Metals in Biology
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Lecture - 35
Quick summary on O2 transport

Hello, welcome back today we will quickly summarize for you the Oxygen transport. You have seen these oxygen transport before, but in different places let us put it in one basket and try to make things easier for you for exam purpose ok. You have seen oxygen transport happening in case of hemoglobin; where we have the heme ion center. You have seen it is happening in hemerythrin, where you have 2 non heme iron centers and of course, you have seen in hemocyanin where you have 2 copper centers present.

Let us try to put it in one place and see their differences; we will also see a very little example or some selected examples of the synthetic model system that exists to mimic these oxygen transport enzyme.

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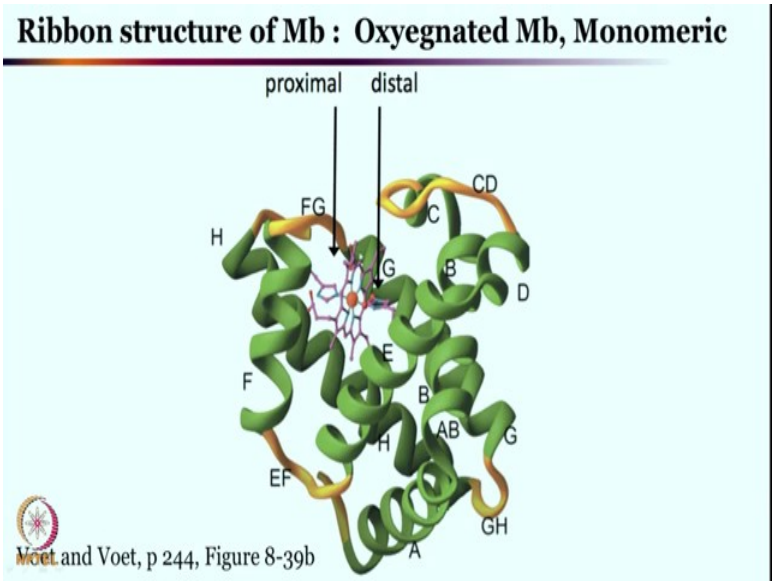

Hemoglobin and myoglobin is first of; so in myoglobin you have seen there is a porphyrin center and there is an axial site which is bound with the porphyrin site porphyrin iron center and at the distal center oxygen binding is happening right.

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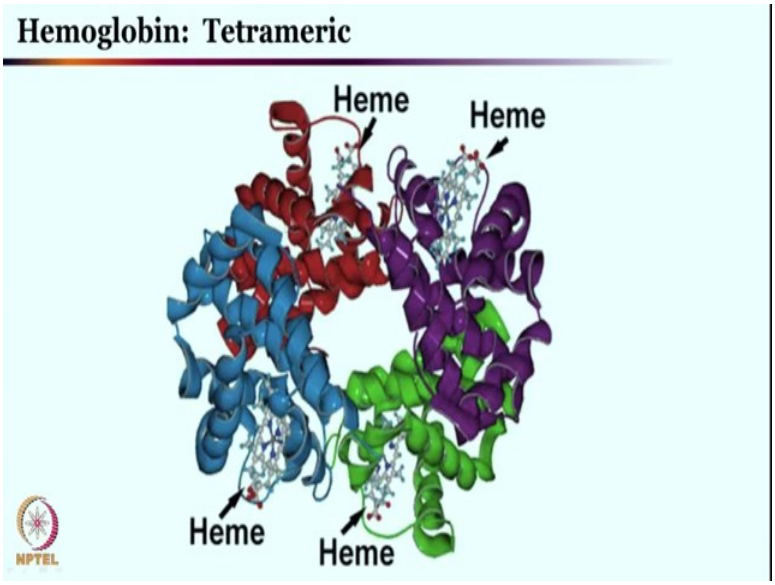

This is one heme center in case of hemoglobin there is four such heme centers present there is a allosteric effect or one oxygen binding is affecting the oxygen coordination of the others ok. You have seen the sigmoidal curve for the oxygen binding into the heme centers.

So, that is all fine heme hemoglobin versus myoglobin difference is it is four iron center in case of hemoglobin and one iron center in case of myoglobin that is fine. You have seen a tabulated format of which differentiate hemoglobin and myoglobin in the previous class ok.

