Inorganic Chemistry of Life Principles & Properties
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Lecture - 46
Role of Molybdenum in life - Oxidoreductases

Welcome you to the next class on Inorganic Chemistry of Life Principles and Perspectives. The last two classes; we have been looking at the molybdenum based enzymes and we will continue with that and try completing the molybdenum based enzymes in this particular class. I already explained to you nitrogenous aspect the complete, then started with a oxidoreductase properties of the enzymes these are nothing, but their pterin containing cofactors molybdenum cofactors the cofactor that is present in nitrogenous is called the ifemoco. It is called iron molybdenum cofactor and the cofactor that is present in this called as a mocos; that is all in a simplistic form.

Now, I have mentioned to you the enzyme in the oxidized form which is a molybdenum 6 form can convert the substrate to oxidize to oxidation when the enzyme in the reduced form is 4 plus; it can reduce the substrate to the reduced one. So, therefore, these enzymes act as a oxidoreductases. Now let us look at that a little more clarity.

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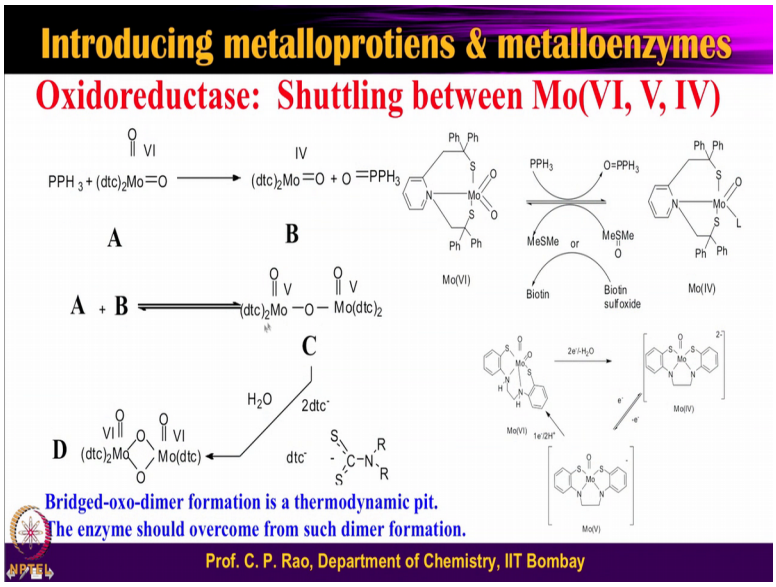

What does it mean? So, take an enzyme a modal compound. So, a modal compound is shown over there.

So, you have dioxo molybdenum with dithiol carbonate dtc is nothing, but dithiol carbonate as you can see from here and this is a molybdenum 6 compound and this molybdenum 6 compound in presence of their triphenylphosphine will react and triphenylphosphine will try to reduce this. And plucks out the one of the oxygen to become triphenylphosphine oxide, you know, the in triphenylphosphine you have a an empty orbital, it is basically a Lewis acid which can pick up the oxygen over there.

So, therefore, triphenylphosphine has got a pair of a electrons which will accept the accept the oxygen in the form of a not along with the electrons and the electrons will go to the molybdenum because triphenylphosphine will go to the triphenylphosphine oxide where the phosphine in the phosphine the phosphorus is in 3 plus oxidation state and the phosphine oxide the phosphorus is in the plus 5 oxidation state. So, the 2 electrons lost. So, those two electrons are gained by oxygen.

So, therefore, triphenylphosphine oxide; so, so, therefore, it O is taken not as a O 2 minus, then O as a 2 plus the 2 electrons are coming here so; that means, the 2 electrons left over here are given to the molybdenum and the molybdenum becomes molybdenum 4. Now you can see the out of the 2 oxos; one oxo is gone and now you have a only one oxo, it is molybdenum 4 and molybdenum 4 is a very reactive species and little amount of a molybdenum 6 present in that can react. So, you let us call this molybdenum 6 as a species molybdenum 4 as a B species, then this A plus B will give a 4 oxidation 6 oxidation and together is a 5 5; so 4 plus 6; 10; 5 plus 5; 10.

So, you get a molybdenum 5 molybdenum 5 with a bridge oxo and this is also very susceptible for water reaction, if we when the water is present it can even form dioxo and it can go back to the molybdenum 6. So, you can see molybdenum 6 going to molybdenum 4 going to molybdenum 5 going to molybdenum 6, etcetera. So, this is what I mean by the shuttling between the molybdenum 4, 5, 6, this has to be done in a very controlled fashion by the enzyme, if it does by controlled fashion by the enzyme, then it can do oxidation reaction in one stage reduction reaction other stage.

But if the enzyme is not surrounding the thing, then it can happen this one. So, this is a thermodynamic pitch. So, formation of this formation of this, once it forms mu oxo a di molybdenum 5 or mu dioxo di molybdenum dimer, both of these are dangerous so; that means, protein will bind to this and prevent the formation of such dimer, either this kind

of dimer a mono bridge dimer or di bridge dimer that is the beauty of the enzyme in this. So, that is where we can see with the modal complexes. So, in the modal complexes how would you try to make the protein mimic you make a bulky groups and that is where you can see that.

