**Metals in Biology**
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**Lecture - 07**
**Oxygen transport and activation**

Hi welcome back to Metals in Biology. Today we will discuss Oxygen transport and activation, the book to follow is that of the Leapard and Berg's Principles of Bioinorganic Chemistry. We have taken also the notes from different references, as well as class notes by Professor Leapard.

Well, I am sure you are familiar with this topic one way or the other that we are alive because we can inhale right. The moment our oxygen supply from air is stopped; we will be dead. Well for keeping us alive this oxygen transport at different parts of our body is done by blood. As you all know there is hemoglobin in the blood which carries oxygen and upon oxygen binding this porphyrin ring of hemoglobin porphyrin histidine coordination of hemoglobin makes the blood red.

We know in certain degrees these things, so I will not be getting into too much of what you have known, but I will try to known so far, but I will try to give a give an overview of this oxygen transport.

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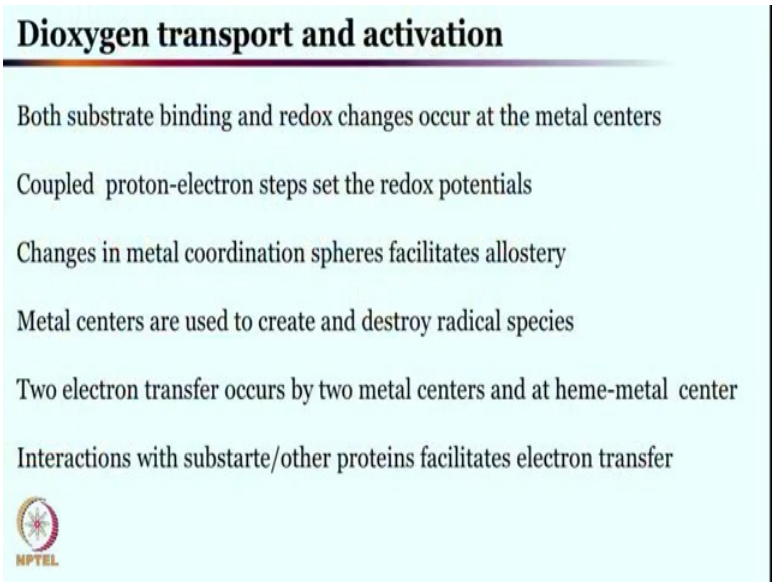

Or it is also true that that dioxygen transport and activation of oxygen are interlinked. Lot of chemistry is done by utilizing oxygen molecule that we inhale therefore delivery is important also activation is important by doing so the lot of metal enzyme can do their activity.

So, just to look at some of the summary part that both substrate binding and redox changes occur at the metal center. Essentially saying that metal enzyme is at the core of all the activity, not only substrate can be hydroxylated for example or other reaction can happen with substrate as well as the redox changes that can happen at the metal center, but all of these are happening at metal center.

Often, we see that proton transfer or protonation and electron transfers are coupled with each other and they are the one which is responsible for the redox potential. Well, we will see that metal coordination sphere facilitates a number of processes including allostery. Metal centers are used to create and destroy radical traces essentially saying that metal are at the center of the activity, it can create a radical center, it can initiate a radical process and also it can terminate a radical process.

Two electron transfer occurs by two metal centers that is important to understand; that if two electrons are required for a process, it is two metal center not one metal center that is usual involved. So, one electron transfer process is still predominates in the biological system and at heme centers obviously. Interaction with substrate and other proteins facilitate electron transfer that we have discussed briefly in electron transfer; that electron transfer to occur we need to have the substrate and the protein and or other part interacting with each other.

Well, we will come back to these over the courses, but let me discuss or let us discuss the oxygen transport today ok. Not only the hemoglobin that we have in our blood is the only oxygen carrier, there are other different oxygen carrier right.

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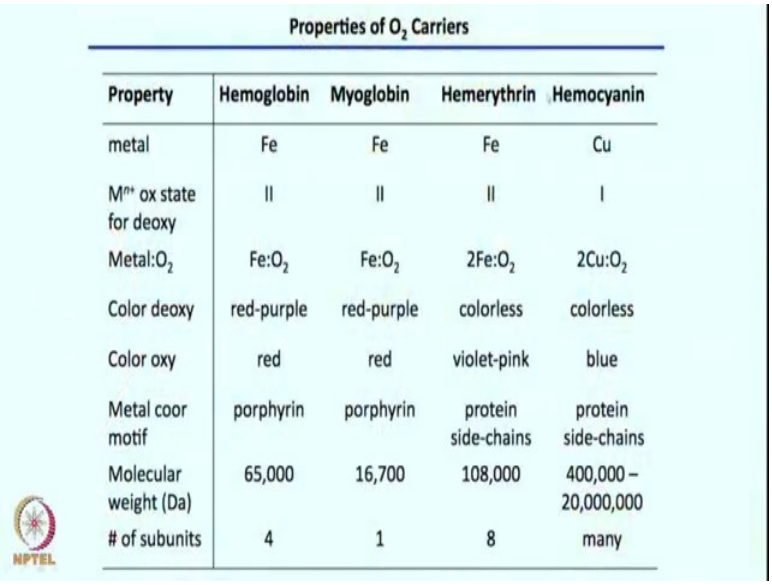

