Inorganic Chemistry of Life Principles & Properties

Prof. C. P. Rao
Department of Chemistry
Indian Institute of Technology, Bombay

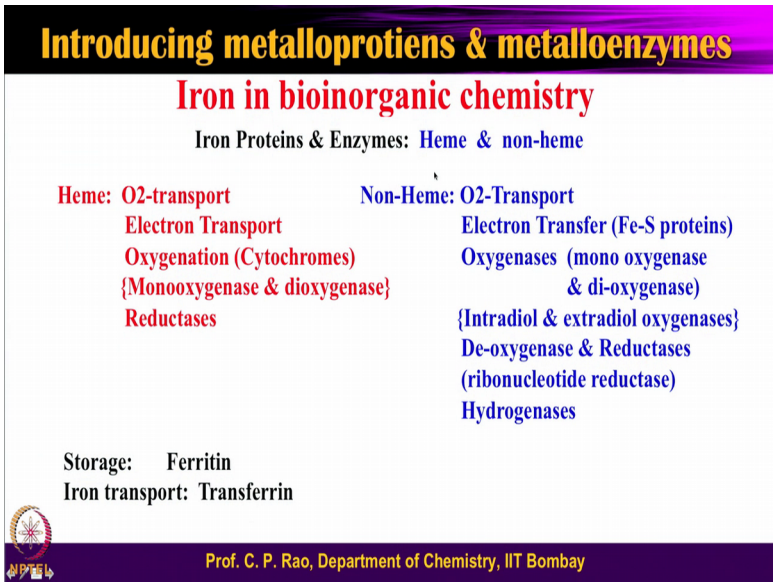
Lecture – 24
Role of Iron in life – General perspectives

Good morning welcome you all to the next lecture on Inorganic Chemistry of Life Principles and Perspectives. In the previous couple of classes I have tried to introduce the enzymes, based on vanadium and based on manganese. In case of vanadium we have seen vanadium bromoperoxidase, glomoperoxidase, heloperoxidase and also we have look at the inhibition of the vanadate in ATPG cycle.

And on the other hand in case of manganese case, we have looked at manganese superoxide dismutase which is 1 manganese based 1, then we also look at the manganese catalyst where there are 2 manganese ions are there, then we have looked at the oxygen evolving system of the photosystem 2, which is tetranuclear manganese cluster. So, we have looked at all these three.

So, for these cases we have look at all the mechanistic aspects and more details. And the rest of the items were just covered as an information into that. Now, let us smoothly get into the next topic that is on the iron in the biological inorganic chemistry. So, here we may be taking bit longer time because, the iron case is quite huge there are a huge number of enzymes represent in the biological systems, both based in the heme and non heme. So, therefore we will be spending a bit several more lectures on the iron biological inorganic chemistry ok.

(Refer Slide Time: 01:40)

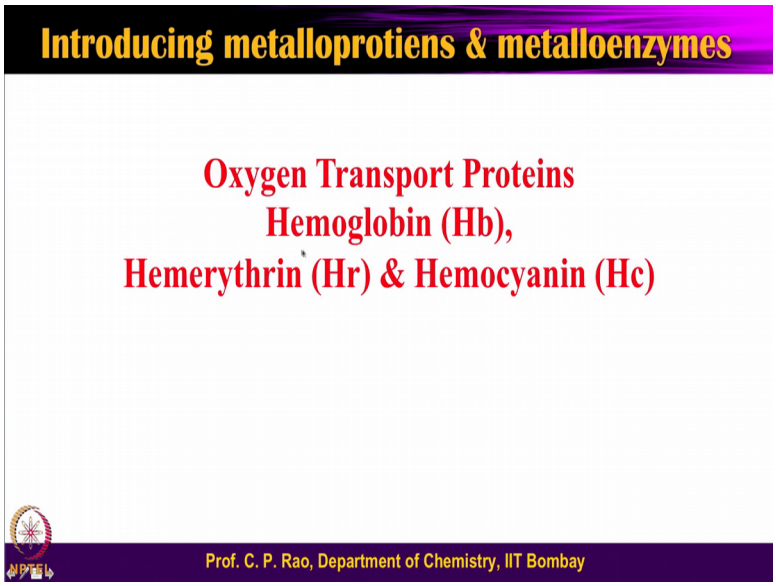


Let us look at this particular slide as you can see the iron is very special because, there is something called heme, which I have already introduced in the introductory courses, a classes. And then we have a non heme. Look at the heme proteins, they can do oxygen transport, they can do electron transfer, they can do oxygenation, they can do reduction many kinds of things variety of oxidations reductions everything.

Look at non heme where there is no heme, they can do transport, they can do electron transfer, they can do oxygenase ok, they can do deoxygenase, they can do reductase, they can do hydrogenase variety of functions are there ok. So, iron case we also have the ferritin which is iron storage protein and transferrin which is iron transport protein ok.

