Inorganic Chemistry of Life Principles & Properties
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Lecture – 36
Role of Nickel in life – Hydrolase, hydrogenase & SOD

Welcome you to the next class on Inorganic Chemistry of Life Principles and Perspectives. In the previous class I have introduced the different types of nickel enzymes variety of reactions, I have also started going through the details of the one of the enzyme urease, which is a hydrolysis type of enzyme. Where the urea is broken down into ammonia and the CO 2 and in presence of water it will be H 2 CO 3.

And we were looking at in the previous class about the mechanistic aspects already I have talked to you, some aspects like binding as a carbonyl moiety, when the flap is open the urea comes in and, then the flap turns where it binds to the other NH 2 group through the hydrogen bonds, the distal NH 2 groups. And then you have an attack of the hydroxide moiety and the from the neighbor hydroxyl group that is present, or that is activated by the nickel center.

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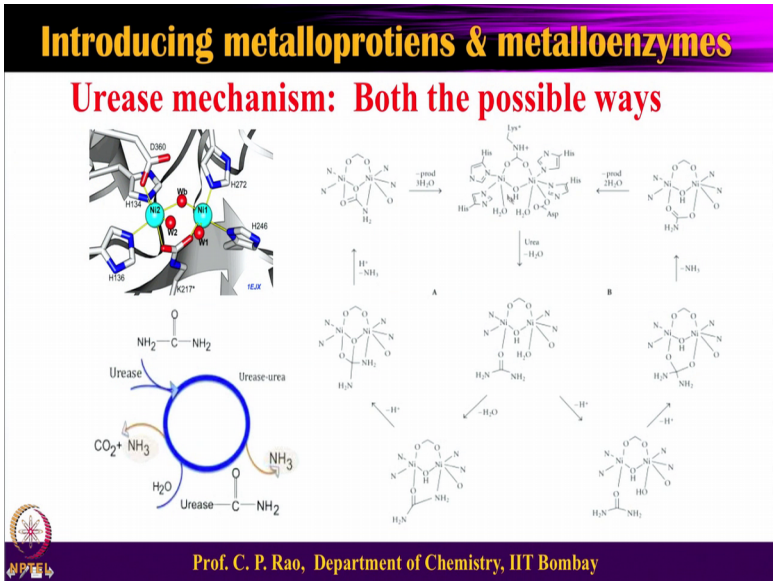

Now, let us look at the same as I mentioned the previous class there are 2 sets of mechanistic spots are being proposed, having some very close by kind of a similarity in terms of the intermediate so, based on this. Now, in the in the first case that you have the

enzyme in the in the state prior to the activity in presence of the urea, the enzyme becomes active and at that stage you have an open core towards for the urea to enter into the active site, and the urea binds initially to the carbonyl moiety.

And then the other aspect is that as I mentioned to you earlier, the NH twos are getting stabilized by the hydrogen bonds, and one of the group of the NH 2 will be more like a hydrogen bond donor kind of thing, the other NH 2 will it will more like a mode acceptor and, but overall it gets locked up as I told you locking up as the whole thing.

Now, in this particular b type of mechanism that you have your hydroxide and the nickel, the hydroxide a showing a nucleophilic attack and the carbonyl center, will lead to this kind of an intermediate and, loses the ammonia with appropriate kind of amount of water etcetera, from a carbonate intermediate. This carbonate intermediate again in process the water will break down to ammonia at H 2 CO 3 and, the enzyme is regenerated ok.

In the other case after the CO is bound to the nickel, when the flap is closed, it is possible that one of the NH this other NH 2 could be coming closer to the second nickel center. And this upon transfer of the proton of the proton, you have the bridge hydroxyl group attacking on the carbonyl moiety.

So, in the earlier case it was the nickel hydroxide which is showing a nucleophilic attack here, and here it is a bridged hydroxyl which is showing the nucleophilic attack after the proton is transferred and, this gives a slightly different kind of an intermediate and, then after that the same kind of thing. So, you have a ammonia, that in presence of the water, it will give back to that.

So, the two both two possible kind of a mechanism have been proposed for these a once, but nevertheless the common factor in all these things is binding through CO and, then flap closing and then followed by nucleophilic attack, then at the end water cleave cleaves the thing it to the making the one.

