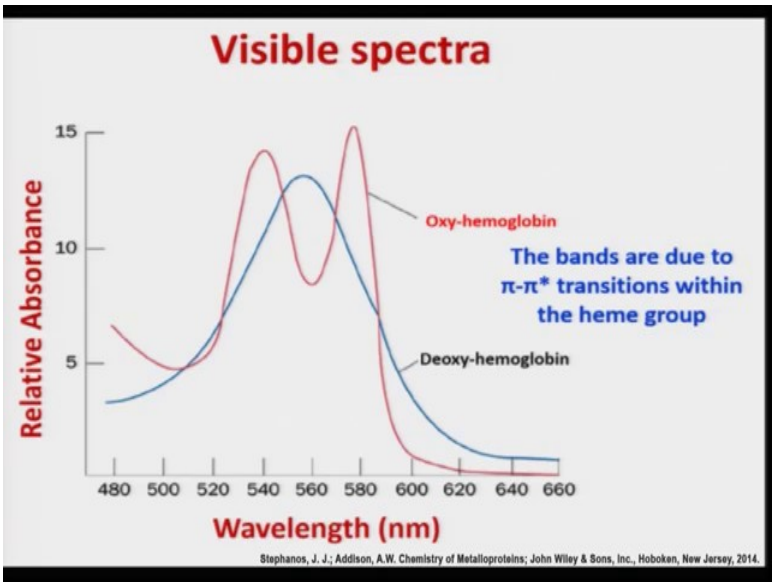

Now, during this transformation from deoxy to oxy, there is a huge spectrum change and that visible spectra is shown over here.; As one can see that, the red one is the visible spectra of oxy-hemoglobin. You can see there are two bands and because of deoxy, it was only one band which is blue in color. So, just by looking at the visible spectra one can easily see whether it is an deoxy form or an oxy form., because deoxy to oxy there was a one band converted to two bands and this bands are actually due to π-πtransition within the heme group not from the protein.

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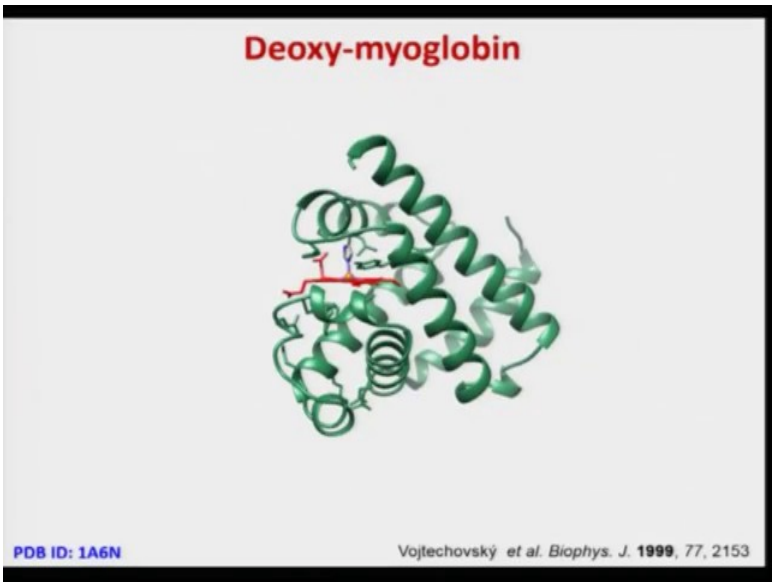

The protein structure of deoxy-myoglobin is shown over here and as one can see that the huge protein chains wrapping around this heme unit. And this iron is placed above the plane which is clearly visible, and the fifth position is ligated with the histidine residue.

So, the sixth position is still empty, some people think that it is maybe the coordinated with weak water molecule. Whatever it is, this water molecule can easily be replaced by dioxygen. So dioxygen, if it wants to bind it has to bind on the sixth position and we will see that what has happened in a our previous slide that dioxygen binds at sixth positions.