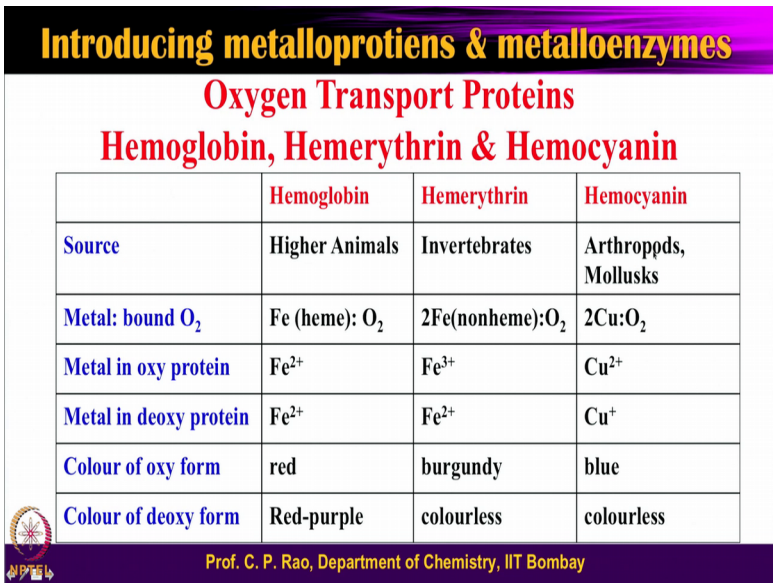
So, I think we will try to start with transport systems first, then we will go to the other cases ok. Oxide transport proteins.

(Refer Slide Time: 02:55)



So, oxide transport proteins which we are very well aware in humid system is hemoglobin, which we are not so commonly aware is other things like hemocyanin not for humid systems, but these are for other kinds of things I will show you in the next slide. So, the short form for hemoglobin is used as Hb, a short form for hemerythrin is called Hr, a short form for hemocyanin is referred as Hc.

(Refer Slide Time: 03:24)



	Hemoglobin	Hemerythrin	Hemocyanin
Source	Higher Animals	Invertebrates	Arthropods, Mollusks
Metal: bound O_2	Fe (heme): O_2	2Fe(nonheme):O_2	2Cu:O_2
Metal in oxy protein	Fe^{2+}	Fe^{3+}	Cu^{2+}
Metal in deoxy protein	Fe^{2+}	Fe^{2+}	Cu^{+}
Colour of oxy form	red	burgundy	blue
Colour of deoxy form	Red-purple	colourless	colourless

Let us look at this particular table, which tells you the various properties various aspects of these transport proteins. A 0 hemoglobin is from the human higher animals,

hemerythrin is in invertebrates and hemocyanin is arthropods and mollusks. So, as you can see there will hierarchy of the life changing, from the higher life to somewhat intermediate somewhat to lower kind of thing.

So, these let us look at a few of the properties, the there is a ion, iron is the form of heme, hemoglobin, in the hemerythrin there is no heme ok. And there is a 1 iron 1 oxygen hemoglobin, 2 iron with 1 oxygen hemerythrin, 2 copper silver oxygen in hemocyanin; so, coppers not irons.

So, metal ion in oxy proteins oxygenated one. In case of hemoglobin it is iron 2 plus in case of a hemerythrin iron 3 plus, in case of a hemocyanin is a copper 2 plus. Deoxy form; that means, where there is no oxygen bound, then there is again Fe 2 plus in the hemoglobin and Fe 2 plus in case of hemerythrin and, copper plus in a hemocyanin, you can see that is one electron difference in this.

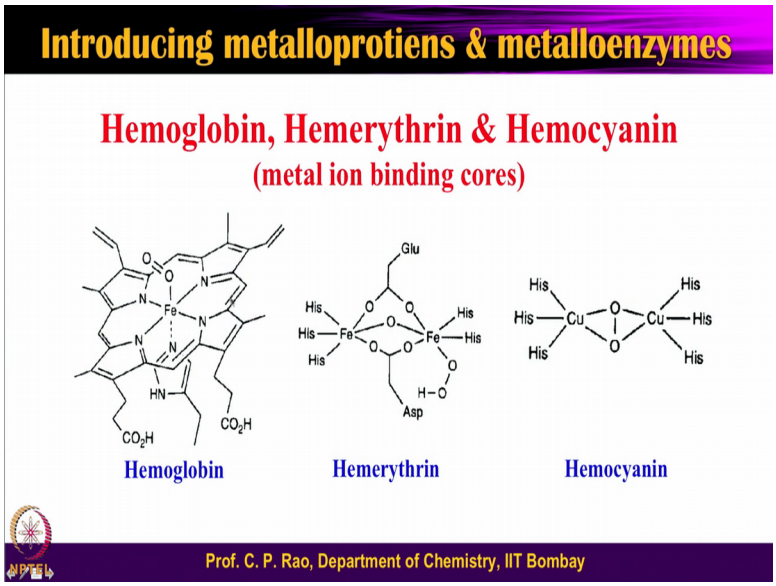
One should think of where does this one electron go. So, this one electron must go into the oxygen, the O 2 that is bound whereas, here there is no change in the number of electrons in the oxidation state; therefore, O 2 does not accept any electrons, or straight away one can one can derive such information from here ok.

