Inorganic Chemistry of Life Principles & Properties
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Lecture – 25
Role of Iron in life – Transport systems

Welcome you all to the next lecture on Inorganic Chemistry of Life Principles and Perspectives. In the previous class, we have been looking at the oxygen transport system; towards the end we were trying to look at the oxygenation curves, difference between the myoglobin case and hemoglobin case just let us recapitulate that one.

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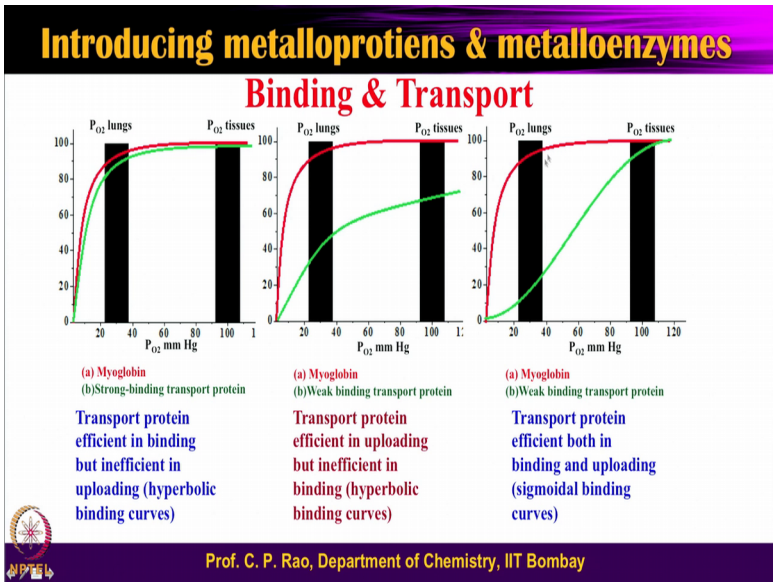

And look at this is the myoglobin clase is completely hyperbolic.

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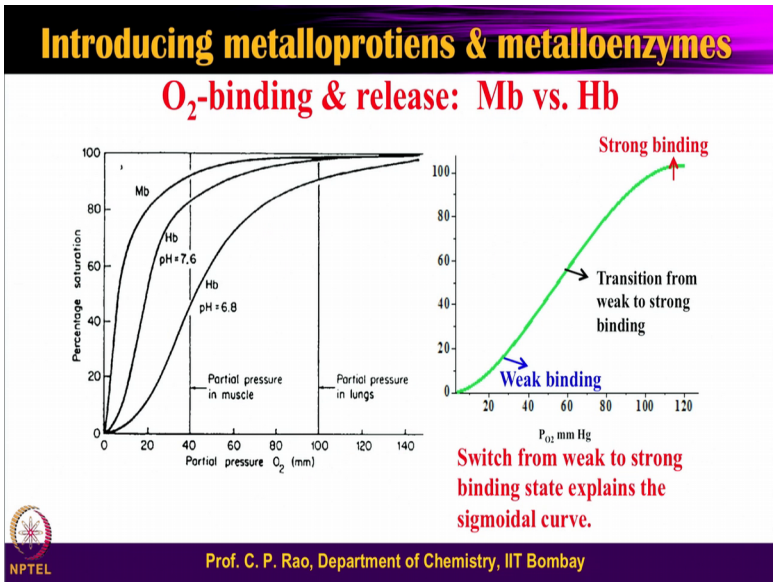

And if we were to have a protein which should they have a optimal binding as well as the transport property of this, it should have a transition kind of a model and that transition model relate to this what you have here is a sigmoidal.

And if you compare the hemoglobin curve is exactly like a sigmoidal kind of thing. So, you have a weak binding, you have a strong binding reason there is a transition. So, therefore, the myoglobin is can only oxygen whereas, the hemoglobin can pick up as well as the transport. So now look at various events that we studied regarding this one.

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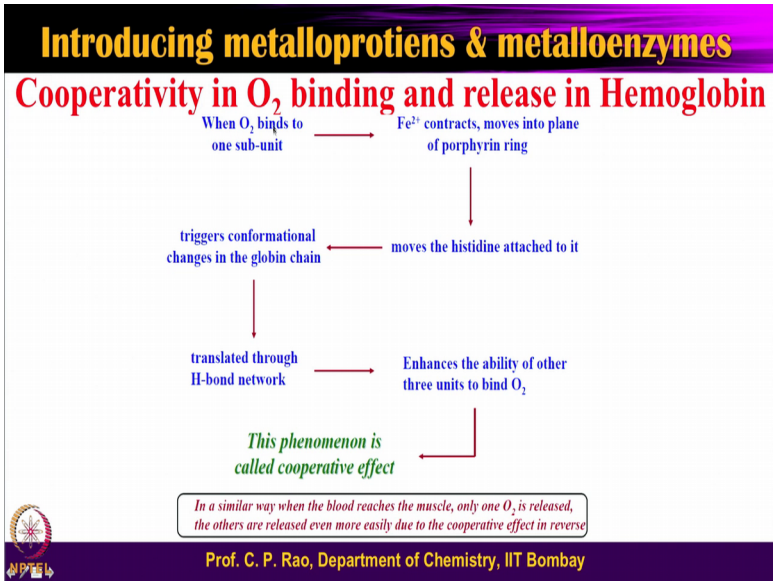

So, first thing is let us say you bring the oxygen and bind it to the sub one of the subunit; that means, when one when O 2 binds the one of the subunit what will happen? We have seen already the iron 2 plus goes from high spin to the low spin and then iron 2 plus size contracts and then goes more into the plane of the porphyrin ring; that we have already seen it goes more on there.

But then what will happen there is a histidine attached to it so; obviously, as a movement from in which was about 0.6 angstroms above the plane. Now after the oxide is bond, it will it comes to about 0.2 angstrom above the plane; that means, more or less into the plane that will disturb the connectivity that you have or it will move the histidine bond that is attached to the iron.