So, bulky groups are put over there and therefore, you can do phosphine reaction tryphenyl phosphine oxide etcetera and then to reverse this put dimethyl sulfoxide will go to dimethyl sulphide you can see that. So, in ones direction; it will go phosphine to phosphine oxide and this one in the other direction you put the dimethyl sulfoxide that will give the oxygen and give this one too. So, this is basically explained over there by this kind of a reaction 1 electron reduction one electron on oxidation another electron reduction another electron oxidation.

So, essentially this we would like to say that the molybdenum 6 to 5 to 4 and the same as. So, here it is say 4, then 5, then the 6. So, if you put the reverse 6 from 5 to 4. So, when you have a dioxo it is 6 when you have mono oxo, it is 4, if it is mono oxo plus a sulphide double bond sulphide, again it is plus 6. So, it is understandable that now you understand that.

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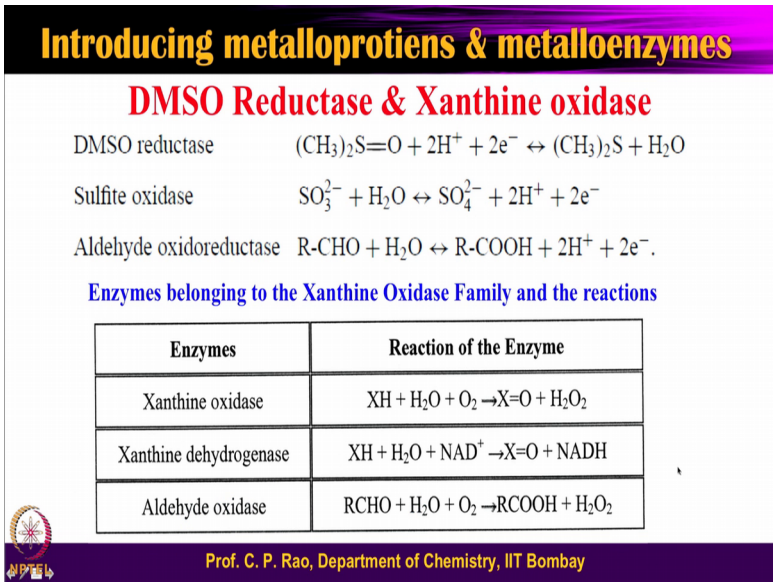

How these enzymes are able to act as a oxidoreductases, they are able to act as a oxidoreductases because the enzyme can easily convert from 6 to 4 molybdenum 6 to

molybdenum 4 and back to molybdenum 4 to molybdenum 6 by giving away the oxygen or by taking out the oxygen from iron two the substrate.

But this is the essence of the whole thing. So, this essence; now I will explain you through few examples, I have already given you essence. Now dimethyl sulfoxide 2 plus 2 protons 2 electrons give dimethyl sulphide and you can make the reverse also if the enzyme is a reduced state enzyme is a oxidized state, you will go in that this direction enzyme is in the reduced state will go in this direction sulphite oxidase that is enzyme in the oxidized form sulphide go to sulphate, if the enzyme is in the reduced from the sulphate to sulphide similarly aldehyde oxido reductase. So, aldehyde two carboxylic ok.

So, you can go from carboxylic to aldehyde. So, in a all these cases, what I want to tell you is that the enzyme can undergo oxidation and reduction commitently the substrate will go reduction and oxidation. So, the enzyme if undergoes oxidized the substrate will be reduced if the enzyme is reduced a substrate is oxidized that is all. So, same things which I have told earlier to xanthine oxidase xanthine dehydrogenase aldehyde oxidase; these all I have explained to you earlier.

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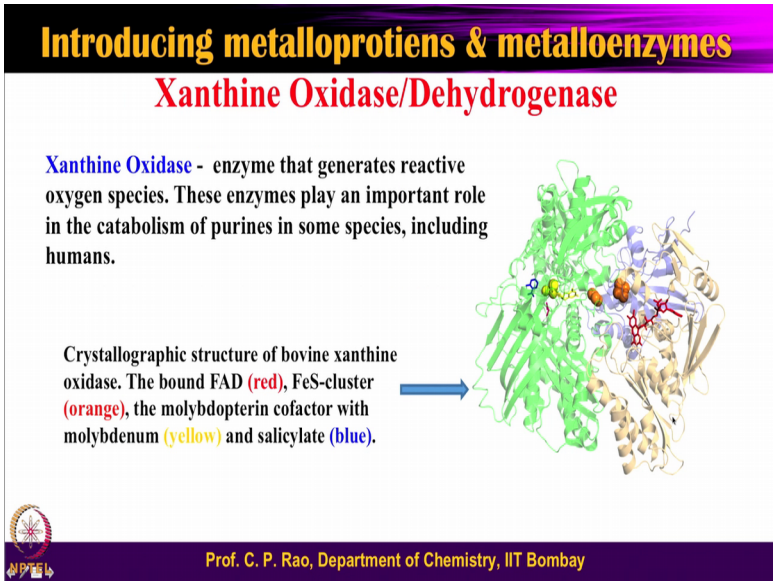

