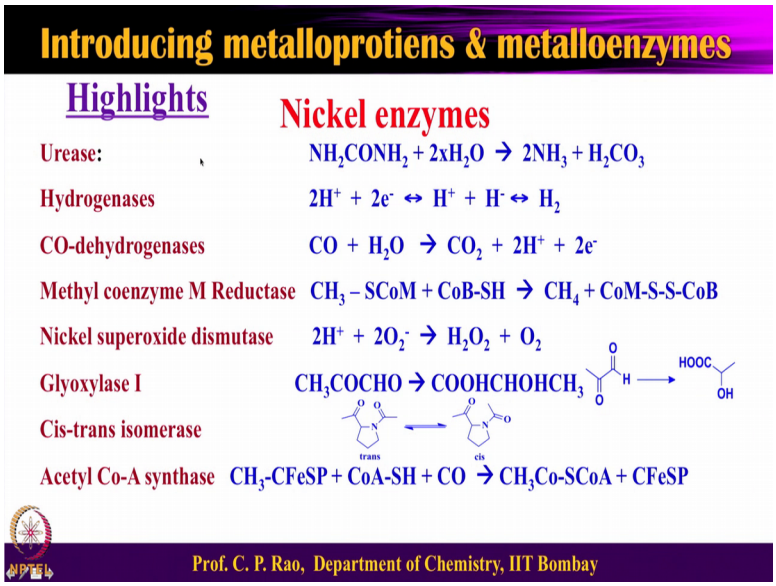
Inorganic Chemistry of Life Principles & Properties
Prof. C. P. Rao
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture - 56
Highlights of the course - Part IV

Welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. During the past three classes or so, I have been trying to revise what all I have taught, which I put under the title called highlights. And towards the end of the previous class, I was talking to you about the nickel enzymes. Anyway I will bring back the highlights of the nickel enzymes once again now here, and then try to complete the rest of the enzymes associated with it under the highlights category in this particular class. And then quickly start the tutorial parts from the next class onwards ok.

(Refer Slide Time: 01:02)

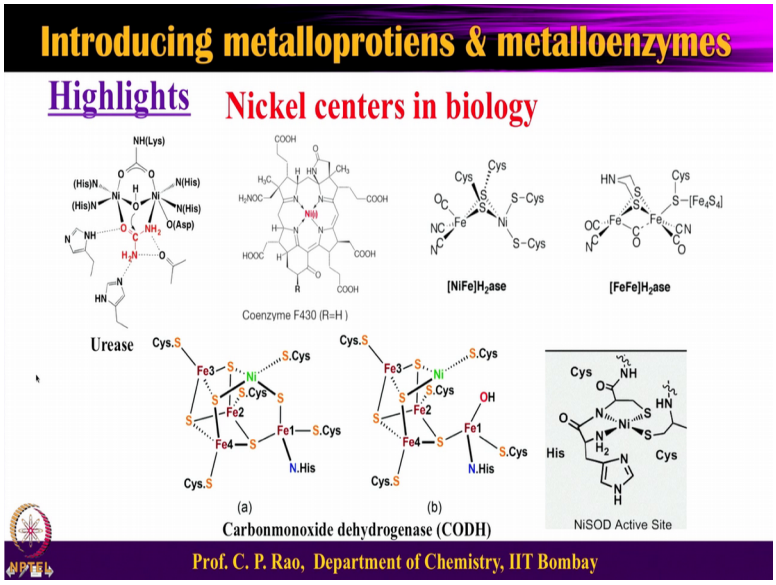


You see that in case of the nickel enzymes, nickel is involved in variety of reactions. Reactions such as a hydrolysis like a urea hydrolyzing to give ammonia kind of thing, hydrogenases, where the 2 H plus plus 2 electron giving 2 H 2, and it is a kind of a reversible kind of reaction depending upon the nature of the enzyme, whether it is in the oxidized form or the reduced form also carbon monoxide dehydrogenase going from C O to C O 2. And things such as the methyl coenzyme M reductase, a nickel superoxide

dismutase, superoxide dismutase I have told you earlier to I will just show some highlights.

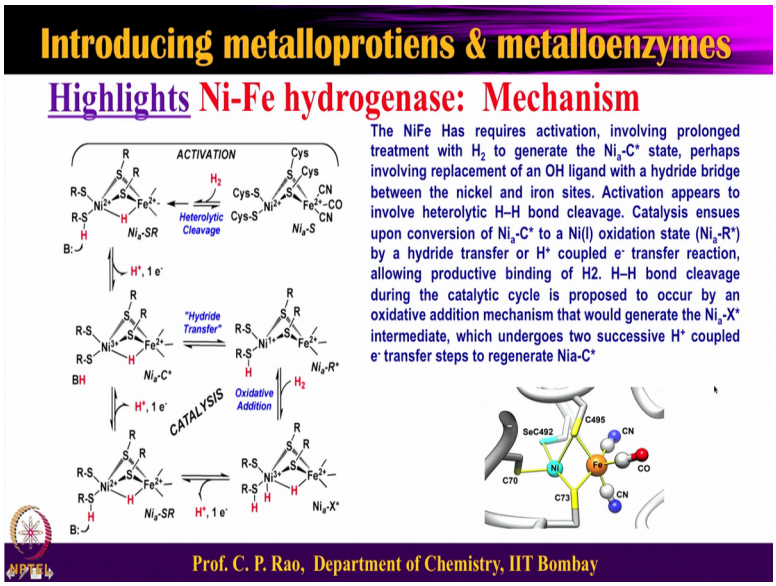
Glyoxylase, of course, I will not be discussing about this. Cis-trans isomerase, I will not be discussing, because we have not done even in the main lectures and acetyl coenzyme synthase the methyl part of the coenzyme a becoming the transfer into this methyl co enzyme Co-A. So, these are some of the things. And we have looked at in the regular course urease, hydrogenase, dehydrogenase and the methyl coenzyme reductase and glyoxylase not glyoxylase, I think probably a superoxide dismutase etcetera ok.

(Refer Slide Time: 02:23)



Coming to the kind of a nickel centers that you find this is the dinickel center which is, present in the urease enzyme which is bound to histidines, and aspartic etcetera bridge by a carboxylic group etcetera. Then you have a coenzyme factor F 430 nickel iron hydrogenase iron iron hydrogenase, and then you have the carbon monoxide dehydrogenase carbon nickel carbon centers and this is in SOD kind of thing ok.

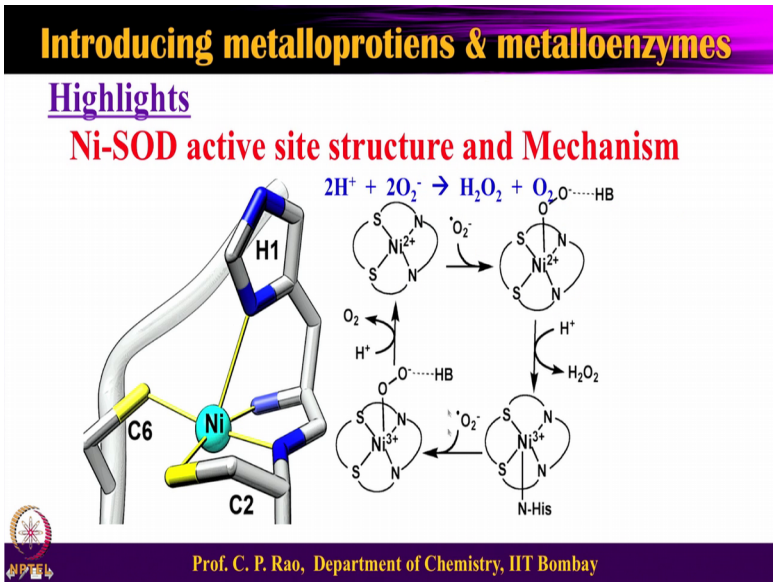
(Refer Slide Time: 03:01)



Coming to the highlights of the hydrogenases you seen; there are two parts of the steps, one is called the activation step other is called the catalysis step. In the activation step in presence of the hydrogen gas it gets activated, because of this S H gets protonated and more of the hydride is bridged. And this is the active species, this active species further undergoes a reduction and the see it gets oxidized to nickel 3 plus, and by removing losing one electron and the proton. And then you have a hydride transfer going over there, and then you have a hydrogen again oxidative addition then losing another proton.