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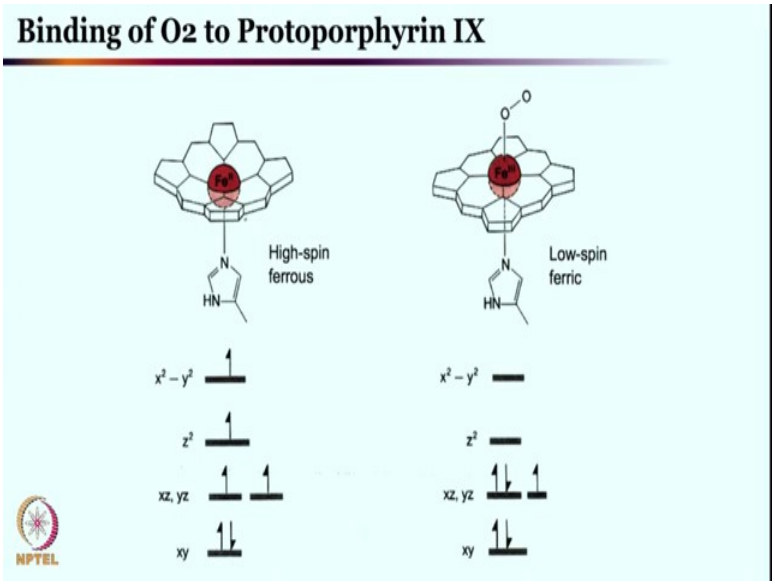

Now, binding of oxygen to protoporphyrin IX; we have seen it is also quite exciting. You have seen the spin change from high spins compound to low spin compound; you are forming. You have a ferrous means iron II plus oxidation state and you have ferric; that means, iron III plus oxidation state. Upon oxygen binding this porphyrin iron species goes to iron III plus superoxo intermediate which is then forming the low spin ferric compound right.

So, this is iron II plus it is outside the plane; it is not really inside the plane, high spin ferrous and this is this is the histidine binding. Remember for cytochrome P450 where oxygen activation is coupled with substrate activation as well as substrate oxygenation chemistry is happening there; it was a cysteine not the histidine. So, that cytochrome P450; here we are discussing about the hemoglobin and myoglobin cases, this is histidine histidine axial chain. As you can see the high spin ferrous; that means, Fe 2 plus d7 iron system high spin would mean t2g4 and eg2 right.

So, iron II plus means this 6 electron system sorry and this 6 electron system would be this t2g4 and eg2 system fine. Now let us look at the low spin ferric; of course, this is a iron III plus; that means, it is a d5 electronic system; since it is low spin; it is going to be t2g5. This is a superoxo; how do you know it is superoxo? Very simply if you want, you can record every visible spectrum which is characteristics of it and you can also record resonance Raman; which is kind of definitely proof that it is superoxo species.

You have seen for myoglobin cases it would be 1105 wave number for the oxygen stretch in resonance Raman; that means, that it is without doubt this is the iron III superoxo species right. That is fantastic that is a superoxo species right over there and this oxidation state change, as well as spin state change you have seen very clearly ok.

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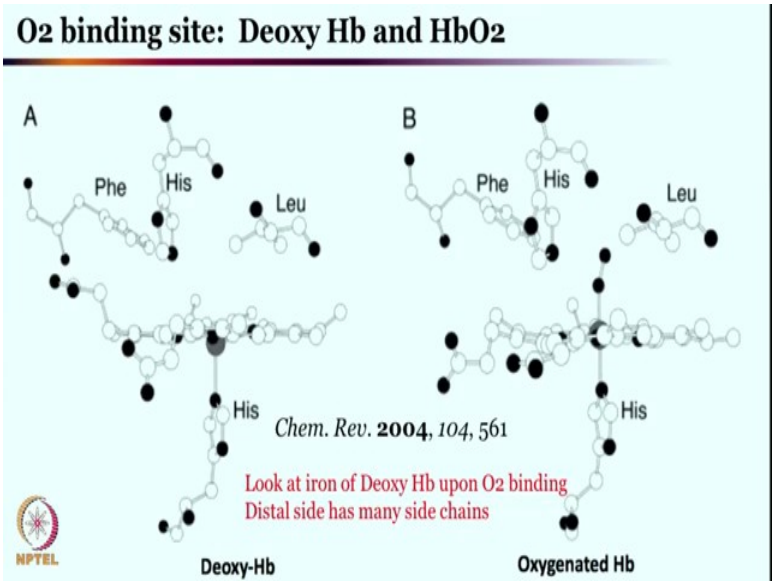

Now this phenomenon you can follow in the enzyme in the form of; let us say crystal structure. This is a nice crystal structure showing that iron II plus is outside the plane histidine is bound. Once again if it was one cytochrome P450, here you would have cysteine bound with it right.

There is a; there is many protein residue or these backbone from the amino acid backbone of the protein residue which is coming into the picture in the distal site. All these are appended towards this active site giving rise to a great feeling for this oxygen binding ok. Once the oxygen is binding with iron of course, at the first step there will be oxygen binding without any electron transfer.