Now let us look at this basic principle or concept that we understood. Now by using a few examples one of them is xanthine oxidase or xanthine dehydrogenase oxidase and dehydrogenase are one of the same. So, xanthine oxidase during the reaction, you will see in the next slide, it will generate some reactive oxygen species like the superoxide,

etcetera. So, these enzyme play an important role in the catabolism of purines in some species including humans also and you can see the whole structure of this one this is a bovine xanthine oxidase.

So, in this bovine xanthine oxidase, the red one is what this is the molybdenum this flavin adenine that cofactor the flavin containing cofactor and then the one which is in the orange color, this is the iron sulfur cluster and this one which is in the in the form of a molybdenum cofactor and this is the cell is like power. And this is before an enzyme in the resting state.

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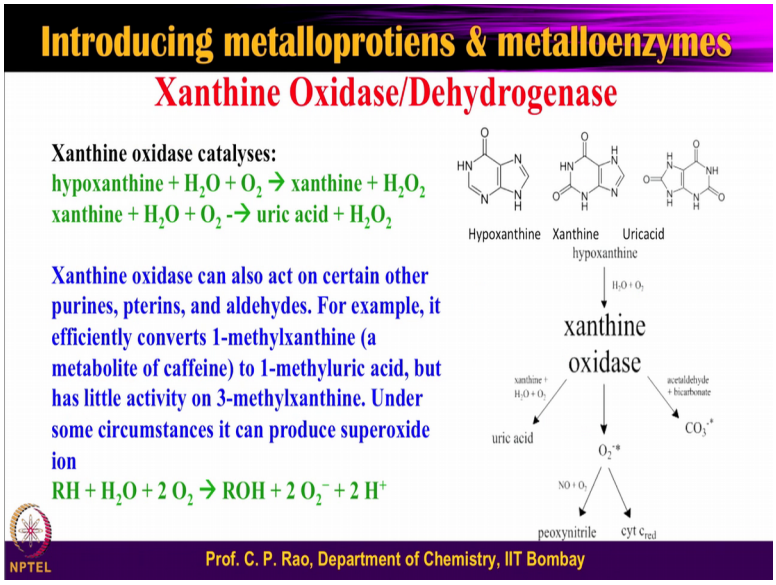

So, this is the structure in enzyme resting state, let us recall or recapitulate; what does the xanthine oxidase do xanthine oxidase catalyzes hypoxanthine to a xanthine, you see that hypoxanthine to xanthine; what will happen there is no CO here; a CO has come.

And then it can do one more reaction also xanthine to uric acid. So, you see the xanthine and the uric acid. So, uric acid has one more CO coming here. So, that will become xanthine to uric acid. So, from hypoxanthine to xanthine an oxidation by one oxygen and xanthine to uric acid is by oxidation by one more oxygen totally 2 oxygen oxidation from hypoxanthine to uric acid and this is where the xanthine oxidase hypoxanthine and xanthine oxidase can generate the O 2 minus and this O 2 minus can react with no and give the peroxynitrite ok. So, as we have seen xanthine oxidase can give a superoxide radical and the superoxide radical in turn can react with the no give proxy nitrate which

is the all these are dangerous species and of course, they can also get redox things by other things.

And on one side, you can have a uric acid as you can see over there ok. So, therefore, xanthine oxidase can also act as a certain other species like purines pterins and aldehydes. So, for example, it efficiently converts one methyl xanthine one methyl xanthine is a metabolite of caffeine in the body caffeine when you consume, then it will it metabolizes that will give one methyl xanthine and that will convert to one methyl uric acid.

But suppose if the system has got 3 methyl xanthine, that will not get reacting because the orientation is also important so that is where the enzyme reactions are both position specific as well as stereospecific and that you once again you can see that. So, so under certain circumstances this can create the superoxide and that is where the reaction is shown over there ok.

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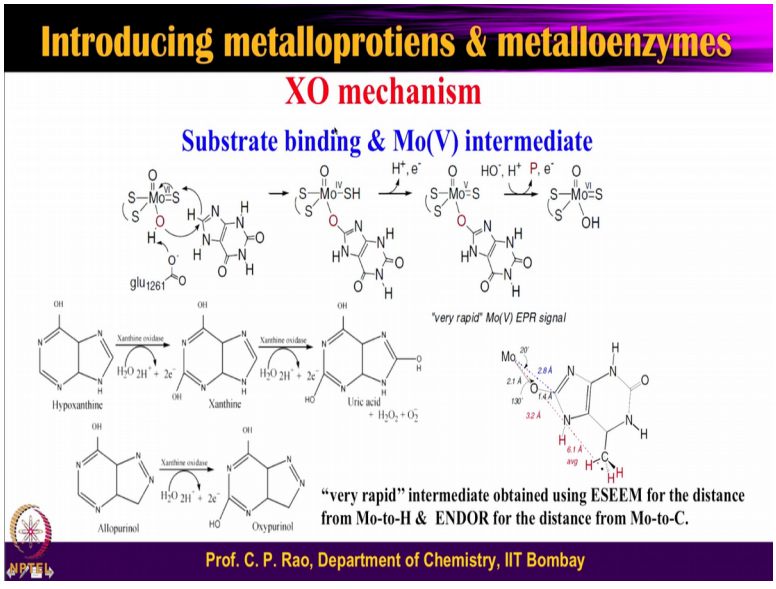