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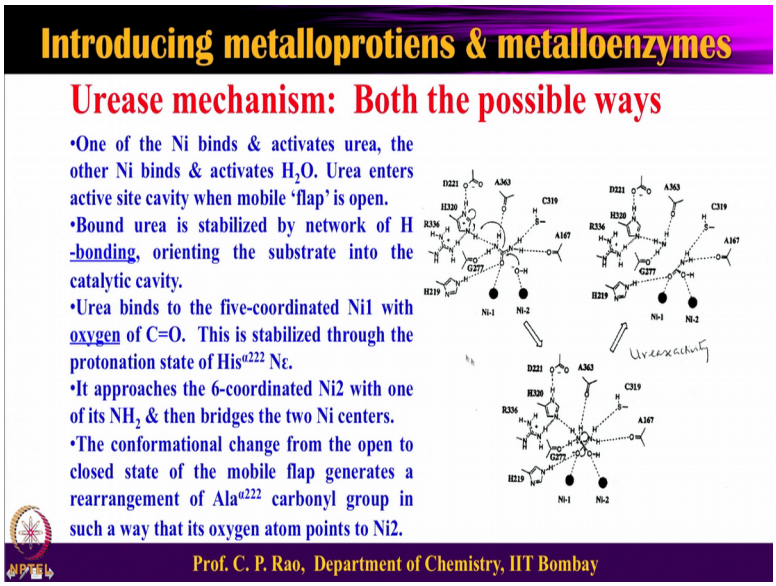

So, the same thing whatever I talked to you we put in the form of some points over here. So, one of the nickel binds and activates urea and, the other nickel binds and activates the water that is point number 1 ok. So, that is when the urea enters into the active site center, or active site cavity, and there is a flap I mentioned to you that is a mobile flap, flap can be changing can be turning and that flap is the open form will now turn into the closed form of that.

So, the bound urea now is stabilized by a network of hydrogen body I mentioned to you that too, orienting the substrate into the catalytic cavity. So, therefore, all these units are utilized number 1 to recognize urea number 2 to lock it the urea both. And this one is helping in the proton protonation part of it. Now, urea binds at the five coordinated center, nickel center and this is further stabilized by the various groups that you have.

So, the other NH 2 part will approach in the nickel 2 center that is what and of these where the nickel and other nickel those two are the bridge it centers. So, this all these things will bring a conformational change; that means, entry of urea in to the closed center of the reaction center, or catalytic site will be associated with a conformational changes, because the flap open, flap closed, that brings a lot of changes. And these changes will bring these carbonyls pointing towards the amine group of the urea. So, you can see again that too.

So, therefore, you have the groups of the various residues the alanine 170 alanine 366. So, all of these turned their CO groups towards the NH 2 and therefore, they act as a hydrogen bond acceptors because, CO cannot act as a hydrogen bond donor, it can only act as a hydrogen bond acceptor towards the NH 2 ok.

And this all helps that the binding of NH the urea towards the nickel centers. Now, as you know that the urea is a very weak ligand in terms of the chelation, or poor chelating ligand and, because it has a low basicity and in other words Lewis base character.

This Lewis base character is been modified by the hydrogen bonds that you have. So, these hydrogen bonds will modify the Lewis base character of the urea the urea binds somewhat quite ok, with all these hydrogen bonds ok. All this will help the NH 2 and it allows to bind to the nickel 2 center.

So, the position of the urea in the active site, as I said earlier is associated by structural changes conformational changes and, this change is also included in the neighbor residues on this ok. So, that will turn into a hydrogen bond donors, as well as hydrogen bond acceptors. So, on one center you have a hydrogen bond donors and the other close to the other nickel center, you have a hydrogen bond acceptors. So, all that means, is this particular thing.

So, these are the kinds of the two different types of the possible mechanisms, they are present in the literature. Let us continue with what we were looking at. So, we have the urea moiety getting locked up in this particular system, where the carbonyls are turned by making this flip of this ok. So, essentially what we are saying is the binding of the urea molecule is associated with a number of conformational changes and, thus you have two different kinds of a mechanisms.

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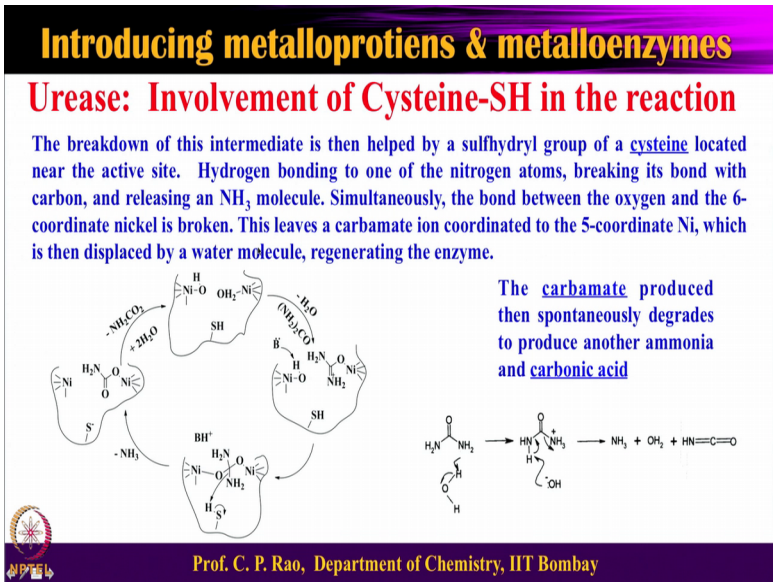

Now, there is another aspect that also is part of this particular enzyme is that, where you have the thiol function which is close to the dinucleur center. As you can see from here the there are 2 nickel centers, the nickel center, one where you will urea bound the nickel center the other one where the water can be picked by the port, or by the nearby base, which could be carboxylate based most of the times, and that could be aspartic glutamic which will take away the proton, and then that you have a hydroxyl moiety.

