Inorganic Chemistry of Life Principles & Properties
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Lecture – 45
Role of Molybdenum in Life – Nitrogenase

Welcome you to the next class on Inorganic Chemistry of Life Principles and Perspectives, just immediately previous class we were looking at the Nitrogenous Molybdenum containing enzymes of which we have entered to the Nitrogenase arena. So, as I said that nitrogenous has got two major components in the form in its enzyme; one is called the iron protein and the other part is called the Molybdenum iron protein.

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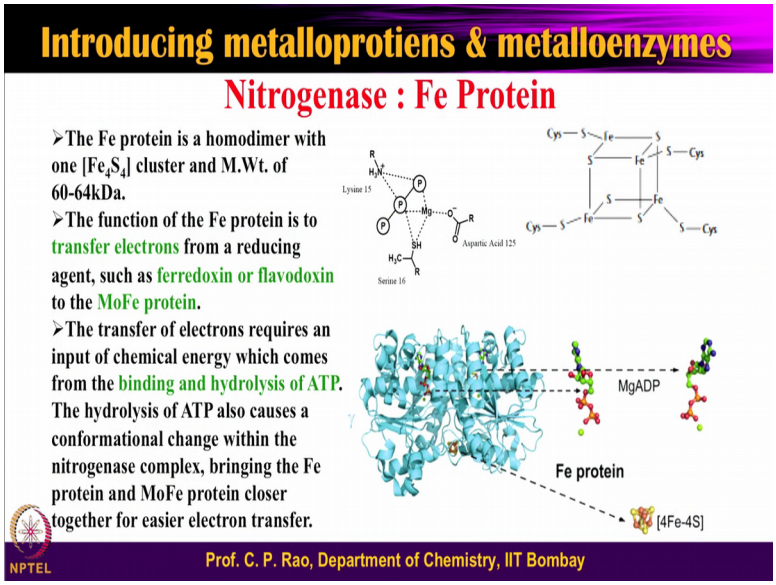

And this iron protein, has again components in it and iron sulfur cluster and a molybdenum ATP as well as molybdenum ADP bound system. These are the very important essential components.

Now, this is essentially an iron protein is essentially a dimer which is called the gamma 2 having about 60 kilo Dalton molecular weight. So, what is the role of this? The role of this is the functioning is to bring electron electron transfer, and it uses from reduced ferredoxin or flavonoid, and then to the molybdenum iron protein that is the role. And this does not happen just like that there is energy involved as I mentioned earlier, and that goes by the energy hydrolysis of ATP.

And that is where we are talking about the magnesium ATP, the phosphrylation basically. The Phosphorylation, phosphorylation Dephosphorylation, in other words, hydrolysis of ATP will bring a change in its conformation, such that the iron protein will come in good contact with the iron molybdenum protein or molybdenum iron protein such that the electron transfer takes place effectively from iron sulfur cluster present in iron protein to, a different kind of an iron sulfur cluster present in, molybdenum iron protein that I will explain just in a while in the next slide itself.

So, this particular thing is nothing, but the top portion of this enzyme as I mentioned.

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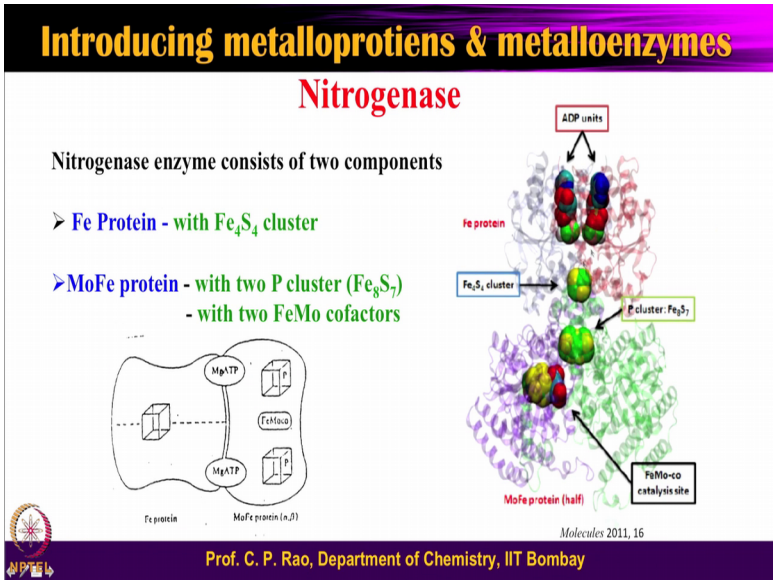