A xanthine oxide ah; so, let us look at the mechanism. So, first of all the xanthine oxide the oxidase and xanthine oxidase; what do you have xanthine oxidase has got one to one one pterin and one molybdenum center, this will react with the substrate what is the substrate hypoxanthine you see that the way that it reacts. And there are a certain groups which I will show you in the next slide, how they are supporting and this undergoes a reaction over there from this oxo and this will bind to the molybdenum.

So, when it bind to the molybdenum its starting from 6 will go to the 4 and with one electron and one more electron will go back to the 6 and releases the product and releases the product when it goes here the P; the P is nothing, but the product ok. So, product is what oxidized form ok. So, hypoxanthine to xanthine then xanthine to uric acid; so, this is we know already; so now again back to this. So, therefore, you have a enzyme is ready for the next cycle. So, I just show some examples you can see and look at hypoxanthine to xanthine to uric acid.

So, allopurinol to oxypurinol and all of these are going from the substrate to the oxidized substrate oxidized product to a further oxidized. So, in this how do we know this binding thing this binding thing has been determined by using endor experiment electron nuclear double resonance spectroscopy, because it has the electron and there is a proton nuclei; you look for a coupling from that you can get the distances. So, from the molybdenum to this carbon you can get the distance.

So, therefore, you can fix how via how far it is from that you can get whether it is bound at the molybdenum center if. So, through what etcetera can be obtained. So, you can take it as center.

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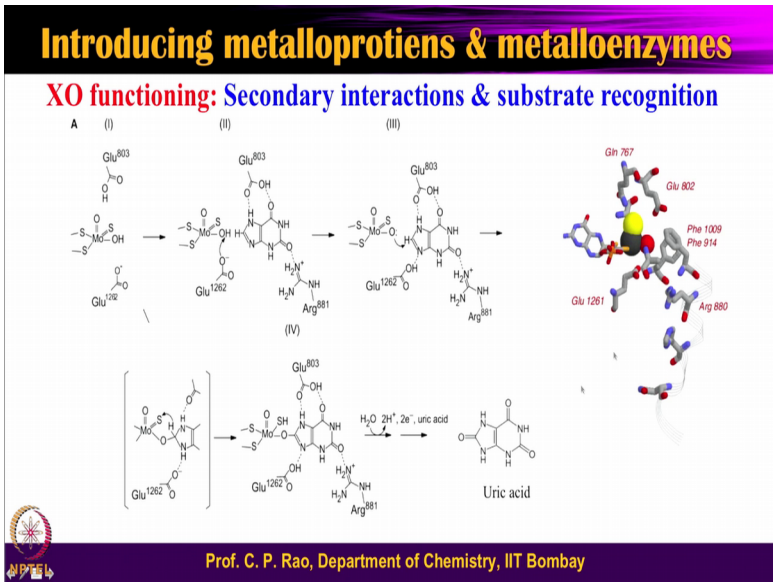

So, now let us look at a little bit more with clarity the same thing, but the reaction you can see that this is the molybdenum center having. So, many groups nearby and this is your cofactor part of it and this is the dithiol thiolene thing. So, there is a glutamic

another glutamic phenylalanine ariginine. So, so many kind of glutamic residues are there.

So, these are all having some role or the other we will look into that. So, you have the xanthine oxidase. So, therefore, this will activate by this glutamate to knock out this proton and therefore, this becomes O minus and that will that will be attacking the center and this when it attacks center then; obviously, this will undergo. So, how does it know this position this knows this position because this particular substrate is held by this glutamate on one side arginine on the other side.

So, through all through the hydrogen bonds; so, the hydrogen bonds extended by the side chain of this glutamate and same side chain of this arginine will recognize and position the substrate exactly at this place. So, that O can attack on this. So, let us take that O attacked on this and when it is O attacked, there is one more activity that this glutamate will again giving a proton provide a proton. So, you get some kind of an intermediate and that will lead to the kind of a species you see that. So; that means, now the oxygen is ready to go from molybdenum to the substrate then go from hypoxanthine to xanthine and this can happen with the two proton two electron.

And in this case, it already a xanthine; so, xanthine to uric acid you have. So, xanthine to uric acid; so, the xanthine recognized by like these kind of a hydrogen bonds and they attacked by this will give. And then finally, the first one is the attack this oxygen at this particular center the molybdenum center and that is activated by removal of the proton and that will bind to this particular center; and how does it know because it holds this particular substrate, but in this particular fashion.