But immediately after that immediately after oxygen binding one electron from the iron center transferred to the oxygen to make it iron III plus superoxo. You start with iron II plus, you went to iron III plus superoxide species. This species is also affected by this by this protein side backbone or protein side chain, which overall helps in giving the geometry it is having that is the bend geometry and this is particularly why we see that

the role of this histidine phenyl aniline and leucine are quite crucial for the oxygen binding ok.

Now, we also understand that this histidine will move in as soon as this iron center moves in the cavity; this histidine also moves in side. This pool or this trigger will cause a trigger for the other iron center; other three iron center that is present in case of hemoglobin to make them activate, to make them ready for the oxygen binding.

So one oxygen binding at the one of the iron center helps binding other iron in with the oxygen center. So, this movement from this outside plane to inside plane; this overall upward movement for the histidine is quite important. As you have seen for in case of cytochrome P450; here it would be cysteine, now this cysteine thiolate will help you overall in breaking the oxygen-oxygen bond in case of that hydro peroxo OOH that is being formed.

And then overall this iron oxo species subsequently after oxygen oxygen bond cleavage whenever it is happening that is in cytochrome P450; this iron IV oxo or this high valent iron oxo species that can be generated in cytochrome P450, it will be stabilized or it will be really easy for cytochrome P450 to have this cysteine which can stabilize such an intermediate ok.

So, once again these there are similarities between hemoglobin and cytochrome P450; cytochrome P450 not only activates the oxygen, it actually also activates the substrate to do the chemistry. In case of hemoglobin and myoglobin cases; it is purely oxygen binding right, and you do not see any other reaction happening at the hemoglobin and myoglobin.

And that is partly because and of course, the partly because of the presence of these protein side chain and absence of any substrate and the substrate binding pocket; has there been a substrate binding pocket and they substrate available then we perhaps will not be living here. Because these are the reason why we inhale oxygen and how we take up those oxygen and deliver at different part of the body. If this oxygen was involved in to activity such as substrate hydroxylation chemistry, we will not have been getting these oxygen at different part of our body which is essential for surviving different cells. So, therefore, with this whole system perhaps our whole system biological system perhaps would have collapsed.

So, as you see nature has designed everything perfectly that in this case, it puts or it engages a histidine as opposed to the cysteine. And more importantly substrate binding pocket and non availability of substrate makes it perfect for it for hemoglobin iron center protoporphyrin IX center to act as just oxygen transport agent or center. It is not really participating in anything else, its role is to reversibly bind; so, whenever it is required it can bind and it can release oxygen.

So, if this is the porphyrin center oxygen can come in and binds with it of course, there is a superoxo formation after electron transfer, but wherever it is required it can release the oxygen. So, it is almost keeping the oxygen, watching the oxygen, but it does not engage oxygen too much into the reactivity. It is not like it cannot it is capable of doing it, but overall architecture is such that it is just participating in oxygen binding and removal.

This reversibility is so good that; that you do not see any loss of activity in these cases. If you want to do the same chemistry in synthetic setup, it is not that very easy we have discussed that will briefly mention that again. So, the problem that happens with the synthetic chemistry is well the moment one iron center is bound with one oxygen, another iron center is also approaching it to form the iron III peroxo iron III species.

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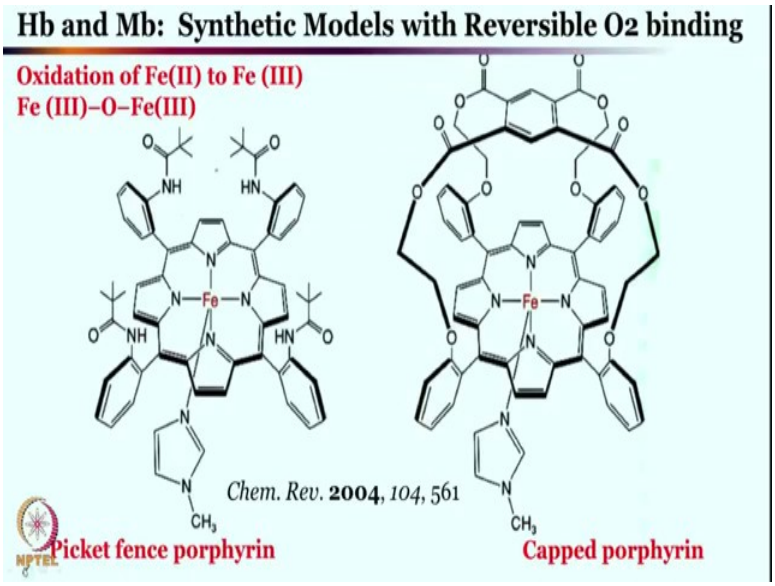

So, it would be in between as you have discussed earlier; it will cause problem let me briefly mention once again and that is you will end up seeing.