So, because iron 2 iron 2 here is iron 3 iron 2 obviously, 1 electron difference copper 2 copper 1. And you know that there are 2 ions so, 2 cases 2 into 1 electron here, there are 2 coppers 2 into 1 electrode. So; that means, O 2 is not O 2 in these things possibly O 2 with some 2 electrons or something of that kind. We will come to all those details a bit while just hold on your breath to understand more details.

Now, come to the form of the color aspects of an oxy form, in case of blood we know very well blood is red and hemerythrin case burgundy and in case of hemocyanin blue. So, these are called the blue blood animals, you know the arthropods and mollusks are referred as a blue blood animals, we are red blood animals. Color of deoxy form red purple in case of hemoglobin and almost colorless in case of hemerythrin and, almost colorless in case of hemocyanin, there is a this is in copper 1 and this here is copper 2, but due to some other reason the color is lost here and the color is still there ok.

So, let us look at what kind of a binding course that these have we mentioned, 1 iron 1oxygen.

(Refer Slide Time: 06:32)



In case of hemoglobin you see that this whole thing is heme and this is the iron center, which is connected through the 4 nitrogen porphyrin. And there is one more nitrogen coming from histidines, which is called proximal histidines, which is coming from the protein chain side chain of the protein of the hemoglobin; so, globin chain. And at this side you have a 6 coordination where the oxidase important.

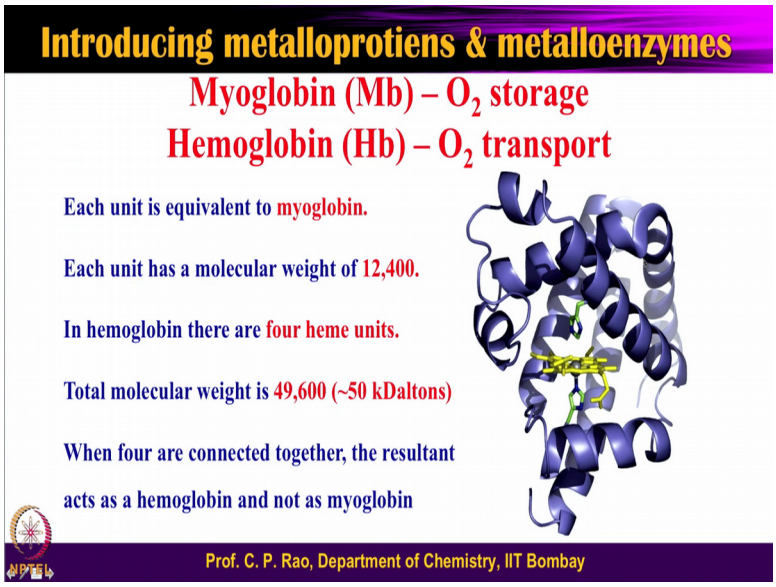
Now, in case of hemerythrin the oxo form, the oxo form is bonded to the O 2 is not like O 2 like this here, it is already. What is this? This is like peroxo or hydro peroxo. And you know that the peroxo and hydro peroxo formed from O 2 after 2 electrons production. And that is what I showed you on the previous slide just by looking at the iron oxidation state in the oxy form versus the deoxy form very easy.

And of course, the 2 ions are bonded by 3 histidines 2 histidines respectively and, these are bridged by the carboxylates. So, we will come to more details later on ok.

And the in case of hemocyanin in the 2 coppers, again O 2 this O 2 O 2 2 minus. So, then again 2 electrons copper 1 copper 1 becomes copper 2 copper 2 therefore, 2 electrons that will become this one. So, the way the oxygen is present in human blood is more or less unperturbed of O 2, or least perturbed O 2 and whereas in case of these hemorythtin and homocyanin the one of the bond is almost reduced from the double bond of the O 2.

So which means where it releases it should gain back that; so, therefore, there is a quite a huge mechanism involved in these delivery of the oxygen which are not very well understood. So, we will not go into more details, but we will compare only the features, but we will go into more details of this of the hemoglobin ok.

(Refer Slide Time: 08:45)

