Inorganic Chemistry of Life Principles & Properties
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Lecture – 33
Role of Iron in life – Reductases & Phosphatases – continuation

Good morning welcome you all to the next lecture on Inorganic Chemistry of Life Principles and Perspectives, in the previous class we have tried to look at the Phosphatases and of course, rib nucleotide, reductase etcetera. And we just started towards the end of the class the nitrite reductase.

So, we continue with some right nitrite reductase. The reason that I have taken this reductase afterwards is this is a heme based one, the one which I have talked about the rib nucleotide reductase is non heme based place. Even, the phosphatases purple acid phosphatase was also non heme based one, it is only that the classification otherwise there is no other problem.

Now, let us look at once again I will re explain of the couple of things that I have done already, in the previous class on the nitrate reductase. Nitrate reductase is an enzyme which converts the nitrite group into nitric oxide kind of a moiety NO 2 to NO.

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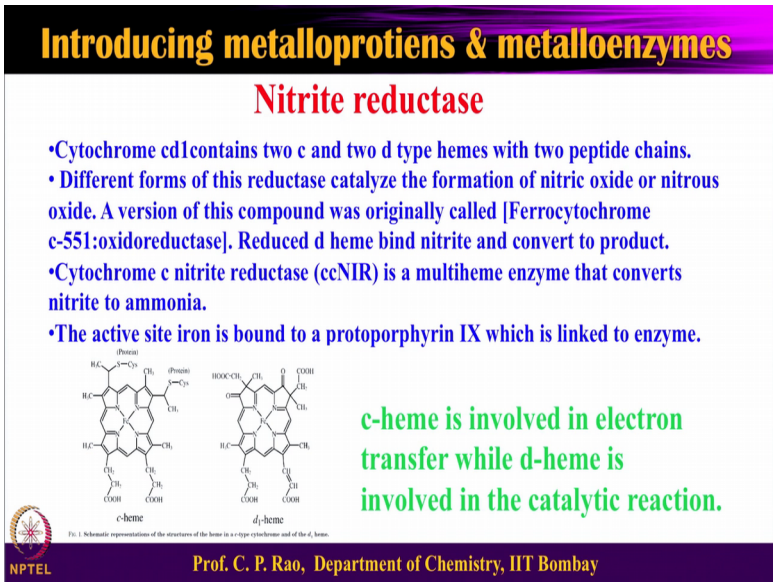

So; obviously, what it requires NO 2 to NO? It requires one of the OO 2 nitrogen bonds should be taken out. So, how do you take out? We have already seen earlier.

So, whenever you want to take out you add the electrons into the anti bonding orbital, and then you add some protons that O will go as H 2 O or you have a substrate it can add to that too. So, it is very similar kind of analogy will work out here, so which means this enzyme requires some you know the substrate has to bind substrate has to reduce. So, therefore, some electron transfer should take place and then finally, you break one of the NO bond etcetera.

So, therefore, in this enzyme we could see there are two types of a heme based centers are there, which is called the c type heme c and heme d ok, and there are two each of this because there are two polypeptide chains in that. So, therefore, you have a dimeric kind of a protein.

So, there are different forms of these enzymes are there, and they do different levels of the reductions. Some of them do the from nitric oxide to nitrous say NO 2 to NO. And some of them can even do from NO 2 to ammonia as well we will study both of these cases as we go along in this particular class. The one which does from N O 2 to NO is a kind of a thing which is known as a Ferro cytochrome c 551 oxidoreductase.

So, in this case you have a reduced heme of the d type which will bind a nitrite group and then converge into that so; that means, the d part of the heme is catalytic type and they c heme is the one which is the one which is involved in transferring the electron ok. So, therefore, cytochrome c nitrite reductase cc nir is a multi heme enzyme that converts nitrate to ammonia which we will take up after completing the NO 2 to NO kind of an enzyme.

So, as you can see below here there are two types of heme c and then heme d; what is the difference you can see that this there is oxidized part of that; that means, some of the portion of the skeleton of the porphyrin is oxidized in this particular case as you can see that a non-oxidized and then oxidized.