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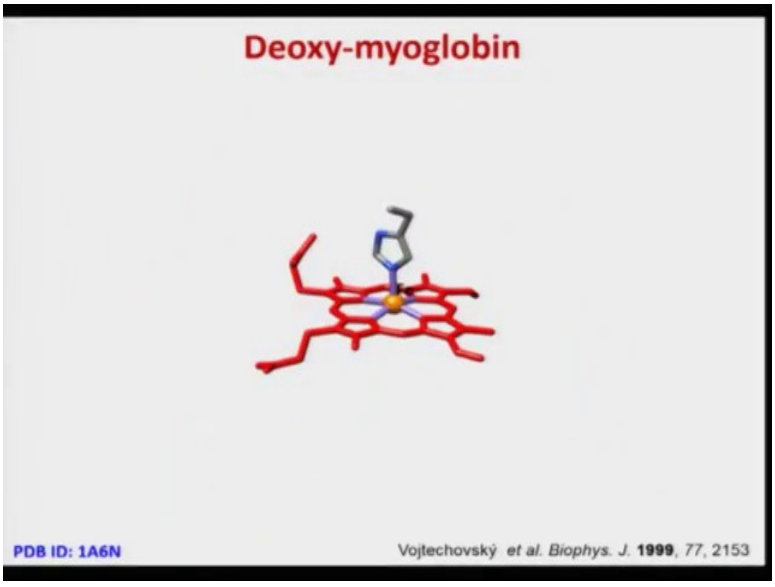

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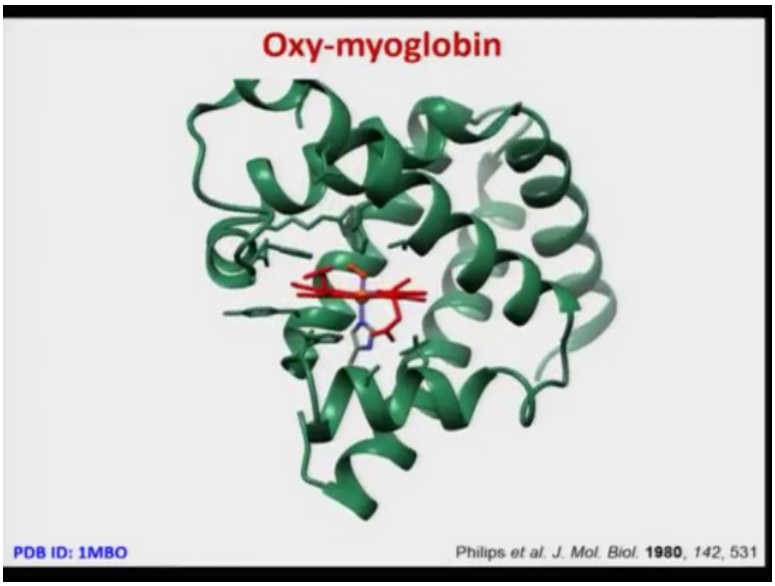

So, this is the protein structure of oxy-myoglobin and again there is a huge protein chain and dioxygen binds at the sixth position only and only at the iron center. It is interesting to note over here is that although there is a huge protein chains but only the iron center where are these all these dioxygen binding or enzymatic transformations happens only on the at the metal center not at the protein chains.

So, that shows the beauty of an inorganic element, here it is an iron.

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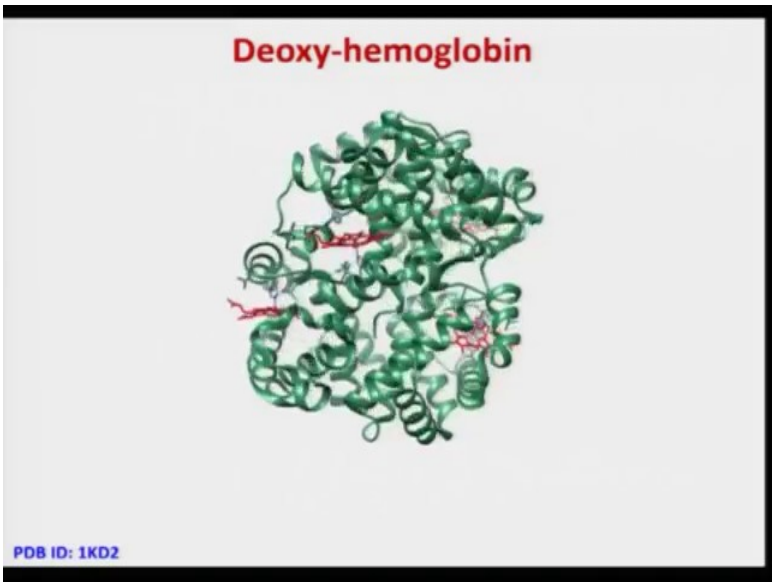

In case of deoxy-hemoglobin, as I have already said that hemoglobin is a tetrameric unit. There is a huge protein chains around this heme center, but there are four heme center you can see yourself 1 2 3 4, four heme centers. And there is hole where dioxygen goes inside the protein and binds at the iron center and responsible for carrying the dioxygen in our body.