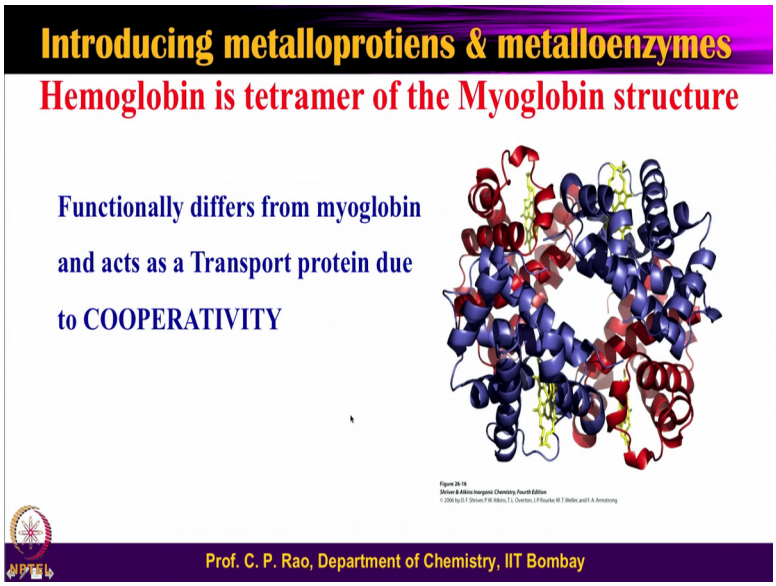


Look at this particular structure shown over here on the right side ribbons you know alpha helical structures etcetera, now ribbon this kind of a helical structures, there are some random coils very little of the beta sheet will be there. And in the center there is a core here there is a core protein from the core in this core, you have the heme that is the porphyrin and center metal iron and this histidine is coming from this particular helix. This particular thing is myoglobin it has only 1 unit and, it is molecular weight is 12.4 kilo Dalton.

Now, on the other hand if you connect 4 such units, I will shown in the next slide, how they are connected. The 4 such units, then it becomes hemoglobin. So, myoglobin is 1 unit; hemoglobin is 4 such units. So, 4 into 12.4, 49.6 and 150 kilo Dalton is the molecular weight.

So, but what happens when the 4 are of these are connected, the result in protein which is called hemoglobin, I mean it is not a myoglobin. And there are changes in it is properties too as we will see in the next one.

(Refer Slide Time: 09:53)



So, let us see that how hemoglobin looks like. Now, hemoglobin looks like you can see first, one heme here that yellow portion, there is a one more him here, one more heme here, one more heme here that means, the pro four such units 1 on this side one on the backside, one on the front, one on the rear. So, totally four very symmetrically connected is called quaternary structure and, 4 myoglobins are joined together to form a hemoglobin.

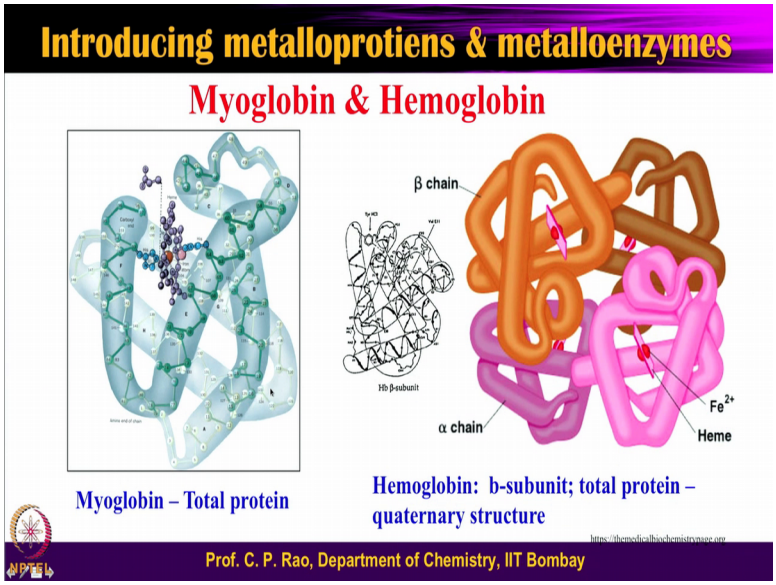
But the myoglobin now let me tell you myoglobin means myo is muscle. So, it is present in the muscle, and it is involved the myoglobin is involved in mostly in the storage the oxygen whereas, the hemoglobin heme hemoglobin hemo referring to the blood. So, hemoglobin is involved in transport. So, when we have one protein unit it acts like a storage, when you have four such units are joined together, it acts like a transport protein.

This looks like very interesting is not it? How come 1 unit of the protein is just a storage and 4 such units joint together there is something called cooperativity that means, these four units which are joined together do not behave as an independent or individual units, rather they jointly work as a cooperative unit. So, it is this cooperativity, which brings in the transport property for hemoglobin and, storage property for the myoglobin.

So, the storage versus transport we are going to discuss and debate in the next several slides, why is that what is the transport a storage alone, other is the transport ok. So,

whatever I said till now let me let I have summarized in the form of structures on this particular slide.

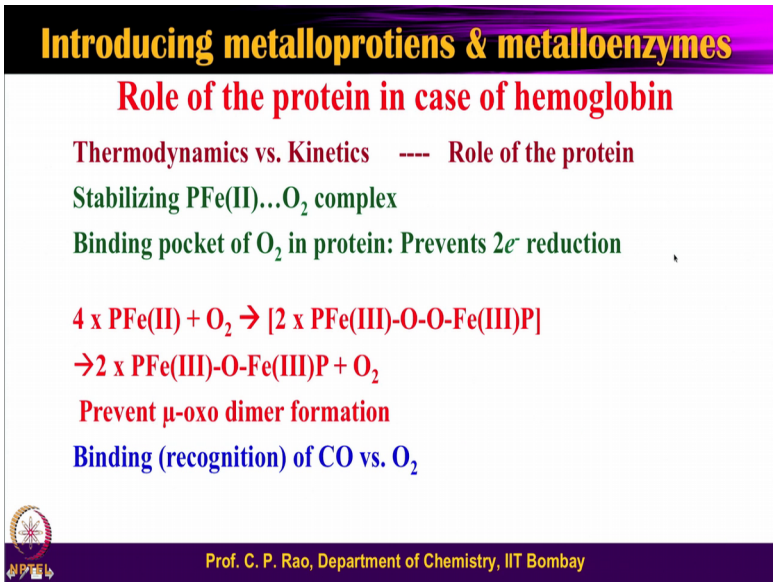
(Refer Slide Time: 11:57)



This whole thing is your myoglobin and this is your heme portion ok. So, you can see the myoglobin and this is the hemoglobin; one such unit one such unit one more and one more four units are there. And if you expand any one of this unit and you can see this so, this one and this one looks exactly alike exactly alike no difference it almost, but each of these is interconnected.