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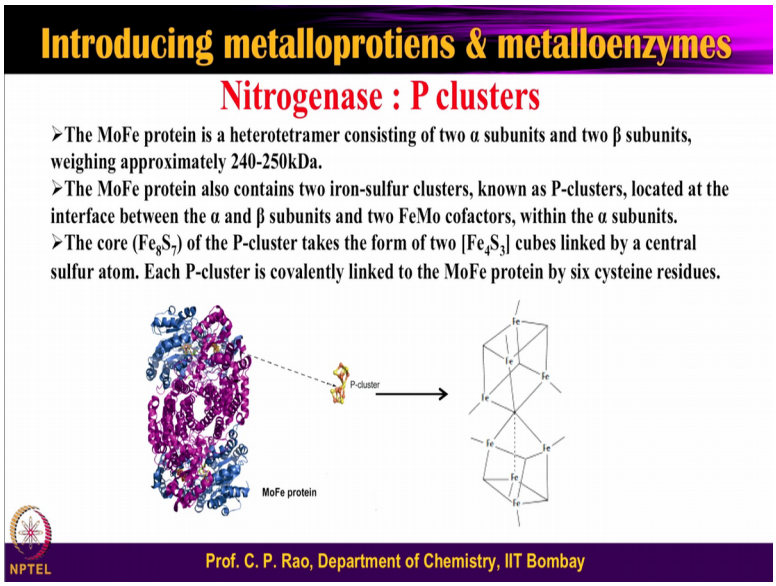

So, let us look at the other part the molybdenum nitrogenase, molybdenum iron protein. Molybdenum iron protein is a tetramer they is basically a hetero dimer of alpha, dimer of beta alpha, two beta two so which is around 240.

So now, if you combine the total molecular weight of 240 plus 60 in the previous case, that is the iron protein totally; the molecular weight of this nitrogenase enzyme which is a complex enzyme is around 300 kilo Daltons.

So, very huge protein, so; that means more than 3000 amino acids etcetera etcetera. So, this contains the iron sulfur cluster, which is shown here and its needs named as P-cluster. So, in this P cluster what you have is the Fe 4 S 3 another Fe 4 S 3, so the two Fe 4 S 3, are fused through one sulfur. So therefore, the whole thing is Fe 4 S 3 plus, Fe 4 S 3 plus 1 S, that will be Fe 8 S 7; Fe 4 S 3, Fe 4 S 3, and then sulphide in between. This is whole thing is called the P-cluster, besides this you have another unit which is a catalytic unit, this is only electron transfer.

So, this P-cluster takes the electron from the iron sulfur cluster present in the iron protein, due to the hydrolysis of the ATP that brings a conformational change and transfers. So that means, this mg ATP and the this is iron sulfur cluster or at there at the juncture of the iron sulfur protein with that of the molybdenum iron sulfur protein.

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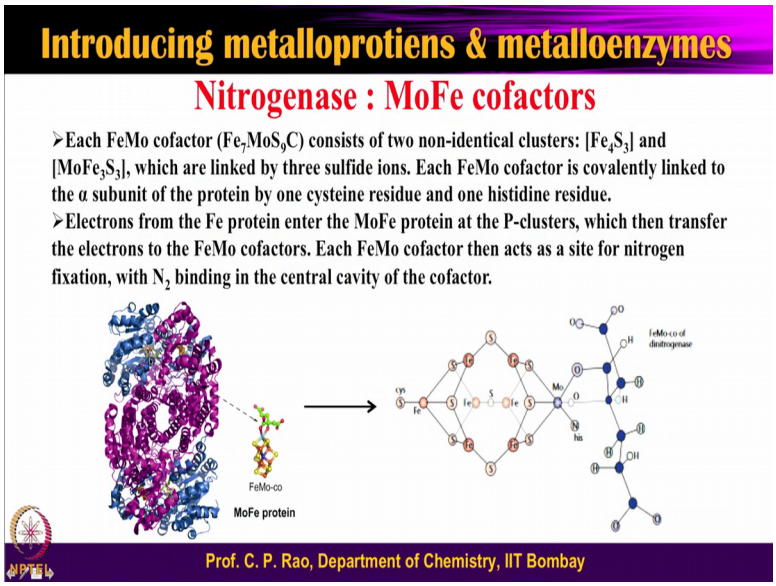

As I said that the, the second component present in this particular alpha 2 beta 2 part is a cofactor called the molybdenum iron, molybdenum cofactor iron.

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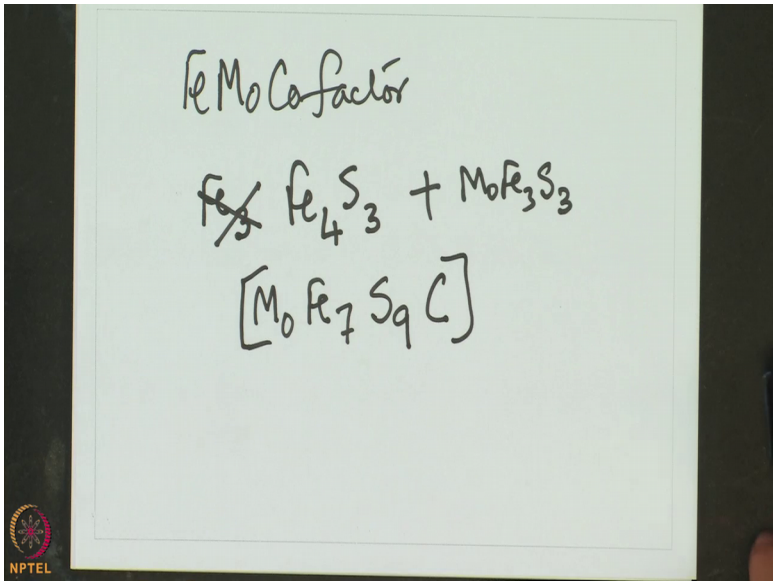

So, this is basically you have an iron 3 S iron 4 S 3, and molybdenum Fe 3 S 3. So, this is the Fe 3 as sorry Fe 4 S 3which is combined with; instead of another Fe 4 S 3, MoFe 3 because you always have a 4 corners; other carbons are by sulphide. So therefore, 8 corners always and Fe Mo Fe 3, S 3 and these two are there, which are connected

together to form with the bridge kind of a center there with the 3 sulphide groups will be there.