So, you have a totally two protons coming out, and two electrons coming out in the reaction here, one and they are one more here one hydrogen plus 1 H plus. Be leaving the pre step activation step. So, therefore, you can see the H 2 giving 2 H plus plus two electrons is completely over there. This is the kind of a center which explains the catalysis of this ok. So, so during the process you have a redox also occurring for the nickel center 3 and 2 3 plus to 2 plus. So, this is the kind of thing.

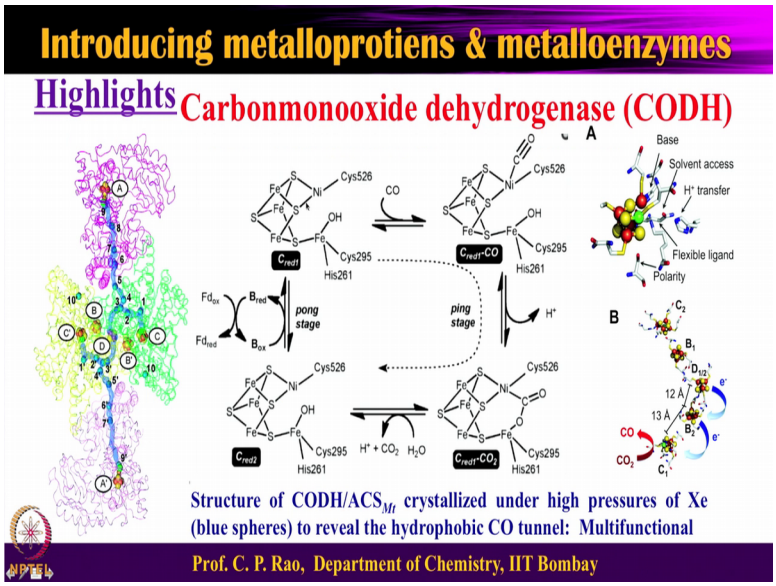
(Refer Slide Time: 04:29)



In case of a nickel superoxide dismutases just like the way that I talked to you about the manganese case. In the first stage the 2 plus ion goes to 3 plus as you can see here by the by the removal of H 2 O 2. And the next step again it will go back to 2 plus with removal of water. So, so first step is the nickel gets oxidized, and O 2 minus not gets reduced. In the second step nickel 3 plus gets reduced, and O 2 O minus gets oxidized to O 2. So, you can see that nickel 2 plus in presence of the 1 mole of the superoxide, and then this will bind here by replacing the water or other coordination. And this undergoes redox to nickel 3 plus, and in presence of the proton it will throw away the hydrogen peroxide.

And then you have your one more mole of the superoxide one more mole of the superoxide again get bind over here gets activated by the base from the protein. And this in presence of the proton will push the O 2 out, and then you have the cycle being done in the SOD. So, main thing is that you need to remember is in the first step, nickel 2 plus go to the nickel 3 plus, and then becomes H 2 O 2. In the second step the nickel 3 plus goes back to nickel 2 plus, and then gives out the 1 O 2 ok.

(Refer Slide Time: 06:01)



Now, looking at another enzyme which is, carbon monoxide dehydrogenase. Carbon monoxide dehydrogenase is a very you know involved kind of an enzyme or complicated enzyme as you can see enzyme with the A centers, B centers, C centers and D centers. And you can see several of the B, and several of the C and then the A centers etcetera here. So, all of these are involved in some activity or the other particularly the electron transfer finally, the reaction takes place over there. The electrons are generated, and the protons also then these are translated through this or transported through this C 2 then B 1, and then D and then B 2 prime or C 2 C 1 prime etcetera etcetera etcetera. Here at this cluster the reaction of the C O 2 to C O which is a reverse in the reaction.

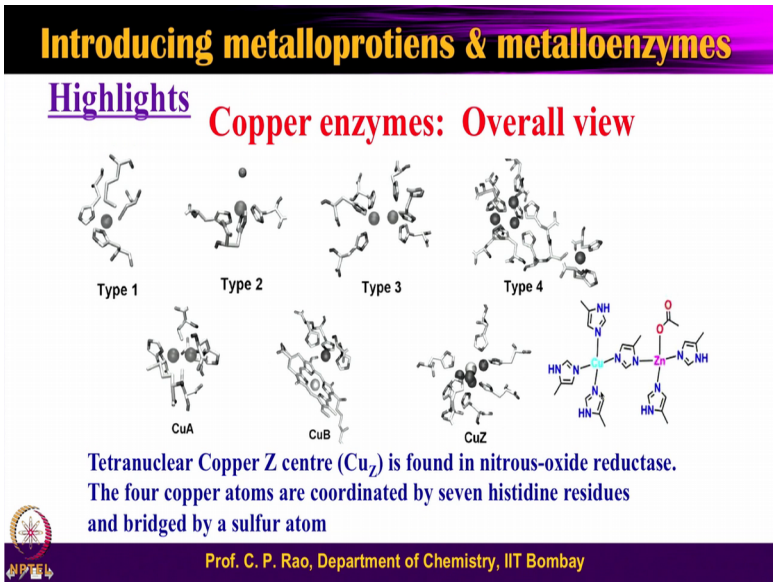
And otherwise you have the C O 2 to C O 2, and you can see over here that is the cubin like cluster, but it is not cubin, because this particular part is broken. So, you have one one nickel other is the iron. So, the nickel center is connected to the protein through the cysteine moiety. And the iron center is also connected through both cysteine and a histidine moieties over there. This in presence of the C O, the C O binds at the nickel center. The C O binds at the nickel center and this in presence of the O H, and this O H will act as a kind of a nucleophile at the C O here as you see that.

So, that means, this proton is out. This proton how will it be out? There will be some protein base near base protein based base, which will pull out this particular proton. So, it will make and you see the intermediate here, the intermediate is the kind of a

carboxylate, which is bridge kind of a thing through carbon and the oxygen kind of thing. So, this in presence of the water will give you a C O 2 out, and the protein is more or less obtained, but in a different oxidation state, so they again. So, this needs to be read this is the reduced kind of thing. And therefore, it will go back to the oxidized form of that, and this is done by the external proteins here over. Internally B oxidized will go to B reduced ok, and the reduced of this will go to the oxidized.

And this is this is triggered by the ferridoxin. So, ferridoxin oxidized to ferridoxin reduced then B reduced by B oxidized, and this will in turn will you know convert this particular thing back to this particular thing. So, this part is the kind of a the carbon monoxide binding, the carbon monoxide ejecting, and here the redox state is coming back. So, ping and the pong ping pong kind of a mechanism that one can see that.

(Refer Slide Time: 09:08)

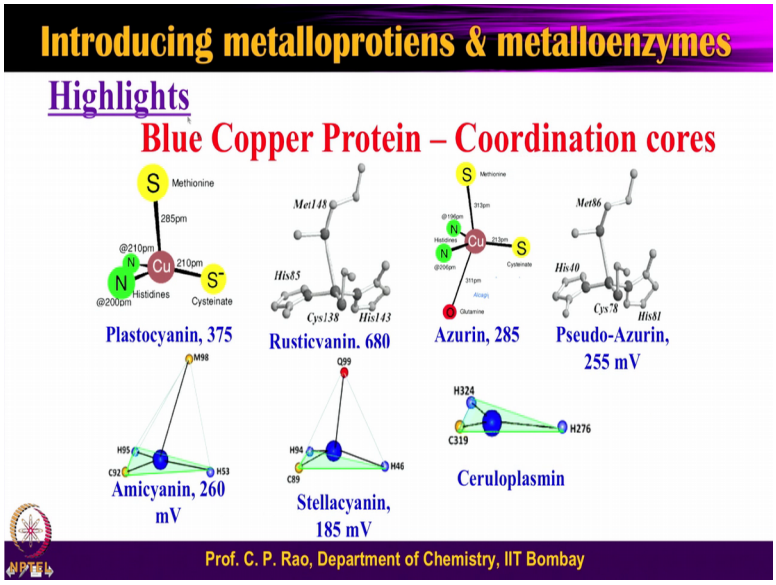


In fact, this whole electron transfer path was identified by xenon, filling that xenon with that these are some of the highlights of the nickel. Now, look at the copper enzymes, the copper enzymes it is called type 1, type 2, type 3, type 4, and type 2 both have got one copper. So, type 1 has nothing to do with the copper 1 or type 2 has nothing to do with the copper 2. And this is completely involved in the electron transfer, and this electron transfer is also this gives the blue color to the protein, and because of the kind of a charge transfer that you have from thiolate to the cysteine, that is what is involved.