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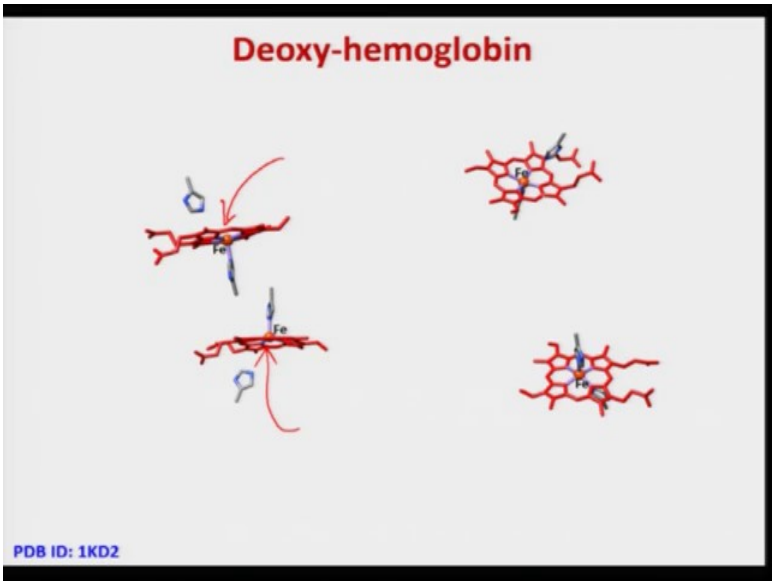

Now, once you move this protein chains as you can see these are tiny four heme centers, the dioxygen can bind in the sixth position. As we have seen in case of myoglobin they are all these six positions are empty where indeed dioxygen bind.

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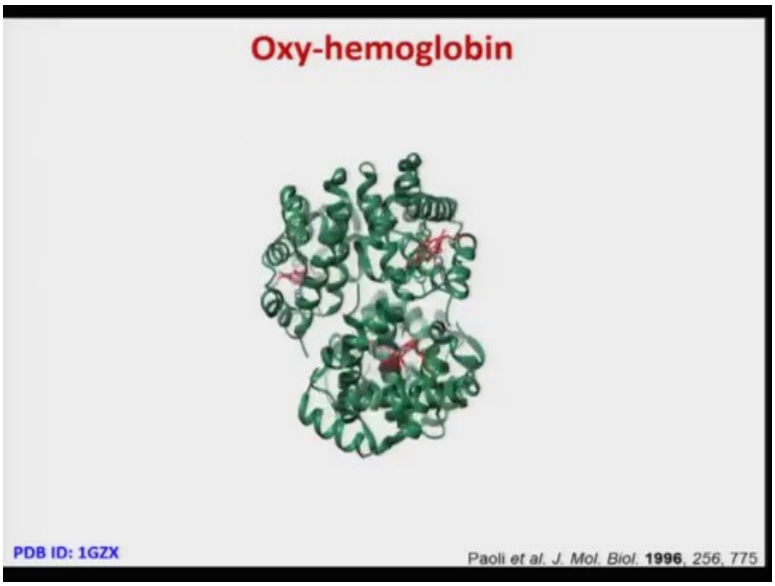

Here you see that protein structure of oxy-hemoglobin that, this dioxygen is bound on the iron center and this is in an angular fashion. And you can also see there is a histidine which are at the top of this iron center which is we call it as a distal histidine. It is clearly visible in the protein structure if one look at very carefully but this is not bound to iron, this is just above this dioxygen, we will see the role of this histidine moiety.