So, what will this differ? Obviously, this will differ on the way that this it is redox potential is lying ok. So, the redox potential, so the c versus the d are going to be different and as you can see the c is involved in electron transfer while d is involved in the catalysis or where there where the NO 2 binds and then for the reduction or catalysis takes place you know this.

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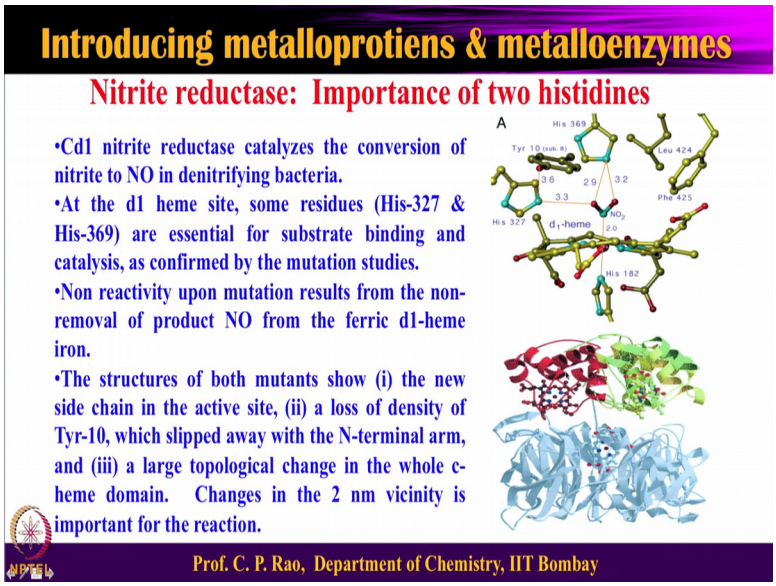

So, let us look at more details of this enzyme and the more details of this enzyme as I said that this Cd 1 nitrate reductase catalyzes the conversion of NO 2 to NO, in the denitrifying bacteria. So, if you look at as I mentioned in the previous slide that the d heme is the center where the reaction occurs or where the NO 2 binds.

Now, if you look at that the same thing here as you can see this is the iron center this is the kind of heme and you know this nitride group binds. Let me bring one point clear to you in terms of the kind of a heme that we have lets go back to the previous slide and sorry this one yeah here you can see that. So, there is a kind of a heme c type heme d type oxidative type and therefore, the redox potentials vary yes. So, therefore, that is an important point to be noted; so therefore, that kind of a center which is d is very well suited for binding to the NO 2.

Now, what is to be noted here is very interesting thing is this NO 2 has some interaction or secondary interactions with this kind of histidine with this histidine in this particular enzyme this is called histidine 327, and the histidine 369. So, therefore, these two histidines are absolutely important they have a very important role to play. How do we know? Whenever we say that a particular amino acid residue plays an important role; what should be the trick what should be the tact? Trick and tact in this is you replace that amino acid residue by a different amino acid. What is this process called; mutation.

So, you mutate the protein or enzyme at that particular center to a different amino acid, which does not have the same side chain. Therefore, that kind of process is called mutation. And when you do the mutation in this particular case either at the histidine 327 or at histidine 369 or at both these what defined is the outcome of this is there is NO product that is coming out of this particular enzyme, the particular enzyme does not give the product.

Actually this is not that the reaction does not occur reaction occurs to reasonable extent, but the product does not dissociate product does not come out of this particular system. So, therefore, therefore, in when whenever you mutate and change these histidines what you will have essentially is reaction will happen, but catalytic cycle does not complete because, the no does not dissociate that is a very important point.

So, and one more thing that could be realized when you do the mutation is that there are certain things you can see there is 1 tyrosine moiety tyrosine 10. So, therefore, this tyrosine 10 also gets perturbed when the mutations have taken place. And also we need to see within 1 to 2 nanometer vicinity the protein environment is also important.