Then you have a type 3 with that two copper centers which will act like a bridge for activation the oxygen, therefore, it will be found in the oxidases. Then you have a type 4 kind of thing then copper A copper B, these are found in the cytochrome oxidase, then copper Z which is not, so well known which is a I think the four tetranuclear cluster.

And then you have copper zinc which is in the superoxide dismutase. So, tetra nuclear copper Z center copper Z is found in a nitrous oxide reductase, which I have not explained to you. The four copper atoms are coordinated by seven histidine residues, and the bridged by this sulfur atom in these words.

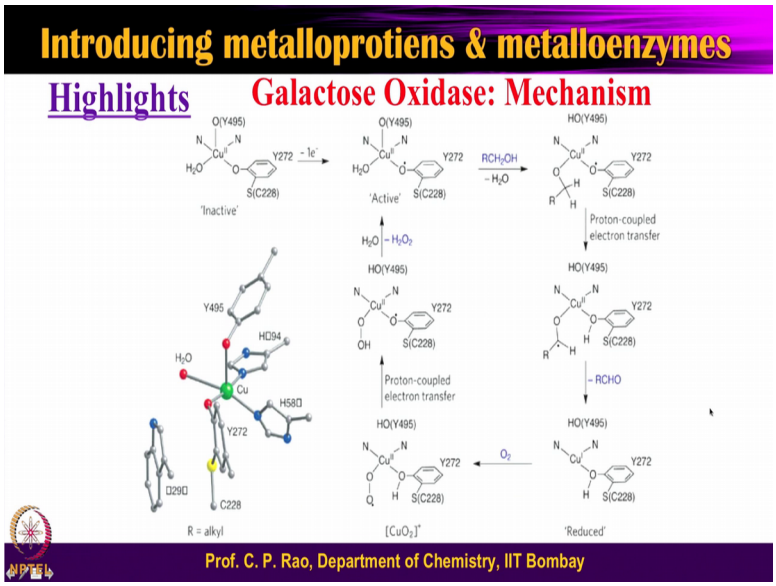
(Refer Slide Time: 10:30)



So, as I said is kind of things are copper blue, copper proteins. Type one copper protein you can see in all of them the copper moiety is that the one of the bond is very very long and distorted geometry, distorted from the regular geometries. Why is it distorted regular geometries, because copper 2 will go to copper 1, when the electron transfer. And copper 1 goes back to copper 2 during the electron transfer.

So, therefore, the protein should be able to accommodate both the copper 1, and copper 2 simultaneously during the during the electron transfer. Therefore, the nature has chosen a very asymmetric kind of a geometry in such kind of a blue copper proteins. And that is a one way blue in moon in disguise. And even these proteins will exhibit a potential as well as 200 to as a high as 700 millivolts. Because of a primary coordination differences not so much so, but secondary coordination differences which are most important in this.

(Refer Slide Time: 11:33)



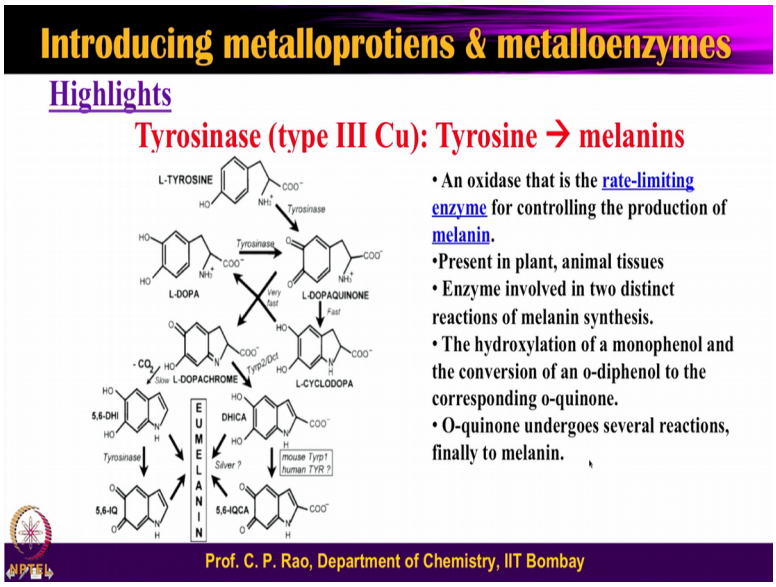
So, type 2 one of the type 2 activities is galactose oxidase. Galactose the primary alcohol of the galactose that is C 6 alcohol of the galactose is converted to the aldehyde. The protein is an unusual aspect that is this particular thing. This is a tyrosine, this is a cysteine tyrosine and cysteine are joined together after the translational events. That is after the protein is synthesized this is called post translational event, and this acts like a cofactor here ok. And this protein center the copper center is activated when you have an electron is lost therefore, you have a radical is formed. This radical is the one which is involved in there.

Now, this is very well poised for the alcohol to bind here, and alcohol binding you can see and then followed by redox. So, once you have the redox then this gets oxidized, because this O dot radical versus it will generate a radical at the carbon center. This carbon center and you can see here and therefore, you have a R C H O is coming out therefore, the reduction oxidation the substrate reduction at the copper center. So, copper has got to copper 1.

Now, to bring back the copper 2 then this needs to use the oxygen. See in this oxygenase oxidase oxygen is not used for binding to the substrate. Oxygen used for to bring back a protein to the normal state, is a copper 1. So, then (Refer Time: 13:09) oxygen O 2, then you have again O 2 bind here. One electron transfer that will be pick up the proton O O H, and this will replace by the water and then goes back to the normal. So, this is an

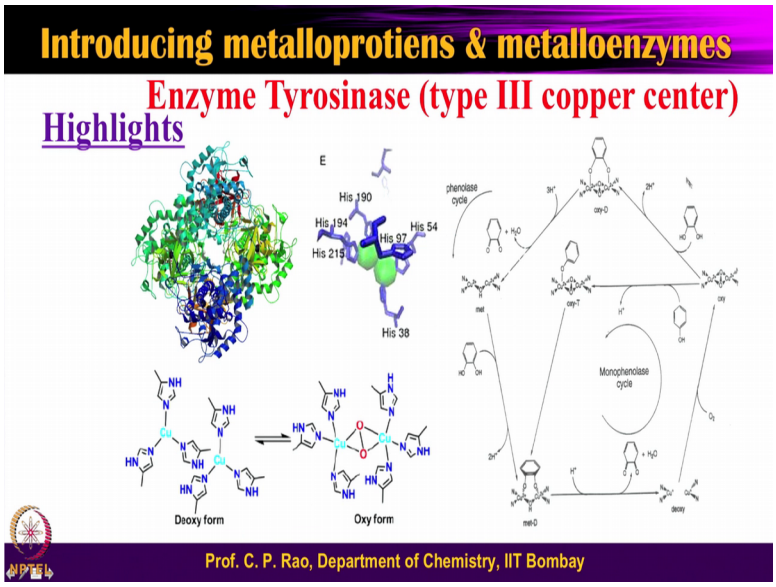
oxidase unlike that happens in case of the iron centers or iron iron enzymes. O 2 is not involved in the beginning, O 2 is involved only when the enzyme is reduced at that stage to get back to the normal.

(Refer Slide Time: 13:38)



Also I have talked to you in case a type 3, type 3 is involved converting tyrosine to through various oxidative processes to finally to melanin kind of thing. The huge number of reactions as you can see over there is possible.

(Refer Slide Time: 13:56)

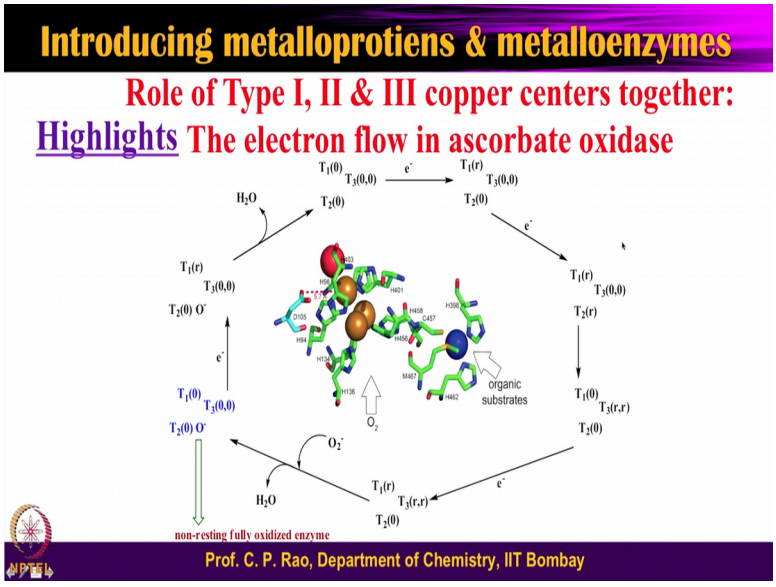


And of course, one can take this as the kind of an enzyme tyrosinase, which can add hydroxyl moieties. So, let us see the in the tyrosinase enzyme you have a two copper centers. These two copper centers are copper 1 state, and both are connected by three histidines, but no bridging ligand at all.