So therefore, you have Mo total together, Mo Fe 7 S 3 S 3, 6 plus 3, bridge sulphides so that will be S 9 and there is some other species, which is probably as a open center, so this is the all molybdenum cofactor. Let me just go back to the previous case where the iron sulfur cluster is involved the P cluster, as I mentioned this P cluster is not a suspended moiety in the protein, each of the iron is connected to the cysteine group.

So, there are 4 cysteine, 4 cysteine, 8 cysteine, groups are there; in case, the simple iron protein where the Fe 4 S 4 cluster is there again, it is bounded by the 4 cysteine, here it is bonded by 4 plus 4 8 cysteine residues.

So, this is your mlybdenum iron cofactor, as I said, the one is Fe 4 S 3 component, Mo Fe 3 ES 3 component, and the in between bridging sulfides and this is the country 100 molybdenum center, you see molybdenum center has got a lot of coordination here. 3 and 3 coordinations over there, and there is; the kind of a citrate based a group is also involved and.So these are all very important kind of a things.

So therefore, each iron molybdenum cofactor is covalently linked to the alpha subunit of the protein, by one cysteine residue and one histidine residue in this case, unlike the iron Sulfur clusters or P cluster, where all our cysteines.

So now, what is the story? The electron going from the iron sulfur protein, which in turn gets from other sources as I will show you more clearly in the next line to the iron molybdenum protein and from there, to the P cluster and from the P cluster to the iron molybdenum cofactor.

So finally, the reaction occurs at the iron molybdenum cofactor, where the nitrogen fixation takes place so basically, 8 electrons first 2 electrons are involved in converting the H 2 plus, A 2 H plus, plus 2 electrons, H 2 ok. So, we have a case,where the 8 electrons, at the molybdenum center will convert the N 2 to N 2 NH 3 and 2 H plus 2 H 2 and that is what is happening at this particular center. So now, you can see so this is called the catalytic unit so molybdenum iron cofactor is nothing but a catalytic unit basically in this ok.

So, now, we have seen iron protein having Mg ATP Mg ADP binding region, as well as the Fe 4 S 4 cluster, then we have seen the Mo Fe protein, where there is a P clusters and there is a catalytic Fe O Fe Mo CO.

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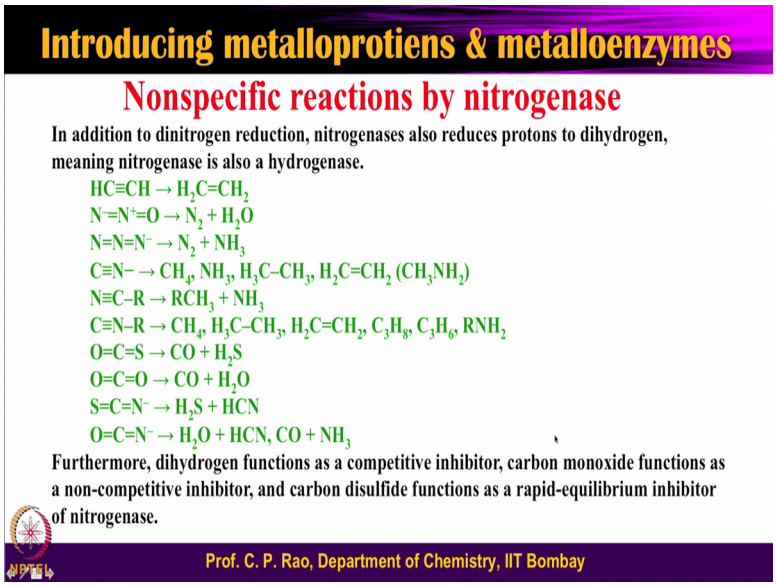

So this means, we have looked at the most of the general features or compositional features of the nitrogenous.

Just before we go into more details of the electron transfer just look at this reactions, which are Nonspecific by the nitrogenous, so I have already showed the acetylene to ethylene N 2 O 2 N 2 etcetera earlier. I am just showing again cyanide giving the methane plus ammonia intermediate things can give other kinds of things too.

So you have, isothiocyanate kind of thing a carbo carbondioxide, thiocyanate all these kinds of things can be reduced these are all known specific reactions, so what does it mean? it means if you take this enzyme into a test tube and use any of these use their regular conditions for the enzyme to function and then use any of these as a substrate you get the product ok.