And that kind of a movement in that will trigger a conformational change; that conformational change go through that particular protein and to the neighbor translated through the hydrogen bonding network. And then that will enhance the ability are the other units or neighbor units; either to bind to the oxygen. And this is the kind of phenomena what we are saying is when one oxygen binds at one unit, the binding of the oxygen binding affinity of oxygen at the second, third and fourth becomes absolutely very high. And therefore, such a phenomena you can call it as a cooperativity effect. This effect is happening between the 1 heme of one of the sub unit to the other heme of the another sub unit to the other heme of the another sub unit and the fourth; so, all this.

So, and similarly when it releases also it goes exactly the reverse way when 1 mole of the one of the oxygen is released then the rest of the oxygens from the rest of the heme sentence will also get easily released too. So, the oxygen binding then it contracts the size of the iron; iron gets more into the porphyrin ring that will give a kind of a movement on the iron histidine bond and that will create a conformational change. And this conformational change is transferred from one unit to the other and which results in the cooperative effect.

I hope now you understand how the oxygen is cooperatively bound in case of hemoglobin, and this is what is the responsible. There are many other facets to this how much are the conformational change, which region of the conformational change? These are all not part of this particular course; that will be something comes from the biophysical aspects etcetera. But we just say that there is a conformational change of the

of the protein moiety because, the iron is coming into the close to the plane and then iron to the histidine bond is moving.

So, we have looked at aspects corresponding to oxygen binding, what kind of a protein characteristics you need to have? It would favor the oxygen complexation, it should not favor be oxo dimer formation, it should not favor electrons transferred into the O 2 and all these kinds of things; then it should have both the binding in the weak to the strong by transition having a sigmoidal kind of a oxygenation curve, but not the hyperbolic kind of a oxygenation curve.

So, all of these are perfectly valid with this and therefore, you have a cooperative effect of this the oxygen transport of this. Look at one another aspect you know there is a lot of demand for synthetic blood. You know why? Because lot of patients have to be injected lot of the leukemia patients have to be refilled remove all the you know the old blood and refill with the good and fresh blood etcetera. So, therefore, there is a huge demand for synthetic blood.

So, there are some affords; efforts in the literature, there are several efforts in the literature to go towards this.

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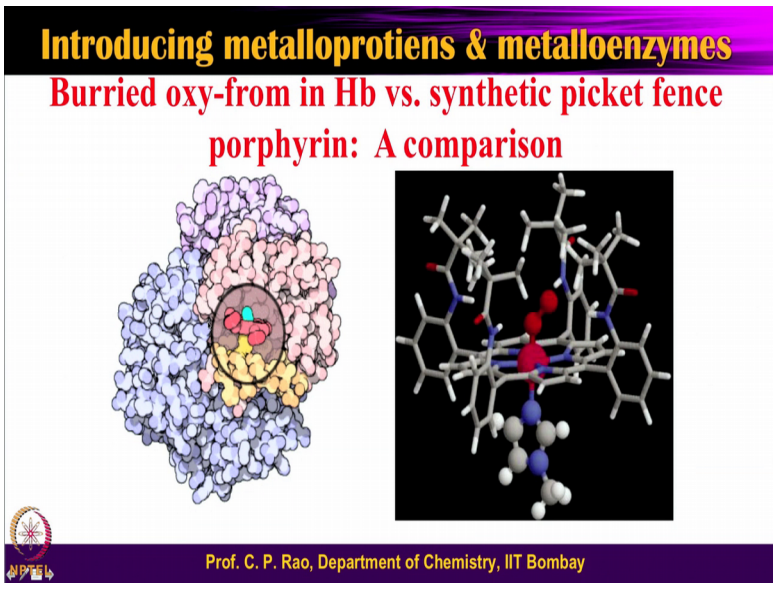

Just look at this particular thing these are all entire thing is a protein and you have a heme, this is the oxygen you can take it as. So, it is basically a heme, the oxygen and the

heme is buried inside the protein. And as a result of that the mu oxo dimer is not formed, as a result of that it is able to do perform many things.

Similarly, people have looked at can we make some kind of a mimic to this. So, what they done was this is the just a simple porphyrin is connected to the histidine ok. And of course, there is a O 2 here and then on the periphery they have built certain units, so that it is a kind of a fence. So, this is a fencing around that; so around the O 2 and in fact, even interactions also being given over there.

So; that means, this Fe O 2 would not go to form Fe O Fe kind of a mu oxo dimer kind of thing. So, people have there is a huge number of different developments in the synthetic; synthetic porphyrin, synthetic heme chemistry, but we cannot go into all of them; just I brought to your notice that the synthetic chemists are smart and they have tried to mimic this.

Because it protects this one they also try to protect by using this fence and this is how the picket fence kind of a structures have come. Of course, still we have many other aspects are required to make this as a synthetic blood a bit not so, immediately close to that. So, let us not too much worry about, but efforts are always all towards the making the synthetic blood ok.

Now, we have looked at more or less exhaustively as a biological inorganic chemists need to know the role of the iron, the role of the heme in a myoglobin and hemoglobin. Now let us look at the other ones where you have a hemerythrin invertebrates and hemocyanin in mollusks.