And now this is associated with an SH moiety which is enclosed by and, this is the reason precisely why two different kinds of mechanisms have been, been able to provide in this particular thing. So, therefore, for the intermediate that you have here, there is a nucleophilic attack of the nickel hydroxide in this, the protonation is coming from the thiol function. And this thiol function is involved through thiol function is involved in the proton, and then they will leave the ammonia and, then goes back in the same manner as the other case too.

In fact, there was some experiments done there in the literature, people have looked at modified versions of the urease enzyme, where they have modified this SH moiety and, when you modify this SH moiety the reaction is being basically modified, or inhibited rather, the reaction is basically inhibited. So, therefore, we understand that the thiol function utility is also important you know this and, that is what is more explained over there.

So, essentially even a urea and even hydroxyl attack on this breaking the ammonia and, the intermediate and this intermediate further with the water we will go with another molecule of ammonia and the H 2 CO 3. So, this is up to the intermediate part and, then further goes. And because breaking of this carbamate intermediate in the presence of water is a very fast reaction ok. So, the initial attack is the slower reaction. So, the initial attack followed by breakage of the carbon nitrogen bond of the carbon urea that is what is the slower kind of aversion.

So, now, we have looked at all the possible things that are there, in the urease mechanism the urea where the binds initially through the carbonyl, moiety through one of the nickel and, then that drags the molecule closer to the active site. And in this process that brings that is associated with a conformational change and, then the flap closes, when the flap closes all the carbonyl moieties turned towards the I mean moieties.

And then lock in the whole system, and then you have a the hydroxyl giving a nucleophilic attack on this. And then the in one mechanism, we have used the use the SH moiety in the other mechanism we have used the (Refer Time: 12:09) is all for proton transfers therefore, two different mechanisms have been postulated, or proposed for this kind of thing ok. Now, that is what about the urease reaction.

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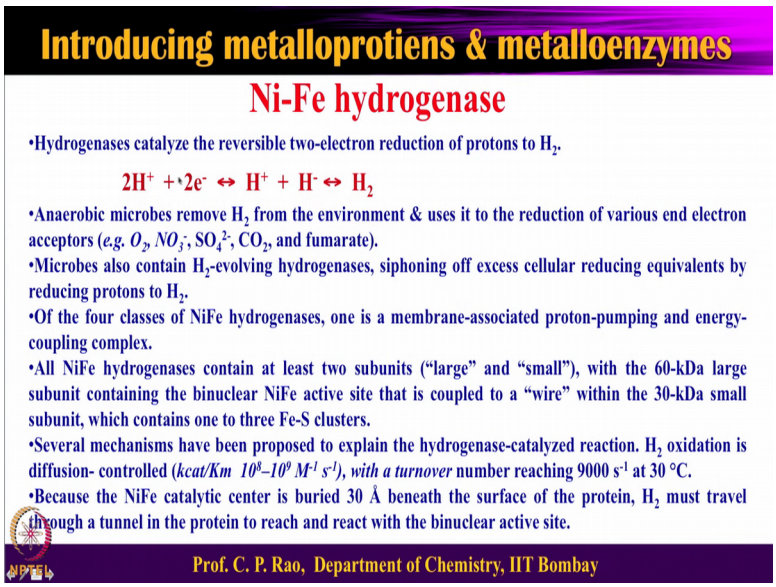

Let us look at the next enzyme is hydrogenase, hydrogenase is is an enzyme as I mentioned already earlier, the reduction of H plus 2H 2, or oxidation of H 2 to H plus ok.

So, therefore, in the in anaerobic microbes, they continuously it will convert or utilize this H 2 to reduce the end acceptors like O 2, NO 3, SO 4, CO 2 etcetera so, these are all reduced. So, therefore, the microbes also contain the H 2 evolving reducing equivalence and, they were all utilized by these ones in terms of the reducing the protons. So, the protons are to be reduced to H 2 ok.

So, there are four different classes of nickel ion hydrolysis, and one of them is a membrane based associated proton pumping and an energy coupled type of a system. And that is what we look at in this particular case. Other nickel other hydrogenases are mostly based on the iron. So, we are not going to look at those ones. So, we will look at the nickel iron containing 1.

