Inorganic Chemistry of Life Principles & Properties
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Lecture - 32
Role of Iron in life - Reductases & Phosphatases

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. So, in the previous class we have looked at reductase, dinuclear cases and ribonucleotide reductase and although we have looked at the (Refer Time: 00:37) which is which decomposes the H 2 O 2 to water both of these been looked at.

So, having looked at some reductases and we have looked at already oxygenases, now probably it is a logical to get into some hydrolase or phosphate hydrolysis, which is known as phosphatases. So, these are one of the things reasonably well understood or called purple acid phosphatase why there is why the term purple.

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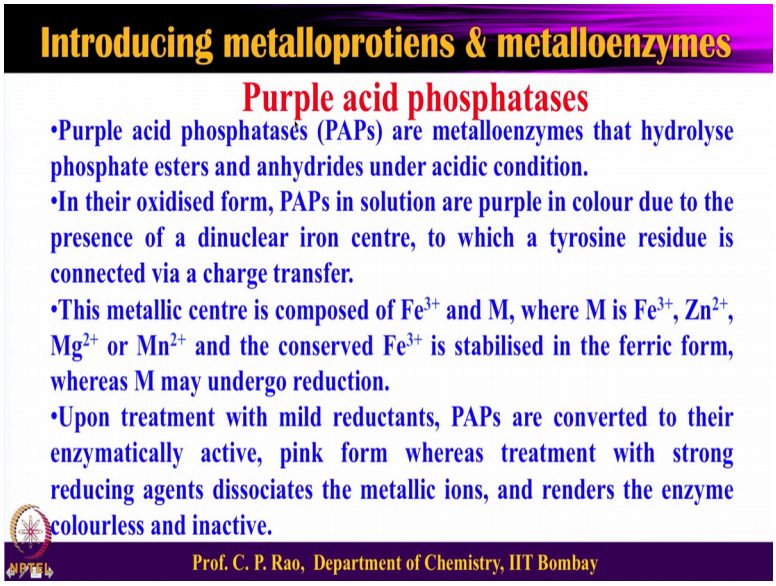

So, the purple comes from the color acid from the pH. So, acidic conditions or these are purple acid phosphatases. And these are the of course, the metal of enzymes which are involved in hydrolyzing the phosphate esters under acidic conditions and ok.

And if you look at their oxidized form they are in purple in color and particularly so, because you have the color is coming the origin for the color is the charge transfer, and

the charge transfer is from the tyrosine to the iron center or oxidized iron center. So, these are the kinds, and if you look at the centers of these, they are all composed of iron 3 plus and the second metal ion could be iron 3 plus could be zinc 2 plus, could be manganese 2 plus or magnesium 2 plus and these are all found in different kinds of a species. So, and the iron 3 plus of course, is the one which is conserved in all, the second metal iron is being replaced either by zinc or by manganese or by magnesium or by iron iron 3 plus 2.

So, generally when you take this purple acid phosphatase and try to use a mild reducing agent, they get into active form; so that means, active form has some reducing. But if you use a strong reducing agent, then they will break down these dinuclear centers the dissociation takes place enzyme becomes inactive and the solution becomes colorless so; that means, they are totally broken down.

So, these are enzymes which are involved in hydrolyzing the phosphate ester bond etcetera etcetera and I said there is they are dinuclear one of them is iron 3 plus other than could be the either the iron 3 plus magnesium 2 plus or the manganese 2 plus any of these things are possible depending upon the type of species.

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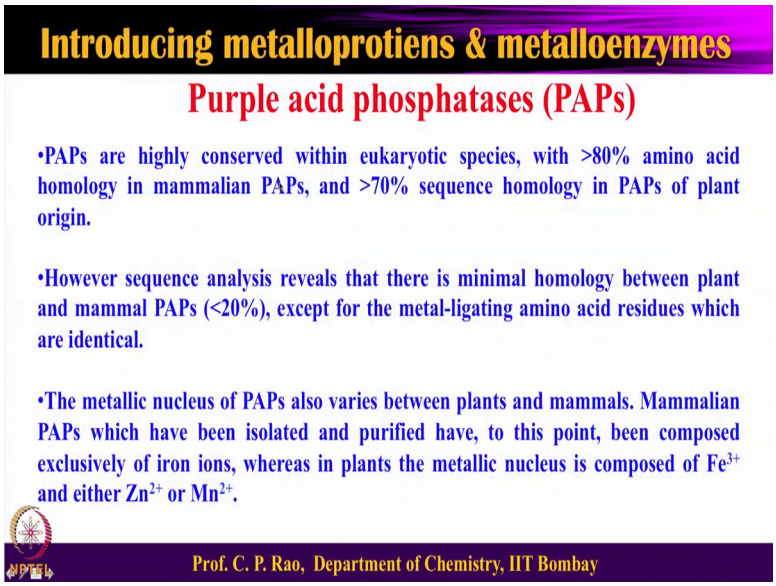