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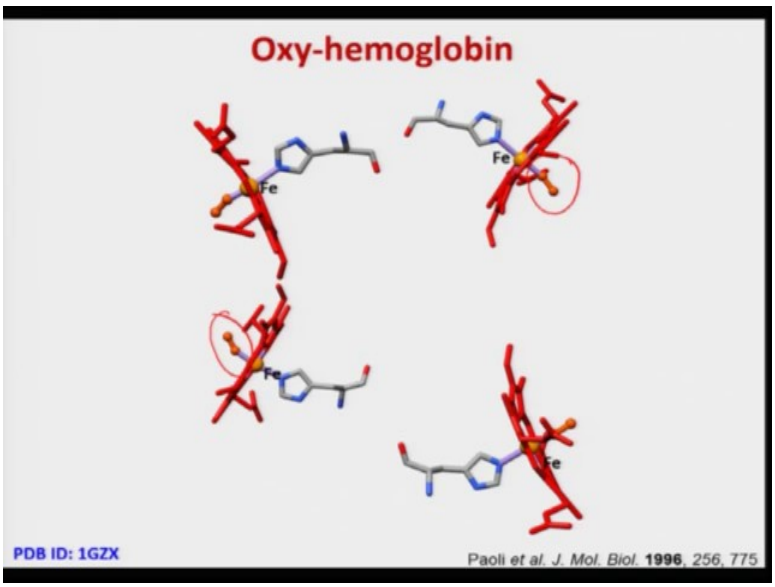

Now, once we remove this protein chains, you can see that that four heme units and dioxygen is ligated at the sixth position in a center. So, this is what is we call it as a oxy-

hemoglobin because oxygen is bound and you can see that oxygen bound in a angular fashion with iron but this is also you can see from the X-ray structure of this oxy-hemoglobin.

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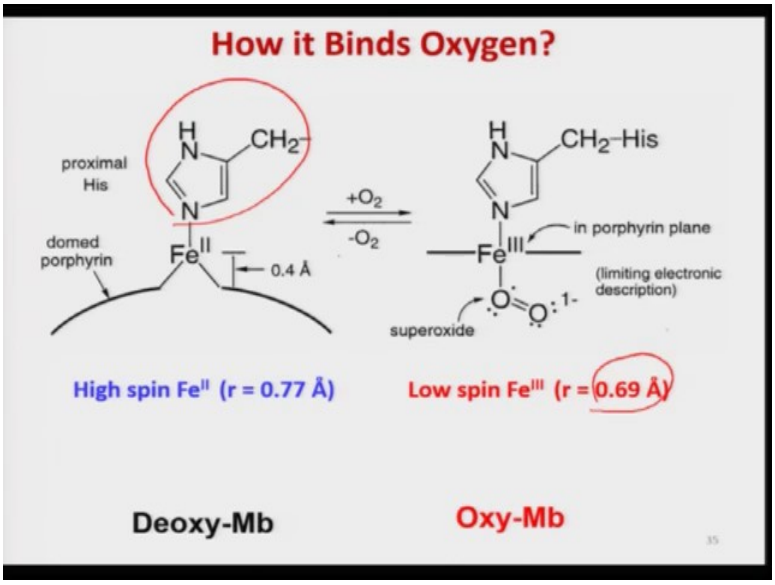

What is exactly happening in case of deoxy-myoglobin and oxy-myoglobin which is after dioxygen binding what changes is happening? Now let us look at deoxy-myoglobin or even one unit of deoxy-hemoglobin very carefully.

As one can see that the fifth position it is ligated with a histidine group, we call it as a proximal histidine and iron is sitting above the main porphyrin plane. So, porphyrin is no longer a planer, it is in a domed shaped and from this mean plane of the porphyrin the iron is sitting around 0.45Å . Why it is so? Because Fe(II) if you look at the radius of Fe(II) it is around 0.77Å and which cannot fit exactly inside the cavity. So, it has to sit outside of the porphyrin cavity. And also the fifth coordination which is also pulling out this iron center towards outside and thereby increasing displacement of iron around 0.45 to 0.5Å .

Now what would happen when a dioxygen binds to a iron center? First thing, as one can see that this dioxygen is actually getting reduced to superoxide whereas, Fe(II) center getting oxidized to Fe(III). So, metal is getting oxidized and dioxygen is getting reduced to superoxide.