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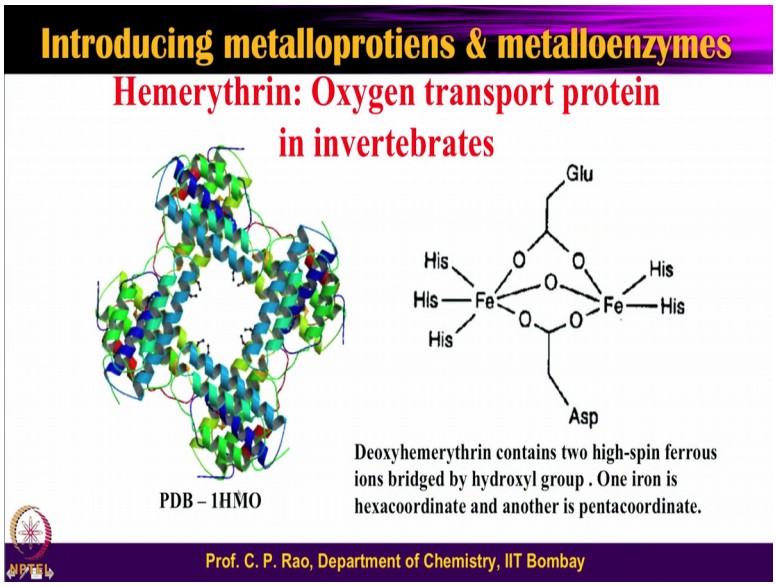

Let us look at this and you say that; this is the total protein and protein in stretching 1 2 then 3 and then 4; it is a kind of a tetramer you have. So, it is a tetrameric kind of a protein and in each of the unit you have this di-iron center ok. So, in the hemerythrin you have a di-iron center one of the iron is a 6 coordinated, another iron is 5 coordinated and this has a 3 histidine, this has 2 histidines.

And these are bridged by the carboxylates of one is aspartic, another into glutamic. And then there is a bridging; the bridging is not O it basically OH and how this OH shuttles I will explain, when we go from deoxy to oxy. So, this is a deoxy form as such then how the oxy form etcetera, then we will look at just in a while ok.

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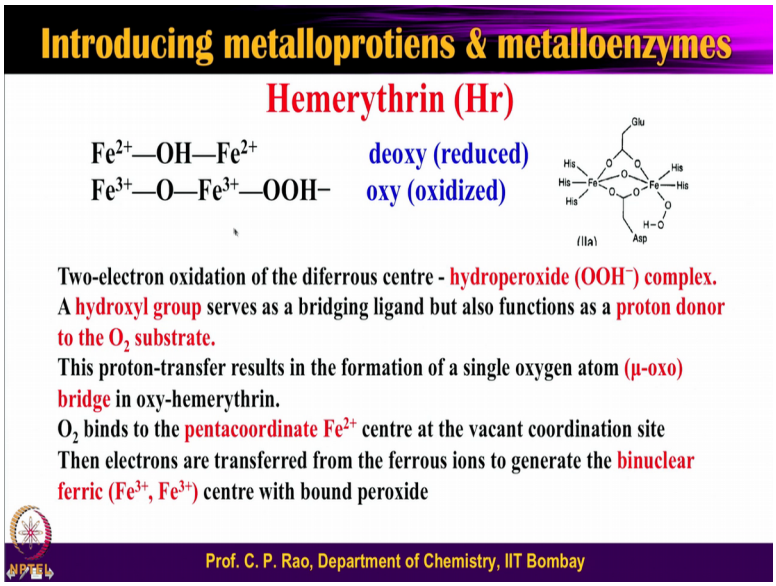

So, in this particular case the deoxy form you have 2 iron centers; both the irons are a 2 plus and there is a bridging is OH. Of course, these bridging are besides the carboxylate bridging; besides the carboxylate bridging; carboxylate bridging is there, but in addition.

Now in case of oxy hemerythrin; in the case of oxy hemerythrin the irons are oxidized. As I told in the beginning itself you can see the 2 irons becoming 1 electron here oxidized 1 electron totally 2 electrons; obviously, you should think the 2 electrons must have gone somewhere; where they have gone? They have gone into the O 2 and once they go into the O 2 they becomes a O 2 to minus. And the OH transfers the proton; the OH the bridging OH transfers the proton that will become OOH peroxy kind of a thing; so, oxidized.

Now, you can see there is a huge difference between the oxy form of hemerythrin and the oxy form of hemoglobin. In the oxy form of hemoglobin nothing happens to the oxygen-oxygen bond order to not much extend it all; whereas, in the hemerythrin the oxygen O-O bond order is reduced; this is all I explained to you.

So, this is besides the carboxylic bridge etcetera. So, therefore, those are something important.

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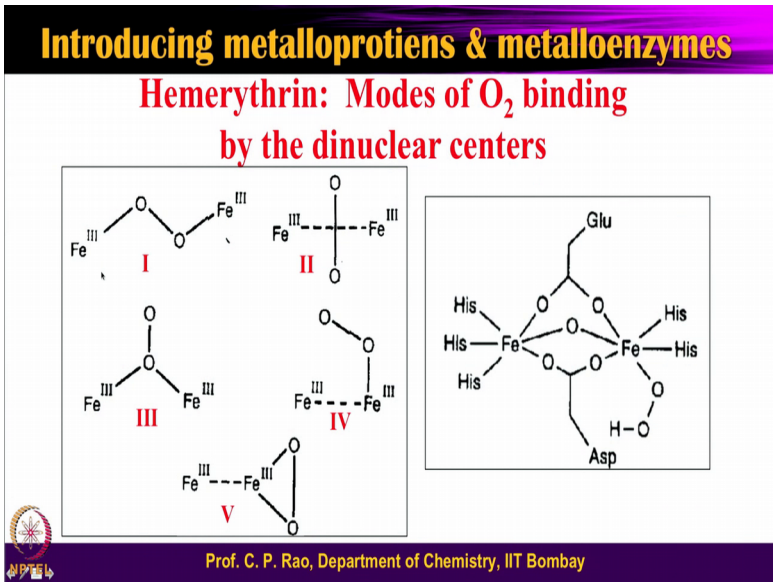

Now, so is a di-iron center; so, one can make some kind of a guess between the 2 iron centers what different ways a O 2 can bind? So, one of the thing it can be binding like this or one of the thing it can be binding like this, one of the thing it can be binding like this and one of the thing it can be binding like this or it can be binding to only one iron, but like this there are possible. How will we know these things, how will we differentiate, can anybody think off what are we looking at?