So, what happens when you do the mutations there is some new side chain at the active site or some of the tyrosine things will go away? The tyrosine slips out of this region and sire tyrosine density will not be seen that much. And a lot of topological changes also occur in the vicinity of this. So, therefore, what we can say is within 1 to 2 nanometer area surrounding this particular region is of the protein residues are very important, because you have to have electron transfer you have to have a reduction process removal of the oxygen 2.

So, therefore, the crucial thing of course, the catalytic activity comes at d side and the electron transfer comes from the from the c type and this can be seen very clearly in the next slide similarly you can see the this things are involved over there ok

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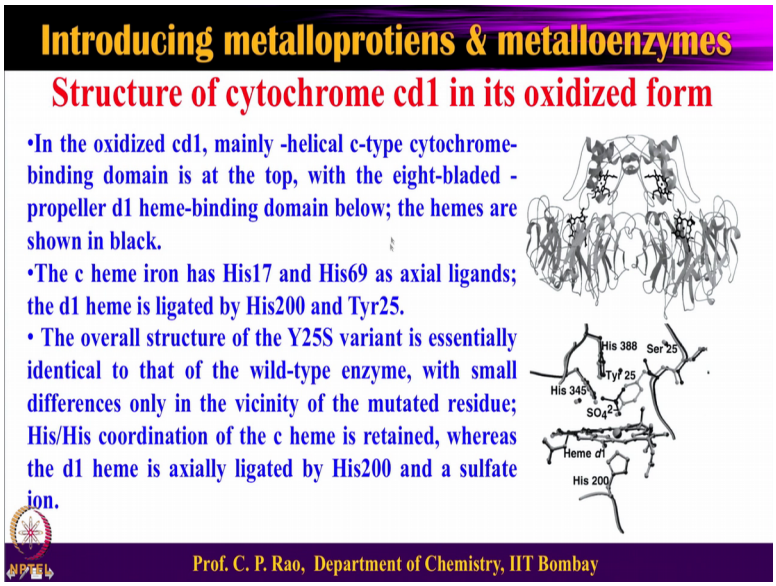

So, let us look at the some structural aspects of this cytochrome Cd1 or nitrate reductase enzyme Cd1, in its oxidized form in the oxidized form or the Cd1, the mainly helical c type cytochrome c domain binding the one domain that is which is the below, you have two types of domains.

The domain one which is the d 1, which is over there and then you have the cytochrome c type which is coming from, it is the cytochrome c which is involved on the top and the d is which is over here, and that is how it is involved ok.

And if you now look at the close proximity how do you find which is cytochrome c type one is d type you look at the binding. The binding of histidine 17 and histidine 69 as axial ligands that will qualify the cytochrome c and in the other case it will qualify this cytochrome d will be have histidine 200 and hist histidine 25.

Now, let us bring up one the mutation; the mutation is one of the tyrosine is converted to the cysteine ok serine. One of the tyrosine is converted to serine Y 25S in this variant it is essentially like that of the wild type, but there are some differences in the vicinity of the mutated residue ok. The c type does not get perturb both the hemes are there both the histidines are there, but what get perturbed is the d type, the d type one is ligated to histidine 200, but one of the ligation is lost the other ligation which is tyrosine 25, is lost and in this position a sulfate ion binds an anion binds.

So, this also gives a clue that this is very well suited the cytochrome d type is well suited for binding to the anions or to the substrates and because earlier it was the tyrosine 25. So, therefore, if you modify this tyrosine 25 then; obviously, that is not going to bind and therefore, that can be seen very well and this is the position where the anion or other substrates can bind and can be very nicely seen from the crystal structure sulfate binds over there, that gives a an explanation for how it binds.