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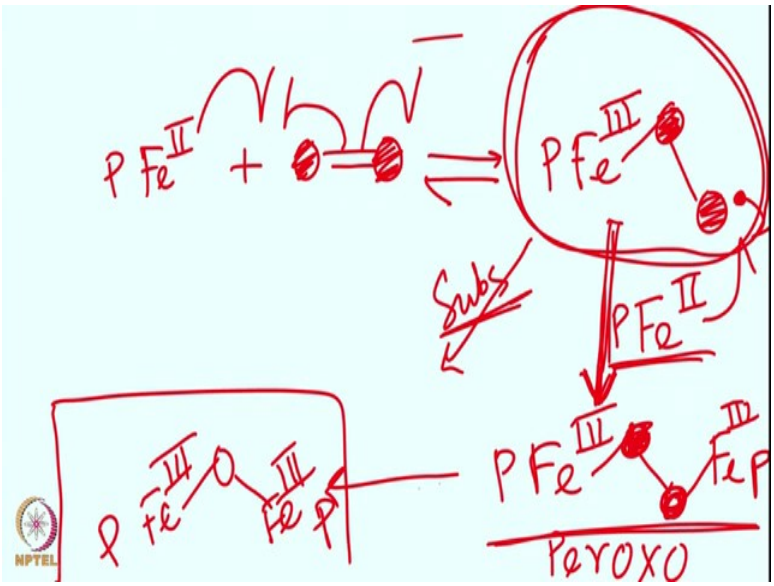

So, the moment you have a iron II porphyrin system let us say P; it reacts with oxygen to give you; let us say this is leveled oxygen if you are leveling to give you iron III superoxo species that is the first form species, this is the superoxo species radical, cation. So, it breaks it breaks to give you iron III superoxo or species subsequently from their another porphyrin iron II species comes in. And it gives in one electron to it and essentially it gives porphyrin iron III oxygen oxygen and iron III porphyrin species right.

So, this species is now then can further react. So, in case of enzyme, it stabilizes over here; there is no problem because there is not the second iron center or second porphyrin center is not really available because enzymes are protecting every site very perfectly. But in synthetic studies when you are trying to do this chemistry in laboratory; you can get this chemistry, but you can of course, get this chemistry.

But this is not limited or this is not staying right over there, it can further react with another equivalent of porphyrin iron III which is present into the solution and can give the peroxo species. So, this is the iron III oxygen oxygen and iron III peroxo species; this species of course, also reactive further it can go on and react. And finally, it will perhaps stop reacting at this point when you have this iron III ; iron III oxo or muoxo porphyrin iron III, this is relatively stable compound.

So, it goes there you can see that this is kind of a thermodynamic sync right. This is not what you want, you want this to be reversible and it stays over there that is what is

happening this all this reversible chemistry is happening in enzyme. The fast in enzyme case this is where its it gets stabilized, but in synthetic chemistry set up; you end up getting the side reaction with which causes the majority of the problem.

And therefore, mimicking this chemistry in synthetic setup is extremely difficult because we want to mimic just the reversible binding we do not want to get any reactivity out of it. We do not want to react it with any other substrate or anything because we want to see the hemoglobin chemistry, which is the reversible oxygen binding right. In synthetic setup most of the studies that has been done so far fails to mimic this reversible oxygen binding completely.

Because it not only does this chemistry, but it over reacts right this is the problem. Of course, it is possible to mimic, but it over reacts and therefore, it leads to the side product formation right. So, let us go back ; so to prevent these over reaction or even the auto oxidation of iron II plus II; iron III plus which is again another problem. Because this iron II plus stabilizing them in iron II plus is very difficult because it is very sensitive; its air sensitive and moisture sensitive all these species need to be recorded or need to be stabilized stored in glovebox right.

This is very sensitive chemistry; now to prevent the that second iron center to come close to the first iron center where a superoxo species is forming; it has been successfully designed to have this picket fence porphyrin which is essentially helping you in getting the mononuclear. So, mononuclear iron superoxo species; these oxygen binding are reversible in nature. In this case you see that axial ligand is also there in the form of (Refer Time: 15:37) which is similar to what we see in histidine right.

So, this picket fence porphyrin can mimic the reversible oxygen binding of the hemoglobin. Also there is this capped porphyrin as you see; it is a really nice drawing and really nice molecule where as if there is an umbrella in front of the iron site which is protecting from another molecule of these to come very close to each other.