We are looking at the way that the O-O is bonded to either one iron or both the irons; that means we need to get a click in our mind that we can look at the O-O stretching vibrations. That means, vibrational spectroscopy or infrared spectroscopy or FTIR will be able to tell us the whether the binding mode is like this, whether the binding mode is like this, whether the binding mode is like this etcetera. This have been done with a small molecule and they have been compared with the protein systems.

So, we are not going into the all those details and ratify; all that I want to tell you is the O-O stretching frequencies differ; if you go from type I to type II to type III to type IV to type V. And that itself is a good message for you to say that; yes this is the FTIR spectroscopy can be used ok. This is not so, much of importance here because I will be explaining this later, but since that binding modes are there I have showed it over here ok.

So, what is happening here? The iron which has a 5 coordinated will bind to the O-O and both the irons will give 1 electron each; then O-O minus and there is OH hydroxyl proton is transferred to this. In fact, it is stabilized by some kind of a hydrogen bonding interaction as well between that oxo bridge and this OOH part of it; oxo bridge and OOH part of it; so, this is stabilized.

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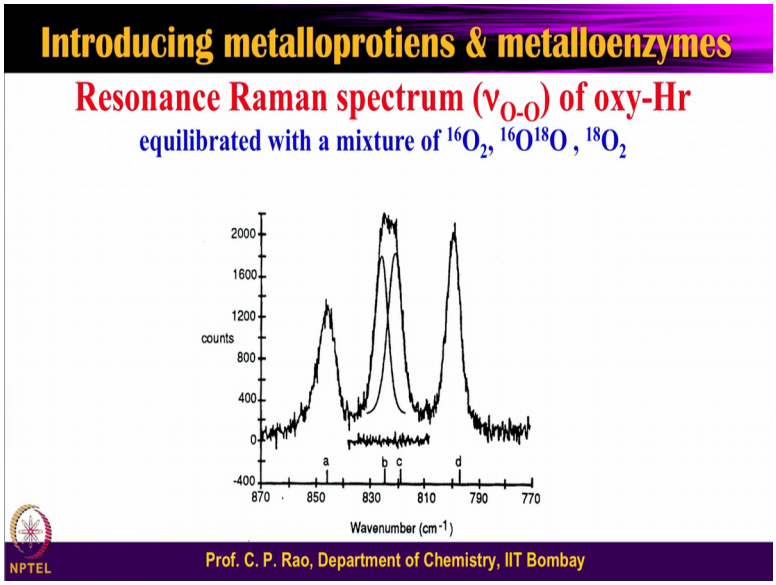

And now so, as I said since this is possible to be differentiated from that so, we have now here vibration spectrum in the new O 2, but this vibration spectrum is not used just to meant for differentiating all the 5 forms.

And this is used to meant to identify whether the kind of a binding is in this particular mode or not. So, therefore, just to identify that it is in the form of the species IV; type IV species that is what you. And if you look at the O-O stretching region which is you will find the O-O stretching y vibration.

Now, you can have a natural abundance of O-O then 16 O 2; or you can partially change into 18 or completely change 18. So, as you go from 16 to 16 and 18; there will be a frequency shift and frequency shift will be in the lowering side. And you go to the 18; the frequency shift will be (Refer Time: 14:03) why the frequency will lower?

You know in the infrared spectral stretching vibrational frequency and the numerator; you have a bond order or bond strength or force constant and the denominator you have a

mass unit; here the mass is O-O mass. So, in one case 16 16 mass reduced mass you to bind that is 1 by 16 plus 1 by 16 or 16 18 or 18 18 so; obviously, they are they reduce mass are different. So, therefore, you find peaks accordingly, we do not need to analyze much more than this.

So, therefore, we can try to understand that very nicely and let us look at the process in a simplistic manner. Because this protein is a very tough protein, a huge protein and studying all the phenomena is not that easy. So, we will just take only comparative a kind of a approach here; now you see that.

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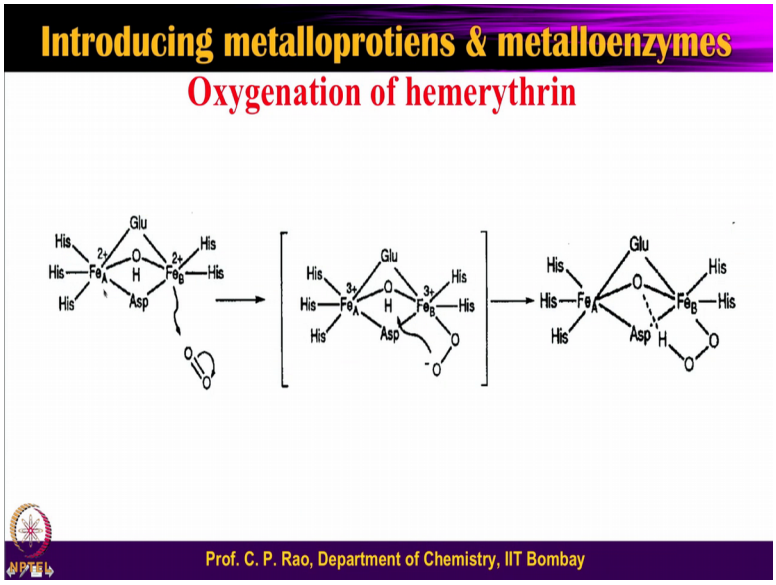

See that the iron A is having 3 histidines, iron B has got 2 histidines and in the deoxy form both are 2 plus and the bridging is OH. And one of the carboxyl bridging from a aspartic acid, other carboxyl bridging is from the glutamic acid perfect.

Now, if the O 2 comes in proximity where will the O 2 bind? Because there is a vacant site here, the O 2 will have an interaction over here. So and when the O 2 binds; there is an activation and both the irons will be 1 electron each and then this is what is formed. And this one will have this is the OH plus and that will form a kind of a hydrogen bonding; you see that bridging oxo the hydrogen which is present here has transferred to this; therefore, you have form a kind of a its metallacycle O iron O-O H O cycle.