Properties of $O_2$ Carriers

| Property | Hemoglobin | Myoglobin | Hemerythrin | Hemocyanin |
|---|---|---|---|---|
| metal | Fe | Fe | Fe | Cu |
| $M^{n+}$ ox state for deoxy | II | II | II | I |
| Metal:$O_2$ | Fe:$O_2$ | Fe:$O_2$ | 2Fe:$O_2$ | 2Cu:$O_2$ |
| Color deoxy | red-purple | red-purple | colorless | colorless |
| Color oxy | red | red | violet-pink | blue |
| Metal coor motif | porphyrin | porphyrin | protein side-chains | protein side-chains |
| Molecular weight (Da) | 65,000 | 16,700 | 108,000 | 400,000 – 20,000,000 |
| # of subunits | 4 | 1 | 8 | many |

So, we have of course, hemoglobin and myoglobin; in addition there is hemerythrin which is responsible for oxygen carrying also in let us say marine invertebrates. There is hemocyanin where we have arthropods and molluscs where hemocyanin is involved into the oxygen transport.

So, we so far I guess you are familiar with both hemoglobin and myoglobin ok, they combined with oxygen. But when we do not have hemoglobin for example, where invertebrates; invertebrates there we have hemerythrin and also orthopods, molluscs let us say crabs these short of species. Once again they do not have the blood like red for us, but their blood can be blue that is thanks due to the copper oxygen chemistry ok.

We will today see mainly this iron oxygen binding chemistry with hemoglobin and myoglobin. Ideally I would like to discuss hemerythrin and hemocyanin subsequently, but just to keep it dilute and spaced out we will discuss him hemerythrin and hemocyanin in some later classes; all it is part of this course anyway.

So, hemerythrin and hemocyanin we will not discuss today or in subsequent in the next class we will come back to this topic later. But just to tell you that hemerythrin has two iron centers which can also bind with oxygen and it can transport oxygen. But more importantly none of these cases oxygen remains just as oxygen O2 ok, in some of the books you may see that are this is remained as O2, but that is not correct; all we are as

we said we are following this book by Professor Leapard, you can follow there, it is written very nicely over there.

Now, these iron oxygen species are interacting with each other to give a species which is in this case will be a hydroperoxo and in this case it would be a peroxo. As you will see over here this is going to be a superoxo species. So, one of the thing you really need to understand that this oxygen that we are inhaling during even the transport at the different part of the body, it is not just iron oxygen complex simple; electron transfer is occurring from iron center to oxygen to make it reduced.

So, oxygen will be reduced in the process by 1 electron in some cases in the hemerythrin and hemocyanin cases it is by 2 electron. But most importantly no matter what happened to the oxygen the process is reversible; therefore, wherever it needs to be delivered still it can be delivered as oxygen, it is not the reduced species that is getting this delivered that is quite important to understand. It is a completely completely reversible process and therefore, or let us say our blood can deliver oxygen at every part of the body as O2; not O2 dot minus not as the superoxide, not as a peroxide, not as hydroperoxide or anything.

So, it is just the O2 molecule gets delivered, but more importantly you must remember and understand that these are not just iron oxygen binding, there is electron transfer happening during these processes ok. Now, as you know that for hemoglobin and myoglobin for these porphyrin iron, oxygen chemistry is these color upon oxygen binding becomes red purple, but for hemerythrin; it is colorless, it is colorless in the deoxy form right.

Upon oxygen binding, it becomes red as you know this becomes violet pink and for the hemocyanin it becomes blue ok. Of course, another thing I am sure you have noticed or you know already that hemoglobin is like 4 times of myoglobin right.