So, there is another very interesting observation as you can see that Fe(II) which was sitting above the mean porphyrin plane nearly around 0.4 to 5.0Å is now comes down and iron is almost fitting inside the cavity. This is because of two reasons one is that the radius, Fe(III) low spin radius is much less 0.69Å and which fits perfectly inside the cavity. And also this is an sixth coordinations environment which also helps iron to sit inside the cavity and fits perfectly on the plane of the porphyrin ring.

There is also another thing has happened, say this Fe(II) five coordinated species in deoxy-myoglobin is high spin in nature. I will show in my next slide more details about the electronic distribution. However, this high spin converted to low spin after dioxygen binding and again when this dioxygen is getting released it goes back to deoxy, Iron is converting Fe(III) to Fe(II) and again it is five coordinated and where dioxygen comes in and binds convert to six coordination.

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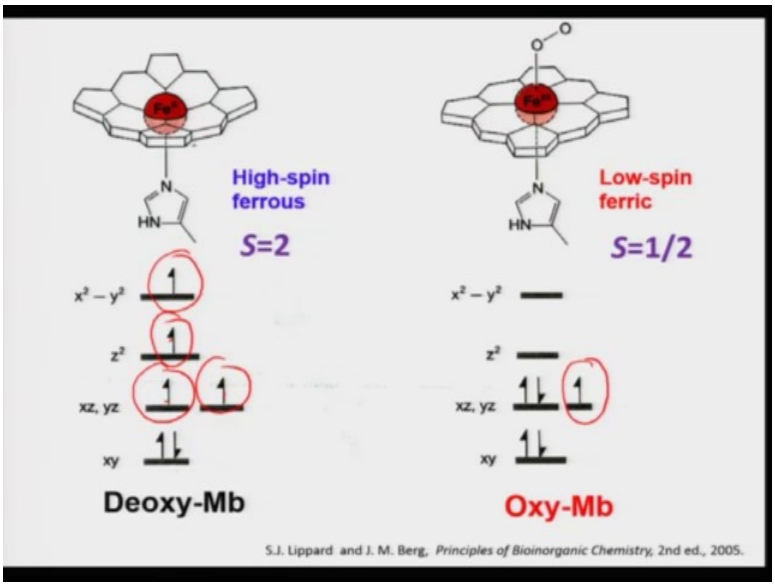

So, as I have said that deoxy-myoglobin which is d^6high spin system and the d orbital is getting filled d_{xy}^2, d_{xz} and d_{yz} there will be two, one-one electron, d_z^2 contains one electron, and $d_{x^2-y^2}$ contains one electron. So, this is 1 2 3 4, four unpaired electron and this is what s=2 it is a five coordinated high spin Fe(II) complex.

In contrast, when dioxygen binds to iron center inoxy-myoglobin what happens? Now Fe(II) converts to Fe(III) so, d^6 converts to d^5 system and high spin becomes low spin. Now if you look at this electronic distribution, it is five d electrons of iron d_{xy}^2 d_{xz}^2 d_{yz}^1

two are paired and one is unpaired electron ok. And so, what you see there is only one unpaired electron in deoxy. There were four unpaired electron 1 2 3 4 and here it is one unpaired electron, and that is why s= 1/2.

So, there is a huge structural changes happening once dioxygen binds to iron, first is one iron to converts to Fe(III) and dioxygen converts to O_2^-. There is also change in the spin state from high spin to low spin state. And also that iron which were placed above the main plane of the porphyrin, now comes back mostly on the plane of the porphyrin ring. So, these are the changes happening from deoxy to oxy form.

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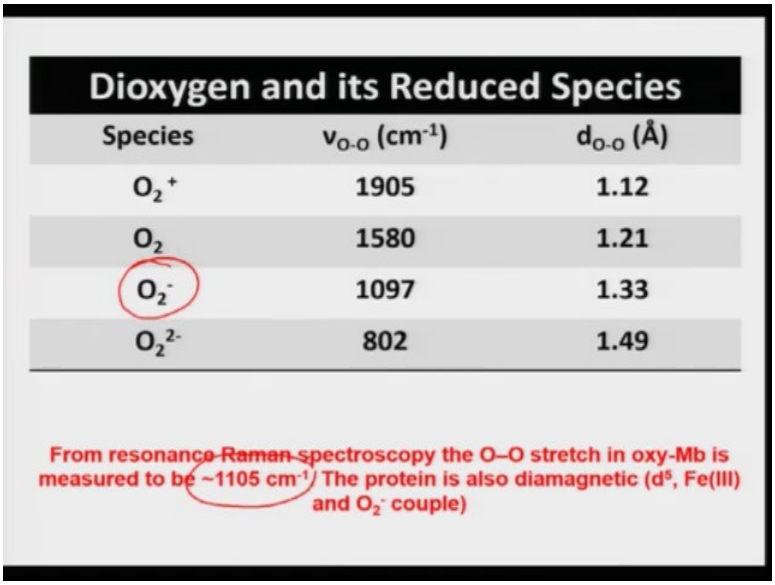