So, in among all these the hydrogen, dihydrogen functions as a competitive inhibitor where that 9 2 will replace that and the carbon monoxide will act as a non competitive means cannot be replaced and carbon disulfide, can function as a rapid very rapid equilibrium kind of a innovation for the nitrogenous in these things too.

Now, so whatever I told earlier about the iron protein and the Fe 4 S 4 cluster Mg ATP or Mg ADP.

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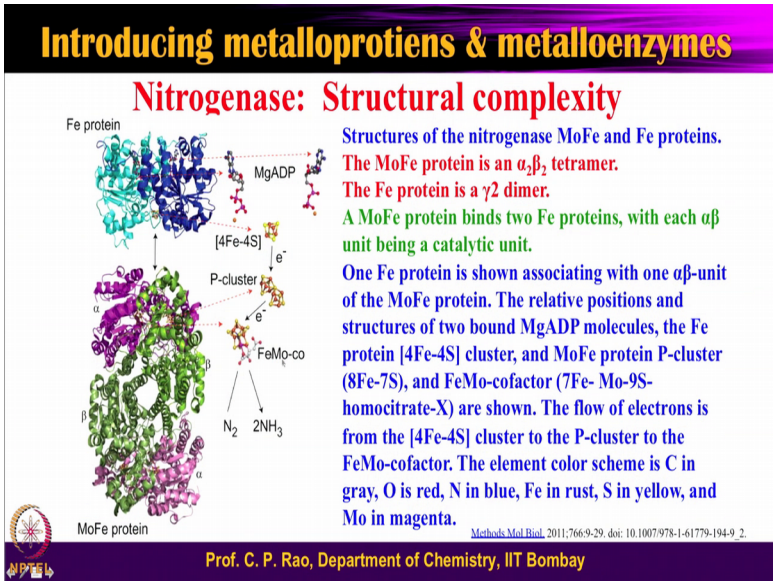

And then P cluster and the F Fe Mo Co is seen here, you can see very nicely this portion is the iron protein this portion the iron molybdenum protein and this protein has got the 4 iron 4 Sulfur cluster it also has mg ATP Mg ATP binding site.

And this particular part of the enzyme has got the P cluster, P cluster is nothing but the fusing of the 2 Fe 4 S 3 clusters with a bridging sulphide and then this is the catalytic center and this catalytic center, is has got basically iron 7 molybdenum, Fe 7 Mo kind of a cluster where Mo is the center where the reaction occurs.

Now you can see, the electron coming from outside to the Fe 4 S 4 cluster will transfer here, by using the Mg ATP, the hydrolysis and this goes into the P cluster, P cluster in turned delivers to the Fe O Fe Mo Co and at the Fe Mo Co, it goes to the molybdenum center and at the Molybdenum center it will react with the nitrogen to give ammonia.

So this is the kind of thing, so this is therefore, it is a alpha 2, beta 2, gamma 2 kind of a protein, in this alpha beta is a catalytic power and the gamma is the electron transfer part of course; there is electron transfer also present PSP clusters in this one too. So, this is the kind of a thing that we have so now, we are we got all the information required to

understand the flow of electron, so path of electron how from where; how from to where it goes.

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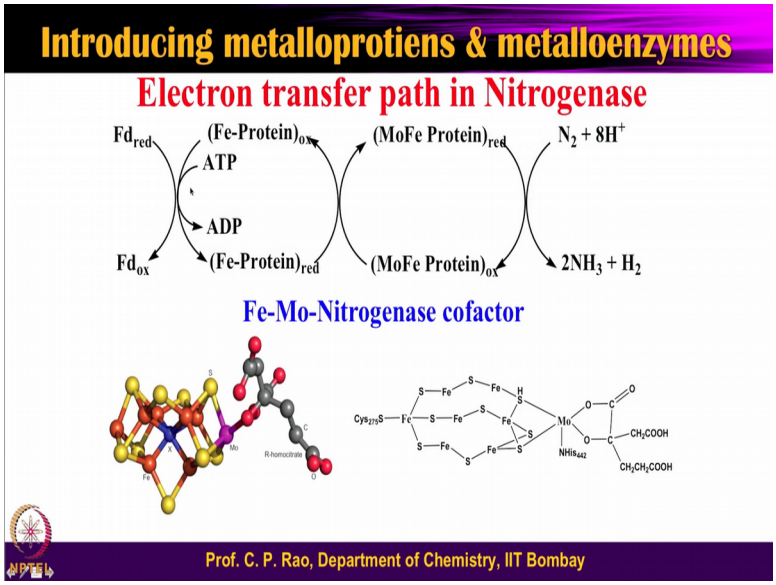

Now, this is the iron protein part and this is the Molybdenum iron protein part, so iron protein part couples with the molybdenum iron protein part, at the end of the molybdenum in the nitrogen will get bound and then converted. Now, how do the iron sulfur protein of the Fe 4 S 4 cluster iron sulfur protein gets electron, this electron comes from the Ferredoxin reduced.

So, when the ferredoxin reduced goes to ferredoxin oxidized, gives electron and that redox potential is very well suited to add to the iron protein oxidized or Fe 4 S 4 oxidized.