So, the 5 membered metallacycle that you have formed here and this will have; obviously, a different kind of a O-O stretching vibrations or O-O vibrational spectrum as compared to the other modes that I have shown on the earlier one, that is how we are able to show all these things.

So, here we have not gone into so, many details because these proteins are very huge. So, therefore, we have restricted ourselves strictly to the metal center and then we tried to understand that phenomena. Now for a while, let us move to the third case which is the hemocyanin; so, the hemocyanin for from mollusks and arthropods it is a blue blood.

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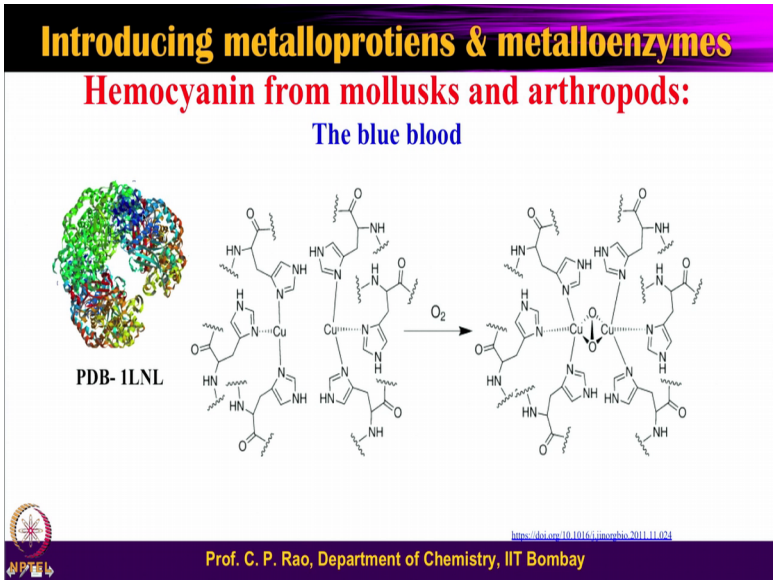

So, let us look at the protein is also huge here, not that simple and this is a quaternary kind of a structure. Now inside each of the sub unit; you have it 2 copper centers. I think these 2 copper centers are further apart by about 3 and a half angstroms or so, 3 and a half to 4 angstroms or so.

And each one of it is 3 histidines here also 3 histidines; you see that? 3 histidines here; 1 histidines, 2 histidine, 3 histidine. Similarly 1, 2 and 3 here and the copper is in copper 1 4 this is called the deoxy.

Now, you add the O 2 to this and very instantaneously; it will form a kind of species like this; how do we know from the again from vibrational spectra, you can make out the way that it is being binding. So, vibrational spectra is really an instrumental and key type of a

method to identify the way that the O 2 is bound, in what manner that that it is bound; so, this is the important aspect.

Now, here what happens? copper 1, copper 1 will go to copper 2 copper 2; there are 2 electrons peroxo. So, here the peroxo is bridged very symmetrically here and in the previous case the hydro peroxo is not bound symmetrically, this is an unsymmetric peroxo. And this is a very symmetric kind of a peroxo therefore, your vibrational spectrum will differ and that is how the things are formed.

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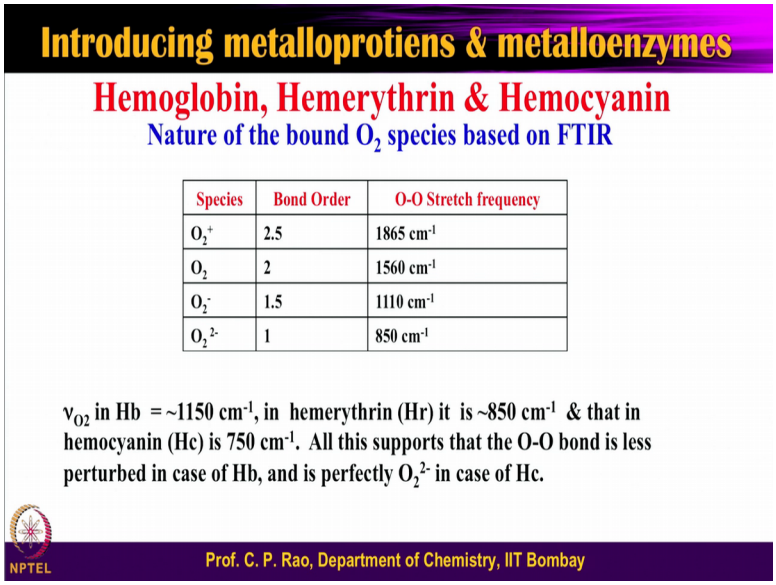

Now, let us look at all the cases; if you look at the hemoglobin, then you find a stretching vibrational around 1200 or so, centimeter inverse and a hemerythrin around 850 and in the hemocyanin 750; this is the O 2; O 2 as a bond order 2 gives around 1500 range the vibration. Remove 1 electron from this because O 2 plus bond order should increase because where are you; why the bond order should increase?

Because you have taken 1 electron out of the anti bonding orbital; so, what is bond order? Bond order is the number of electrons in the bonding orbital minus the number of electrons in the anti bonding orbital by 2. So, since you taken out 1 electron from the right side subtraction which is the anti bonding orbital; obviously, you are increasing the bond order and therefore, your frequency will go up ok.

And the other hand you put 1 electron, when you put 1 electron what will happen? This electron will go into the anti bonding orbital and the anti bonding orbital has more electrons then the difference will be less by 2 will be 1.5. So, therefore, the partially reduced and then if you put 2 electrons; it is further reduced. So, 1.5 electrons around 1100 kind of thing or a bit lower and this is around 850.

Now, if you compare with this; there is a least perturbance in case of oxygen bound to the hemoglobin iron. And there is a intermediate perturbance when it bond to the hemerythrin; as you can see the from the structure, it is not centered between the 2 irons. And when you go to the third case; hemacyanin you can see the O-O is exactly centered is exactly you know binding to both the coppers and that is what is happening in these cases.