So, let us look at the enzyme part nickel iron enzyme hydrogen is contains at least there are 2 subunits, one of the subunit is a large one, other is a small one having the 60 kilo Dalton for the large and 30 kilo Dalton for the small one ok.

And, we will see just in a while in the next slide what these are and how they are. So, let us look at another aspect is this is; obviously, as I told earlier in the case of urease the nickel enzymes, expedited the reaction to 10 power 14 and in this case the hydrogenase, turns the reaction to very high with a kcat add by km 10 power 8 to 10 power 9 ok, with the turnover number of 9000 per second. So, it is very very highly turnover kind of a enzyme.

The other thing is if you look at the enzyme, which I am going to show you the next slide, nickel iron center is present very much deep inside it is about 3 angstrom inside the protein, when you count from the periphery. So, this means that the hydrogen has to travel to this particular site.

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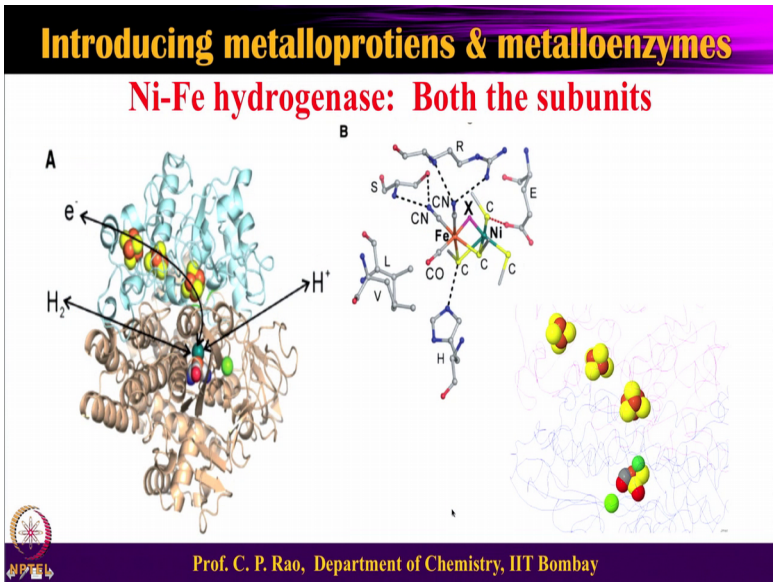

So, as I told you nickel hydrogenase as I mentioned earlier has two parts, what is a smaller version of the molecular weight subunit, another is a large subunit. So, you have about the 30 kilo dalton and around 60 kilo dalton you will see this is roughly doubled.

Now, so the bigger one what do you see you see a kind of a cluster, what is this cluster you go this side you will see that, this cluster is nothing, but iron nickel cluster. So, iron nickel cluster have got the sub binding ligands likes iron, carbon, monoxide and the iron site at the tie of fragments of the nickel site. And these are all secondary interactions giving, these are all coming from the protein and this is the actual active center or catalytic center.

Now, this catalytic center cannot simply function, if it does not have a proton source and the electron ok. So, you can see here a set of a clusters the yellow or orange, yellow orange, yellow orange kind of clusters, you can see more clearly over there these three. So, these three can anybody recollect this these are the iron, sulfur, clusters iron sulfur clusters electron transfer clusters, they can take the electron and give it somewhere, they can give the electron and take it afterwards too.

So, both ways reduced form of the cluster oxidized form of this cluster ok. So, oxidized form is the 1 where it can take the electron, reduced form where it can give the electron. So, therefore, these are the involved in the electron transfer.

So, you have a electron transfer funnel and you have a the active center. This active center if you count from the from the surface, this is something like 30 angstrom. So, therefore, the hydrogen which is an input for this, has to travel through this particular protein by 30 angstroms and reach this one.

So, this is where this kind of a nickel hydrogenase enzymes forms a kind of a tunnel structure here, which is not the main important agenda of this particular course, in a biophysical chemistry you will look at that kind of thing, but take it as granted there is a kind of a funnel, or tunnel and funnel.

So, you have where the hydrogen is passing through that, that you have a sequence of the electron transfer that, I explained to you earlier where I explain to you the electron transfer by the iron sulfur clusters, depending upon the redox potentials they are arranged.

So, you can see and this is your nickel iron cluster. So, this is the total the enzyme configuration in that and, where this unit is you know attached through some interactions like the kind of a weak interactions you know. So, this entire thing is associated for the activity of this. And the hydrogen enters through one of these channels which have to shown over here and interacts with this.