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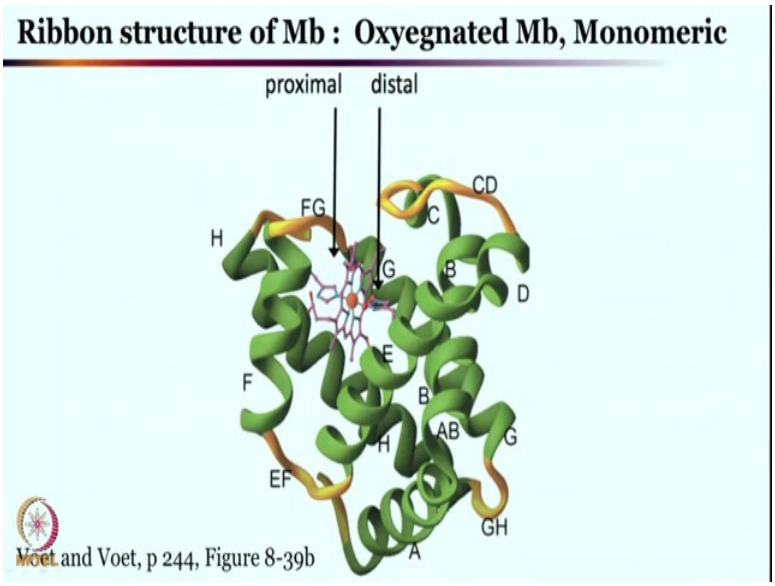


So, let us look at the myoglobin once very quickly. So, what you see is a ribbon structure of myoglobin oxygenated myoglobin, where oxygen is now coordinated and it is monomeric in unit. So, in myoglobin you have this protein backbone all those nice ribbon structure. Now there is a porphyrin ring appended with it and from the axial position there is a histidine and this site is vacant.

Now, with the oxygenated myoglobin you see that oxygen is attached with it; this site is called the distal site and the histidine binding site is called proximal site. So, you have very nice structure, very nice orientation myoglobin which can bind with oxygen and there is porphyrin ring. You should practice drawing the porphyrin ring; it is not trivial, you should practice multiple time and then there is this histidin which is bind on an in an axial form with the iron center and then there is oxygen that is bound with the iron center. So, this is center is called the distal site and that center is called the proximal site ok.

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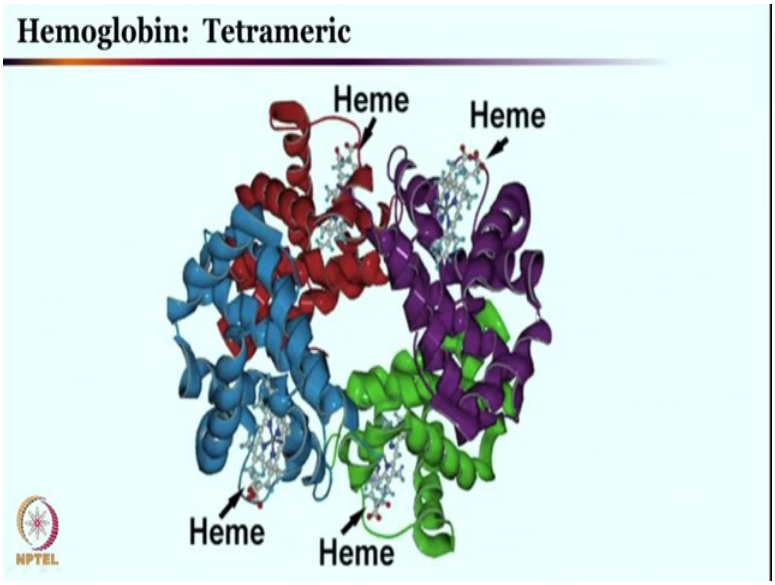


Now, let us look at the structure from U. C. Davis where it shows that the hemoglobin is nicely tetrameric. Previously, you have seen one porphyrin center now you see 1, 2, 3 and 4 porphyrin center; that is quite interesting.

I think that that is a beautiful structure it is showing that hemoglobin is tetrameric in nature. You can see that these four different subunit of the hemoglobin which are nicely colored. And as you can see 1, 2, 3, 4 hemoglobin are there or sorry 4, 4; these porphyrin units are there in this hemoglobin structure which is tetrameric in nature right.

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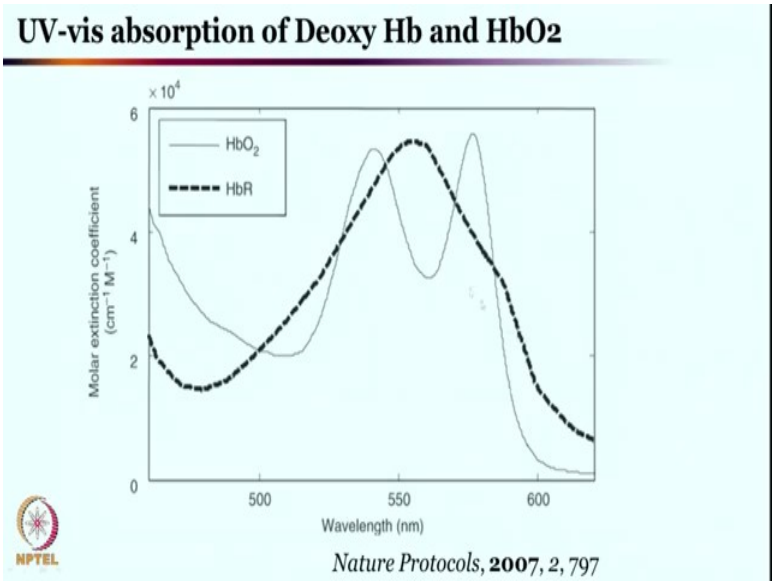