So, therefore, that will be making much more reduced form and that gives the 750. So, you can see that the O 2 in the hemoglobin is not that much perturb O 2 in case hemerythrin is quite much perturb almost about 1 and a half kind of thing. And then when it comes to the O 2 2 minus or in the hemecyanin it is a very much like the O 2 2 minus perfect O 2 2 minus what we will find.

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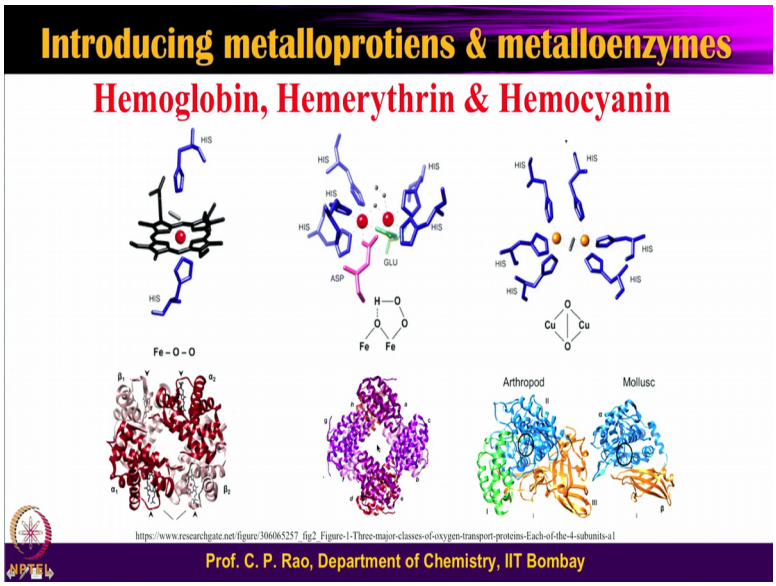

Now, whatever I said till now, let me summarize putting into these one. This is the hemoglobin part of it porphyrin and center metal and this is the guy which is the

proximal directly bonded and this is a distal which will recognize the substrate and it binds Fe-O-O.

Now, you come to the hemerythrin there are 2 O irons that there are one iron here, one iron here and in absence of a O 2 these are all in iron 2, but once the O 2 is approached both the irons will turn to iron 3 because this iron 2 iron 3 can be differentiated by using EPR Mossbauer spectroscopy, there are a lot of studies are done using EPR Mossbauer spectroscopy; wherein you can identify not only the iron that is a 2 plus or 3 plus and we can also identify whether the iron is in the low spin or high spin.

So, therefore, using the combination of the Mossbauer and magnetic aspects EPR; one could fully establish and these have been done in this. I am not taking the details of this into this particular course; only I am taking the results out of that and using it. And here it is bounded to one of the oxygen, one of the irons and therefore, it is not symmetric and therefore, the entry of the 2 electrons is not as good as in the case of hemocyanin.

See that hemocyanin there are 2 coppers and then you have the O 2 here; it is exactly symmetric and think it looks of that kind; these are this kind of course, this is not linear this is bent and this is like this. So, all of these can be very nicely studied by the vibrational spectroscopy and the oxidation states and the spin states of the iron can be studied by EPR, can be studied by Mossbauer and magnetic measurements.

So, one should be able to answer these things when we have some questions coming in this particular direction. So, that takes to the end of the oxygen binding, oxygen transport; oxygen transport in a higher organisms like human and the lower organisms and much lower kind of a invertebrates and the mollusks kind of thing. So, iron; 1 iron in the human system, 2 irons in these ones and in the mollusks you have the 2 copper centers ok; so therefore, you can see all of them.

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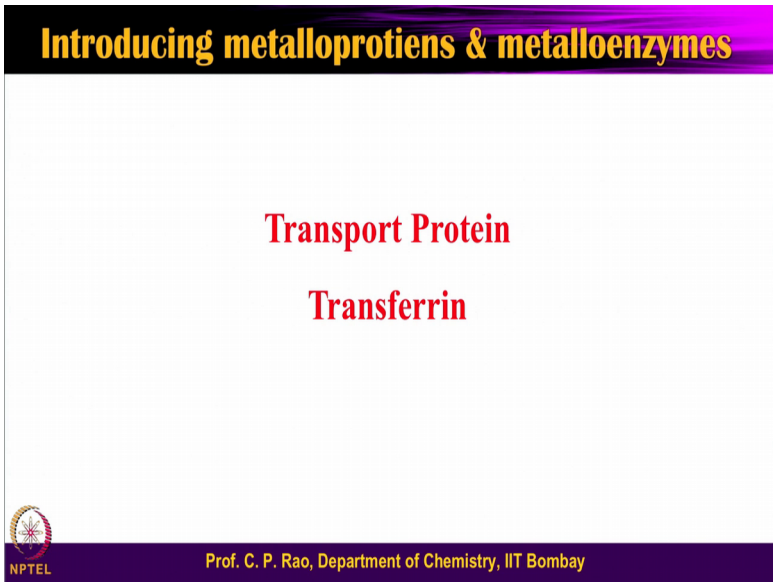

Now, let us move on to the transport, but not of the oxygen, but transport of some ion; let us say. So, transferrin; transferrin is an enzyme which transports the iron ions. Because iron is required in the entire body for a variety of cellular functions and this is being transported by a protein called transferrin. And this transferrin is a blood protein and it will take to the different organs to the body and delivers there. So, therefore, the iron based enzymes in turned or enriched in the cells of each of the organ of this body and that is what is going to be the important for the functioning of the each of the organ.

So, let us look at the ion transports a general aspects of it; general aspects of the ion transport.