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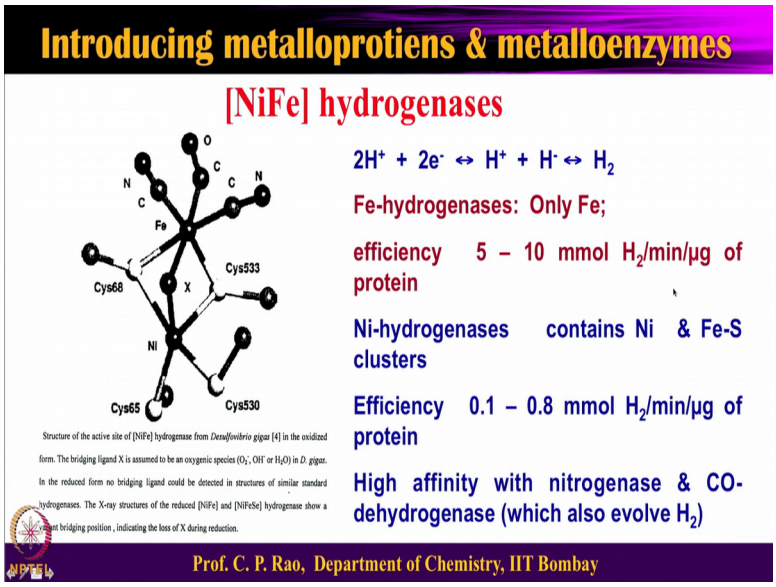

So, let us let us move on here, so, just that at center a load is shown over here, even iron center even nickel center the iron has got CH, CO, CN the nickel has got cysteine and another cysteine. And these are bridged by 2 cysteines. And there is a X bridge it could be OH it could be water, it could be O 2 minus etcetera. Depending upon the situation in which the cluster H X, what situation could be oxidized be reduced.

In the oxidized 1 you will find the bridging in the reduce would you will not find the bridging. So, in the oxidized one you will have the bridging in the reduced NiFe hydrogenases, you will not find that ok.

So, these hydrogenases or they have a very the iron containing hydrogenase have it a very high efficiency, they can may convert 5 to 10 millimolar of hydrogen, they can generate per minute per micro gram of protein whereas, nickel containing hydrolysis, or somewhat lower the efficiency and, they give 0.1 to 0.8 milimolar hydrogen per minute per microgram, when you are looking at when you are looking at the H plus going 2H 2 reaction ok. So, these are or the kinds of the hydrogenases that, we have how does this hydrogenase work.

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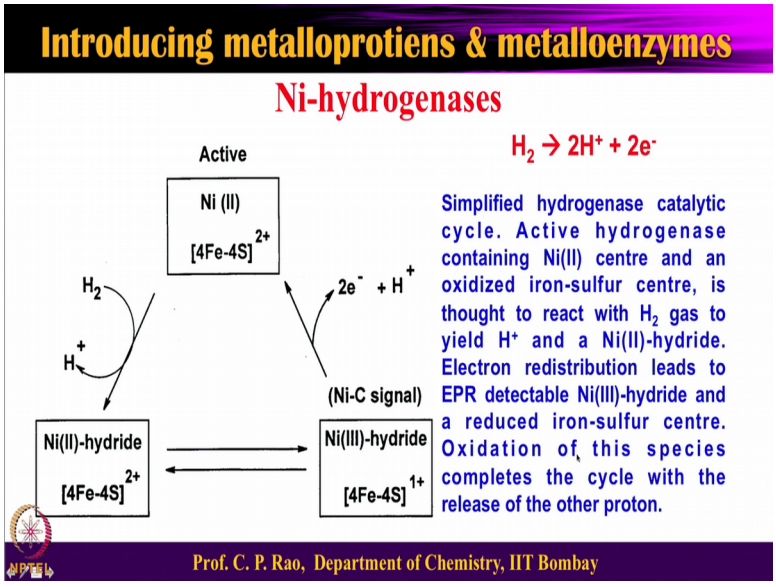

Let us look first at a simplistic view. Simplistic view no we have seen in the previous slide there are two possibilities, there the 2 unit cells there is 2 you know subunits what are the sub unit having the iron sulfur cluster, other subunit having the nickel iron cluster

ok. So, therefore, we will look at so, we have a nickel in the nickel 2 form and the iron sulfur cluster in the 2 plus which is the oxidized form.

And this is sufficient in nature to accept the hydrogen and, this hydrogen will convert the nickel into nickel hydride species, nickel hydride species is can be in turn with the exchange in this can be in the resonance forms nickel can undergo oxidation and, this can undergo reduction. So, therefore, you have a redox between this and that (Refer Time: 20:47) you the.

So, the species could be either this, either which can convert into this, and this particular thing will get oxidized and then you look proton. So, 1 proton has come here, 1 proton is come here, 2 electrons are come here; that means, from H 2 you are taking away 2 electrons therefore, you brought 2 protons and that is what it is.

So, important thing is a nickel 2 with the oxidized iron sulfur cluster and, the nickel hydride and this cluster. This can be inter the you can have a the 2 other the other form is nickel 3 hydride to ok. And this is gives a kind of a EPR signal. So, the EPR signal can confirm this 2.