So, this proximal site is blocked by the histidine and the distal site is open where oxygen binding can happen. And oxygen binding once it happens this intermediate which is very reactive intermediate despite having high reactivity of this iron III superoxo, it still does not really react with another equivalent of this molecule due to the steric hindrance or the bucket safe of these of these species right.

So, this overall umbrella thing or umbrella design helps in helps in stabilizing; stabilizing the intermediate perfectly; now that is what we have seen. So, in hemoglobin and myoglobin cases you know that there is no substrate activation; it is purely oxygen binding right.

It is a reversible oxygen binding after much effort synthetic chemist can mimic those features of hemoglobin and myoglobin by mimicking or by copying the activity of the hemoglobin myoglobin. It gives an opportunity to better understand what happens in hemoglobin and myoglobin; even at a molecular level things can be done which were not otherwise possible.

The problem of the dimerization or the dinuclear iron species formation can be prevented somehow by putting this big picket fence or cap for firing design.

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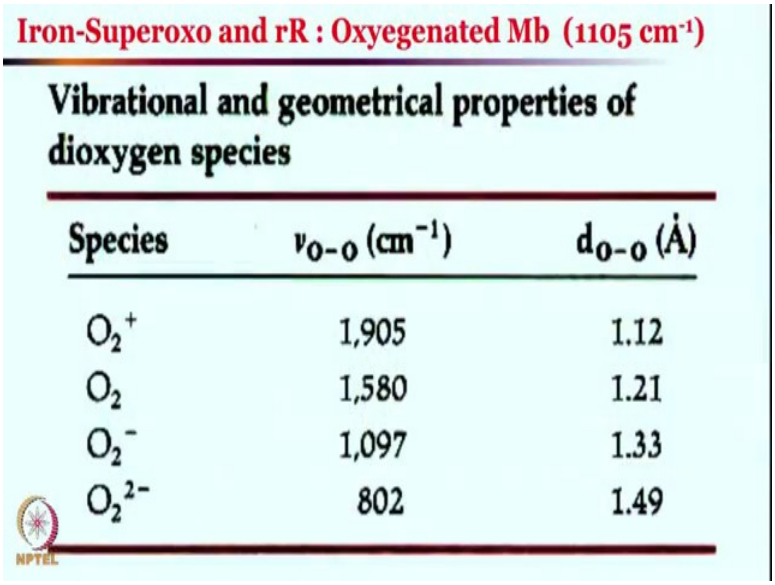

Let us look at another enzyme that is hemerythrin before that just to mention once again this is 1105 wave number for oxygenated myoglobin, this is matching quite well for the superoxo species ok. If you have a crystal structure that can also give the oxygen oxygen stretch, in this region which is quite exciting in any case ok.

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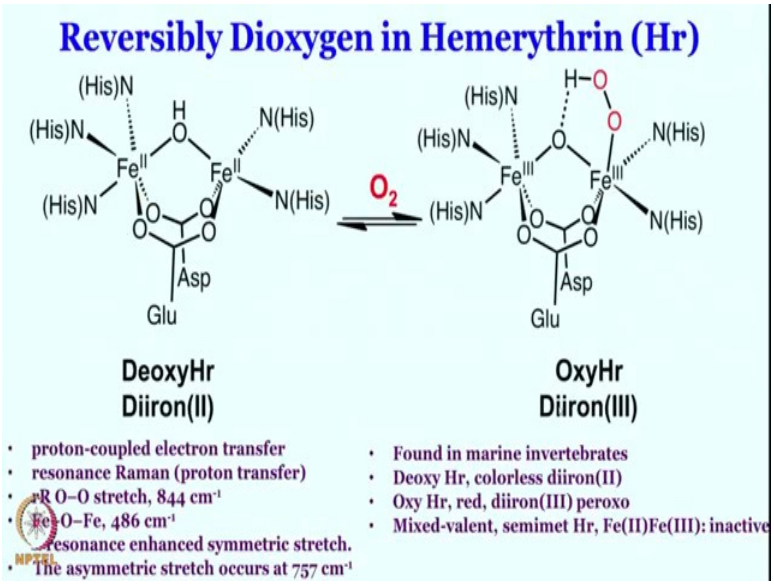

Let us look at the hemerythrin and you have seen hemerythrin is a diiron center ok; these are non heme center. So, hemoglobin is found in some like let us say in higher and higher species such as us, in all other all other mammals in you know majority of the species are having hemoglobin.

But then there is these marine invertebrates and some other species, where you do not have the hemoglobin cases where you have you do not have the hemoglobin iron oxygen binding center and this is where these iron II di iron center will be coming into picture; these are not porphyrin ring or these are having the histidine simple histidine ligation.