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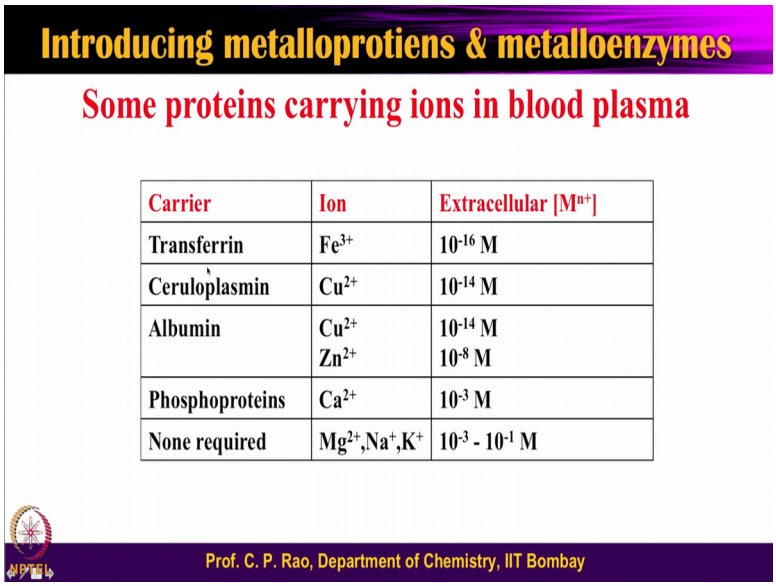

There are different common proteins like transferrin, ceruloplasmin, albumin, phosphoprotein and there are some generately it can go without even; ions like iron, ions like copper, zinc, calcium variety of these ions. In fact, many more are there this is just a few and their cellular extracellular concentrations are some of them are extremely low, but they are very important 10 power minus 16 molar, 10 power minus 14 molar, albumin 10 power minus 14 and you will say and phosphoproteins and so, all these kinds.

So, these are all proteins of present in our body, in the blood and in the blood these ions are picked up and then transported to various organs to the body. And particularly we will look at only one example that is the transferrin example in this ok.

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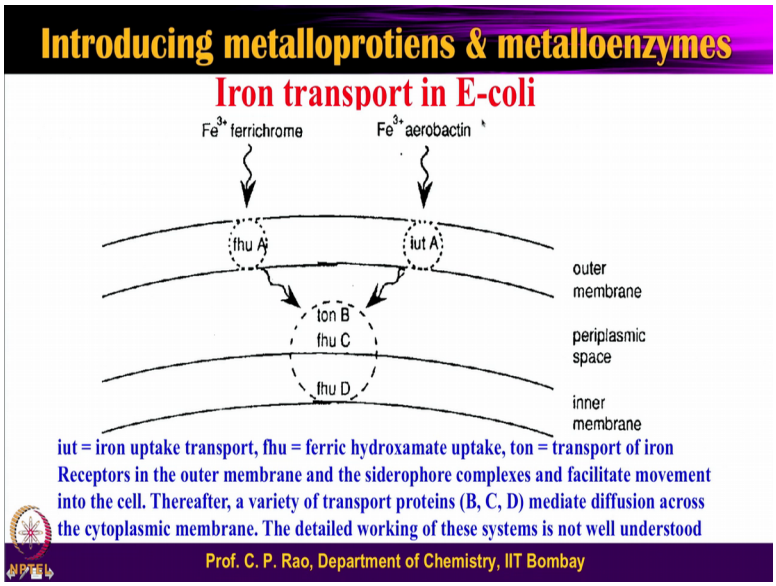

So, just before going into the transferrin story; let us look at some more general features of ion transport particularly in a small organism like E-coli. So, look at this you have an outer membrane and you have an inner membrane.

Now, iron comes as a iron chelator, iron complex etcetera and this is transferred at the surface to these iron proteins which are called iron uptake protein, ferric hydroxamate uptake transport of iron; ton, so all of these.

So, it is transferred to this and these in turned or carried and transferred to this in the inner and this further goes and releases in the blood ok. So, you have a one level of capture of the iron, transfer into another level of things and then transferring into the blood. The outer membrane and the siderophore complexes facilitate the movement into the cell thereafter a variety of transport proteins.

So, these kind of proteins are all there and they take into the cytoplasm oh yeah in this here, they will take in to the cytoplasm and that is an important aspect to be lend.

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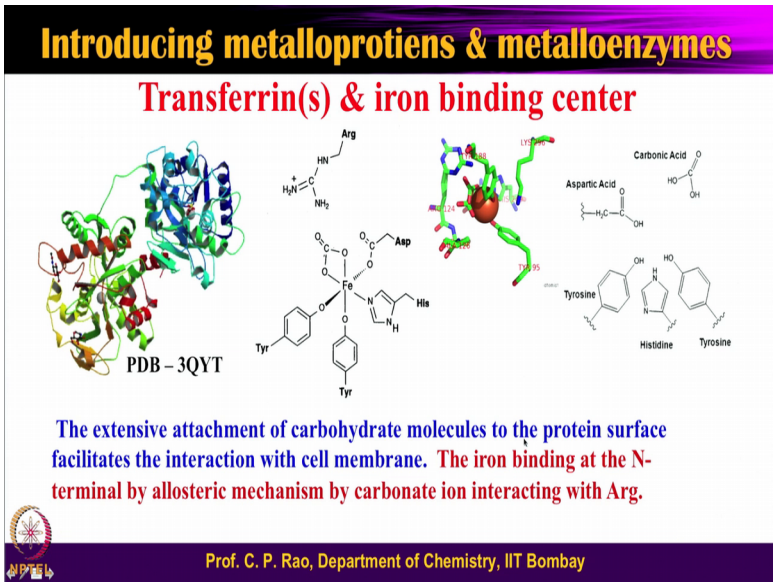

Now, let us look at the transferrin; transferrin as I said, a protein looks like this a huge part here, a huge part here. So, one of them is an N terminal, other is a C terminal and there is one iron center here, there is another iron center here.

And if you look at the iron center in both the cases very much similar; whether it is a N-terminal, whether it is a C-terminal. The only difference is that they are binding strengths C-terminal is bound bit more stronger than that of the N-terminal iron ion; this is one thing we need to be ok.