Well upon oxygen binding this is a quite simple thing to say that upon oxygen binding that UV visible spectra changes. If you record the UV visible before oxygen binding; it is the dotted line over here around 550 wavelength; 550 nanometer by the way this is the (Refer Time: 10:41) band; the there the SORET this is this is the Q band, there is a (Refer Time: 10:45) band which is much more; much more intense and we are not showing over here and that is very intense. It is not that very very easy to see the shift over there, but that is why we are zooming on only 500 to 600 nanometer region, where upon oxygen binding this hemoglobin O2 binding, you see the UV visible spectra changes clearly.

So, as you have seen before the binding of oxygen; it is the red purple color, upon oxygen binding it is really the red color and that is due to these absorption spectra as you can clearly see. So, this is upon oxygen binding ok; the dotted line over here is the reduced form of the hemoglobin ok, that is once again very fantastic.

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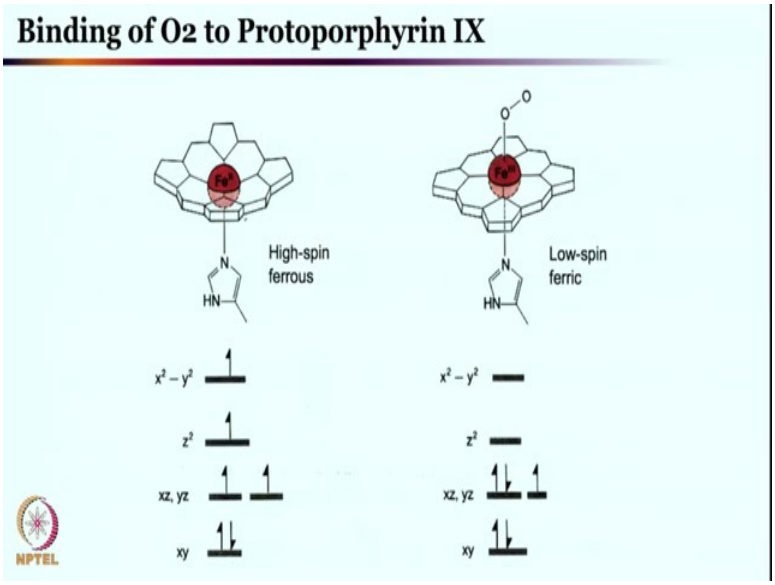


Let us see what happens when oxygen is binding with the protoporphyrin 9. So, the porphyrin ring over here of course, with the substituent is called protoporphyrin 9. You should again practice the drawing of this porphyrin ring that Chemdraw off it ok; we have seen in the last class the Chemdraw of the porphyrin right. So, you should start practicing that.

Now, in the context of cytochrome c right; we have discussed the electron transfer. Now in hemoglobin over here or the porphyrin iron center; protoporphyrin 9, you see you have a iron 2 plus center which is large in size it is not fitting into the cavity of this porphyrin ring, it is outside the porphyrin ring and the and the proximal site is having this histidine.

It is high spin ferrous; despite this porphyrin being a strong field ligand still this is a high spin complex, not the low spin complex. And iron is bigger and not fitting in iron 2 plus is big; it is not fitting into the porphyrin ring and the histidine is attached to it. Despite of these still this is a high spin ferrous high spin iron II plus not a low spin species. For a high spin iron 2 plus species you see that these t2g4 and eg2 orientation is there and this is what is the electronic conference configuration looks like.

Once oxygen is binding with the iron 2 center; this becomes iron 3 plus as we have mentioned that iron gets oxidized to iron 3 plus and it gets reduced oxygen gets reduced to super ox. Of course, before this happens iron 2 will just bind with oxygen that is one of the stage, but right after binding; it will end up transferring electron into the oxygen moiety. So, iron 2 plus is now oxidized to iron 3 plus size decreases.

And now it is fitting perfectly in the porphyrin cavity and with these all these six coordination it becomes low spin ferric in nature. So, t2g5 electronic configuration we have in these cases. So, it is very clear that high spin to low spin configuration occurs for the oxygen binding and oxygen is not just binding with iron; one of the electron from the iron 2 plus is transferring into the oxygen molecule to make it iron 3 superoxide species. So, this is the iron 3 superoxide species which is low spin ferric state right. Let us move on. So, there you are having 1, 2, 3, 4, 5, 6 electron, here you are having 5 electrons due to the d5 of the iron 3 plus ok.