So, so this kind of a this kind of a center in presence of the oxygen. Here you can see the oxidant gets activated to O 2 2 minus, because there are two copper centers, each one can go from 1 to 2, 1 to 2, to electrons that. And this is now good enough for converting the substrate so substrate for example, if a catechol. So, catechol can bind here and then you get the oxidized product or the catechol. Out the enzyme is still not in the normal state, this can do one more catechol be converted into these, so its binds and then catequinone are then back.

So, it can do two consecutive steps of the catechol reaction to get back to the normal. At the other hand if you have a mono O H, that is like a phenol. So, at this stage if we have a instead of the catechol, you have the phenol. The phenol will bind to one of the coppers, one of the one of the copper centers, and the other copper central will hydroxylate to the second the neighbor ok. And then and then in the second step this gets oxidized quinone. So, either you get from catechol to catequinone one more catechol to catequinone or phenol to catequinone.

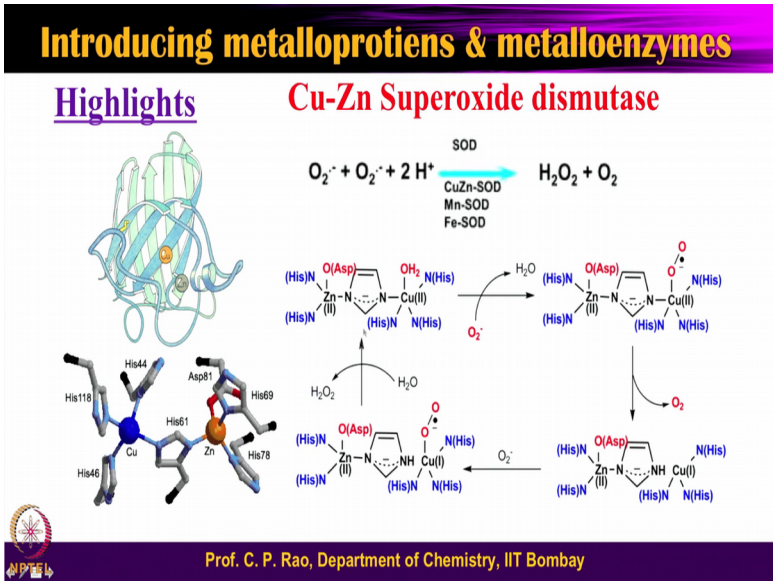
(Refer Slide Time: 15:47)



So, this is the kind of A. So, these are the things, which are involved in the melanin formation also. And there are some cases where the copper centers where you have type 1, type 2, type 3 all together in one, this example is ascorbate oxidase type 1 is shown as T 1; type 2 is shown as T 2; type 3 is shown as T 3; type 3 has got two copper centers. So, in this case it is not 0 it is O O oxidize oxidize oxidize oxidize oxidize. So, all the four coppers are in the copper 2, and the first electrode will enter into the type 1.

And the second electron will will enter into the type 2, and these two electrons are added to the type 3. and one more electron will activate the species into the O 2, kind of a O 2 minus that will add, and that will be thrown out, because already the electron is added to that and at the cost of the water. Now, you have a T 1, T 2, T 3 with 1 O minus, because out of the O 2, 1 O is trapped here 1 O is gone out as a water. At this particular O is a kind of a species which is bound between the T 1, T 2 and T 3 centers, and then one more electron will reduce the T 1. And then that that will be the totally the four electron, and that will become the water and then goes back into this. So, you have a type 1, type 2, type 3 altogether also.

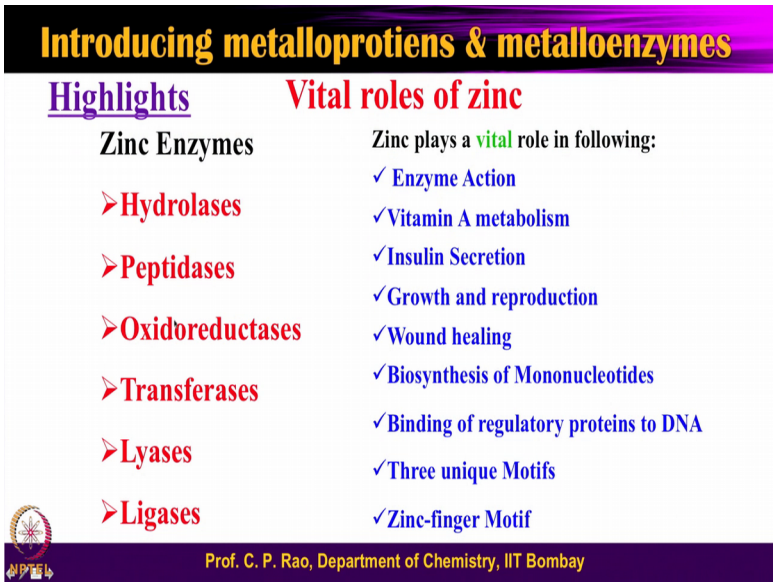
(Refer Slide Time: 17:00)



And this is superoxide dismutase, it is superoxide dismutase here it. So, this is also bimetallic, but it is one is zinc, other is copper. Not both are zincs; not both are coppers also. So, first O 2 minus will attack at the copper center, and this will undergo redox that it will break. And this will give the O 2, then second case O 2 again binds over here, and

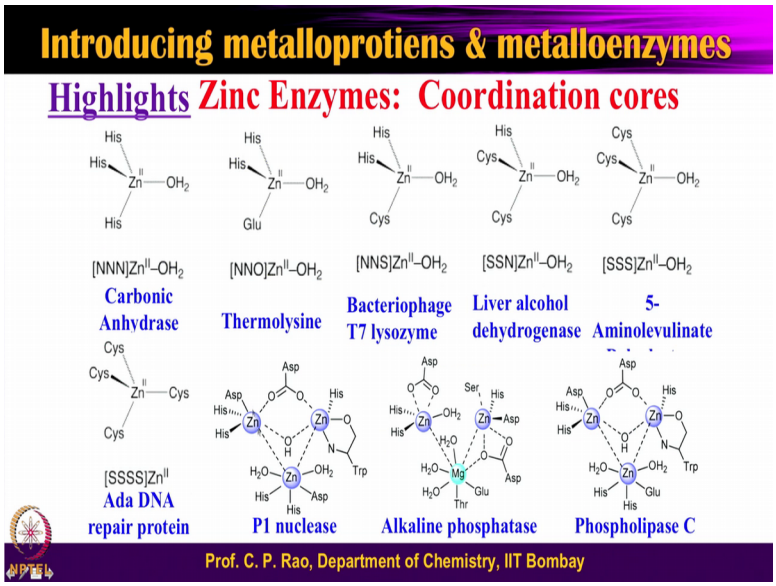
it will kick out the H 2 O 2. So, it is exactly reverse to what you fight for the manganese. The manganese first step will give H 2 O 2, second step will give O 2 in the copper zinc superoxide dismutase, first one is O 2, and the second one will be the H 2 O 2, so the similar kind of thing.