So, this is a crystal structure and see very close to the iron; you can see that what is bound over there. And this is nothing, but these ions sorry groups tyrosine and these are the 2 tyrosines and the histidine. And in addition you see something like this is not a part of the protein, but this component irons a very essential form to be very essential if the component is not there the enzyme is not complete.

So so I will explain just in a while that one and, so therefore for an apoprotein iron and the carburetor required to form the whole enzyme, but without the carbonated lone. So, there is for to the carbonate is a kind of a allosteric a factor in this, but essential factor. So, you can see crystal structure from this and if you look at an apo form, you see that the tryosine, histidine, imidazole etcetera and you have. So, this is a kind of a core where the iron comes into picture. So, this is an interesting phenomena that one can look at.

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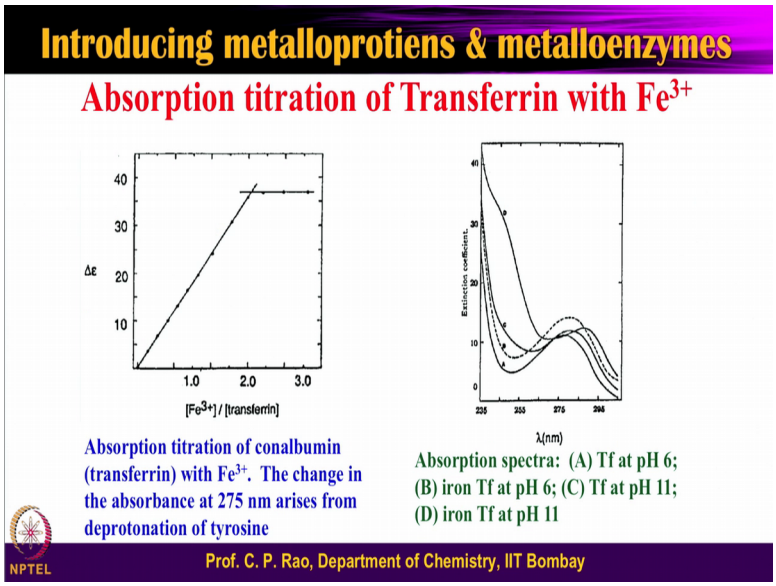

Now, you take as I mentioned the transferrin apo form you take; the apo form of the transferrin start adding the iron into that. So, if you start adding iron you can do iron as an iron 2, you can do is in presence of the air or you can do with the iron 3 also.

So, let us take this one of the apoprotein of the transferrin and add iron to that. So, if you start adding iron different mole equivalents of the iron you see the absorbance at around 275 nanometer; slowly increasing and then saturates and it intersects at this particular region. So, this particular region is basically responsible and explains that there is a complex formed between the pro iron and protein is what here you can see that intersection is 2; and why 2? Because this one iron center at the N-terminal, other iron center at the C-terminal therefore, 2; so you are getting 2 absolutely right.

You can also get this spectra at different pH for the transferrin and presence of the a pro transferrin as well as in presence of iron and these are what is shown over there; you can see very nice complexes form; this is transferrin at pH 6 iron transferrin as at pH 6. Similarly transferrin, free transferrin at 11 and iron with the transferrin at 11.

So, you can see very nice bond and in fact, you can do a very nice titration and try to find out pH variation also you can find out at what pH the complex is stable etcetera, but there is all becomes only the additional things, but no new information.

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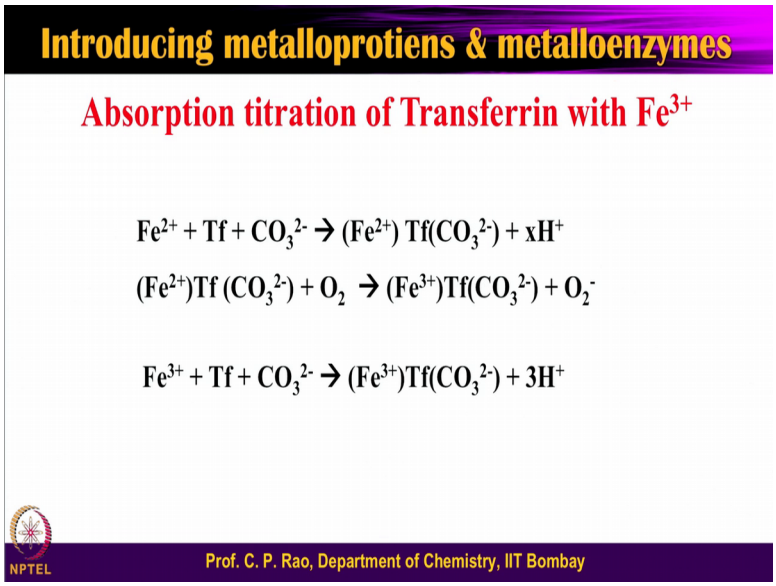

So, the information that we get and this is in this is that it binds, binds in the 2. So, when it binds it binds the iron 2 if suppose you add transferrin carbon is essential as I told you, it will if you form initially iron 2 and then get oxidized to iron 3. Or you can take the apoprotein and add iron 3 as well and then this iron 3 will be straight away no need of a oxidation. So, the apoprotein if you take and you can use the transferrin apoprotein transferrin and add the iron. So, therefore, the iron is basically added; if it is iron 2 initially the iron 2 complex forms and then followed by in the presence of oxygen oxidation takes place, but if it is iron 3 directly you have a iron 3 bound thing.

So, as you can see that we have a system of the iron transport system which is transferring having 2 centers of the iron binding with the iron at the C-terminal and the N-terminal. And so, we could see very nicely how the iron binds, how will find out iron binds, how many irons are bond; we could see all of them.

Now in the next class, we will look at how this particular iron is being transported and what are the other characteristics of the iron transport protein; that we will see in our in the next class.

Thank you very much.