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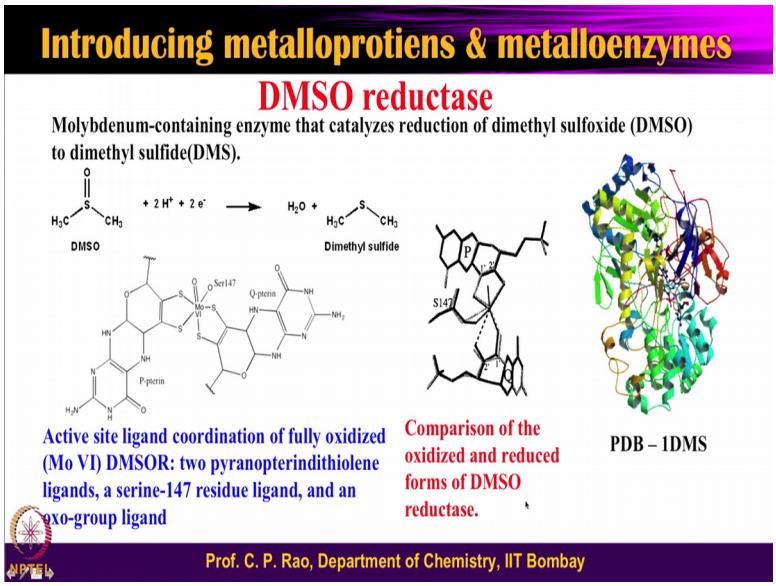

So, finally, uric acid; so, you see that the there is a lot of role of these amino acids. So, they are not directly bonded to your cofactor there the outside. So, that is what we refer as the secondary interactions. Now, let us look at another enzyme in this oxidoreductase category molybdenum containing enzyme that catalyzes the reduction of dimethyl sufoxide to dimethyl sulphide. So, dimethylsulfoxide dimethyl sulphide; so, this particular thing is a one is to two one pterin other pterin and the moo with one of the ligand. So, as I said these enzyme can undergo oxidative oxidized for molybdenum 6 can undergo reduced form molybdenum 4.

If you take both the centers under overlay you see that is. So, perfect with very little changes with very little changes that is why the enzyme is not taken too many changes and time is the enzyme is able to accept both the molybdenum 4 molybdenum 6 in that core and undergoes exchange between 4 and 6 very facile therefore these enzymes are able to act as a oxidoreductase enzymes.

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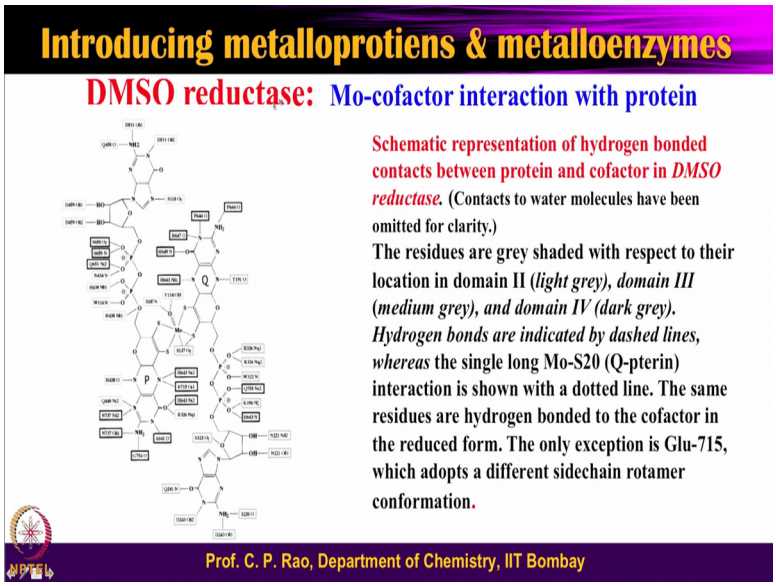

So, this is the how the cofactor is inside buried in the D DMSO reductase. So, this part is a cofactor these are all interactions etcetera you can sit and have a look at this is not very important than this.

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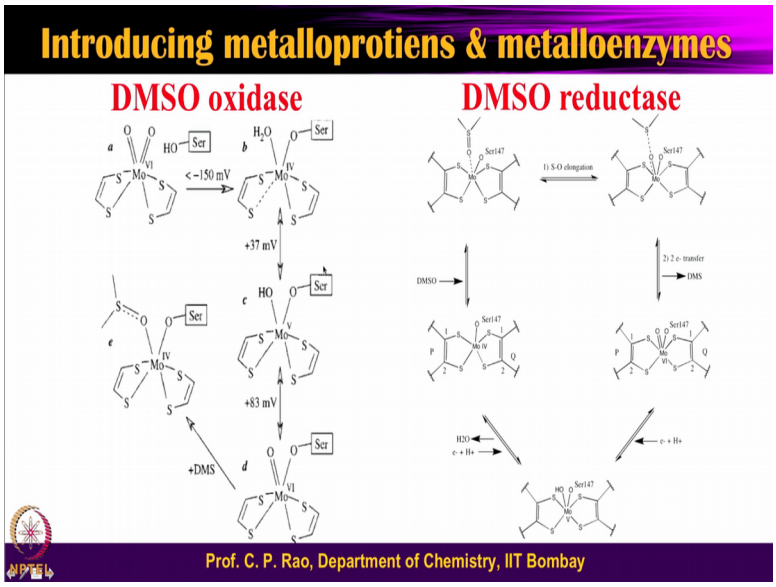