So, Fe 4 S 4 oxidized and that goes to the Fe 4 S 4 reduced, when the ATP hydrolysis is triggered, so therefore, this electron transfer triggers the ATP hydrolysis to and that hydrolysis will pump in the electron into the molybdenum iron protein.

So, the molybdenum iron protein and this electron can give it to the molybdenum iron protein and reduce and that goes into the oxidized. So, when this reduced protein goes into the oxidized the electron that is generated will go into the N 2, so like this you have to have one electron and one more cycle 2 electron, one more cycle 3 electron, 4 electron, like that you need, so electron path is from the Ferredoxin reduced to the iron

sulfur protein oxidized from iron sulfur protein oxidized to the molybdenum iron protein reduced.

This is nothing, but the cofactor and then the cofactor it goes to the molybdenum center and in the Molybdenum center it goes to the nitrogen and the nitrogen goes to the ammonia, you already seen in the previous slide, how it looks like? And this is a molybdenum center these are the iron sulfur clusters with the triple abridges thyroid and there is one cysteine and one histidine.

So, the whole cluster is connected to the protein by two connections one cysteine and one histidine, cysteine is coming from these iron, histidine is coming from this particular molybdenum.

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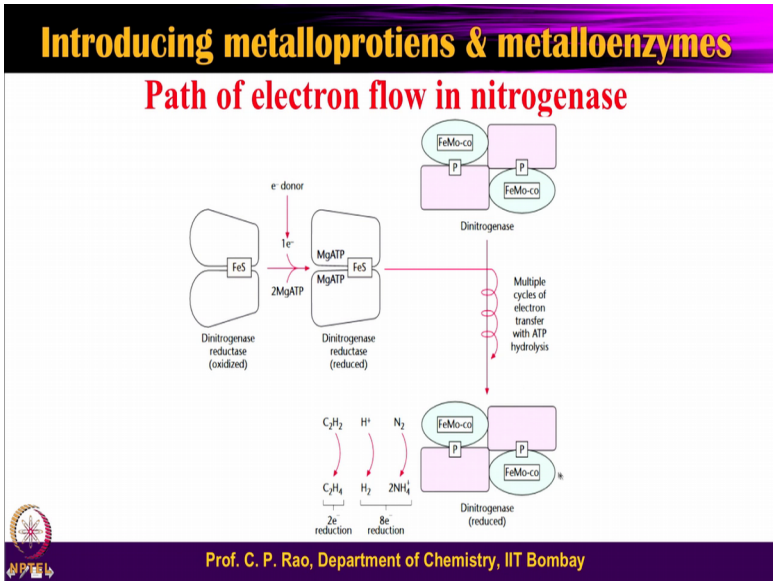

So now, we have seen that, we can see in another way same information, I am showing in a different format, but the message is nothing new.

So, this part is your iron sulfur; now containing iron protein where the electron coming from the Ferredoxin and that will activate the Mg ATP hydrolysis and pumps in the electron and this electron goes into the Fe, Mo Co or Molybdenum iron protein, at the Fe Mo Co and then from there it goes to the molybdenum center and then it can go to the nitrogen or hydrogen plus or acetylene etcetera etcetera.

So, there is no new information I have explained here, except I am showing it some kind of a figure based kind of thing. So, you have the iron sulfur protein over here iron molybdenum protein here, and then and molybdenum cofactor here and then the reaction of this.

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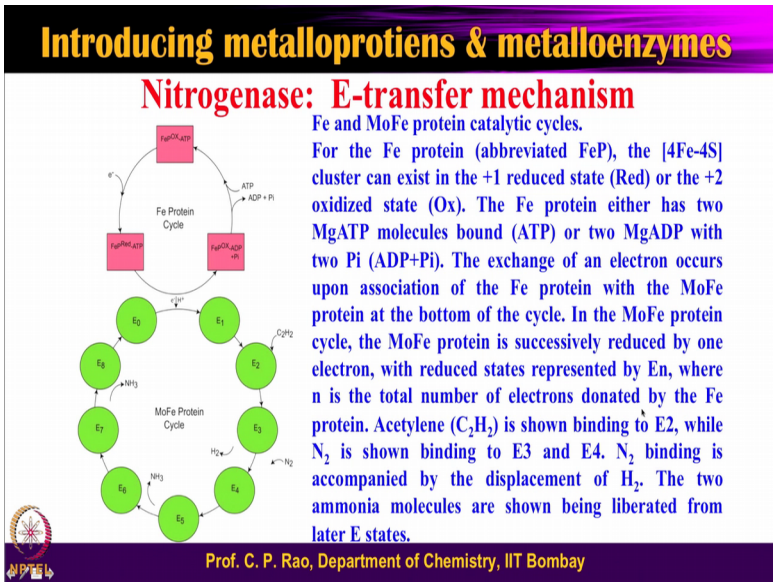

Now, this let us try to understand a better in terms of the electron transfer why? I told you earlier in the previous case I mentioned to you, this does not happen in one short this happens in a several shots, each electron at one shot. So, one electron it takes in goes through this goes through this adds here, yeah 2 electron goes through, this goes through this acts here etcetera etcetera. Of course, regeneration will also take place so that means, we have an electron, electron, electron, electron one by one is added.