So therefore, you have this is what we were trying to say, that 1 unit is not good for transport is good for storage, when you connect the 4 of these units, then it is a the protein has a transport phenomena and, it shifts from the storage to the transport kind of a system.

(Refer Slide Time: 12:46)



Now, first of all look at some features, if some protein has to be a nice you know transport protein, or for oxygen. What kind of qualities should it have? What kind of quality should it have? Number 1, it should bind the protein must bind to O 2 number 1 and number 2 is the O 2 should not get perturbed at all that means, O 2 should not lose it is bonding double bonding character, or O 2 should not receive electrons to itself ok. And there are some additional things are also there ok.

So, let us look at this one. So, you need to have oxygen binding to the iron center that is one of the quality and then the protein should stabilize this complex in this form not in the reduced form. And in the binding pocket of the O 2 in the protein, it should not allow the oxygen to get either 1 electron reduced 2 electron reduced, or whatever it is a; it should not happen; that means, O 2 should not get activated, or O 2 should not get reduced by electrons either of these terms are one and the same, they are all one and the same.

And one more thing which we do not know generally, but when you think of the chemistry, then we will suddenly know. Iron to irons you know very well, you take an iron 2 salt let us say ferric ferrous chloride, and dissolve with water and, just go out for a cup of tea and come back what you will see? You will see quite dark brownish color sometimes even a precipitate. If your tea time is too long you might even do see a precipitate too, or your water is bit of a alkaline also you can see that.

So, there is an oxidation, there could be hydrolysis all these kinds are possible. Similarly, when an iron 2 in hemoglobin or heme binds to oxygen why should it not get oxidized yes, it should it would get oxidized. So, therefore, iron 2 P refers to power of iron, if we add O 2 temporarily; obviously, it will make iron 3 with a kind of a peroxo, because 2 electrons will go and over a period of time it will lose one of the oxygens etcetera, there is a mechanism not shown over here, and that will form iron oxo iron 3, iron 3 oxo iron 3, this is called mu oxo dimer.

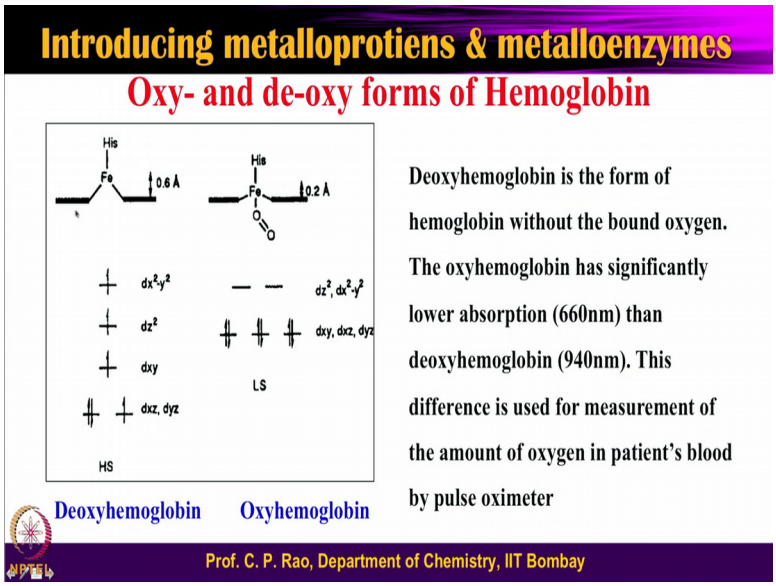
Once this is formed there is no reversible so; that means your oxygen is fully activated, O 2 to peroxo kind to the mu oxo. So, I mean O 2 is completely destroyed.

So, such kind of things should happen. So; that means, your hemoglobin or even myoglobin for that matter to store O 2 here, in it is real form you require to maintain the bond order and should not. So, therefore, the protein should make a complex, with O 2 protein should not reduce the thing. And iron 2 plus should be protected so, that there is no mu oxo dimer is formed.

One more difference is required for the protein, because the substrate for this protein is O 2 not the CO. And so, therefore, there should be recognition of CO versus O 2 by the protein. So, CO is a diatomic molecule O 2 is again diatomic molecule and, how does protein know where is a CO as O 2, there is the only difference is only very small difference, how does that happen. So, if does not recognize, then always the carbon monoxide will bind and, that will fascinate your protein.