Now, let us look at some other species which are let us say eukaryotic species and the higher level which is mammalian. There is a large proportion of the overlap of the amino acid between the eukaryotic species for purple acid phosphatases and mammalian purple

acid phosphatases. On the other hand if you try to look at the plant based purple acid phosphate with that of the mammalian purple acid phosphatase there is a large difference. This is only 20 percent or the overlap you will find. So, between the eukaryotes and the mammalian is a huge percentage of overlap of the amino acids and the sequence homology which is 70 to 80 percent whereas, there is only 20 percent of the sequence homology when you compare the purple acid phosphatases between the plants and the mammalian. Just like we have used the short form RNR for ribonucleotide reductase let us use the short form or the PAP purple acid phosphatases ok.

Again if you look at the metal centers of these ones between the plants and the mammalians there is a difference. So, mammalian pap is been isolated and purified at this point have been composed of iron, where in the plants the metallic nucleus is composed of the iron 3 plus and either the manganese or the zinc and that is where the difference comes diversity.

So, the second iron in mammalians second metal ion is mammalian is still iron and in other kinds of things it changes to zinc and magnesium and manganese 2, and mostly form of zinc and manganese in case of the in case of the plant the things. So, we have tried to look at the compositional metal ion compositional aspects, and we also look at the protein overlap and amino acid overlap. So, this eukaryotes and the mammalian has a large overlap of amino acid, and there is a very little overlap between the plant and the mammalian one.

And the greater difference is that the dinuclear centers is primarily iron 3 iron 3 in case of mammalian, and in case of the plants is iron 3 and magnesium or manganese or zinc. So, mainly zinc and manganese are being formed. So, we got some reasonable amount of introductory aspects of the purple acid phosphatase, purple coming from the color in the oxidized form acid coming from the acidity this medium and the phosphatase activity is a hydrolyzed phosphate ester hydrolysis or this one. So, therefore, these are very well understood purple acid phosphatase.

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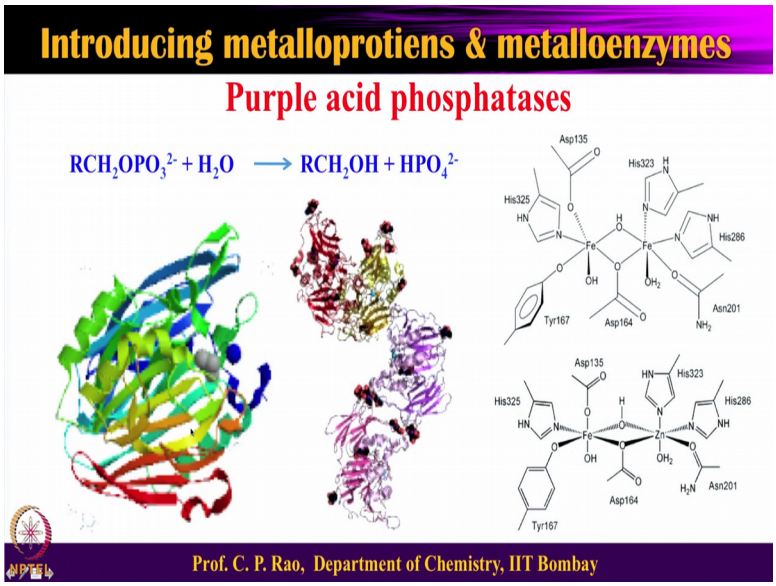

So, you can see the reaction here, this is the phosphate ester and this hydrolyzed to the alcohol and phosphate HPO 4 2 minus there is a phosphate counterpart and the alcohol counterpart and when you condense them you get the phosphate ester and this is what is happening.