So, let us see how that kind of a thing can be understood so, that is what I am referring it as a electron transfer mechanism, electron and this is hydrolysis that will pump in the electron into this chain, the chain of the molybdenum iron protein in that molybdenum iron or iron molybdenum cofactor.

So, you can see that the cofactor, we are referring in the form of E 1, when one electron is added E 2, when 2 electrons one more electron is added E 3, when one more electron is added, one more electron E 4 one more electron is added E 5, E 6, E 7, E 8.

So, that is how it goes all these things, so therefore, you have the in the 9 regenerates back. So, you have an electron flow happening from the iron protein to iron molybdenum protein stepwise by one electron at a time, once you have completely 2 electrons are added to the molybdenum center. These 2 electrons are you to convert H2 H plus, ions to H2 and at this stage the substrate can bind if it is a nitrogen it is N2, if it is ethylene in the C H C 2 H2 in some other context, this was there it is written C 2 H2, but you can consider this as N2 also ok.

So, at this stage your N 2 will come and hydrogen will go. so somewhere around E 3 case, when one more electron comes then nitrogen comes and nitrogen, or you can have these C2 H2 also, that so if it is C2 H2 in the initially it will go to C2 H4, if it is H plus, then it will go as H2 and then nitrogen coming, .so the nitrogen coming, so 1, 2, 3 electrons are given so E3 E4 E5 or NH 3 comes out.

So then E6 E7 E8, one more electro, or one more ammonia comes and then it returns back, so therefore, you have electron transfer cycle very well, so this can be checked by adding injecting acetylene, the ethylene coming out all these kinds of things can be done so N2 basically binds at around E3 state.

So, E3 E4 in between and shown, so once the third electron is there by the time hydrogen is formed, hydrogen is displaced replaced by the N2. So, one by one electron transfer.

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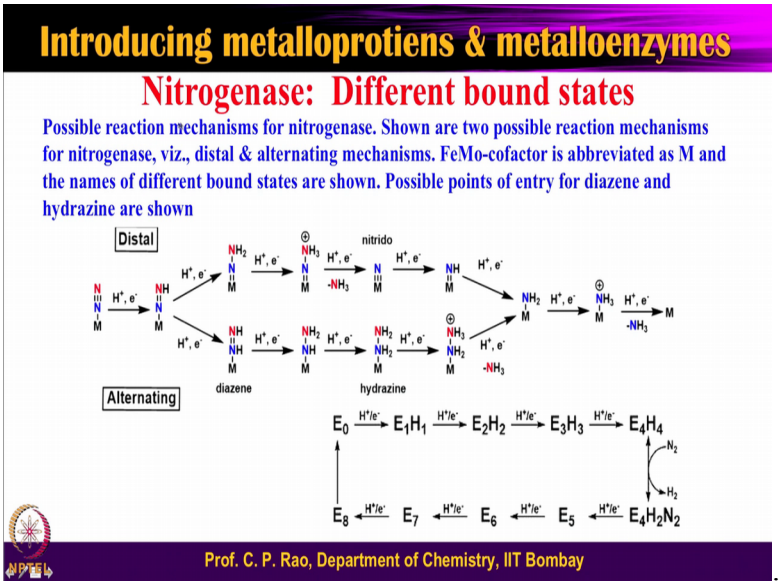

Now, let us look at in the nitrogenous, when such a kind of a one electron, one electron, one by 1 electron and it is not just one electron, one electron, one electron, you have to have also protons that is not shown over here, how the, the H2 is coming out? How the NH3 is coming out? There have to be 2 protons by that time there have to be 2 plus 3 5 protons by the time, 5 plus 3 8 protons, by the time so that is not shown here.

So only the electron transfer cycle is shown here, so therefore, the next slide will show you both the electron, one by one proton, one by one so when you add to N2 one electron, one proton what happens? When you add second electrons second proton what happens? When you add third electron third proton, what happens? So this is what we referred as the different bound states is a mechanism.

So, this mechanism is given in two parts one is the one which is going through the upper part, this is the upper part here and the other one which is going through the lower part.

So, up to here is common, so this lower part is called alternating path and the upper part is called a distal path, so when it goes to this cycle everything is same, so only in this region there are 2 path are proposed, one is called the distal path, other is called alternating path. Now, take the whole enzyme, whole cofactor Fe Mo cofactor as M, so or when there are also assumed this M is a molybdenum center either it is all right. Either you can assume M as a Molybdenum center or you can take it as a total iron molybdenum cofactor now fine.

Now, to this you add nitrogen N2, now you add you need to reduce the one electron one proton will give N N becomes N N H and the triple bond character will also get affected, now add one more electron and one more proton that can become NH 2. So that means, both the previous proton and now the later proton are attached to the outer nitrogen not the bound nitrogen, that is one path, the other path is first hydrogen let us say is bound to the external, nitrogen or distal nitrogen.

The second one the hydrogen is bound to the bound, metal bound so this is the other path, this is the alternating path, take the first path so now, you have first step of proton electron second step of proton electron gave M N NH2.