(Refer Slide Time: 18:17)



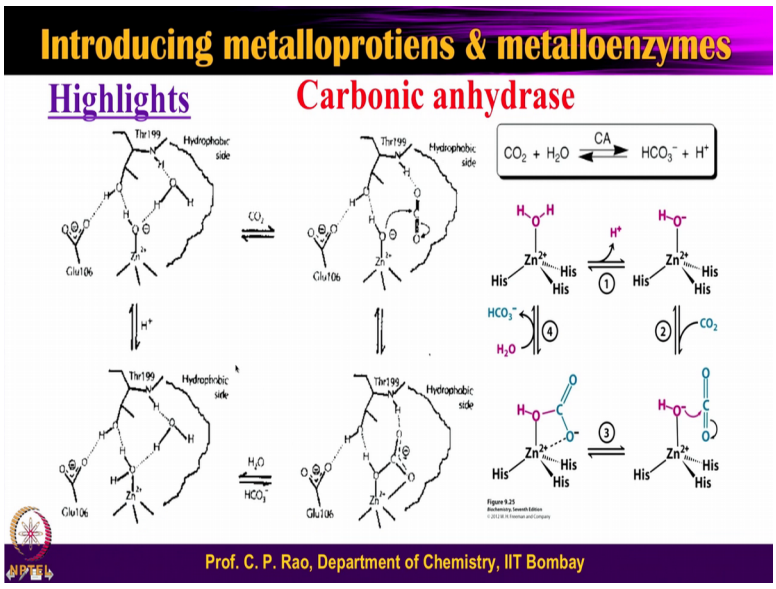
Now, let us come to the enzymes of a zinc. Zinc enzymes zinc is again known redox metal ion, but shows all kinds of reactions; hydrolases which will make the hydrolysis, peptidases which will bring the peptide bonds, oxidoreductases which will bring oxidation reduction of the substrate. You can also add oxygen, and remove the oxygen. Tranferases groups are being transferred lyases, where it is the body is broken without the water, and the ligase the bonds are added when they from a thing.

(Refer Slide Time: 18:37)



So, you can see there are, so many different types of the coordination course in mono. Single zinc center or multiple zinc centers all of these are there. Take one example which is a carbonic anhydrase, carbonic anhydrase enzyme in the zinc center your water gets activated and this is more like a hydroxyl.

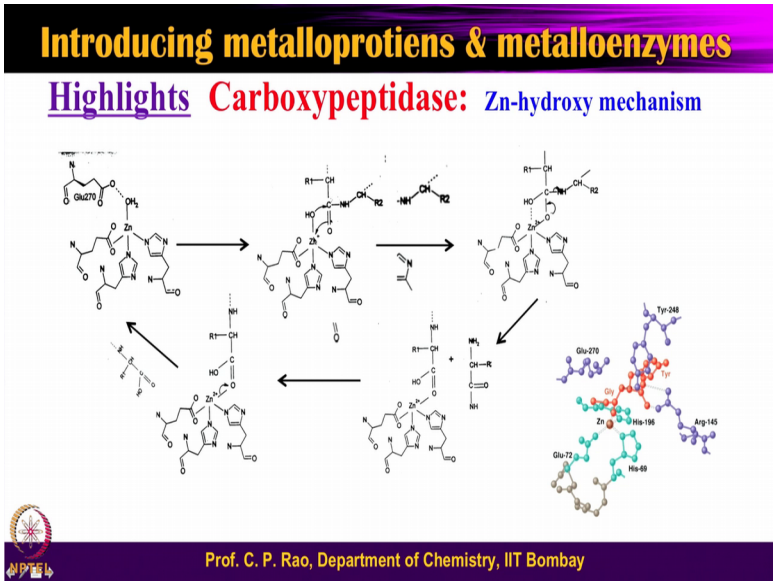
(Refer Slide Time: 18:49)



Now, there is a water present here, this water is attached to the site protein chain, and this particular water is replaced by C O 2 so, and that C O 2 is held close proximity to the hydroxyl moiety. Therefore, this hydroxyl moiety will the oxygen of the hydroxyl moiety

will show will give a nucleophilic attack at the carbon center not at the oxygen center. And that will give an intermediate called carbonate kind of thing metal Z n O C O Z n kind of a metal or cycle. And this will break in presence of the water, and comes out in all this as you can see. This is the way the enzyme purifies the C O 2 from the blood thing.

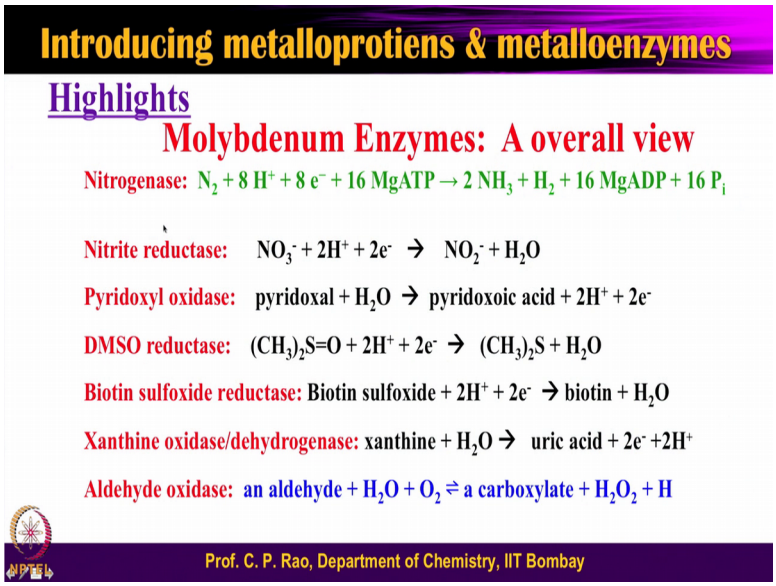
(Refer Slide Time: 19:43)



Similarly, even peptides of various kinds of talk to your carboxy peptidases of C terminal carboxypeptidases of N terminal, this is one of the example first. C terminal the peptide peptide binding here having a nucleophilic attack of the hydroxyl group forming a intermediate. And then this will break with the protonation, and gives the carboxylic part at the amine part of that, and then you have water will be replacing. So, it is very similar kind of a mechanism, so very other things are there ok.

So, now let us come to the other enzyme, which is a molybdenum. Molybdenum is in the case of the molybdenum, we can look at the oxido reductase properties much more. That is why I have not highlighted in case of the zinc, but they are all they are present in both the cases too ok.

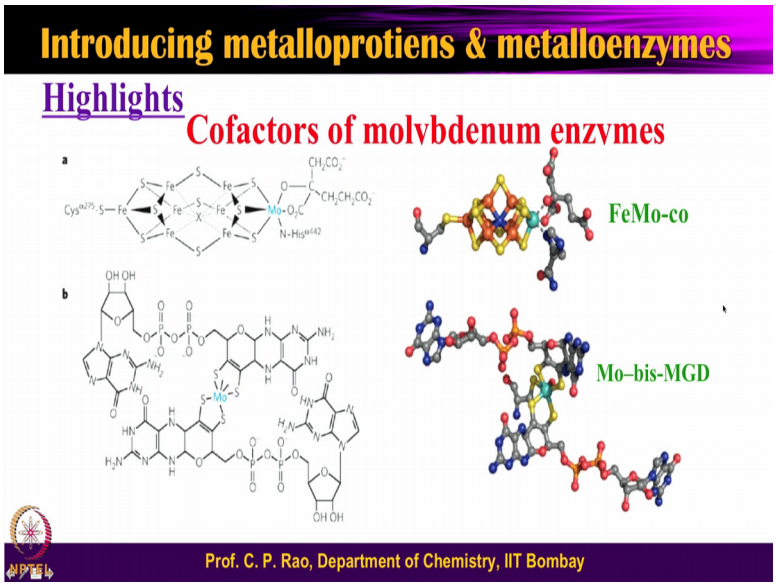
(Refer Slide Time: 20:38)



Molybdenum is very well known enzyme is molybdenum nitrogenase, molybdenum nitrogenase which converts the nitrogen into ammonia. Then you have a host of enzymes called oxidoreductases, it could be nitrate reductase pyridoxyl oxidase D M S O reductase. Biotin sulfoxide reductase xanthine oxidase dehydrogenase aldehyde oxidase. There are a variety of oxidoreductases this is one kind of a reductase, these are all one different kind of a reductase.

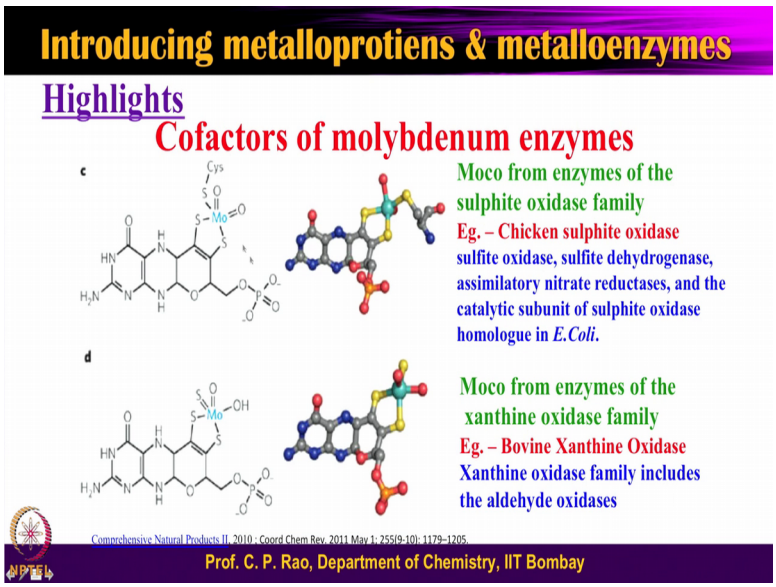
In all these cases, the molybdenum goes through dioxo molybdenum, which is molybdenum 6 to mono oxo molybdenum, which is molybdenum 4 in this process the oxygen is given to the substrate. In the reverse process, what oxide is taken from the substrate. So, when it is in molybdenum 6 with dioxo, it will add the oxygen to the substrate. Whether it is a molybdenum 4 with mono oxo, then it will take away the oxygen from the substrate.