So, therefore, this is an important event, we will come back to that how does the protein, how does the protein, whether it is a myoglobin and hemoglobin differentiate CO verses O 2 that we are going to look at in a while.

(Refer Slide Time: 16:51)



Now, for a while let us focus on two aspects, one is no oxygen bound case, other is oxygen bound case. So, that is called oxy form, it is called deoxy form. The left side is the deoxy form, right ready the oxy form. And take this thick line as the perpendicular view of the porphyrin way; so that means, this is a porphyrin plane is like in line, the iron is bit about that so, it is showing around 0.6 angstrom above the cavity of the heme.

Now, if you put the oxygen this is still above, but not as much it is about 0.2 angstrom. So, almost about 0.4 angstrom drop have happened, from the iron from it is plane. So, the iron is here, palne is here so, and then it comes closer. So, when you have oxygen is bound, then the iron comes closer, when oxygen is not bound it is further away.

So, let us look at in the deoxy form of course, in both the cases iron 2 is the d 6 system and you can see that you have a high spin system. And on the other hand once you make the oxygen ligand then you form the low spin complex and because of that this is iron is able to come and sit in this that I will explain in the next slide, why in the oxy form iron is more fit into the cavity of the heme and whereas, it is not so, much fit in the heme; just wait for a while on the next slide I will be explaining that, or if you think in the mean while it is already good ok.

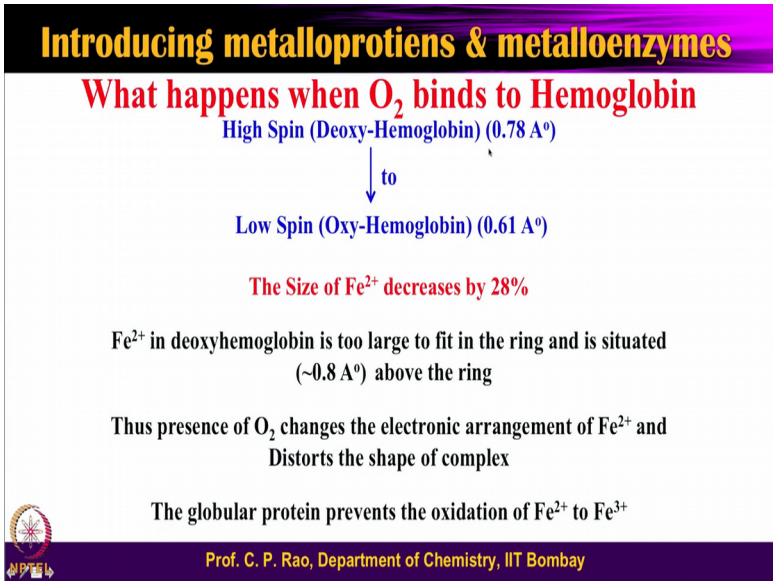
Clue for this is I tell you I will give a clue, this is a high spin iron center this is a low spin irons center, both iron 2 that is the clue. Now, you can understand why it is going into that thing. So, I will explain just unravel that in a while, but you can you are always

welcome to think of on that lines as well. Hemoglobin without the bound oxygen, the oxy hemoglobin is bound oxygen and it has a lower absorption 660 nanometer, deoxidizer as 940 nanometer.

And, this is the difference that is we is used for measurement of the amount of oxygen in present in the patient's blood, by using the pulse oximeter in most of the hospitals and labs, where you go for pathological labs, where they check your blood samples for oxygen purpose they will do that.

Now, you understand that the d 6 configuration is in high spin in case of deoxy and low spin in case of oxy and, I told you to think why the iron is coming closer to the plane in case of oxygen not so in that.

(Refer Slide Time: 19:44)



Now, you see the high spin iron, you know that how do you define a size of an iron. So, we define size of the ion, or even an atom, to the extent from nucleus to the extent to which the electron density is spread. So, therefore, it is a spread of the electron density that you need to look at that. So, in case of high spin the electrons are filled not only in the ground in the other excited level higher energy levels too. So, therefore, the size of the ion is bit growing a longer. So, therefore, it is roughly about 0.8 angstroms in case of deoxy.

Now, the moment you put the oxygen and then oxygen once you put the ion 2 goes into the low spins. And once it goes to the low spin all the 6 electrons are paired into the ground state, you know t 2 g it is a 6 coordinated system octahedral system. So, t 2 g 6 therefore, the electron density is somewhat compressed therefore, the sizes basically compressed.