Now, DMSO oxidase DMSO reductase; So, you have the 6 condition the molybdenum 6 oxidation state which will have about minus 150 milli volts in presence of this serine residue this will undergo a redox facility facile redox to plus 37. So, and therefore, and now that will be easily converted to and then you get into the plus 80; this is very nice

positive potential go back to from 5 and then T 6 and at this stage. So, this is all the resting enzyme stages and this is the actually the active enzyme.

So, from dioxo molybdenum sixth serine bound to one more electron transfer kind of thing to 5 then to 6 ok. So, finally, this is ready for the binding to the DMS because the oxidase oxidase will sub oxidize the substrate. So, DMS can get oxidized to DMSO and the DMS will bind at the metal centre and at this metal centre then you have the oxygen and that oxygen which is this one will go to the DMS and will go as the MSO. So, and the reductase part, let us start from this particular thing where the DMSO is bound and you can see the changes happening or we can take even the pre form the prior form of this the molybdenum is serine DMSO and the DMSO binds over at the molybdenum center ok.

And then this again, it changes its redox potentials also change the electron transfer reaction then it will go to the 6 and then it throws out the DMS and then pulls takes the oxygen and by taking the oxygen it will go into the five. So, that further redox process 5 and then 4 get back to the fourth state. So, oxidized and then reduced. So, therefore, that is where the thing is that. So, therefore, the in one case the DMSO DMS is oxidized to DMSO in the other case DMSO is reduced to DMS ok.

So, as you can see over there the type of a reactions that are going to the 6 then 5, then the 4 etcetera.

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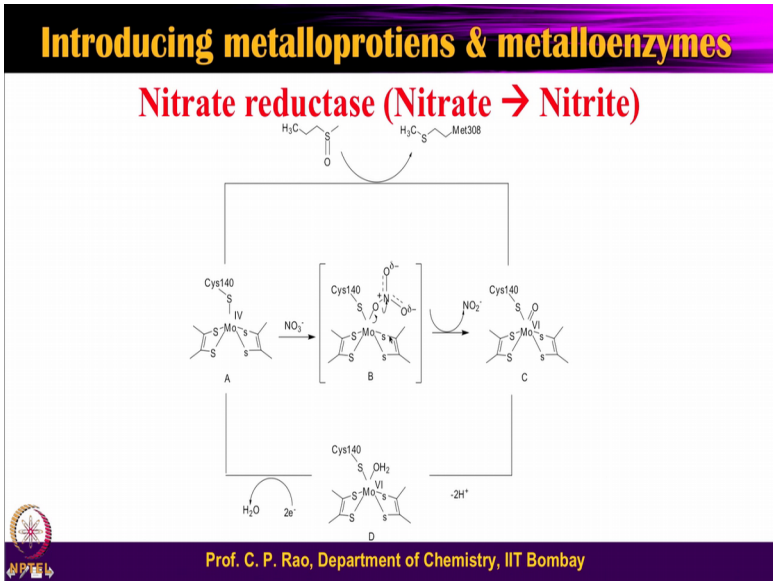

So that you can see we can look at another example nitrate reductase means nitrate or nitrite. So, for a while you do not need to worry about this nitrate to nitrite. So, it is a reductase. So, it has to reduce it has to reduce means the enzyme has to take oxygen the enzyme has to take oxygen means out means it should be in the reduced form that is why.

So, the molybdenum is molybdenum 4 a cysteine nitride see this is no 3 can bind because it is not completely proven. So, just put in the brackets and this will give away the no two and the molybdenum 6. Now the molybdenum 4 is now molybdenum 6, this will not go back to this unless you have nitrite to nitrate part. So, otherwise there are enzymes which will reconvert this from the two electron the two proton back to the. So, therefore, so, you add two electron and this one. So, that will give the reduced form.

So, you can get the reduced form and you can get the oxidized form. So, in the step the molybdenum 4 goes to molybdenum 6 in this redox cycle, it will add two protons and it will add 2 electrons, the electrons are taken by the molybdenum 4 becomes sorry electron taken by the molybdenum 6 and then it will go back to the 4. So, 4, 5 and 6, then it goes back to 4 and 6 as you can see. So, the involved is 2 protons and 2 electrons of the water.