(Refer Slide Time: 21:37)



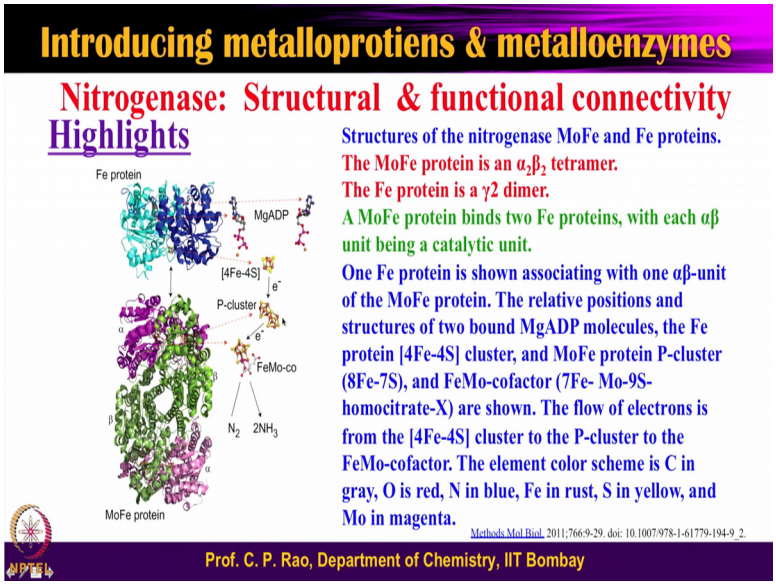
And that if you remember that is the basic mantra of the molybdenum enzymes ok. We talked about the nitrogen fixation or nitrogenous or nitrogen conversion to ammonia, which has got a cofactor of this kind, and you see these all one iron sulfur cluster. And another iron sulfur cluster, which is bridged by these sulfides. And one un identified kind of a ligand, which is close to that of the either the halide or the sulfide kind of thing. It is at this center of the molybdenum where actually the reaction or the conversion of the nitrogen to ammonia takes place, and I will be showing in a while that highlights too.

(Refer Slide Time: 22:34)



Now, when you coming to the cofactors of oxidoreductases, you see this is this moiety is called a pterin moiety, and this is a phosphate; this is a sugar; this is a base. So, it is basically a kind of a nucleotide with the pterin molecule. So, another molecule, so di or you can have also mono kind of thing is another kind of a mono. So, all these kinds of cofactors are present in the molybdenum enzymes.

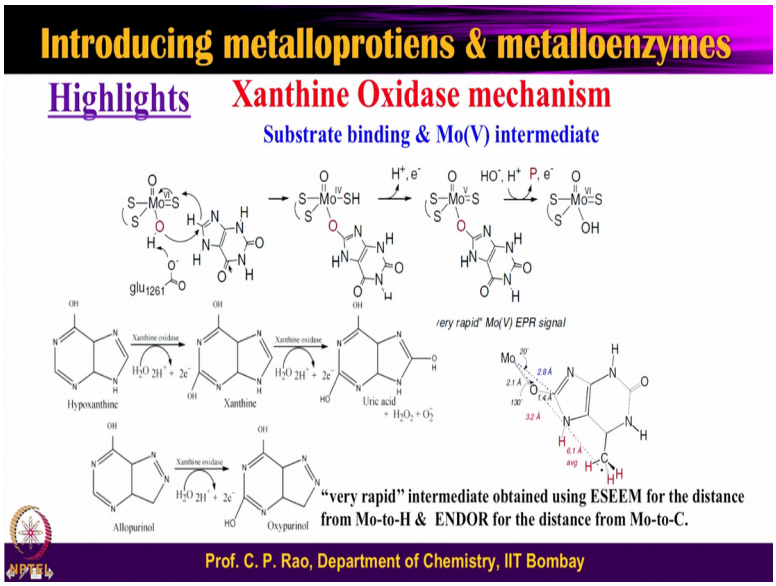
(Refer Slide Time: 22:40)



Let us look at the nitrogenous functioning, it is a very complicated protein you have a iron protein, and this is a molybdenum iron protein. And then the iron protein that has got the iron sulfur cluster, then you have a P-cluster here, and then you have many other cluster, which is the FeMo-co that is called iron molybdenum cofactor.

So, this is where actual catalysis occurs all these are involved in the process of electron transfer at the A D P A D P magnesium A D P is involved in the energy process. Because that is what, the one which couples the electron transfer into between the iron protein with that of the iron molybdenum protein, which couples it is a magnesium A D P hydrolysis will activate the enzyme, and that will connect the electron transfer. As you can see going from here to the 4 iron and 4 sulfur cluster from there to the P-cluster, and the P-cluster to iron molybdenum cofactor, and where the electrons are added to the ok.

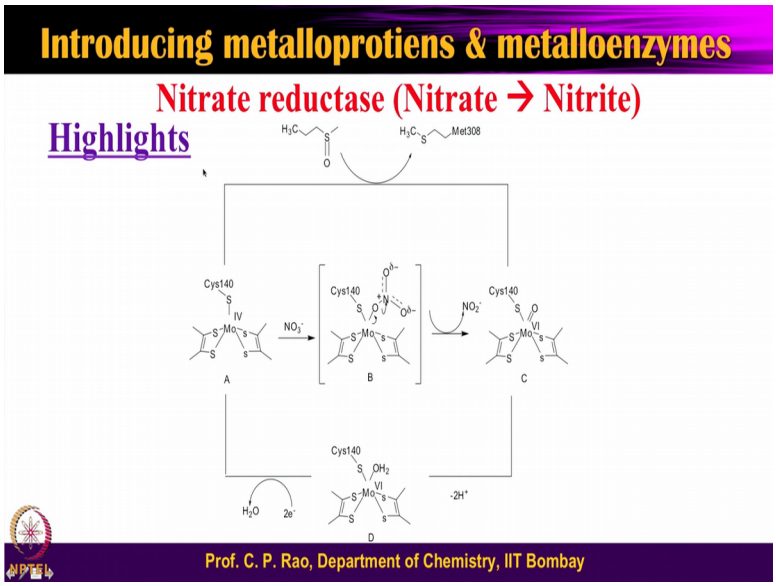
(Refer Slide Time: 23:44)



The oxidoreductases here I have shown the example of xanthine oxidase mechanism. This is the xanthine oxidase mechanism, where you have a only one single dithiol containing pterin moiety, which is bound as here double O O S, and a xanthine. And xanthine basically binds over here, because of the oxygen can give an attack on this particular center, and bind. And in the process the molybdenum 6 will go to molybdenum 4. And the kinds of changes will come to 5 and back to 6.

So, 6 to 4, 4 to 5, and 6 this is done outside the cycle of this one, and this is done in the cycle. So, there are a lot of changes happen xanthine hypoxanthine to xanthine xanthine to uric acid, all of these are done by oxidoreductases of the i of the molybdenum as you can see that over here ok.