So, using this information the beside this have associated the corresponding mechanism.

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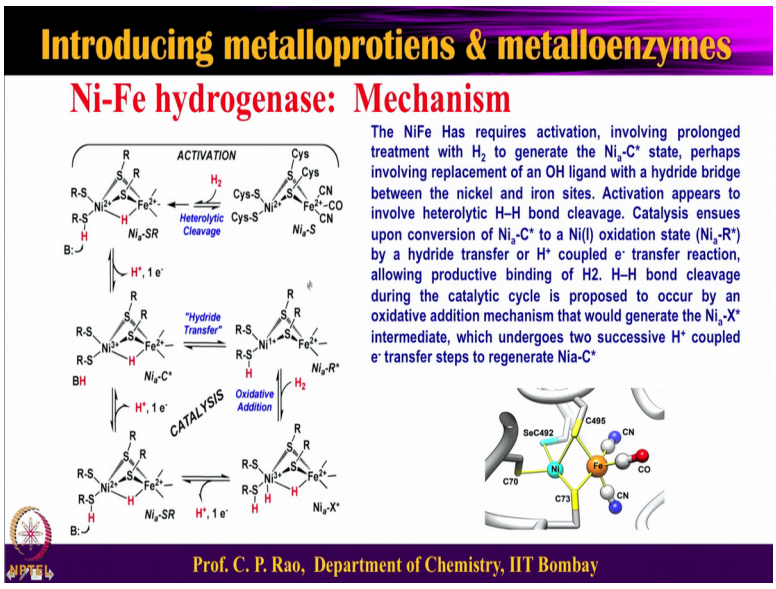

So, the corresponding mechanism you are looking at there are two parts, one part is first of all the enzyme is the passive state, it has to be activated, ones the enzyme is activated.

It will go into the catalytic cycle, it will go into the catalytic cycle here you can see that. So, therefore, we have two parts.

So, once it goes into the catalytic it will continue so; that means, the enzyme is activated, when the resting state enzyme is exposed to hydrogen gas for reasonably long period, then this particular species is formed a kind of hydride and, now this is an active form. So, this particular species can interconvert between this and the this one where, you have a nickel 3 plus catalyst. Now, that is what is showing the nickel signal the EPR signal of this ok.

And, now ones you have here there is a hydrate transfer, as you can see over there from this to goes here, there is a hydride transfer. And then this upon the hydrogen can split into the 2, and that gives 1 H plus 1 electron and the next step use one more H plus and one more electron. And this step is triggered by pulling out this particular proton by a nearby these, as you can see in the enzyme, then the close to this center nickel center, you have other residues. Those residues are actually involved in taking out this one.

So, that is why you have to always see not just the coordination sphere, you have to see the neighboring is the species 2, you see that there are neighboring species of the nickel, which is aspartic glutamic kind of thing arginine, cysteine, lysine, valine and histidine. So, so many residues are they are nearby these are all important ok. So, that is very essential to look at.

So, as you can see that that will be that is the one which triggers the reaction by pulling out the proton and then go to the next cycle. So, you can see so, this is the kind of so, once it gets into the active cycle it can continue to that. So, when it goes through the passive you require to activate by using the hydrogen gas and, this is whatever I talk is written over here you can regionally read and tried to understand.

So, we have made two parts of it when his first thing is the resting state going to activate state and, the second part is catalysis part of it the things are very clear from this particular. So, do not club these two in a single 1 so, that it will be difficult thing.

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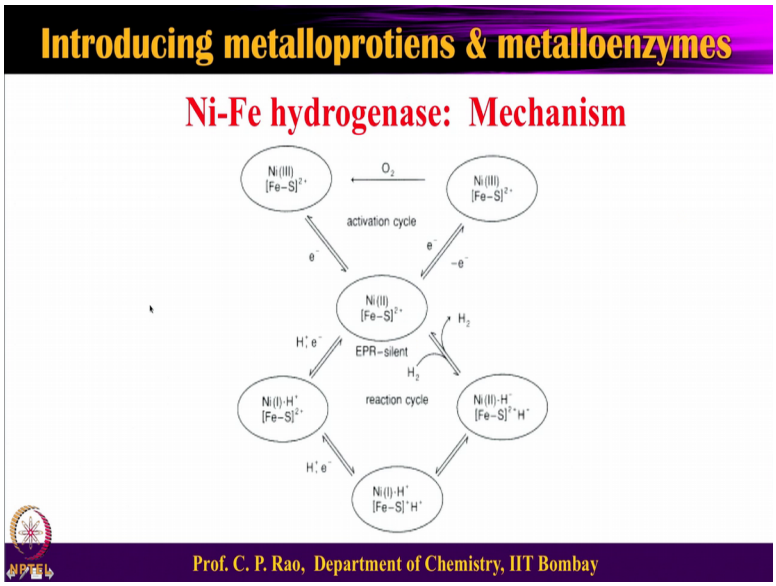

So, same thing I have put in another form here. So, you have the ion nickel to I ion suffer clustering oxidize, it was a hydrogen gas, you get this one nickel going to going the redox part of that and here is the active part.