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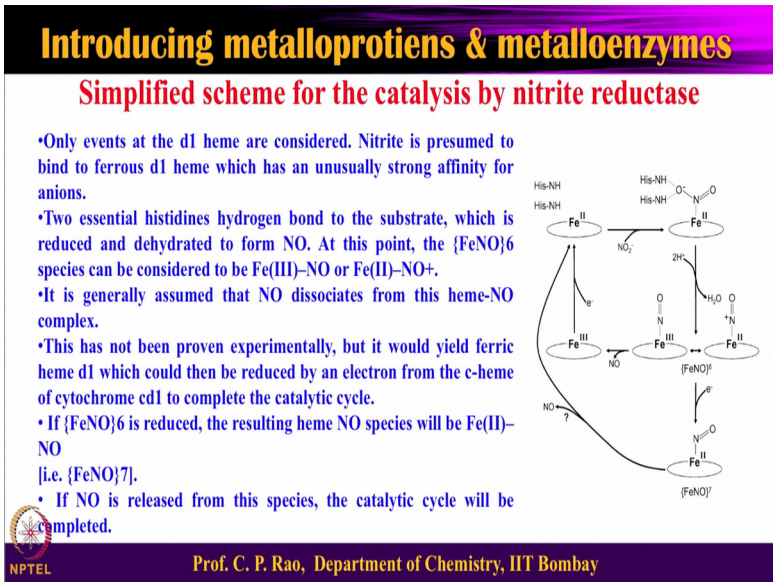

Now, let us look at the catalysis of this the catalysis of this at the d center. So, let us look at the d center here and in the reduced form which is the iron two form, and in this is the center where the NO 2 substrate binds and this substrate has an interaction with the neighboring nearby histidines as I showed you on the earlier slide and therefore, this is what brings recognition for the substrate binding.

So, substrate binding recognition as I told you earlier requires secondary interactions in this particular case these two. And additionally these two histidines have got a lot of importance as I told earlier when you mutate these the reaction goes, but reaction does not complete because the NO does not dissociate.

Now, this NO 2 you know is activated by the iron 2; obviously, and the electron transfer taking place into that in presence of the 2 protons will break down this to the water and the Fe 3 NO, And this could be in resonance structure with the Fe 2 NO, with radical and this is the species which has to dissociate to give back the iron, but the evidences are not

built for this as a bridging on the side, and this iron three is not the active form then there has to be necessarily an electron transfer that electron transfer can come from the cytochrome c part of it and then can go to the iron 2.

So, in at this stage the species is referred as the Fe NO, which is called Fe NO 6 which could be either in this form or in this form. If it is in this form the dissociation has to occur and the dissociation, process is not being proven to this, but the re reduction of this is required on the other hand if it goes why are the Fe 2 with the nitrosyl radical type, and then the further electron transfer, should bring a different species called Fe NO 7, again this should disassociated and no evidences have been shown completely for either of these, but there is a possibility

Since, this can undergo a transformation to the other there is a possibility that either this process can happen though this process has not been shown yet. So, only when they NO is dissociated the catalytic cycle can get reversed here and that will take out the NO and then brings in. So, either it can go through this process or it can go through the iron three, and then further reduced losing the no and then losing the; I think going back to this one.

So, this is also a possible thing because the possibility for this is because the reduced one iron will have a more of strong pipe back bonding stabilization whereas, iron 3 will have much less of such kind of a stabilization. So, there is a good reason to believe that it will break down with the iron 3 and then re reduced to this 2 ok.

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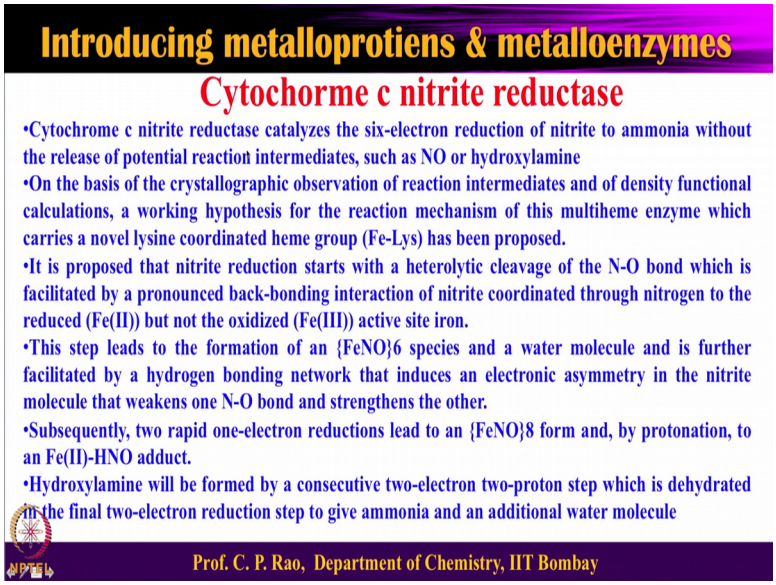