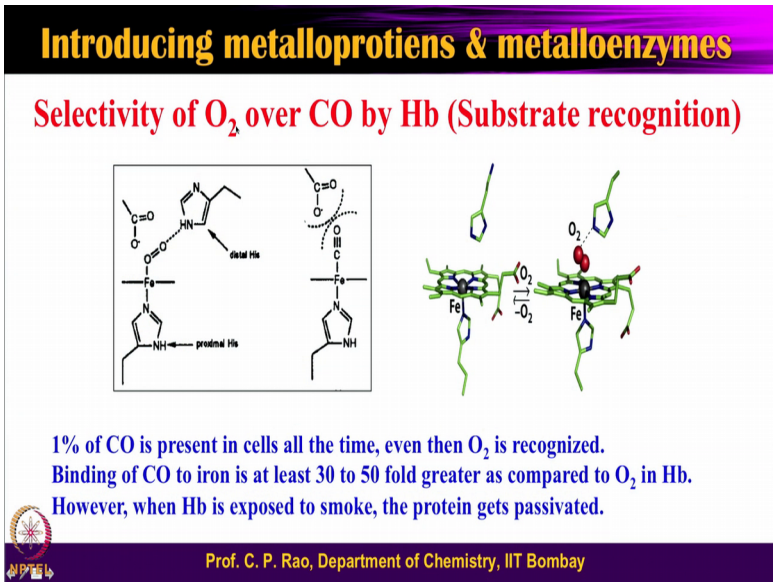
So, almost from 0.8 to 0.6 is a kind of a change which is about 25 to 30 percent change in this. So, you know that from the coordination chemistry principles that what we studied is that we know that the oxy legands are not very strong field ligand there, rather weak field ligands and they generally encourage the system, in the case of as high spin complexes.

But, in case of hemoglobin or the myoglobin when the oxygen is bound as additional ligand, but it prefers the low spin. So, it is not because of the ligand field strength, when this is bound there are some conformational changes come. And these conformational changes indeed, favored the low spin configuration of the electrons in the d system, rather than the high spin it is that part, it is not the oxygen has become suddenly a strong field ligand and no it is not true that.

So, therefore, what we know the coordination chemistry is correct, but there are several things which we do not know because, in the protein chemistry, the protein conformation, protein structures are all also important along with the inorganic chemistry aspects of it. So, together so, here I hope you understand why what happens when the oxygen is bound to the iron size and, why and how does it get fitted into this, I hope you understood all that ok.

So, therefore, in the deoxy hemoglobin it is too large and, in the oxy hemoglobin it is smaller and goes closer to that. So, and we already have seen in the earlier, the protein that is surrounding this heme, it does not allow the oxidation of the iron 2 plus 2 iron 3 plus, it does not allow iron oxo iron which is called mu oxo dimer formation all of these are prevented. And it also does not allow the electrons to flow into the O 2, because of the kind of redox potential that we have for the iron center in the hemoglobin and myoglobin systems ok.

(Refer Slide Time: 23:03)



One another aspect that I talked to you is that this enzyme must recognize O 2 and over that of this CO it is very important ok. So, for this why should it because, if you look at the cells and if you see the cells are always having CO being generated from the dead cells, it is about which is about 1 percent of the CO is always present in cells all the time.

So that means, O 2 must bind to the hemoglobin in presence of the 1 percent of the CO; that means, the enzyme must differentiate the CO versus O 2 at that particular concentration of CO even.

If not what will happen CO will bind. If CO binds, CO binds much better stronger by over 30 to 50 fold stronger and irreversible. Stronger and irreversible and we know the reasons of this back bonding, but in the metal and the ligand all those kinds of things come into picture, which you must have studied in very simple the inorganic bonding studies, a coordination chemistry bounding studies as well. As compared to the O 2 so, therefore, that is one thing.

Now, we will come to that point just in a while, but let us see now, so when it binds to O 2 you see that this is known from the structures of the small molecule as well even from the protein, that the Fe O O is a bend structures around 120 degrees or so. And such a kind of structure is stabilized by two things, there is a kind of a push from this side and there is a kind of attraction from this side histidine, which is called the distal histidine, where there is a hydrogen bond the stabilizers.

On the other hand when the CO binds CO binds like a linear Fe CO and this is a 180 degrees and, this has no stabilizing power into this. So, therefore, the binding nature of these is different, you see that this is the deoxy, of course in the resting state there will be water; water, will be replace by O 2 and then when O 2 comes here, this is 1 binds this is the stabilizing force.

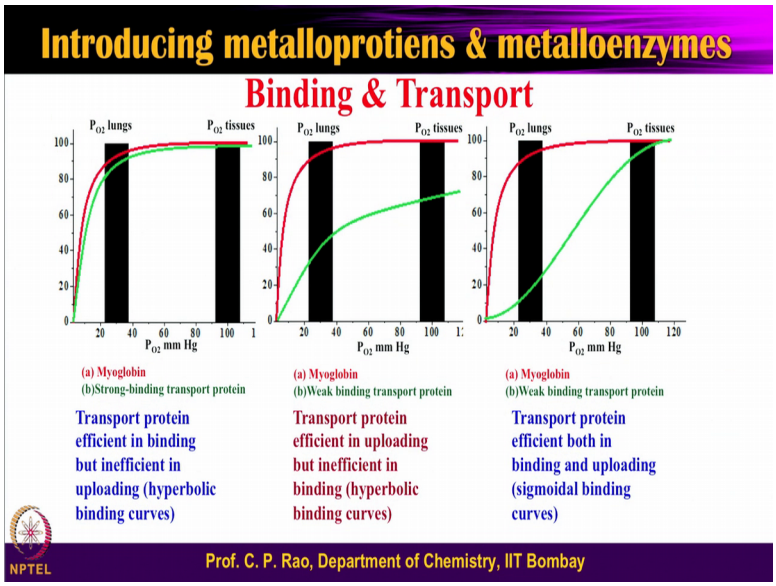
Now, if you look at I am sure you must have heard several cases where there is an accident of fire people obviously, die; you know the main reason for the people dying not because of the if they are burned, but because they are being choked with the smoke. So that is more dangerous and even the fire because, people will run away and they can save themselves, but whereas when the smoke is spread even if you run then smoke haunts you and then the smoke, you breath the smoke and the smoke has got CO most of it and the CO concentration in your lungs becomes so huge, then your my hemoglobin will get saturated with the CO. And once it is saturated with the CO no reversible and you die and that is most mainly the reason why most of the deaths are because of the CO a smoke reason not because of even the burns.