So therefore, as I said that you have the purple acid phosphatases having both the iron iron center or iron with some other metal iron to be manganese or to be zinc. So, you have shown both the cases. The enzyme is shown over there, you have different units connected together and then you have the activity going on at every dinuclear center is catalytically active. Whether this is a iron iron iron dinuclear central or iron m 2 plus dinuclear center, where m 2 plus is zinc 2 plus manganese 2 plus in some cases magnesium 2 plus 2; so all of these now.

Let us look at the iron iron you have 2 histidines on one side and another side one histidine I have already looked at under tyrosine. So, this is the tyrosine which is involved in the charge transfer transition that is happening. And this is again concerned when you go to this the iron, and the zinc has got a very similar kind of a binding centers and therefore, the iron center is preserved therefore the purple color is still retained because of the tyrosine to the iron 3 charge transfer transitions.

. So, there is the core iron core is very well preserved in spite of the amino acid sequence may have a greater overlap or may have a lesser overlap. So, nearly 70 80 percent of the

overlap or 20 percent of the overlap, in both the cases is there. Except the second iron center is replaced by the zinc center in this particular case. So, you can take this from the plants or other non mammalian and mammalian kinds of things, but the reaction is the same ok.

and the 2 aspects that we have stressed now, one is the purple color the other is the oxidized and the reduced forms of this. Let us try to understand this how one studies this purple color how one studies this oxidized and reduced forms of this, particularly when you take the mammalian case.

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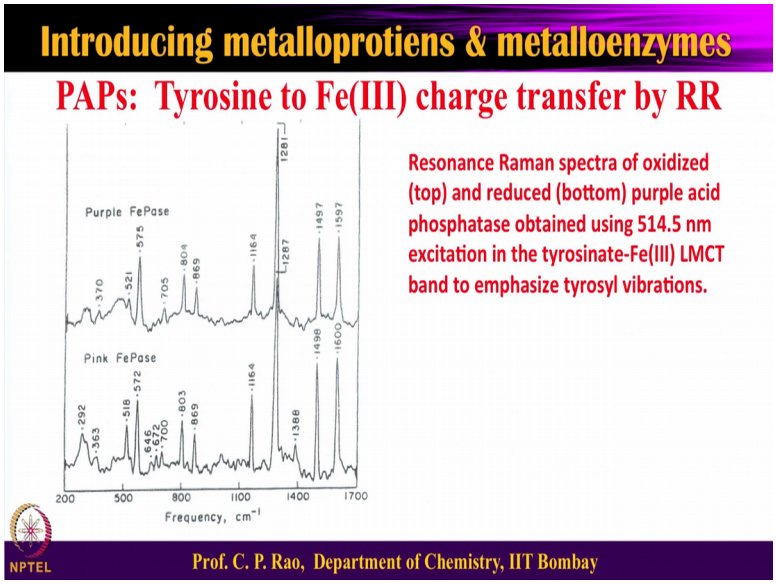

Now, as I said already the purple color is coming from the LMCT Ligand to Metal Charge Transfer. So, this is basically ligand to metal charge transfer is called I am I talk to you in much early stage in this case.

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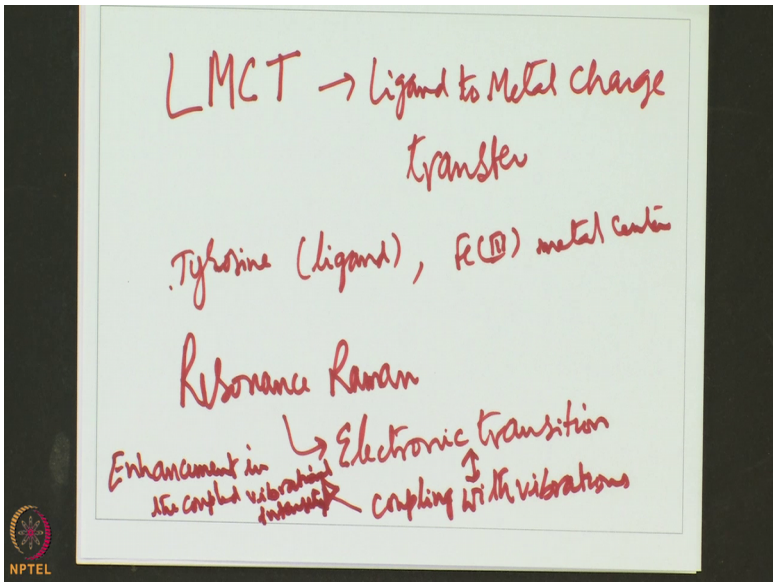

So, this is ligand to metal charge transfer. So, in this case the ligand is tyrosine now is ligand and iron 3 of course, is the metal center. So, that is where we have ligand to metal charge transfer tyrosine to the tyrosine to the metal charge transfer ok.