Now, how we know that what is happening to oxygen, is it a O_2?, is it a O_2^+?, it is a O_2^- or it O_2^{2-}?. So, all this can be confirmed from the resonance Raman spectroscopy. So, resonance Raman spectroscopy gives this O-O stretch of oxy-myoglobin which is around 1105 cm^{-1} and that confirms that this is O_2^- not O_2^{2-} not neutral dioxygen.

Please note that, as we have looked at in case of hemocyanin and hemerythrim there dioxygen is in O_2^{2-} but in case of hemoglobin and myoglobin dioxygen binds as a O_2^- not as a neutral dioxygen, because the resonance Raman stretch of O-O confirms that this is O_2^-.

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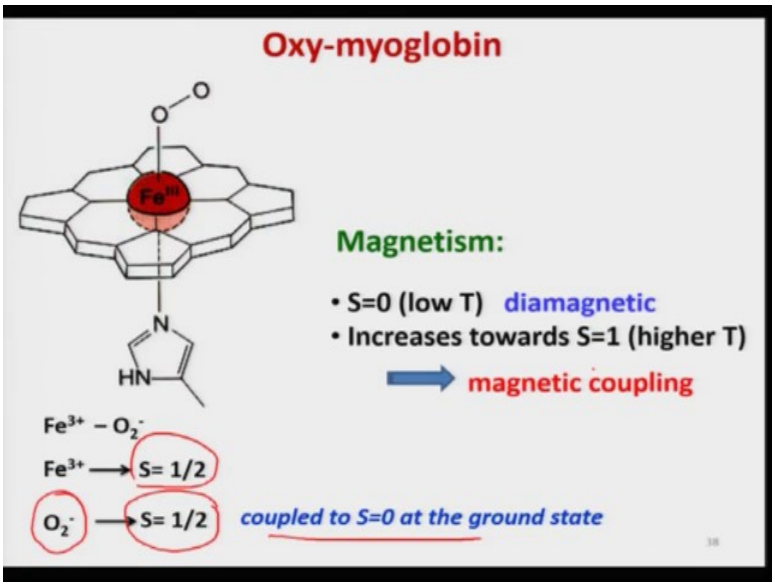

Now, let us look at a little bit more on oxy-myoglobin. Now as I have just shown in my previous slide that the binding is Fe^{3+} andO_2^-. Now Fe^{3+} contains one unpaired electron, so s= 1/2 and O_2^-superoxides have one unpaired electron this is also s= 1/2. So, if there is a strong antiferromagnetic coupling happening between these two unpaired electrons, it results in a strongly diamagnetic system s= 0 at the ground state.Indeed that is what is happening, the oxy-myoglobin is diamagnetic at particularly low temperature. However, at higher temperature the antiferromagnetic coupling will be reduced. So, at low temperature it is a diamagnetic and at very high temperature it is converting to paramagnetic because the reduced antiferromagnetic coupling between these two unpaired electrons at high temperature.

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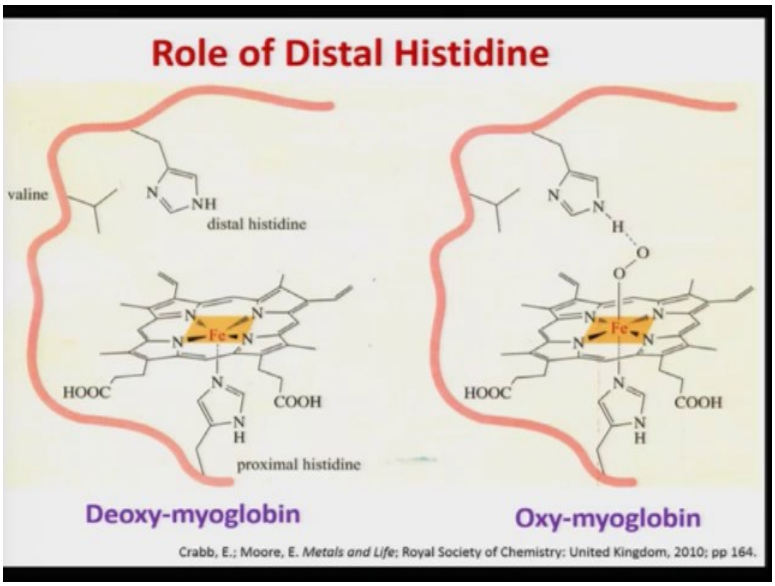