These histidine ligation are quite phenomenal quite important because it provides the opportunity for these species to carry oxygen; invertebrates to carry oxygen. Before oxygen binding, it was a colorless species, upon oxygen binding; so colorless species is this, one upon oxygen binding it becomes red. So, this is a red color species this oxygen binding just like hemoglobin and myoglobin is reversible in nature; that means, it pretty easily can go forward and backward and therefore, the oxygen transport and release can be easily possible right.

If you are looking at this species; so this species will form a peroxo species, let me draw it very quickly.

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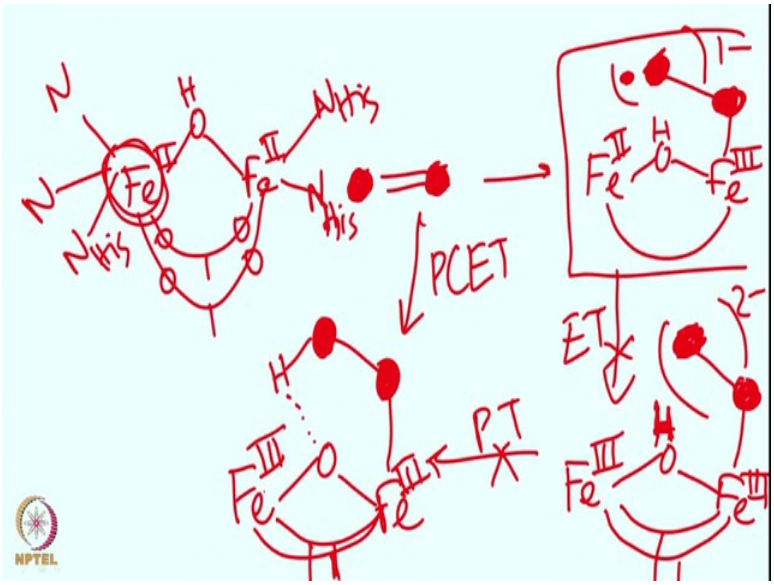

So, what you will see here is; if you are having a diiron center right I am not drawing bridging too much. So, it is a diiron center you are reacting with oxygen; let us say level the oxygen; you get a diiron. Of course, I will draw stepwise, not necessarily these intermediate you form and stabilized very easily; you.

Of course, it is forming, but you cannot stabilize. So, in the first step it is a iron II iron III species. Well only one of the iron site will bind this oxygen because the other side as you have seen is having three histidine nitrogen, nitrogen, nitrogen; these are histidine nitrogen the other side is having only 2 nitrogen histidine histidine right

So, of course, there is bridging I am not trying to draw those; there is a double bridging anyway. So, these are over there oxygen, oxygen let us not try to draw the whole system. So, this is this is unsymmetrical iron center as you have seen over here; you have a iron II plus center with 3 histidine on this side it is 2 histidine right.

And then you have the oxygen molecule in there right. This oxygen molecule binds with it; one of them the one which is unsymmetrical or which is having less ligand this is 3 histidine ligand this is having 2 histidine ligand. So, this is the site where oxygen binds and upon binding the oxygen it can, then through the bridge since it is through the bridge it can. So, of course, this intermediate you cannot see you cannot see such intermediate; this is iron III superoxo intermediate perhaps it is; it is going to be extremely difficult to stabilize.

So, immediately another electron transfer happens to it to gives to give you a peroxo intermediate. So, as you have seen this is going to be 2 minus; the whole thing is going to be 2 minus. So, this was 1 minus superoxo, this is a superoxo, this is a peroxo. Of course, just remember that it is not really possible to see such intermediate just I am drawing stepwise for your clarity or for you to understand very simply.

So, both the iron II plus center give one electron at a time to give the iron III peroxo species. This iron III peroxo species subsequently can be protonated as you have seen over there. So, this is this is going to be a completely proton transfer. So, proton coupled electron transfer perhaps in this step; this is going to be at a step not even this intermediate you will be seen. So, overall you will be seeing the formation of iron III oxo; iron III hydroperoxo intermediate right.

So, this is your oxygen that is coming on, this is your oxygen from the oxygen atom and there is a hydrogen bonding and rest of the things are still there right still there ok. So, I do not think it is fair to draw this intermediate because not such intermediate existence is questionable both the intermediate. So, iron II iron 2 you start with immediately you get iron III iron three hydroxo.

So, this is a proton transfer the way I i have shown you is electron transfer fast and then proton transfer perhaps that is not going to happen; it is going to be proton coupled electron transfer directly it is forming. So, one electron from this side another electron from that side; it makes it iron III; iron III peroxo and then protonation.

So, immediately all of them are happening. So, it could be one electron transfer and one proton coupled electron transfer PCET; that is what is happening. Stepwise drawing these mechanism is not perhaps fair because no such intermediate exists and no and you know we cannot say for sure what exactly happens in a stepwise fashion.