This charge transfer can be really recognized, one is by looking at the absorption spectrum where you have a charge transfer spectrum certain times these charge transfer band will also overlap with other bands therefore, you will not be able to make out. In such a case you can use a vibrational spectrum not a simpleIR vibration not a simple FTIR, but you need to look at the Resonance Raman spectrum. So, or is referred to Resonance Raman spectrum.

So, what is Raman spectrum we know, that it is vibrational those which are not found in the in the IR will be found in the in the Raman spectrum we know all that now what is the resonance Raman.

So, the Resonance Raman is the one where the Raman spectrum is measured under resonance, what kind of resonance? The electron transition. So, electronic transition. So, you electronic transition. So, how would you get the electronic transition? If you know the absorption band, you can excite the sample at that particular wavelength.

So, in this case there is a absorption band at 514.5. So, suppose now you measure a Raman spectrum while exciting the sample at the 514.5 nanometer, then what you get?

You get Raman spectrum which is the vibrational Raman spectrum which is coupled with this IR transition of the electronic transition. So, therefore, you have a electronic transition; so this coupling with the vibrations; so that is what you have in this. So, when it couples what will happen? The vibrational bands will become highly intense. So, when they become highly intense, you measure once the spectrum without this excitation, you measure the spectrum with the excitation you will see the bands which are which are enhanced in the vibrational bands.

So, from the enhanced vibrational bands, you can find out the coupled coupling of that is there between the tyrosine and in neighboring. So, that is what is basically done in this case. So, this the purple acid phosphatase and the pink ion phosphatase, they are all seen as you can see the bands are corresponding to the vibrational bands in that vicinity, identified to be the enhanced one.

So, from this basically you can get the tyrosyl vibrations, and then talk that there is a tyrosine bounded to the iron center there is a charge transfer too ok. To understand the resonance Raman is the Raman spectrum under the electronic excitation being continuously pumped in ion, in this particular case 514.5 nanometer because the absorption takes place at that. So therefore, the in the tyrosine to LMCT and this will enhance the tyrosinal vibrations.

So, coupling with the vibrations and this leads to when it coupled it gives the enhancement enhancement and in the coupled vibrational intensity. In other words the intensity of those couple bands will be increased so that from that we can understand this.

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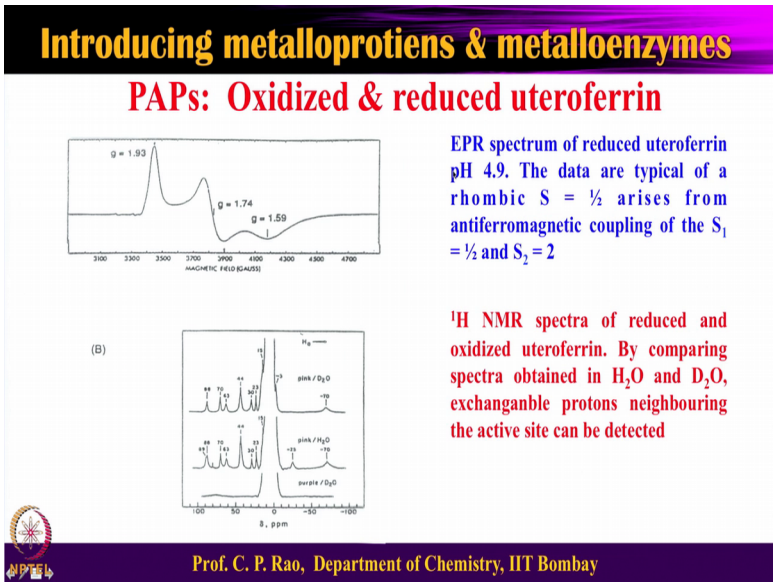

Now, we looked at let us look at the reduced forms of oxidized form etcetera etcetera you can see that this we are of course, referring to the iron iron case, the iron iron case there are 2 ions are there and oxidized form and then into the reduced form 2.