Now, one more electron and proton will certainly add to this one and make NH 3 and this NH 3 can be lost when the next electron proton comes as ammonia, that is what is shown

as a minus ammonia and now what do you have M triple bond N it is called metal nitride, so nitrido or metal nitride so it is a nitrido complex, so you in the distal path you are going through the nitrido complex and you will not go through the same intermediate, when you look at the Alternating path and that is what the difference that units.

So this nitride, now you add one more electron and proton, this only one nitrogen so it will add to that one more electron, one more proton because M NH 2 and one more electron, one more proton become M NH 3, and then it will regenerate back, to M.

So, M can be either the metal center or the molybdenum iron cofactor ok you understand that, so the path one is a distal why the name Distal? The name Distal comes, because the N 2, the outer N is getting protonated first and in the other case, both the nitrogens are getting protonated, so that is what the difference is there.

So let us come to the second path alternating path, first proton electron MN NH, so now, the second electron second proton will be come to the other nitrogen, so now, you have M NH w 1 NH. So, it is a diazene complex of cofactor so diazene, now you put one more proton electron only one nitrogen will get that, put one more electron proton will become NH 2 NH2.

So this is a metal bound NH 2 NH 2 is nothing but metal bound hydrogen, so in the previous case, it is going by a nitride or nitrido in this alternate path, it goes where diazene as well as the hydrazine, diazene as well as hydrazine the kind of a system.

Now, to these we add electrons and protons, so then you get NH 3 and then that will be broken into the one more electron proton will take out the ammonia and then add to this, then it becomes the same ok.

So you have essentially in the nitrogenase as I said, there are different steps of electrons, so the each electron stays we have seen in the previous case. We have seen where the hydrogen comes out, where the nitrogen gets in, here we have look at the species binding. So, we have looked at the later part of the 6 electron story in the previous one, we seen the total 8 electron story there we have seen total 8 electron story.

So, first 2 electrons will be for a hydrogen, next 6 electrons for the nitrogen thing, so in the nitrogen part we have looked at so when the nitrogen binds there are two possible species, one is through the nitrido species or hydrazine, the same thing is shown over here, so these are referred in the previous case, as ah E 1 state, a 2 state, E 3 state etcetera etcetera.

So the same thing is shown here, E 1, E 2, E 3, so by the time into H 2 will go away, so, E 4, E 5, E 6, E 7, E 8, etcetera etcetera, so going back so these are the kinds of cycle that we have, so this is something very interesting as for as the nitrogenous is concerned because very complex enzyme.

So, we started with the story of nitrogenous, which is a reductase enzyme, we have looked at different complexity of the nitrogenase enzyme iron protein and iron molybdenum protein, in the iron protein or the iron sulfur cluster in the iron molybdenum protein both the ah P cluster as well as the molybdenum iron cofactor very nicely.

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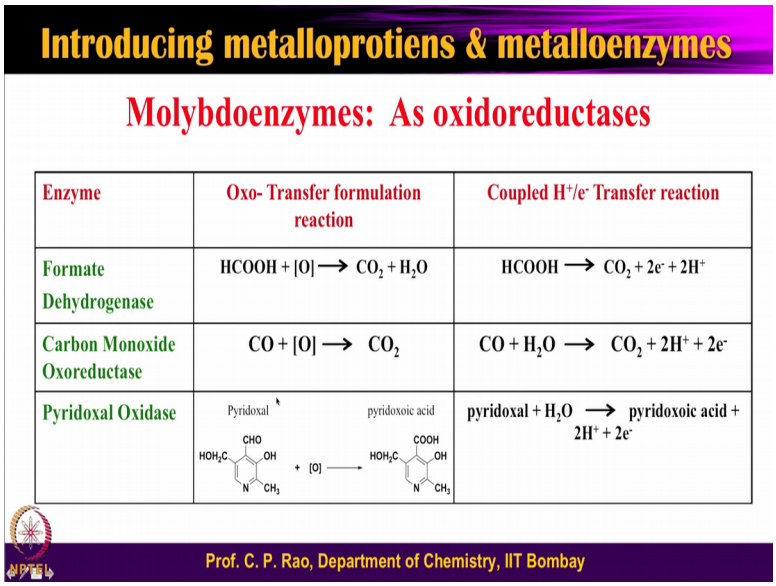

And then we looked at the as a catalytic center and the Mg ADP is the one which is triggering the reaction, by the hydrolysis of ATP and therefore, these two things conformational change, will make that thing to happen.

Now, let us switch over to the molybdenum enzymes and the oxidoreductases I have already told showed some examples earlier. Let us look at before we go into their mechanistic aspects, example one here formate Dehydrogenase, so formate Dehydrogenase goes from formic acid to carbon dioxide and this one CO 2 etcetera etcetera; carbon monoxide, the a carbon monoxide oxidoreductase you can call it as.

So, carbon monoxide oxido reductase is or oxidase you can say ox so CO plus O going to the CO 2. So, this part is for the oxidation purpose, pyridoxal oxidase. So, this is the pyridoxal moiety then you add this oxidation reaction it will add the OH is called pyridoxal acid ok. So, all of these are 2 electron 2 proton any things.