Now, let us look at the role of distal histidine. As you can see in the protein structure, there was a distal histidine which are sitting at the top of this iron center and there is no bond with the iron, just sitting above this iron center which indeed playing a remarkable role. Will now look at, what is the role the distal histidine is playing?

As one can see from deoxy-myoglobin to oxy-myoglobin now when dioxygen binds this distal histidine N-H proton is hydrogen bonded with this dioxygen and thereby stabilizing this oxygenated form Fe(III)-O_2^- . You see that angular fashion and nicely stabilized by this N-H···O hydrogen bonding.

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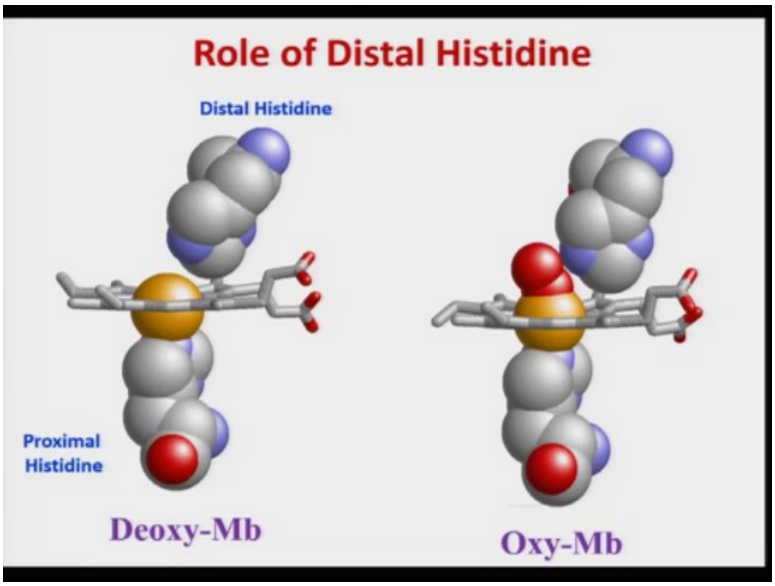

Now, here we have made this distal histidine in a space fill model. The space fill model you can see that this is what is the iron center and this is the distal histidine which are sitting just above this iron center in the deoxy-myoglobin and this is proximal histidine.

Now once dioxygen binds as one can see that it perfectly fits in the cavity, because dioxygen is angular if it wants to bind such a linear, this distal histidine will stop it because it is forcing dioxygen to bind in a angular fashion. We are fortunate that dioxygen always binds in a angular fashion. So, there is no strain. So, it is perfect fitting and indeed we have just seen that N-H proton of the distal histidine actually stabilize this dioxygen binding and they forms a hydrogen bonding and thereby stabilizing that O_2^- binding to iron.

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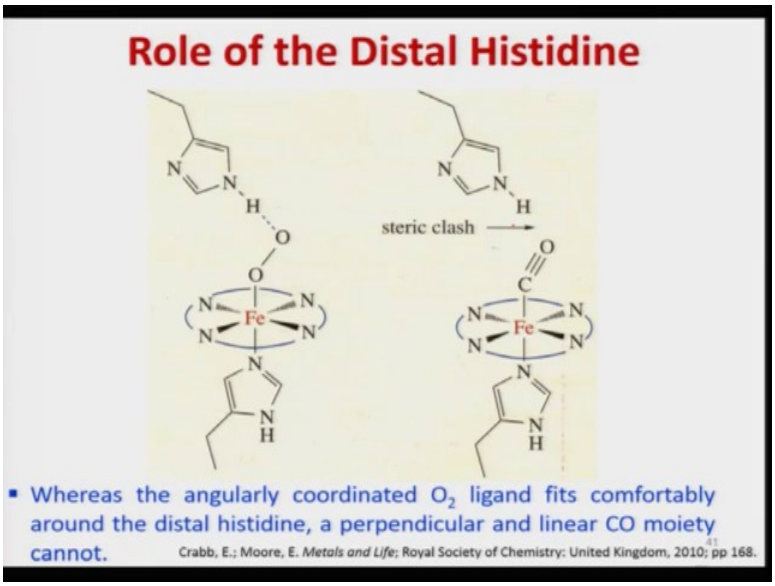