So, if you are not happy with this you can be just happy with the previous slide is good enough you do not need to look at this particular mechanistic thing at all, there is no necessity to look at this, how do we know that the reaction, where the hydrogen is interacting with the nickel center.

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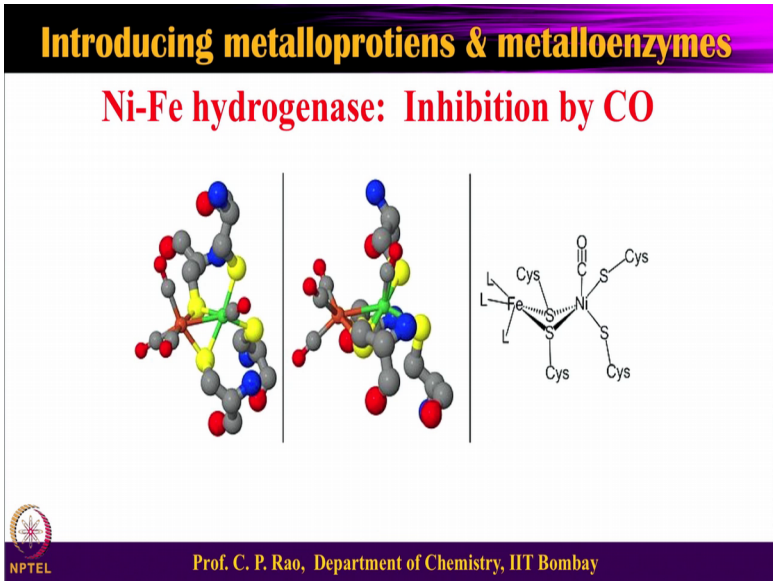

So, people have looked at this by using the carbon monoxide in the system. And you know the carbon monoxide will bind much more strongly, to the nickel as compared to the hydrogen and, then that becomes that will be stops the reaction, inhibits the reaction; that means, nickel center is active important in activating the hydrogen gas initially. And that is where you break, the hydrogen gas into this.

So, what we have studied we have looked at all this information of hydrogen H plus going to H 2 H 2 going to H plus 1 is the reduction reaction, another is oxidative reaction these are done by the nickel iron kind of an enzyme and, that is where recycles of the reactions. And H 2 O converts to 2H plus in 2 steps 1 H plus 1 electron other H plus electron and, that is what is about the hydrogenase enzyme. I will take one another enzyme in this class is that superoxide dismutase.

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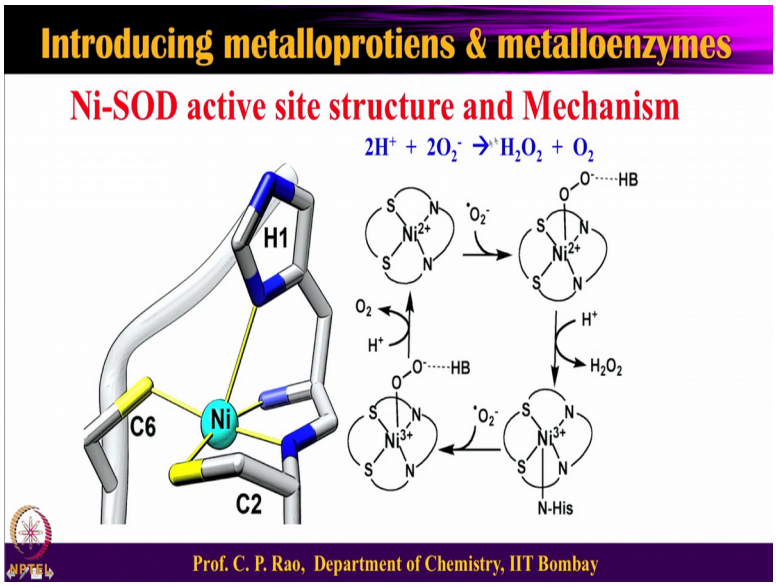

And you know that in the superoxide dismutase the O 2 minus is converted to H 2 O 2. And then just simple O 2; So, this is the active site of the nickel sod enzyme, nickel, and you see that one is the histidine part, then amine thing and this is the amine nitrogen, and this is the cysteine.

So, these are the kinds of things amine bonding that you have and, this particular thing the 2 the sulfur 3 the nitrogen these are the ones which you give get in this. This in presence of nickel 2 plus in presence of O 2 minus the O 2 minus will bind in the nickel 2 plus center and, the and this is recognized by the this is by a group, which has a proton

and when the proton is lot it becomes a corresponding base. And this makes the hydrogen bond.