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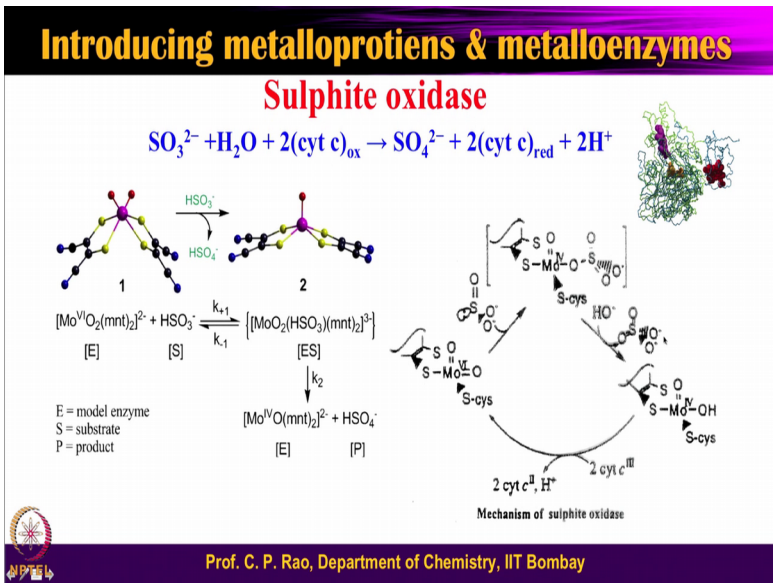

Ok. Now, let us look at another example which is sulphite oxidase ok. So, in the previous case what we have looked at nitrate becoming a nitrite; so in this case sulphide becoming

sulphate. So, it is a oxidase that was a reductase this is from a model complex ok. So, this ligand is referred as a mnt dithionite complex, this is a perfect model for the enzyme and the HS 4 3 minus will give HS for 4 minus and goes back. So, the molybdenum undergoes the redox process and the 6 to the 4.

So, this is a model molecule of course, we have to regenerate back this in enzyme it happens ok. So, let us look at the molybdenum center of the enzyme this is an oxidase keep in mind whenever; it is an oxidase is molybdenum is in 6 form and this is a dioxo form or one double bond oxo 1 double bond sulphite either of this. So, know you have a molybdenum dioxo species molybdenum 6. So, this now you react with the sulphide. So, 3 minus; so, so 3 minus binding over there through its oxygen and it reduces, then you go to the molybdenum 4 and then gives an oxygen to this sulphide goes to sulphate.

And now the molybdenum is in not in 6 it is in 4 and this 4 is brought back to 6 by using a cytochrome C. So, cytochrome C will give one electron 2 times 2 1 electron and the 2 protons. So, this is the whole thing will cycle. So, in the previous case, it has gone to the 6, then you are bringing to 4 in the later case it has gone to 4 you bring into 6. So, therefore, this is done by the external protein systems. So, we have seen the oxidative type oxidation type a reduction type both of these we have seen very nicely in the example.

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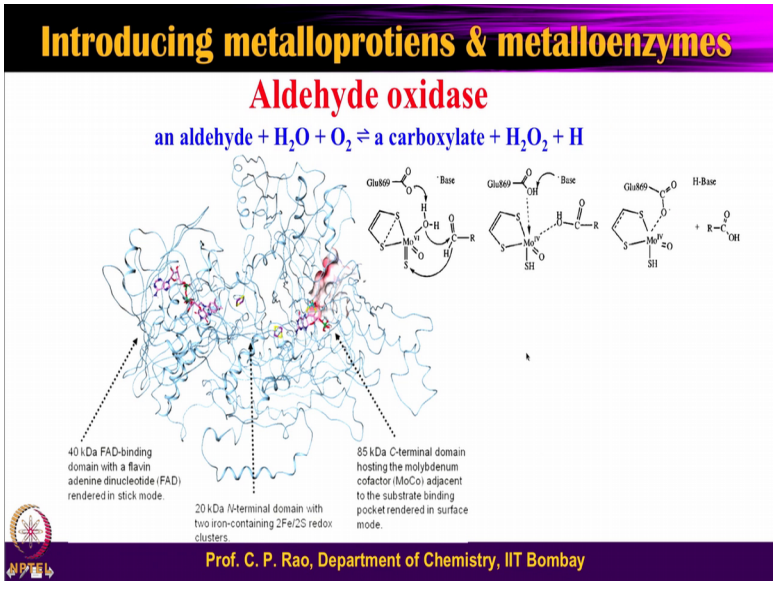

Let us see one last example in this particular thing is the aldehyde oxidase. So, what will happen when the aldehyde oxidizes and when the aldehyde oxidizes what is a oxidized form aldehyde carboxylic acid. So, it is both electrons as well as a oxygen aldehyde has got 1 C 1 O carboxylic acid has got 2 Os. So, therefore, you need to add oxo species to that. So, this in this aldehyde oxidase you can see it is again a complex kind of an enzyme.