This has been clearly shown over here, that the dioxygen always binds in angular fashion and if carbon monoxides likes to come and bind, what would happen?

The distal histidine because of the steric clash force carbon monoxide to bind in a angular fashion, because it cannot bind in a linear fashion, because distal histidine would be blocking that. Now this has a long consequence. So, whereas, this angularly coordinated oxygen fits comfortably around the distal histidine, a perpendicular and linear carbon monoxide moiety cannot.

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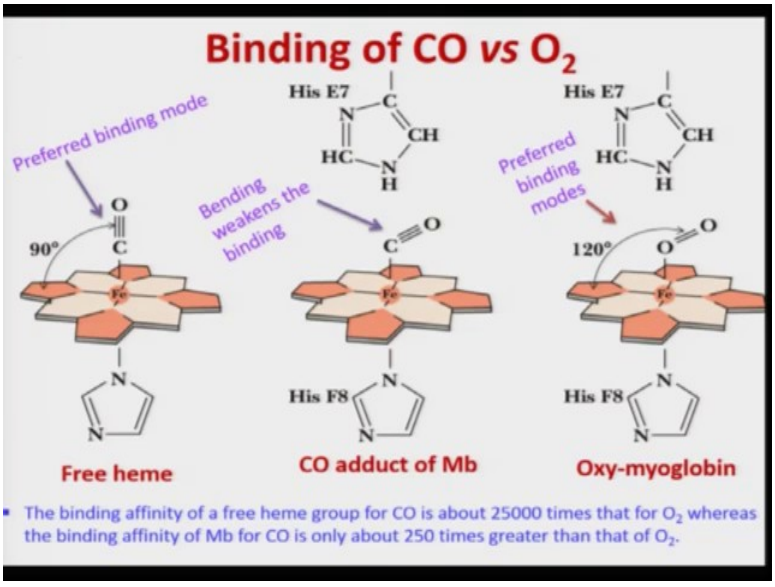

So, what is the result? Result is that carbon monoxide is forced to bind in a angular fashion. Please note that, if you look at any X-ray structure of molecule without protein, you will find that carbon monoxide binds to heme centers in a linear fashion. This is 90^{o}, this is the prefer binding mode, carbon monoxide binds very strongly to Fe(II) center, ok.

However what is happening in case of hemoglobin and myoglobin, carbon monoxide is forced to bind in a angular fashion not a normal binding mode. And what is the result of that? The result is, the binding is very weak. In contrast oxygen also binds in an angular mode, you can see that this is 120^{o} . The binding affinity of free heme group for CO is about 25,000 times than that of oxygen. Whereas, binding affinity of myoglobin for carbon monoxide it is only about 250 times greater than that of oxygen.

Since dioxygen concentration is much more in environment, we are happy that you know dioxygen binds because of weaker binding of carbon monoxide. Otherwise if carbon monoxide concentration increases and if there is no distal histidine to weaken this binding, carbon monoxide would preferentially comes and binds to iron very strongly and not the dioxygen. So, this is very important. So, this actually discriminates dioxygen over carbon monoxides.

Today I have discussed about dioxygen carrying proteins in our own body. Our mother nature has designed hemoglobin and myoglobin, hemoglobin selected for transporting oxygen, while myoglobin stores oxygen. I also have discussed the importance of design in discriminating dioxygen from carbon monoxide just by using distal histidine as a gatekeeper. In my next lecture, I will illustrate how protein chains are actually responsible for reversible dioxygen binding in hemoglobin and myoglobin. I will also highlight how the beautiful design principle makes all this processes possible in sustaining our life.

Thank you.