Let us look at the other enzyme which reduces the NO 2 not just to the NO, but to the ammonia level so; obviously, in the process it has to go through no and then go to this, but the very minimal things are known all this is written here I will be explaining in the catalytic cycle all of these. So, therefore, we do not need to worry you can read after looking into the catalytic cycle or these ones.

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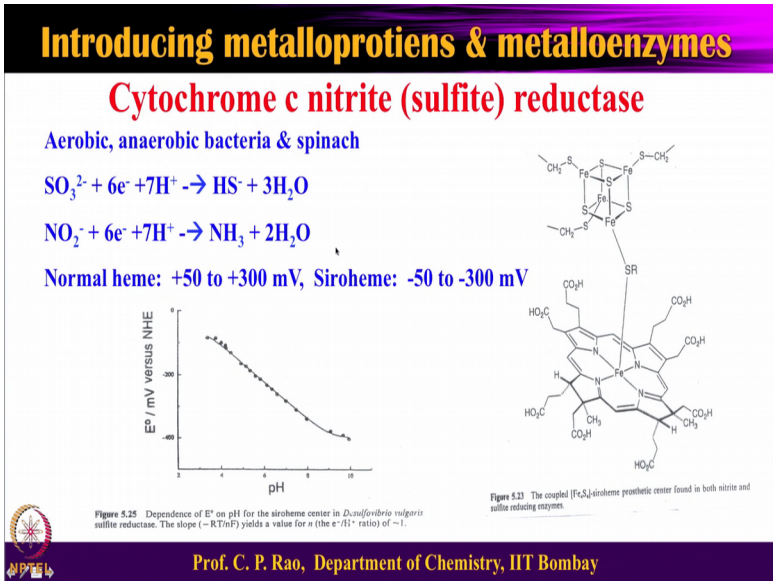

Let us look at some characteristics of this nitrite reductase and this can also do sulfite reduction to, now first let us look at the reactions look at the reaction of nitrite. So, NO 2 minus plus 6 electrons plus 7 protons will give ammonia plus 2 H 2 O; that means, here in previous example NO 2 to NO; therefore, only one oxygen goes as the as the water, but in this case NO 2 to ammonia both the oxygen must go as water and required more electron reduction and more protons because the ammonia nitrogen will take, three protons, two waters will take 4 protons totally 7 protons.

And the NO 2 minus requires only 6 electrons to reduce completely to reduce the oxygen parts to go to this water and then nitrogen apart to get to the NO 3 minus. So, therefore, that is where you have ok. So, the total counting should be perfect and you can see that. So, 6 minus 1 minus 7 minus 7 plus, so that is neutral.

Now, if you go take the sulfide case sulfide case is also very similar and 6 electrons 7 protons it will give HS minus plus 3 H 2 O. So, there is 7 proton etcetera. So, you can see that, so it is very much similar, in this case you have a different kind of a heme center

this heme center is referred as a siroheme, this is a siroheme this heme has got heme has got a iron sulfur cluster both of these got connected together.

So, earlier we studied for electron transfer you have either a heme kind of a protein or you have a iron sulfur kind of protein, here is a case where both the iron sulfur part and the heme part are connected. So, connected through this thiol bridge the iron of the heme to the iron or the iron sulfur protein or iron sulfur cluster is connected through this kind of a thing.

So, this is referred as a siroheme, so you know this the normal hemes and we have looked at earlier we will have plus 50 to plus 300 whereas the siroheme depending upon the group changed here as well as the protein that is surrounding this can go between minus 50 to minus 300.

So, why do you require such a because you are going to reduce the NO 2 minus to ammonia. So, you need a very strong reducing center and that is why this enzyme has taken nature has decided to put a different kind of a reduction center than the normal one and that is what you can see that. So, is it understandable, now we have seen earlier iron sulfur cluster then heme is a combined one you can see that and this is because you require a very high negative potential and this kind of things will show more negative potential minus 50 to minus 30.