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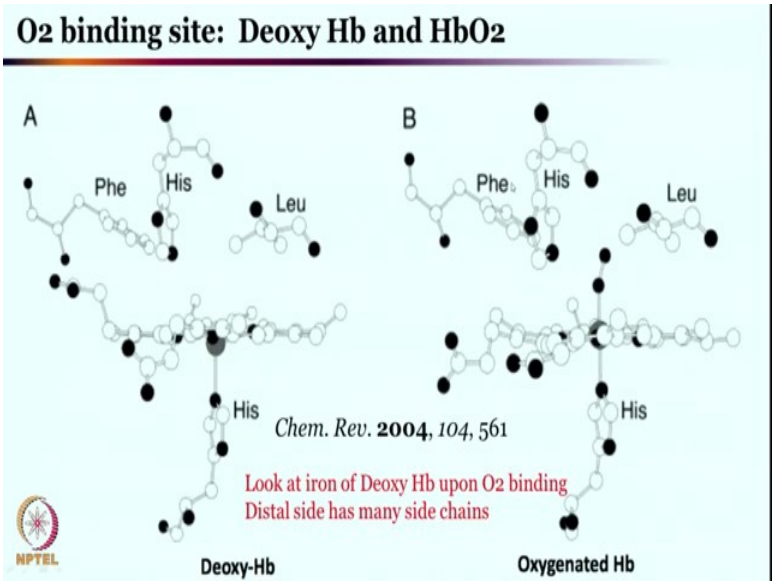


Here is a pictorial diagram from here where we see that in iron 2 plus state, where this is the porphyrin and this is the proximal histidine side iron 2 plus is outside the plane. This is the plane of porphyrin these are different amino acid backbone that is appended in front of the distal site. And, here you see this is iron 3 plus now with bound with histidine iron 3 plus has moved into the cavity of the porphyrin and this movement has a lot of effect in the in the oxygen binding as you will see in case of hemoglobin.

These are the protein side chain or at the at the distal site which also helps binding in the oxygen and hydrogen bonded due to this proximal; due to this distal side chain or distal this protein binding pocket. We will see that this is a bent binding of the oxygen with respect to the iron center.

So, once again look at this iron deoxyhemoglobin; this is the iron deoxyhemoglobin upon oxygen hemoglobin. Now, this is oxygenated hemoglobin or oxohemoglobin now we have moved the iron in the plane and distal side has many side chains as you can see over here.

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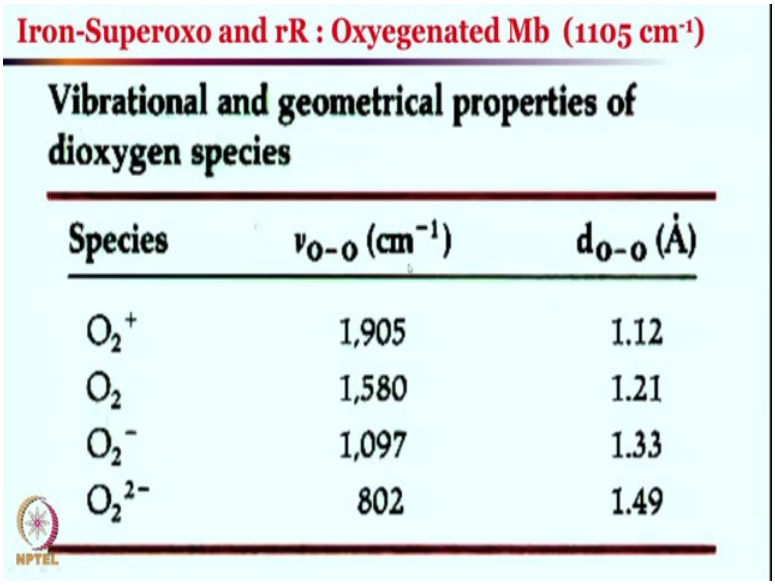


Now, let us move on; upon oxygen binding just what you have seen over here in this structure. This oxygen is getting reduced to superoxide, but now to how to really characterize these species? Of course one of the thing that we can utilize is UV-visible spectra that you have seen. Another thing which we can utilize is resonance Raman spectra of course, you can have crystal structure if you can, but it is not always feasible to get the crystal structure. Therefore, these in-situ spectroscopic technique the solution spectra are very very characteristic of the species.

Quite interestingly resonance Raman spectra of the oxygenated myoglobin gives a 1105 wave number peak. Well just to briefly tell you that oxygen which is O2 without any electron transfer or electron taking away the oxygen-oxygen stretch in the resonance Raman spectra will be 1580 and the oxygen-oxygen bond distance would be 1.21 angstrom. Upon oxidation these oxygen-oxygen stretch become stronger or oxygen-oxygen bond becomes stronger.