So, in this is the uteroferrin, which is for the mammalian case. So, mammalian purple acid phosphatase will have both the metal centers as the iron. So, in this case you can look at the purple acid phosphatase at the 4.9 pH, which is acidic pH and if you look at this particular EPR spectrum, you can see the EPR spectrum is very characteristic of the rhombic type as is equal to half with the anti ferromagnetic coupling the with the as 2 s is equal 2.

So, the one center and the other center say which is s is equal to half, other is s is equal to 2 and that is where you have the coupling happening in this. So, in that coupling can be identified from these EPR spectra, but in this particular case you do not need really know fully well how to analyze this, but just the taking into consideration that is what is happening with these ones and that is why you get the extra bands because of coupling on this.

Now, you can also look at their proton NMR spectrum. So, proton NMR spectrum and you have the there are 2 or these are looked at the pink state and the purple state and the purple state is a completely oxidized state and the pink state will have the reduced also in that.

In this the spectrum are measured in water and with D 2 exchange, to find out those groups which are which are exchangeable and you see this particular resonance or P is disappeared when you add the D 2 O and; that means, that is the one where you have in the vicinity of this the metal center. And all of these are very much shifted and you see in this purple case oxidized you do not see much of a splitting kind of things everything is broadened.

And in the in the pink form with one of the center being reduced, and you can still see, but shifted this is called contact shift. And these are going up to 100 ppm etcetera in a proton NMR going to beyond 15 ppm is nothing, but the paramagnetic shift or contact shift and this is the contact shift.

So, therefore, one can identify the tyrosine you know that term, the charge transfer band the oxidize reduced couplings etcetera all of these can be identified by the EPR and the proton NMR we have already looked at in the earlier case, the proton NMR you can have a contact shift. So, you can have a broadening this all these we have looked at and the proton to d water to d 2 o they put some of the protons will be exchangeable and those protons can be identified; so therefore, their connectivity with this.

Now, we looked at the general aspects the purple acid phosphatase and the purple color the transition and oxidize reduced form, we looked at the comparison between the mammalian versus the plant mammalian versus the other kind of organisms where the purple acid phosphatases are present.

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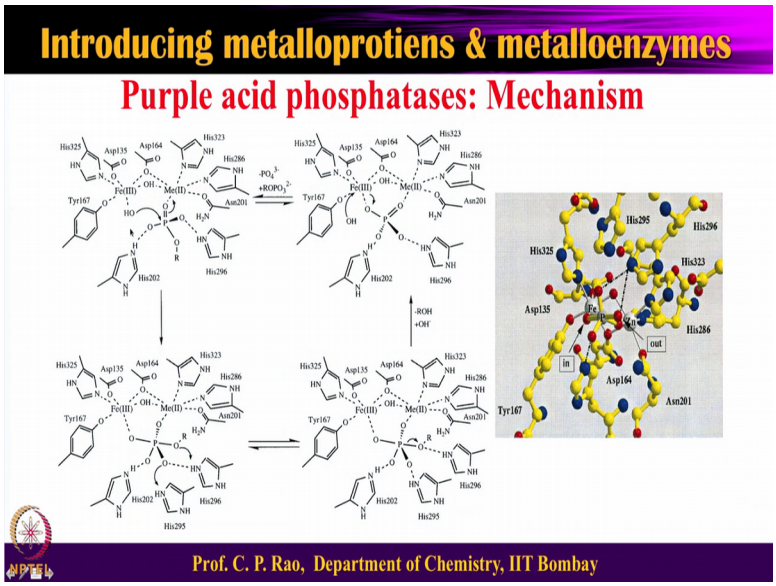

Now let us look at what are what is the role of these di metalic center. So, and here we have iron, the second metal let us say m and what is the role of these and we have already seen this center is very well known. Now this particular thing is involved see to have a reactivity what is required? This particular phosphate ester must be cleaved at this stage at this stage. So, that you get a hydroxyl for alcohol and the phosphate moiety ok. So, for that obviously, you need to have a binding followed by the hydroxyl attack or nucleophilic attack or water attack on these ones.

And that is what happens? So, therefore, you have the phosphate n ester or is bound to the one of the metal center, and the other metal center in the active form there will be water converting into the OH and this oh is involved. So, that is why we saying that that the activity starts from that and this OH is in proximity to this OH is in proximity to the phosphate ester and that will bring a nucleophilic attack.