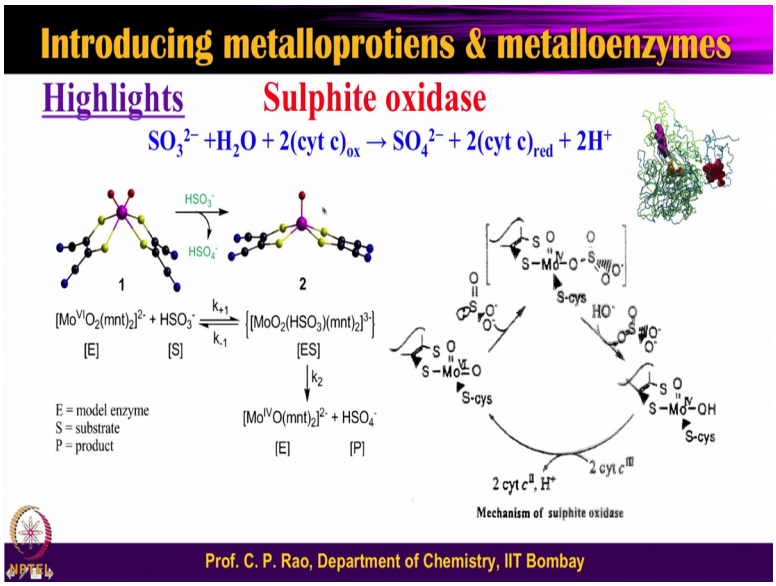
(Refer Slide Time: 24:47)



There is some other things let us look at very quickly, some nitrate to nitrate. Nitrate is a N O 3; nitrite is N O 2. So, you have the enzymes center with the a cysteine in presence of the nitrate, nitrate binds to this particular center. If water is there it will be replaced, and then you can see the nitrate thing. And that will undergo redox from 4 to the 6, and pull out the oxygen from the nitrate.

So, here the enzyme is in the reduced state takes a oxygen from the substrate, here the nitrate is substrate, nitrite is the product, so therefore, it becomes a nitride. Now, the enzyme has gone to the molybdenum 6, then this has to come back to 4. And that is done by the external redox enzymes, by giving the O 2 1 electrons, and then bring back to the takeaway the 2 1 electron two times one electron and give this 1, so 6 to 4. So, you have a continuously kind of a thing that going.

(Refer Slide Time: 25:49)

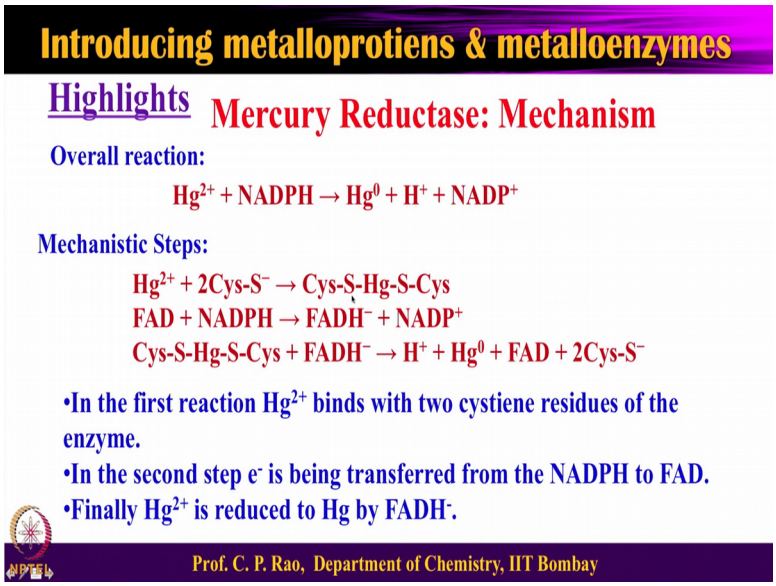


And similarly, sulfide oxidase here in the other way round, because sulfide goes to sulfate. So, sulfate has gone having three oxygen see here, sulfate is having the four oxygens. So, therefore, so you have the enzyme in the form of a molybdenum 6, and dioxo then it binds to the sulfide, and the oxygen. Obviously, this oxygen and the thing itself will give a nucleophilic attack, and this on the sulfur center and give. So, therefore, you got a sulfate kind of a moiety, and this can come out the process the 6 will go to 4, and the this has to the 4 has to be brought back to 6 by cytochrome C 3 3 means the iron 3 plus. So, it goes to iron 2 plus.

So, therefore, takes away electron one, second time takes away one more electron, it will become go to back to 6. So, this is exactly reverse to what happens nitrite to nitrate; and sulfide to sulfates. Sulfate sulfate is a adding nitrite to nitrate is removing kind of thing ok. So, these are the some of the enzymes, that we have looked at based on all these biologically important micro or trace ultra trace elements involved in the in this enzymatic chemistry.

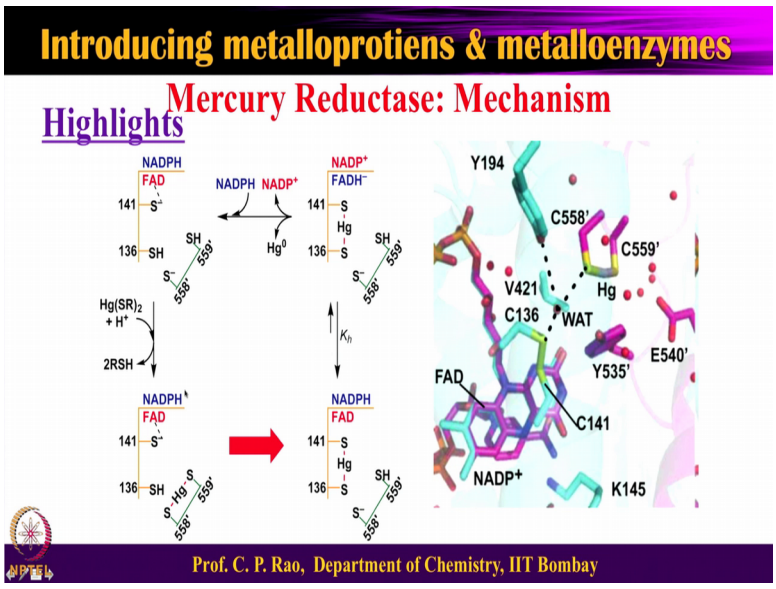
Now, then we also have looked at there are some toxic ions, toxic ions can also be removed. What are the toxic ion? So, is the mercury and the mercury removal we have looked at the mercury reductase enzymes; so mercury 2 plus going to mercury 0. So, obviously, the reduced form of the N A D P H will go to N A D P plus kind of thing. So, how does it happen, mercury 2 plus first binds to the cysteinyl part of this.

(Refer Slide Time: 27:39)



And then form a kind of a bridge kind of a complex these two cysteinyls. And now, this is being reduced by the F A D; F A D ok. So, going into this one an F A D going into this one will give the F A D H. And this will basically reduce mercury 2 plus; mercury 0, then you will get back your F A D, and cysteinyl sulfurs.

(Refer Slide Time: 28:07)



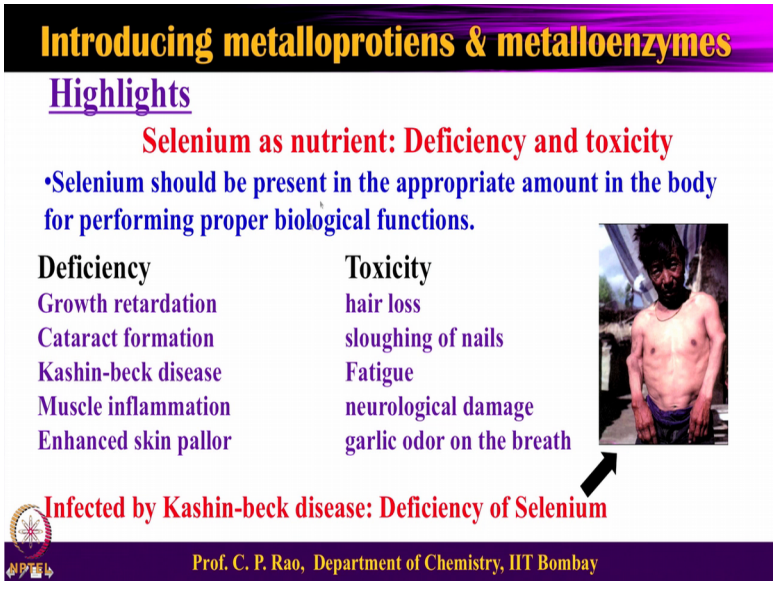
So, as you can see that in all these cases. So, here you start your two centers here, this one is 136 141 there is another center. So, the mercury now, let us see that. So, you have sorry here 141, and 136 you have another 556, 559. Now, this is the this is called resting

and stage enzyme, this will pick up mercury ion from the environment or mercury thiolate kind of a thing, remove this part of it. And that will go into the mercury will go into this particular region first.

And then transfer move by the protein conformational changes to this region. So, from this to this will go here, and from here it will basically come out release out of this by the one more transformation in this conformational changes is going over there. So, from here from N A D P H, and then gives the N A D P plus, and the H is 0 ok. And now, this is again reduced by N A D P H, so active form.