So, therefore, this is in much better or much improved oxygen-oxygen stretch. The distance become shorter which is also reflected in the O-O stretching frequency in the resonance Raman. If you are reducing by one electron that is the case in case of myoglobin or protoporphyrin 9; the structure you have seen a moment ago,

In this case oxygen-oxygen bond is weakened, therefore the length is increased and also the oxygen-oxygen stretch is decreased from 1580 to 1097. So, this oxygenated

myoglobin data of the resonance Raman matching quite well with the theoretical or experimental previous observation with these oxygen, superoxo and the peroxo species. So, in case in these cases 1097 matches very well; so this is the superoxo species without doubt in oxygenated myoglobin; if it was a peroxo it would be reduced by 2 electron. But more importantly oxygen-oxygen stretch would have shifted to 802 wave number; oxygen-oxygen length will be also increased to 1.49 angstrom.

So, the crystal structure UV visible spectra resonance Raman and other spectroscopic techniques can also support such formation of oxygenated myoglobin or protoporphyrin 9 oxygen version HB-O2 version ok. We have learned that now that is clear I am sure you have studied this many times. So, I would not be discussing please feel free to study from any book and that is the book we have also referred.

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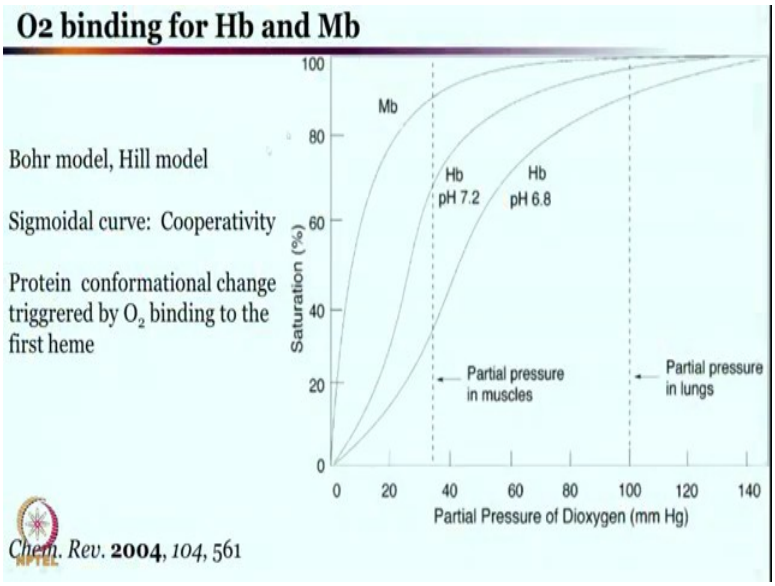


Oxygen binding for hemoglobin and myoglobin; you have seen that how these hemoglobin is binding with oxygen. And and how the curves between oxygen binding curves of the edge hemoglobin and myoglobin varies; these are at different pH spectra, as you can see at pH 7.2 hemoglobin has a sigmoidal curve. That thanks to the cooperativity, the sigmoidal curve over there as you know protein conformational changes that is being triggered by oxygen binding to the first heme; gets translated into binding a binding of oxygen into other hemoglobin side.

Let me go back once more over here, so what we are trying to see say is once let us say one of the oxygen is binding in one of these center that pull as you have seen that iron 2 plus is now oxidized to iron 3 plus; that pull will have effect in all others centers. All other centers now is ready to bind oxygen much more easily, the first oxygen binding is little bit rate limiting once that happens that message or that that trigger that that oxygen binding triggers the binding of the oxygen in other side.

Let me show you in that other picture that we were discussing. So, once let us say out of the four protoporphyrin 9 center; if one of the protoporphyrin 9 center is binding oxygen just like these over here as you can see now this there will be a movement inward movement ok; this movement inward movement by this histidine will be translated through the whole hemoglobin protein because these are part of hemoglobin.

And this movement that pushing in that histidine movement for one of the protoporphyrin 9 will be now transferred to or that signal will go out to the all hemoglobin. And it will be ready; the other three centers will sequentially get ready for oxygen binding and that is what is what we know as the co-operativity, right.

So, the protein conformational changes triggered by oxygen binding at one of the heme center or the first team center will facilitate oxygen binding in other center. Please do read about these, I am sure you are familiar with this from your earlier studies ok. Now the problem; one of the problem that we all have to deal with the oxygen binding at the porphyrin center; in hemoglobin and myoglobin it occurs very nicely and everything is perfected because that is what nature is good at.