Another aspect you should notice here is this phosphate ester is having secondary interactions to this, one of the histidine another histidine etcetera. So, what do you call this? The secondary interactions will basically recognize the phosphate ester and orients the phosphate ester. So, the phosphate ester has to be oriented in such a way that, this binds to the metal center and the phosphorus center is in the close proximity which is in the range of 2 and a half to 3 angstroms.

So,. So, so. So, therefore, you can have a nucleophilic attack taking place in this and that is what precisely happens. So, you can you have hydroxide which is reacting with this. So, and the phosphate ester binding to one of the metal center, the other metal center will provide the nucleophile. So, therefore, one is at a kind of a substrate binding and is the catalytic. So, you can say.

So, within the same by nucleus one acts as a substrate binding, other acts as a catalytic center both to the other. So, we can say this is the structural kind of a one and this is the catalytic one. So, and this provides the hydroxyl attack and in the in the hydroxyl attack you will find the intermediate where the sorry the intermediate where this phosphorus center with the 1 2 3 4 5. You know this pentacoordinated or pentavalent phosphorus center. So, pentavalent phosphorus center is a transition state kind of thing.

And so, attack in this now that will be further breaking this particular group and leaves out this particular OR going out. And for these the proton sources I are coming from these particular residues. So, therefore, there is a one is these residues are helping in recognizing the phosphate these residues in the where neighbor are also helping protonation deprotonation aspects 2.

. So, therefore, secondary interactions in an enzyme or metal enzyme are also very important, its not only primary where the metal ion is bound the secondary interactions are also. So, that will give the ROH out and then which will give. So, you can have. So, in presence of the phosphate you can go back phosphate ester you can go back this 2 ok.

So, you can see. So, one is the binding, another is a nucleophilic attack and transition state and then further hydrolysis and breaking the alcohol and the next step is replacing the phosphate moiety by the incoming phosphate ester. So, we have not shown the other part.

Now, if there is no incoming phosphate ester, then you can add water the water will displace and (Refer Time: 22:12). So, we can do. So, this will be continuously happening. So, that is why it is shown over there ok. Phosphate ester, binding, the transition state for the hydrolysis of this, and the ROH going out and the in entry of the phosphate ester replacing the phosphate moiety HPO 4 2 minus moiety. So, this is continue and you can see one of that particular intermediate kinds of the binding center can be seen over there and this is the center.

So, now, we understand the purple acid phosphatase is a hydrolytic kind of a activity, and there is no redox activity involved. So, although the 2 metal centers one metal center is involved in the binding, the other metal center involved in providing the hydroxide and then stabilizing or favoring through the formation on the transition state and then the water attack and then those things. So, and the secondary these groups are involved in the protonation recognition as well as protonation deportation steps in this ok so this 2 ok.

So, this comes to one of the aspects of this hydrolysis parts of it, then we also have some reductases where the reductases not by the by nuclear iron center, but these are by the heme reductase. So, we have already looked at the non heme reductase which is non heme reductase ribonucleate reductases itself is a non heme reductase because the 2 iron centers, which are present are no heme is there in this.

. So, we will look at in this particular case the reduction of NO 2 to NO nitrite reductase as well as NO 2 to NH 3 where you require more number of electrons. And for nitrite to nite and nitric oxide you require only less number of because you will remove only one O. In the other case you are removing both the os and putting hydrogens and therefore, making into ammonia.

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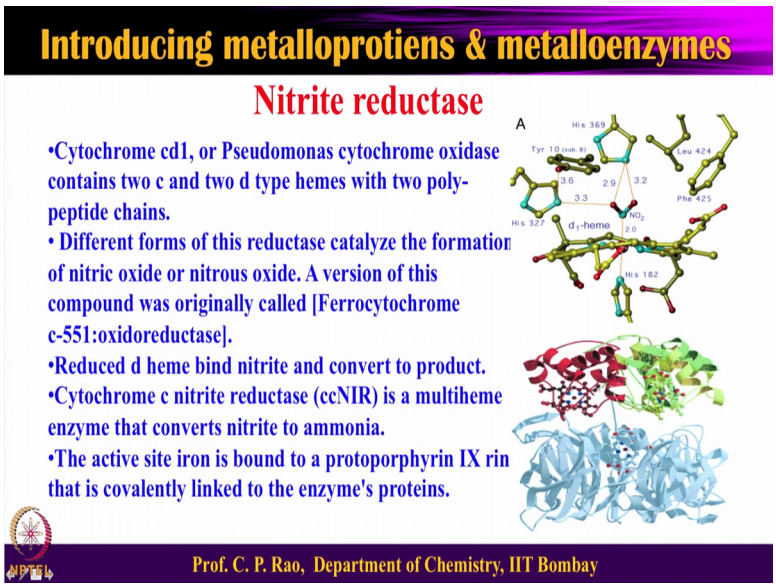

So, these are the 2 cases we will at under the non under the heme reductases ok.