Now, this protein will also have as a function of pH the redox potential vary and as you can see the slope if you look at this slope and the behavior of this you can definitely say the electron transfer is coupled with the proton transfer. So, both the electron transfer and proton transfer events are coupled together, that we will get from this particular kind of this kind of plots are called poor box plots pH versus redox potentials of this. So, the sulfide reduction reaction nitrate reduction reaction to ammonia requires a strongly reducing center therefore, a siroheme is being, being utilized in this particular case.

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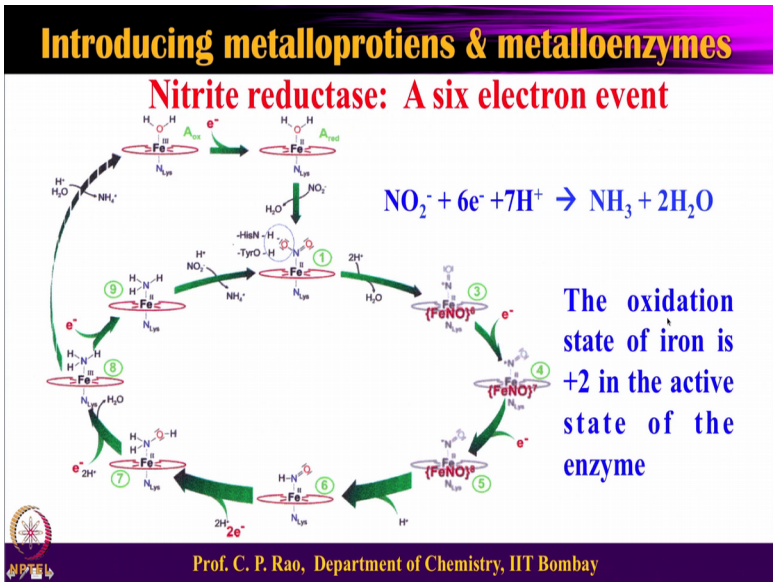

Now, let us look at the proposed mechanism; the reason is that many of these steps are not been experimentally shown, but some DFT computational calculations have, have supported have proven have brought in this one. So, based on that this particular scheme has given ok, first of all we should say that the non-deoxidized form is active center the reduced form; that means iron 2 center.

So, the irons 2 center, so the resting form of the iron three is not the one rest and it is the iron two center is reactive and at this stage that you have a NO 2 binding. So, NO 2 binds here and this binding of NO 2 is facilitated by the neighboring groups one is the histidine another is the tyrosine as you can see over here.

So, this is one of the species and then the protonation to this the protonation through this and since this is bound by this will pull out the NO bond it will stretch the NO bond further, because of these secondary interactions with the histidine and tyrosine. And that favors the breakage of the this O in presence of the a 2 protons as a water and then what you get a nitrosyl kind of a species, and this species has a number given Fe NO 6. So, just nothing to do with the 6 or 6 plus anything it is a species 6 which is computationally derived.

Now, this with further reduction with 1 electron and that electron gets into this particular the part and therefore, the binding of the Fe NO is now become bend, this is again being computed by the DFT computation this species is called you know 6. And then one more

electron will further add to this and that will make the further in the reduced form of it and at this stage the protonation you can see if you O and then one more they 2 elect more electrons will add into this, and the protonation will take place.

And at this stage you have the three protonation the water; obviously, will go out because of these 2 protons and electron. So, these 2 protons and electrons you required, so you have a the water is last and then now this further this species one more electron reduction will perfectly get the NH 3 and in presence of the NO 2 this NH 3 can be taken out as NH 4 plus ion.

Now, you can see that there is the electron counts you could see that from iron 2 plus this 1 electron added over here to one more electron added here, two more electrons added here that is 1 plus 1 2 plus 2 4 plus 1 5 plus 1 6. So, totally you have a 6 electrons and you see there are 2 protons added over here and 1 proton that 3 added plus 2 more 5 added plus 2 more 7 added. So, you have totally 7 electrons added, so many of these species are shown by the computation, DFT computational studies as you can see that whole thing.