But as you have seen; as you have seen over here you can see that this is hydroxyo intermediate. And upon oxygen binding it forms these from iron II iron II a iron III iron three hydroperoxo intermediate which is; which is hydrogen bonded through these new oxo species right.

If you try to record the resonance Raman of this oxygen oxygen stretch you find that it is at 844 wave number which is matching perfectly for the peroxo species. So, in the first

class of haemoglobin myoglobin, you see that it is a iron III superoxo species; it is formed of an oxygen binding, it does not go anywhere after that. But in case of hemerythrin; it is one more electron reduced species and protonation that is iron hydro peroxo species.

It is a diiron species and it is a bridge species that is quite phenomenal I think pretty exciting intermediate. And these species are spectroscopically characterized and you can see the spectra spectral behavior right over here right.

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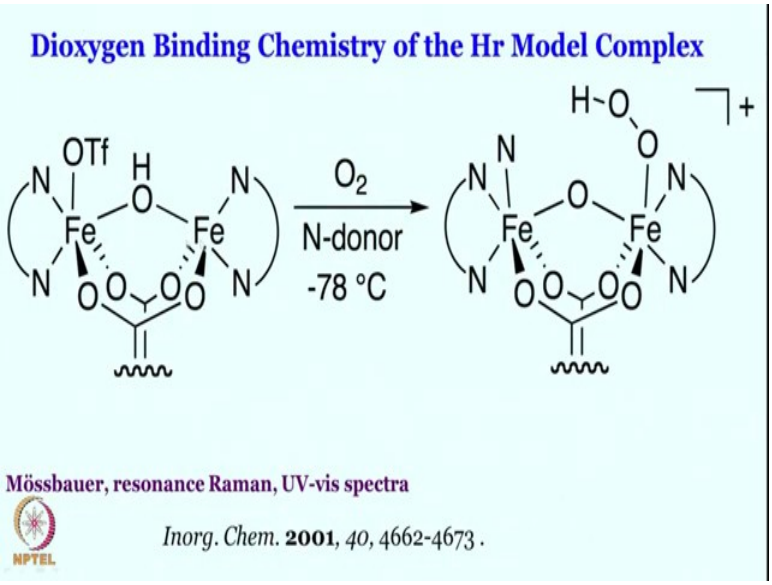

So, let us let us move on and try to see what we can get in case of in case of the synthetic modeling studies. As you have seen in the synthetic modeling studies; it would require the three histidine on one side or three nitrogen binding on one side.

Another side would have 2 nitrogen binding synthetic chemists has been very efficient in mimicking this and it was possible to mimic almost exactly the spectral feature. So, synthetic chemists has been extremely successful in mimicking this activity and we see that it is it is possible it is actually possible to get the exact 3 histidine or 3 nitrogen binding on one iron side.

Another iron side will have the 2 nitrogen binding and the hydro peroxo species formation oxygen oxygen stretching and all the other spectroscopic features UV visible

Mossbauer spectra are exactly similar to what is found in the enzyme; that is fantastic and these are completely reversible oxygen binding in the enzyme that is fine.

So, you have seen the difference between hemoglobin and hemerythrin.

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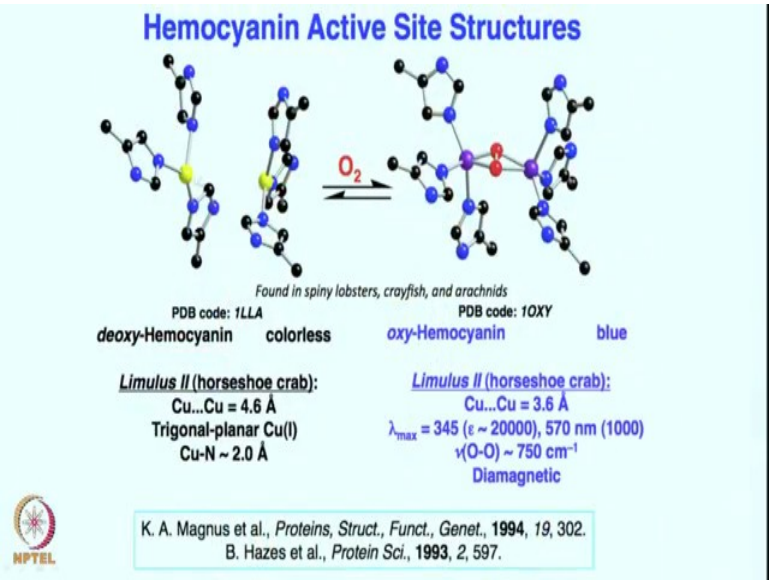

Let us quickly look at the hemocyanin, as you have remembered perhaps it is a 2 copper or it is 2 copper center as opposed to 2 iron center in hemerythrin this is found in orthopods and mollusks.