Let us take the first part is the nitrite reductase in this, the nitrate reductase v uses the 2 the cd one cytochrome oxidase contains, there are 2 types of hemes are present in this not one heme. Just like in the dinuclear center to iron the iron center to ions are there, in this particular reductase there 2 hemes on there. So, one of the heme is the c type of the heme and the other heme is the d type of the heme; and it is the c type of the heme which is involved in the electron transfer, it is the d type of the heme that is involved in the catalysis of this because you require electron transfer you require to remove on of the o, and you require to make the n (Refer Time: 25:16) this.

So, the once it is reduced, the reduced d heme binds the nitrite because its a catalytic center and converts into the product and whereas, a cytochrome c nitrate reductase, is a multi heme enzymes that will convert the nitrate to ammonia and this we will see in is the next example. So, the active side of the iron is bound to the protoporphyrin 9 and that is covalently linked to the enzyme proteins ok.

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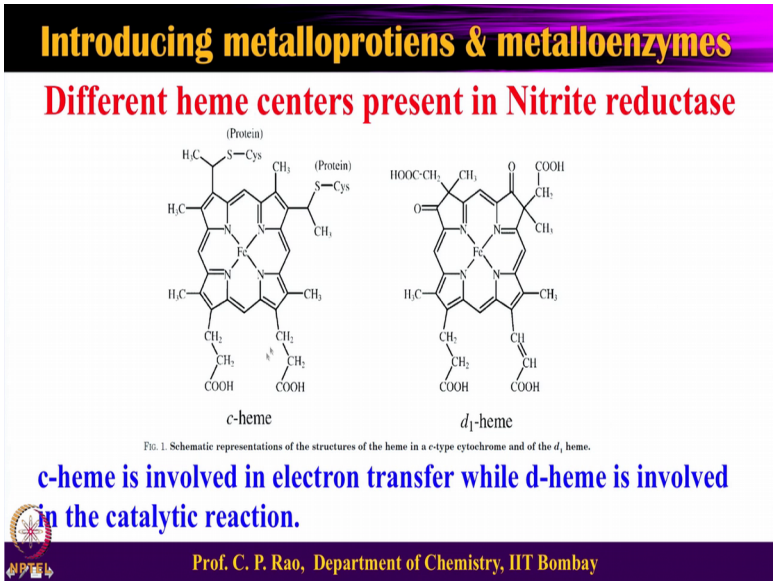

So, you can see that they are there are the 2 types of iron, let us look into this one v the 2 types of hemes, one is the c heme type c-heme c we already studied and then heme d type which has a different kind of a groups. So, here you have a reactivity is going on a catalytic and here only electron. C heme is involved in the electron transfer while d heme is involved in the catalytic reaction ok.

So, you can see that this we will come back to that the nitride, nitride is bonded there are 2 histidines are there involvement of that and this is the bound site with d and these are the other residues providing the electron transfer etcetera. So, we look at the detail in the in the different context in the next slide there are 2 histidines are involved in this particular thing.

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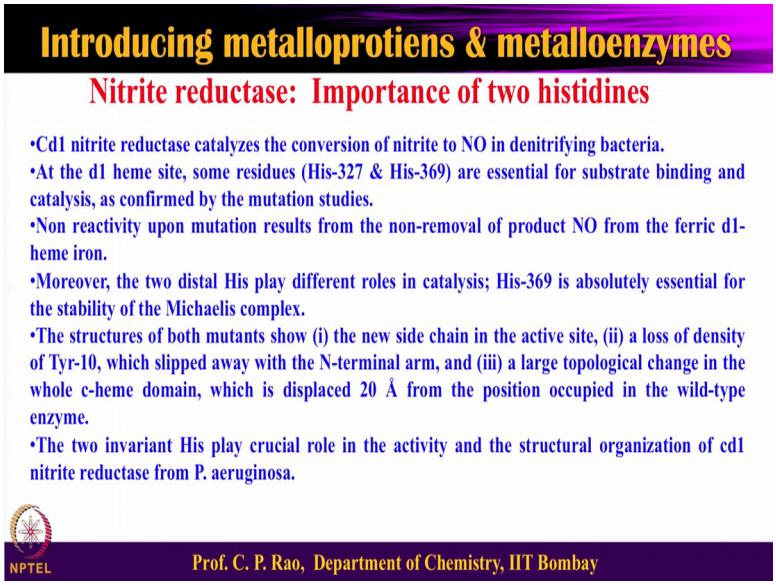