But what if we want to understand this problem in synthetic laboratory ok? Are we going to understand this in a clear cut manner or there is going to be a problem in studying this chemistry in the laboratory? What am I trying to tell here well there is a need to understand what goes on in hemoglobin and myoglobin of course, in the form of enzyme; in enzyme.

But lot of these enzymatic studies are not easy to do and they are complicated and the conclusion at the molecular level; what oxidation state is there what is happening over there all these conclusion and what is the spin state what is the binding mode all these conclusion are not easy to be drawn at the hemoglobin and myoglobin or any other enzymes in that matter and, this is where particularly bioinorganic chemists are quite

useful in synthesizing this molecule in laboratory and then try to see whether these porphyrin; let us say in this case porphyrin iron center can really bind oxygen.

And if they are binding oxygen; how they are binding oxygen ok. This is what is called synthetic modeling studies, the problem with the synthetic modeling study is unlike the enzyme where you have really one iron center or four iron center, but they are separated completely. So, they are not really going to dimerize with respect to each other, they are not going to interfere too much of each others activity.

But in synthetic setup when you are want to study one porphyrin iron center, there are invariably other porphyrin iron center into your solution. You cannot just take one molecule of iron very iron heme centers very precisely, there is always hundreds of porphyrin iron centered the moment you want to take any solution of iron iron heme center right.

So, this is particularly where if you want to study the iron oxygen chemistry in laboratory; one at least one more iron center will come close to each other and then they try to interfere with each other. Let us say this is the porphyrin ring of one center, this is the one porphyrin center ideally they should not have any talking with each other; if we are to mimic the hemoglobin and myoglobin chemistry. But in reality in solution they will be coming close to each other and try to interfere with each other.

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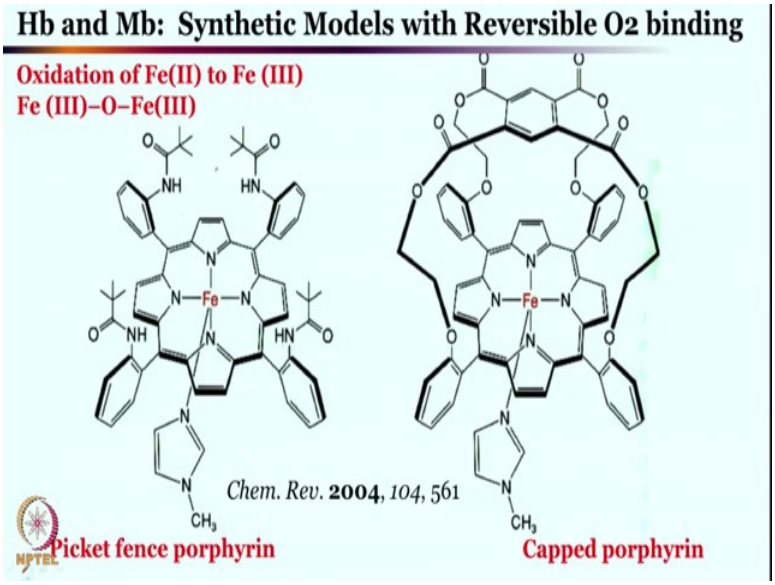

So, the moment one of the iron center is forming oxygen chemistry; then the other iron center will come in.

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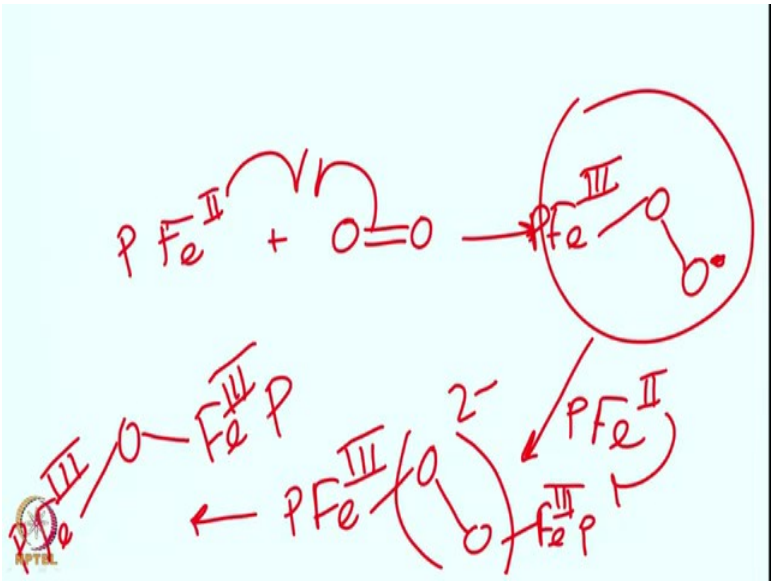

For example, so if you have a iron center over here; iron 2 and you want to react with oxygen over here. What will happen is first as you have seen in case of hemoglobin and myoglobin; this would be iron 3 center and oxygen is getting reduced by one electron and that is your the superoxo species.