So, for example, lobster crayfish arachnids all are having these short of copper center or dicopper center for oxygen binding. 3; histidine is bound with one copper, another copper is having 3, another 3 histidine and the oxygen is getting reduced by 2 electron; one electron each from each of these copper to form the iron peroxo species. You can see the distance between these two copper centers was 4.6 angstrom; it is reduced to 3.6 angstrom upon the oxygen binding.

So these are trigonal planar copper I center and these are super sensitive ok; this can react with oxygen while almost immediately right these are completely reversible oxygen binding and upon oxygen binding it forms the peroxo; that means, 2 electron reduced species which has a characteristics peak of oxygen oxygen stretch of 750 wave number which once again just like what you have seen in hemerythrin are peroxo.

But it is a different type of peroxo its a side on peroxo in the hemrythrin ring you have seen a hydro peroxo and in an almost an end on larsen right. So therefore, these species are all different both hemoglobin hemerythrin are different compared to hemocyanin. But one thing puts them in common box and that is they are capable of transporting oxygen, they do not utilize oxygen for any other activity other than transporting right.

So, that makes them very interesting; this species is colorless in nature this place is blue. In nature in all these cases you have seen hemoglobin bead hemoglobin hemerythrin henocyanin; all these cases oxygenated species are really bright color right you have seen red; red and blue hemoglobin hemerythrin and hemocyanin that makes it quite exciting.

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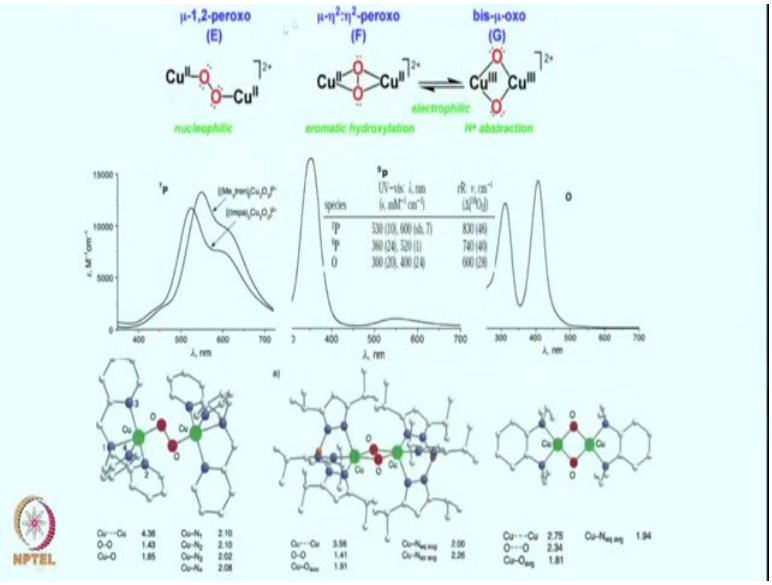

As you know there exist a number of mimic synthetic mimic that that exactly kind of nowadays mimic; what is happening in hemocyanin. The efforts for mimicking with a tetradentate ligand is this one we will discuss we have discussed this before with the bidented mimic is this one.

But the one which is found in hemocyanin is exactly this one which is found with this tridentate ligand backbone right; which is fantastic and it matches exactly the spectroscopic features also both UV visible and resonance Raman. So, let therefore, let me summarize by telling you that as you have seen hemoglobin myoglobin of course, hemoglobin myoglobin hemerythrin and hemocyanin these are all great enzyme.

And they are all capable of binding oxygen in a reversible format and they are not interested in anything else. Nature has designed these in such a way so that there is no substrate available. You know what will happen if a organic substrate is available in front of this you have seen it will summarize that as well in another class.

But if there is a; there is a substrate available in front of this; it will end up reacting because all of these all of these intermediate in hemoglobin, myoglobin, hemocyanin, hemerythrin all of them are reactive intermediate. Since there is the design or architecture does not allow to have any organic substrate present in front of them; therefore, they are sitting pretty and idle.

They are not going to do any chemistry, they will be as if their hands are tied and they are very happy to just transport the oxygen and deliver it ok. So, that is that is all for today, but as you know these chemistry takes quite an interesting turn when you have cytochrome P450 or tyrosine for example, for hemocyanin you see this chemistry becomes much more beautiful, much more exciting in terms of substrate oxygenation chemistry or the synthetic chemistry. We will come back to those in summarizing those in the next class.

Thank you very much, keep studying.