And in the conversion of nitrite to no; so it is at the heme d one site, the histidine 327, histidine 369 or absolutely essential for substrate binding and also to ensure the catalysis is confirmed.

And how do you know? If you replace any of these histidines and both the histidines by other residues, of a which is called mutation no reactivity no, the conversion and release is not happening primarily because in such cases the conversion takes place to some extent, but release will not take place. So, there are some digital historians also play important role in catalysis and therefore, all these things are important for these complex formation binding and then conversion.

the. So, if you look at the whole enzymes and the mutant reactivity, there is some new side chain in the active site which is involved which is important and there is some the tyrosine the density is reduced in that, it will just moves with the n terminal arm so; that means, there is some protein conformational change happening and there is also some topological changes in the in the c design domain. So that means, there is a electron

transfer happening in this area, during the electron transfer we have already seen that the reorganization occurs and that is what we change; so this whole thing.

So, the 2 invariants histidine plays a very crucial role in the reactive activity and structural organization. There are 2 things are there. So, there is a structural organization reorganization where the electron transform, and the on the other case we have the binding and reactivity this is the 2 essential aspects of it.

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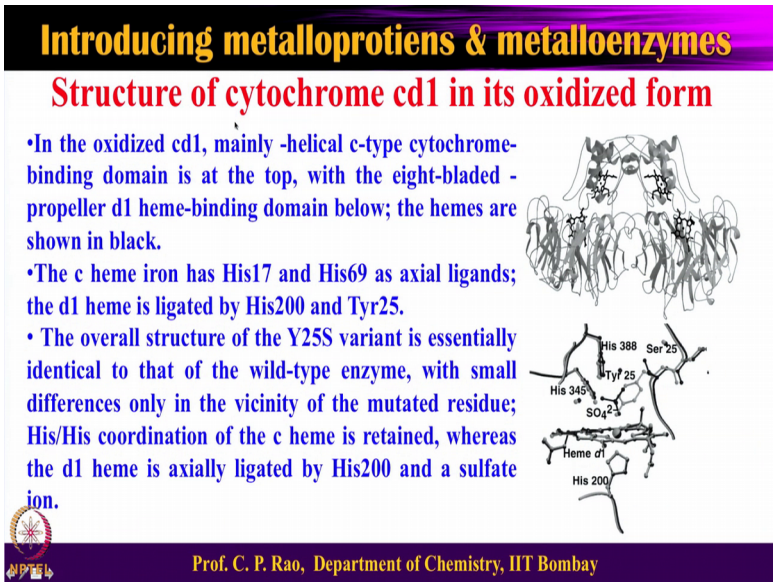

. So, now, you can see the structure the cytochrome c d 1 the oxidized form, there is a helical c type. So, you can see these are all the helices and there is a c type and this is a c type and this is sitting on the top, and the d 1 is sitting like the 8 bladed propeller heme binding ones here, there is one and there are the propellers. So, the c part of it and the d part of it and they are one or the other. So, has the c has got histidine 17 and histidine 19 as the axial ligands, which you cannot see so, much over here and the decays you have 20 and tyrosine 25 close by.

So, the overall structure are the of a mutant when you look at the tyrosine to the cysteine variant, is essentially identical to that of the wild type enzyme with small difference only in the vicinity of the mutant, which case. So, histidine histidine coordination the heme c is obviously retained there. So, there, but the d 1 heme is actually ligated by 200 and when you make the mutation because you are changing the tyrosine and then it picks up

in the solution the sulphate moiety. So that means, we can understand that this group this metal center has an anion kind of a affinity.

So, we will see more details of this enzyme, cytochrome c oxidase for the nitrite reductase in the next class and we will also look at the continuation of that going from nitrite 2 ammonia 2.

Thank you very much.