So, therefore, this hydrogen bonding is a kind of a parameter, which drives the reaction towards these two recognize the species. And this kind of an thing will lead to the removal of the in presence of the proton, this will cleave this particular bond and give H 2 O 2 ok. And this upon one more molecule of the O 2 minus dot are one more species of the O 2 minus dot, it will be bound to the Ni 3 plus. and this one and this particular thing in presence of proton, again use another O 2 molecule and gives back to this.

Let me once again go through this. So, you have nickel 2 center or this in presence of first O 2 minus, the O 2 minus benzia stabilized by the BH, they could CO OH other kinds of things two could be SH all these kinds of things.

And that is being stabilized to over here, and in presence of the H plus this will give H 2 O 2 and, because you are breaking this and proton here break this one; that means, you need to reduce, because you have a O 2 HO 2 minus and then you will take one electron out into this. So, that will become so, when you take out one electron of this nickel O bond nickel becomes nickel 3 plus, the nickel 3 plus will bind to another mole of a O 2 minus dot and that will again be interacted by in presence of the proton.

So, you have a total structure revolving around this particular thing in mechanism. So, 2 moles of a O 2 minus dot which is the field, which is a super oxide is converted into H 2 O 2 plus O 2 obviously, it requires the 2 protons 2 and, this kind of mechanism I explain to you in manganese etcetera. So, therefore, I do need to spend too much of a time.

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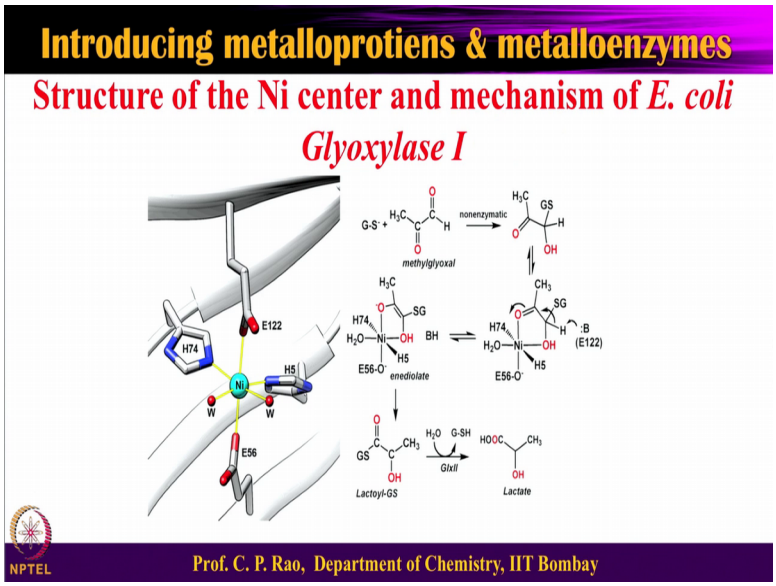

So, now one last thing in this particular case, I would like to get to you is the glyoxylase, as you can see methylglyoxal is converted to lactate. So, methylglyoxal into lactate you see here, the 3 carbon here 3 carbon product therefore, there is no carbon loss carbon gain. And you can see an aldehyde a methyl is as it is, aldehyde is converting into carboxylic acid and the CO is converting OH.

So, out of this you can see one center of the carbon is reduced, other center of carbon is oxidized. So, overall you do not see that much of a difference in that so, that is how. And in this case the nickel is bound to this to waters, you know the aspartic histidine the histidine etcetera, you can see very nice kind of thing 1, 2, 3, 4, 5, 6. So, you have a hexa coordinated kind of a nickel, but with 2 water molecules.

You know when water molecules are there, they can either convert into O H, or they can provide coordination by elimination, they can be easily substituted by the by the substrates. So, you can see that this methylglyoxal the converting into the other form of it keto enol kind of a form, by the enzyme this G S minus is an enzyme.

So, enzyme converts this first this converted form can bind to the nickel center. So, the enzyme a part of the G S minus will convert and, this will bind to the nickel center and takes this particular conversion and, goes to the lactoyl with G S and when the G S is removed as a G SH it will go back to the lactate ok.

So, you can see as such this is being converted by the G S minus and, this binds the nickel center and, then you have internal proton transfers etcetera and then G S will break to G SH impresses, the water and you will lactate kind of thing.

So, now we have seen 3 enzymes in this particular nickel case, in the last class we started with the urease, in this class we have finished the urease, mechanism we also looked at the superoxide hydrogenase, then superoxide dismutase then glyoxolase. So, only one enzyme is left, that we will see in the next class which is called a carbon monoxide dehydrogenase.

Thank you very much.