Now, in hemoglobin myoglobin it stays as superoxide, but in laboratory scale another iron 2 will come in. Let us say this is porphyrin P P P coming and then it would immediately form a iron 3 superoxide to peroxide because, this can also give one more electron to give iron 3 porphyrin.

Now this whole unit is now 2 minus, so porphyrin iron 3 peroxo iron 3 porphyrin; it is a dimeric compound that you are going to get. And from there on it will not stop over there, it can then again go further and give the decomposition product which is nothing, but let us say a **μ** oxo porphyrin species.

So, these sort of reactions are very common in synthetic laboratory and this is why it is always difficult; very very difficult I would say to do the chemistry that is happening in in nature in hemoglobin and myoglobin because it gets much more complicated. In hemoglobin and myoglobin, it is kind of site isolation chemistry is going on in one side

one chemistry is going on. It is not getting affected chemistry is not getting too much affected by another site. But in solution these sort of problems are so common that studying these chemistry becomes very very difficult.

A lot of effort has gone into in mimicking the chemistry that we see over here. These chemistry over here people have tried tried quite a lot of things to mimic these chemistry, but, but a lot of things has failed. To prevent this short of dimerization that we discussed **μ** oxo formation as well as auto oxidation of iron 2 plus 2 iron 3 that is also another problem.

There has been the drawing where in these bulky substituent which are not present in the enzyme, but to prevent these homodimerization and other side reaction these bulky substituent are designed and placed at the periphery of the porphyrin ring. So, that another porphyrin cannot come in.

So, porphyrin was just like this; they were coming in very close to each other and they were having let us say very close contact. But if it is made bulky like this, they will not be able to come very close or the porphyrin center they will not be able to meet with each other very easily. So, the bulky porphyrin are made so which are known as picket fence porphyrin to prevent the oxygen reaction; iron oxygen that reaction that is over here.

That species should not react with another equivalent of these species and this bulk protection protectant; it is like a boundary; this boundary protecting are protecting the oxygen binding to this iron center. So, that one iron and one oxygen can react not two iron and one oxygen can react in case of this mimic ok.

Once again in enzyme that is taken care by the protein backbone; these are bulky, they prevent and anyway in nature has designed in such a way so that other center is not very close to each other which can interfere but in synthetic setup for synthetic bioinorganic chemistry; it is quite a challenge quite an effort that has gone into to understand what happens in hemoglobin and myoglobin.

Another drawing is over here which is a kept porphyrin; as you can see it is a gigantic structure or quite a lot of synthetic effort that basically prevents or protects one of these distal site of this iron center; proximal side is blocked by the histidines or in case of this

synthetic studies, it is an image all unit. Now the distal site is prevented by this fence or the capped porphyrin, which is preventing another porphyrin to approach these iron oxygen species that is being formed ok.

So, in summary what we have seen so far is in hemoglobin and myoglobin; it is the oxygen binding that is happening which is keeping us alive but most importantly these are mono nuclear chemistry; although tetrameric are the four porphyrin centers are there in hemoglobin, but they are not interacting with each other in the form of the oxygen, iron complex formation, they are not forming a dimer or anything they are individually monomer unit.

But the same study when we try to do in our laboratory in the synthetic laboratory; then it does not really happen that very easily because it we cannot control the chemistry this chemistry is very sensitive and very reactive. So, we can do the reaction same reaction what is happening in hemoglobin myoglobin, but it is so reactive so that it goes on and react further right and therefore, we get lot of side reaction.

This reversible oxygen binding is very difficult to mimic that very easily in synthetic laboratory. And it has been done by much affords gone over the decades where now porphyrin is now protected as if like a wall is built around the porphyrin and then therefore, the second porphyrin cannot come close to it; we have seen the picket fence porphyrin and capped porphyrin, how they are preventing and maintaining a 1 : 1; iron is to oxygen ratio and that is quite phenomenal.

We have seen how upon oxygen binding in hemoglobin and myoglobin iron was outside in ferrous high spin ferrous state. But upon oxygen binding and becoming smaller in size to while oxidizing to iron 3 plus, we can then see that it is getting pushed into the plane of the porphyrin. And that push is triggering the histidine movement and that also helps in binding oxygen in other three center of the hemoglobin right.

So, this is what is the cooperativity, you can read from any book you wish. With that let us come back with more discussion on metals in biology in next class. Keep studying hemoglobin, myoglobin and it is quite fascinating what are the things been done. You are also encouraged to read from many different sources; keep studying, we will get back to you soon.

Thank you very much.