So, you have a the fad binding domain with a flavin adelo adenine dinucleotide. So, which is shown in this stick form this is a fifth 40 Kilo Dalton and there is another part of the enzyme which is 20 Kilo Dalton and this has got the iron sulfur cluster and this iron sulfur cluster is a 2 iron 2 sulfur cluster, this is a 20 Kilo Dalton, this is 40 Kilo Dalton and this whole thing is 80 Kilo Dalton and this domain where the molybdenum cofactor is same.

So, this is one of the electron transfer center this is another electron transfer and this is the enzyme center another word say sorry catalytic center. So, it is a basically everything is together is enzyme and this is the catalytic part of the enzyme. So, what is it doing aldehyde? It picks up and converts into carboxylic acid. So, you can see. So, it is a oxidation. So, the enzyme in the oxidized form which is in the 6 plus form and this is bonded to that as we know and in the aldehyde oxidase; this is double bond O double bond as.

Now, this will react with the aldehyde and this is that the water is activated by a glutamate and that will have an interaction with this as you can see here and that is picked up the proton and you have the O, it is transferred to the carbonyl center to make into a carboxylic species here and this will go out and then the enzyme will go into reduced form. Now, this has to be re brought back here by other proteins like other cytochromes, etcetera because cytochromes are well known electron transfer agents.

So, so, we have seen a few examples both oxidative type as well as a reductive type and nicely the enzyme goes to molybdenum 6 to molybdenum 4 and back if you take a small molecule molybdenum complex, it will not do because two molybdenum 5 when it forms a molybdenum 5, then it can interact with another when it forms a molybdenum 4, it can interact with another molybdenum 6 and form molybdenum 5 molybdenum 5 oxo bridge in presence of water it can form even the dioxo bridge.

So, there is a danger. So, small molecules cannot easily mimic this enzyme unless you do a lot of synthetic modification.

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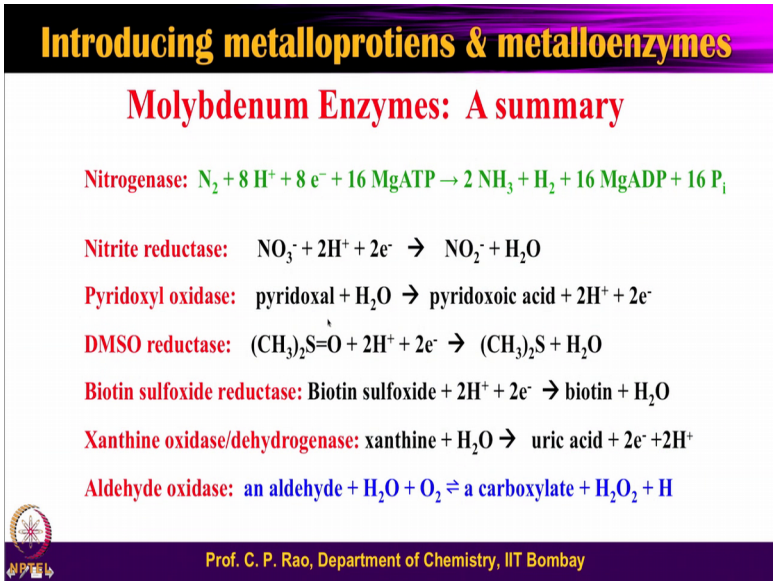

To prevent such dimer formation where did we see earlier one such dimer formation the oxygen transport have a globin there the iron porphyrin very easily gets dimerized if there is no preventing groups. So, enzyme itself prevents in summary. So, let me say that the molybdenum enzymes in summary that we have looked at a one enzyme which is nitrogenous its absolutely a reductase type, it can convert the nitrogen to ammonia and many nonspecific reactions are also there and many places with a two electron two proton kind of a reactions its capable of doing.

So, it can be used for probably if somebody is interested can isolate these enzymes from the natural source arrest on some solid surface and you can start using like a factory for synthesizing the products the second part we have looked at a huge range of reductase oxidases you can see a nitrite reductase part pyridoxyl oxidase DMSO reductase DMSO oxidase also biotin sulfoxide reductase xanthine oxidase aldehyde oxidase. So, you seen nitrite oxireductase, it will give nitrate to nitrite pyridoxyl oxidase pyridoxyl to pyridoxic acid DMSO reductase to DMSO two DMS dimethyl sulphide biotin sulfoxide reductase biotin sulfoxide to biotin xanthine oxidase the xanthine to uric acid aldehyde to carboxylic acid these are the things.

So, the first one is a very complicated that is the nitrogenase and the nitrogenase really requires a large number of one one one electron kind of things the second kind of things are the trick of oxo transfer reactions oxo transfer followed by electron transfer followed by proton transfer. So, basically a proton transfer electron transfer coupled with an octo oxo transfer in other words oxo transfer is coupled with proton and electron so that the molybdenum can undergo plus 6 plus 5 plus 4 without getting dimerized without getting the reaction being arrested in this.

So thus, we conclude the part of the molybdenum enzymes by giving all these details and the mechanistic aspects.

Thank you very much.