Of course, I am not saying there are no deaths or the burns, and what I am saying is the maximum things always because, when the fire is there; obviously, firefighters will come and try to extinguish the fire, but the smoke is not easily extinguished so, easily as the fire get is in is extinguished immediately the smoke goes longer.

So, therefore, people before they can escape out of the danger, probably they get caught in the danger this one. So, that the higher at that stage can this protein differentiate between O 2 and the receiver? No, because, there is a concentration shift huge concentration of CO even that this stability whatever, you have is overcome by that. But within the cell yes where there is once a percent of CO is there, it is always there ok.

Now, let us try to look at so, we have looked at the various properties that such a proteins should make, they are should exhibit. Now, look at one particular aspect of this.

(Refer Slide Time: 27:28)

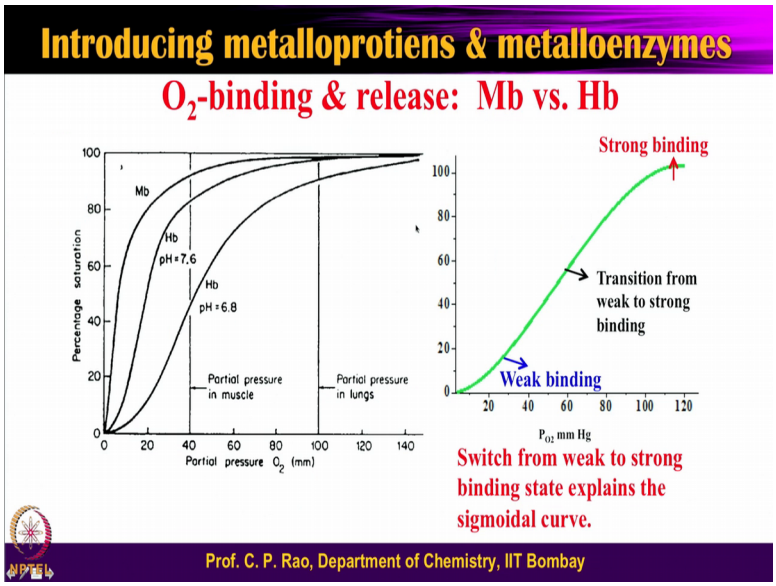


Suppose if a transport protein binds to oxygen very strongly, what level of strength of oxygen binding is important. Those proteins which are strong in binding will have a problem in releasing ok. And you see that when they are strong in binding they will go something like this, which is called the hyperbolic kind of a thing.

Now, if you take some transport protein where is some transport protein, which is strong not strong in binding, but inefficient in binding and somewhat efficient in the uploading kind of thing, then you will find the hyperbolic curve of this kind ok. So, there is a lot of difference.

Now, here if you take a transport protein is both efficient, in binding as well as transporting both binding and transporting, then it will not be neither the hyperbolic of this type, nor of this type, but of this type. What is this type called? This is called sigmoidal, sigmoidal 1. So, what is the myoglobin; the red one is myoglobin here, red one is myoglobin red 1 is myoglobin. So, myoglobin is always going in the hyperbolic; therefore, myoglobin is not meant for the transport, but it is just meant for the storage this ok. And let us try to see the same thing in case of the hemoglobin.

(Refer Slide Time: 29:05)



Now, look at the myoglobin once again here and this is what? This is the partial pressure of O 2 versus the, to what extent the oxygen is oxygenated protein is oxygenated, so, oxygen saturation; see Mb is the myoglobin is going like this, absolute hyperbolic. And forget about this Hb, look at this Hb and here, and you see a sigmoidal.

So, look at on the right side this is a sigmoidal plot. So, in the sigmoid plot what you have? You have a weak binding in this region and, you have a strong binding in this region that means the protein has one kind of a conformational structure here. And the protein has a different conformational structure; that means, protein is going from weak binding one conformational structure, to strong binding to another conformational structure and there is a transition.

So that means, if a protein has the qualities to have a weak, as well as well to have a quality of this strong, then it will be able to function both pickup as well as the transport the oxygen very efficiently. So, it can switch from the weak binding to the strong binding and, I will continue with this in the next class. So, I am trying to explain you the oxygen storage and lase of these difference between the myoglobin and hemoglobin.

So, in the next class I will continue and try to clarify all the things associated with the mechanistic aspects of the oxygen transport in hemoglobin and which is not so in the myoglobin part of it.

Thank you very much.