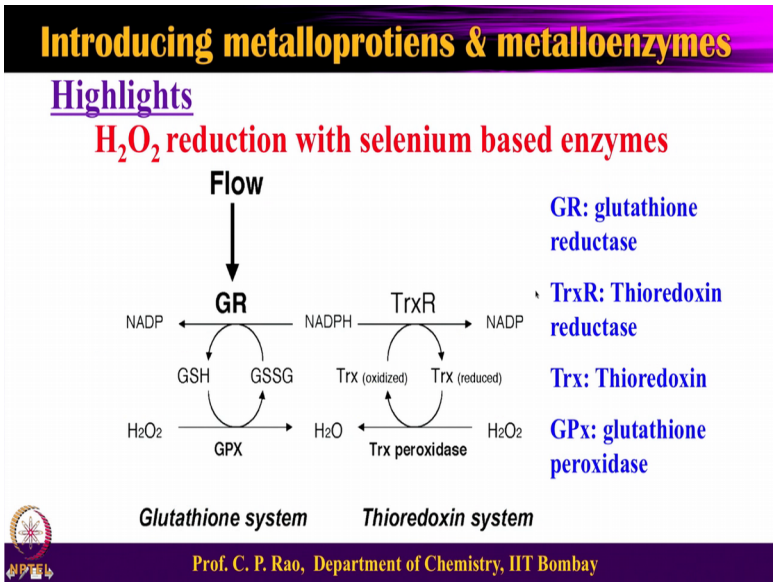
So, active form mercury pick up, mercury shifts from one center to the other, and then rearranges into this and then finally, the because the there is a bit of redox happening between the N A D, F A D to N A D versus this. F A D H minus this N A D P H, becomes N A D P plus, then this is ready to reduce this one, and then the reduction takes place in this two ok.

(Refer Slide Time: 30:00)



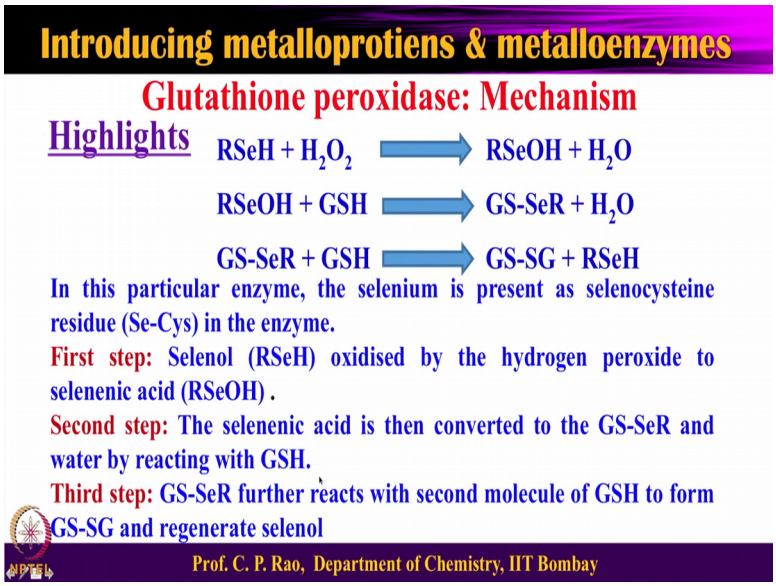
The last part of the highlights is on the selenium, and selenium is present in the body only as a selenocysteine, and no other form, no direct enzyme. But this itself is involved in some of the enzyme. Selenium is a one of the important micronutrient deficiency, and toxicity you can see the kind of a kashin-beck kind of a disease. This can create a growth retardation, con cataract formation, kashin-beck disease, muscle inflammations, enhanced skin pallor many kinds of things.

(Refer Slide Time: 30:20)



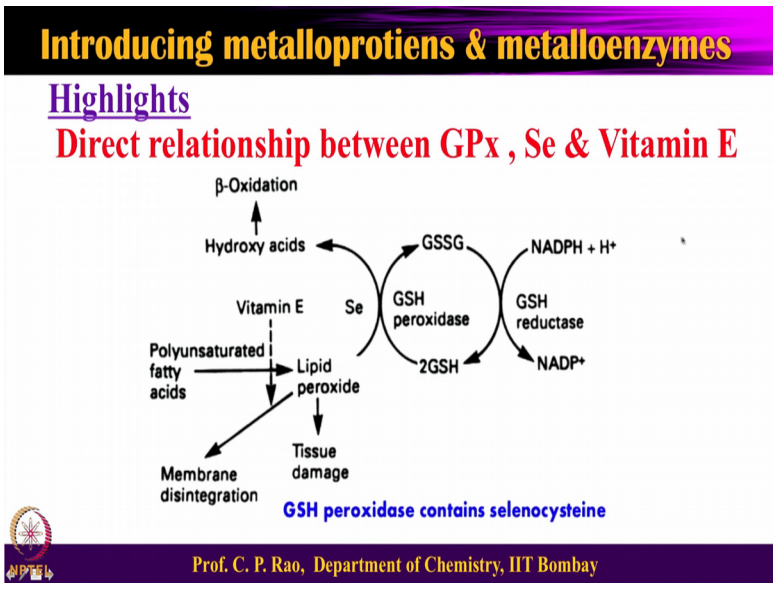
So, if you see that the H 2 O 2 reduction with the selenium based enzymes, as you can see is one set, this is another set this is called glutathione reductase. And this is called the thioredoxin reductase and thioredoxin; so glutathione reductase glutathione peroxidase sorry glutathione reductase, and glutathione peroxidase a cycle G P R G R, and G P X ok. And here the H 2 O 2 for example, and then G S S G goes to G S H, and H 2 O 2 will go to H 2 O. Another end the thioredoxin are and thioredoxin factors the H 2 O 2 goes in thioredoxin reductase will take the N A D P H, and converts the thio redoxin oxidized to a thioredoxin reduct reduced. This reduced one will act on the H 2 O 2, and will go back to the oxidized and give the water.

(Refer Slide Time: 31:25)



So, basically R S e H plus, H 2 O 2 giving the selenol, so this is like a thiol, and this is the equivalent to the acid, and this will make a S S C the oxidized form of G S-S e R. And this is further reduced by the glutathione; so glutathione and glutathione oxidase G S-SG plus R S e H. So, therefore, glutathione peroxidase selenium is involved in all this.

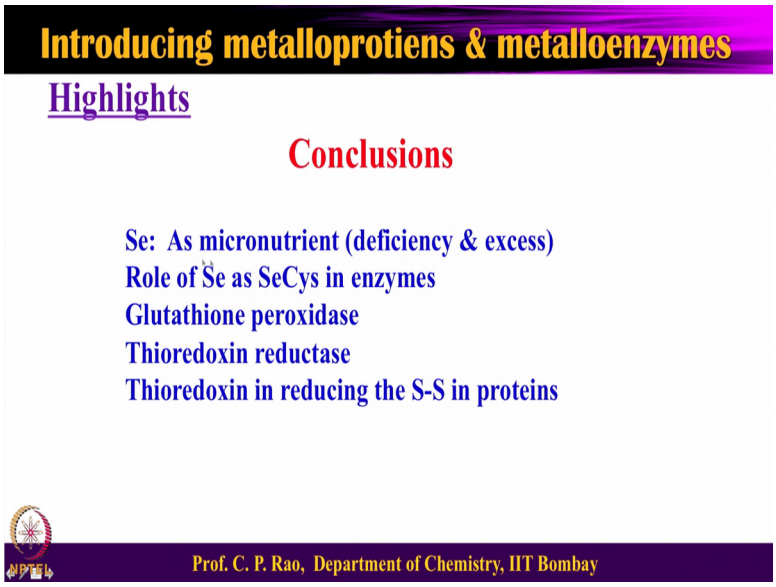
(Refer Slide Time: 31:57)



As you can see that the NADPH going to NADP plus will generate the oxidized glutathione to into the reduced glutathione. Glutathione this will be acted by the glutathione peroxidase, and then this will oxidize through that and in the process. This

will get the selenium the lipid peroxides etcetera with the with the presence of the selenium enzyme. They will go into the hydroxy acids and the oxidation of all this, so glutathione peroxidase contains.

(Refer Slide Time: 32:34)



So, selenium is a micronutrient role of selenium, and selenium cysteine in enzymes, glutathione peroxidase, thioredoxin reductase, and thioredoxin reducing the S-S in proteins.

So, I have more or less covered almost all the highlights of all the enzymes, the initial parts of the introductory part, and then alkali alkaline earth everything in thus. So, from next class onwards I will be taking the tutorials.

Thank you very much.