So, the active form of this one is not the iron three it is the iron two. So, therefore, the cycle can continue with it and the reduction can take place go back to that thing So, now let us go back to the earlier slide where I said, so all these points that you can see.

So, that based on the density functional theory this has been very well worked out the species kind of thing of course, the cleavage requires the electrons to be pumped in protons to be there. And a pulling on the no bond by the neighboring groups the first one happens and that is where Fe NO 6 species has been shown and that is what we have explained to you then it goes to the Fe NO 7, then goes to the Fe NO 8, these kind of a further reductions reduction followed by this one.

So, the totally, so this enzyme not lot of the species have been worked out I have been experimentally shown, but DFT computations have clearly favored this kind of a species, the species is 6 type, 7 type, 8 type and the reactions become very fast after this. So, this, so the active form is plus 2 totally you have to have a 6 electron reduction, activity comes from the iron 2 ok.

So, in the kind of a reductase based on the heme we have looked at two aspects NO 2 to NO stage then we have looked at the NO 2 to ammonia stage there are 2. So, to have the NO 2 to NO then we have a c type and d hemes whereas, to have NO 2 to NH 3 ta 3 we have another one which is called siroheme, siroheme means heme which is connected with a iron sulfur cluster through a thoil bridge. So, is that clear everything is there.

Now, this brings to the to the to the end of the different types of the enzymes that we are trying to look at which are all based on the iron some of them are based on the iron heme type some of them are based on the non heme type.

So, let us look at the summary of what I have done in the past 8 to 10 classes or 7 to 8 classes or so, which I have consumed or utilized for the iron enzymes is kind of a recapitulation let us look at old summary.

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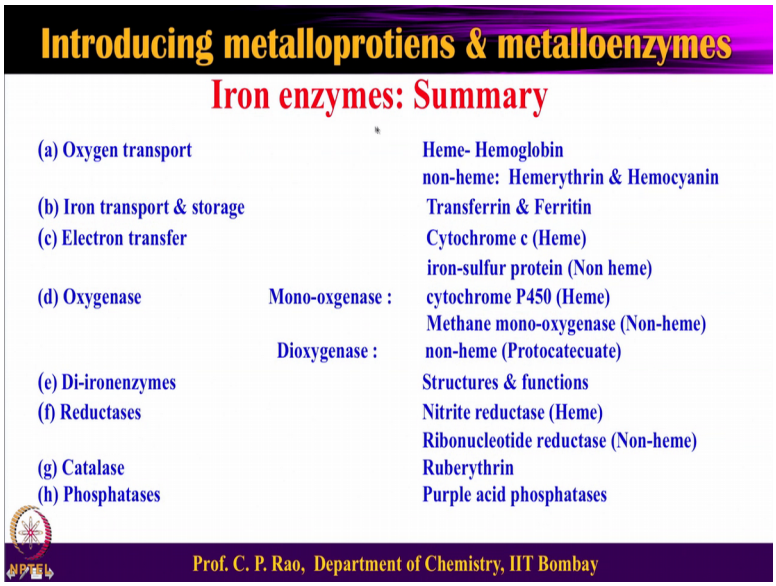

So, the iron enzymes or I have talked to you about the oxygen transport one in the oxygen transport one, I have talked to you the heme based hemoglobin and myoglobin hemoglobin does the transport myoglobin does the storage etcetera. Then I have also looked at some other cases the mollusks and other kinds of lowers species where nonheme which is a hemerythrin which is di iron center hemocyanin which is dicopper center

Then I compared how the oxygen transport takes place in these versus it takes place in the hemoglobin, hemoglobin more or less o two is not reduced much at all whereas, in hemerythrin and hemocyanin they reduce and bind in differently all these things have been explained. And they can very nicely be understood from even simple iron spectrum by looking at the OO stretching frequencies we can explain.