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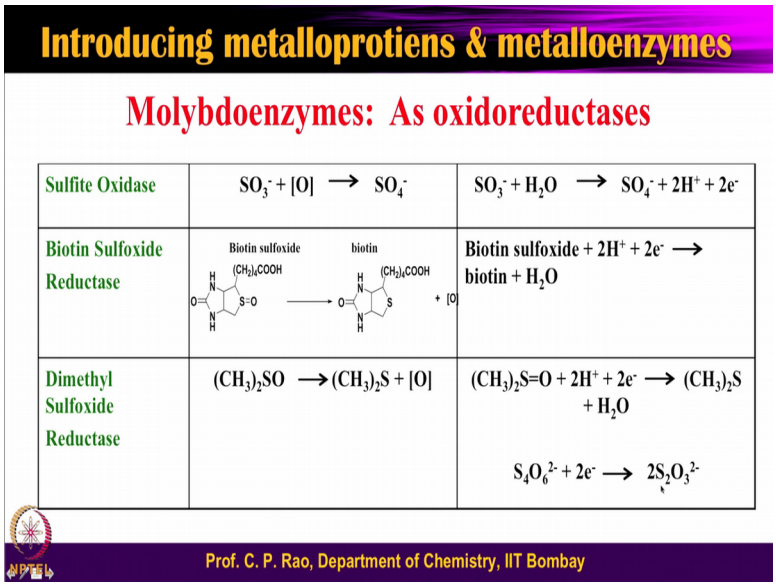

Now, sulfite Oxidase, that is a SO 3, going to SO 4, again 2 proton 2 electron, and one oxygen biotin sulfoxide reductase. So, reductase means, you take out an oxygen from here.

So this is Biotin sulfoxide, SO this is the sulf oxide you see this is just simple biotin no oxide will be there and this oxygen will go as a water and that will go as a water, you see here in the reaction biotin sulfoxide plus 2 H plus, plus 2 electron means biotin plus H 2 O, so dimethyl sulfide reductase.

So, this is a reduction of the dimethyl sulfoxide, there is dms 0 and becomes dms dimethyl sulphide, so that means, that oxygen is pulled out by 2 protons and 2 electrons, to the water and then dimethyl sulfoxide will go to simethyl sulphide.

It can be from S 4 O 6, 2 S 2 O 3 also. So, you have a number of the O O the oxido reductase reactions.

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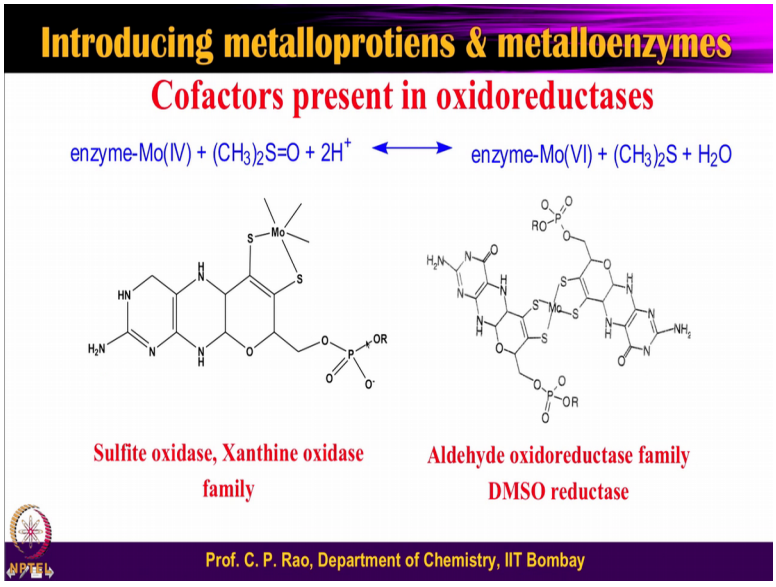

And as I have already told you earlier, there are two types of a molybdenum cofactors are present and one of the cofactor is the one where a molybdenum center with one tenon, and the other cofactor is there in the molybdenum center with the 2 pterins and these pterins are arranged in a somewhat like a trans orientation. And this particular the one which is containing one is called the sulfite oxidase family some of them, some of them are xanthine oxidase family differences in one case have a S CH 3, in an S cysteine I am sorry.

S cysteine in other case, double bond S; So, you have a sulfite oxidase xanthine oxidase, so it is another family, why it is another family? Because in this family, we have one molybdenum center with one terry the second family is called the aldehyde reductase family, in this case we have 2 pterins are involved, so one pterin and 2 pterin are involved in all this kind of things.

So now, in the next class, we will fully try to look at how all these oxido reductase enzyme? So, as I said in the beginning of this particular topic of the molybdenum, that there in the enzyme in the oxidized state, which is molybdenum 6 it will be able to oxidized the substrate, when the enzyme is in the reduced rate that is multiple before it can reduce the substrate.

Therefore, it can act both as a oxido reductase kind of an enzyme, and that both of these things are there in the next class I will demonstrate that property, what I mentioned? Oxido reductase property.

Thank you very much.