The followed by that I have explained to you iron storage and iron transport iron transport is by transferrin and iron storages by ferritin and in this ferritin again storage iron is brought by transferrin and then transfer to the ferritin and again you take it out. So, I have explained to you how the ferritin is loaded how the iron by iron and how the iron is taken out mobilized.

So, when it is brought it will comes in the form of iron 2 plus and then it is given inside to the iron ferritin and in the ferritin it gets oxidized and form a ferri hydride kind of a species with phosphatespecies and with a bridged oxo phosphate kind of a bridge and iron three then when it is to be released then this iron three has to be reduced by the by some kind of a reducing enzymes some kind of even electron transfer heme enzymes which will reduce and then the iron is released.

So, these are all I have explained to you, then I have gone to the electron transfer the two types of electron transfer one is on the heme based cytochrome c I have fully explained cytochrome classes I have explained then a cytochrome c and how it functions all these things have been explained. Then there are nonheme ones which are iron sulfur protein where the iron sulfur proteins are also involved there one iron four phthalates there is one iron sulfur protein called the two irons and two sulfur and then four iron four sulfurs.

In these things it is these are the inorganic sulfides inorganic sulfide it can be taken out by adding the mineral acid as H 2 acid comes and if the inorganic sulfide is not there NO H 2S, will come which can be made out otherwise you have this and then during the electron transfer there is a the core of the iron sulfur core which is a cubin will contract and expand, contract and expand that is the kind of thing which brings concomitant changes in the in the protein structures.

So, having explained this transport storage electron transfer etcetera then I have taken into the oxygen is prop properties means or adding oxygen you can add one oxygen of the O 2 or you can add both the oxygens of the O 2, which are referred as the mono

oxygenase or the dioxygenase. And under the mono oxygenase I have explained for the heme case cytochrome p 450, completely how the reaction occurs what is the catalysis how the catalysis occurs etcetera species like ferryl oxo species form which is very strong.

I have also talked to you the O 2 activation will take place after the substrate not before the substrate please remember that because there is it will be a great danger. Then I have also explained one another example of the mono oxygenase by taking non heme enzyme which is called methane mono oxygenase. It is just methane to methanol which is not that simple you need very high pressure high temperature catalyst if you want to do in a lab, but in enzyme does which so here nicely and such kind of conversion is important because the methane cannot be transported because methanol can be transported very well these are energy based materials.

Then I have looked explained to you what is a dioxygenase, the intradiol extradiol, but I have taken example only for the intradiol which is protocatecute non heme based kind of thing and so, other examples I have not taken purposefully in that because there are much more severe things are involved in that too ok.

Then I explained to you the class different kinds of di iron enzymes various functions in this and so, all those things classification with the different kinds of di iron centers. Then I took up the nitrite reductase with the heme explained to you non heme rib nucleotide reductase both of these are explained.

How the two compartments of this protein in rib nucleotide reductases are involved though there is no the substrate binding at the iron center, but it binds in the other subunit of this. These two subunits are interfaced at the c terminals one is having the iron center which is called hydrolyze other one is having the self-hydroxyl functions which is involved in the redoxy, together the whole reaction.

There is a tyrosine radical which is stabilizing in the rib nucleotide reductase and in these two prime hydroxyl should be converted to two prime deoxy and that is what is deoxyribonucleoside, because deoxy ribonucleotide reductase are not input to the human system. So, therefore, that is required to be made.

So, nitride to NO and I have also explained nitrite reductase to the nitrate reduction even to the ammonia, then I have explained prior to that catalyzed iron center activity ruberythrin and it is been how that binds that peroxide H 2 O 2 and then reduction the this mutation of the H 2 O 2 the water I have also explain one another class of enzymes which is basically hydrolysis and this gives the phosphate hydrolysis phosphate ester hydrolysis in this two.

So, these one of the kinds of things, so transport storage and then oxygenase oxidation properties reduction properties hydrolysis properties and these are there are many other details are involved one hydrogenases of iron I will explain when it comes to the story of the nickel. And I have purposefully avoided that. Many more things are there to be impossible to cover all of the enzymes of the iron in for a particular course. So, therefore, I stop for the iron enzymes at this stage.

Thank you very much.