Inorganic Chemistry of Life Principles & Properties
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Lecture - 35
Role of Nickel in life - General perspectives

Let me welcome you all for the next lecture on the Inorganic Chemistry of Life Principles and Perspectives. In the previous couple of classes we have looked at the cobalt based enzymes. In the case of cobalt based enzymes it is particularly the coenzyme vitamin b 12 is the one which acts associated with a large number of enzymes and wherein we have seen group transfer, hydrate transfers and reactions such as ribonucleated, reductase all these kinds of things we have looked at.

Now, let us take up another set of enzymes the enzymes based on the nickel, ok. So, let us have let me draw your attention to the following slide where we have a number of nickel enzymes have been given here.

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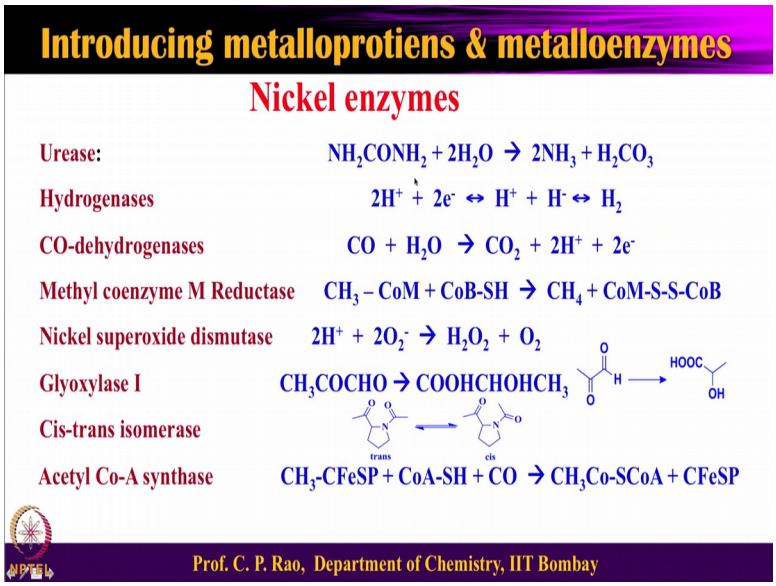

So, I have selected some enzymes where these are all working based on the nickel enzymes. These are urease, I will come into the details of this in a while hydrogenases, carbon monoxide dehydrogenases, methyl coenzyme reductase, nickel superoxide dismutase, glyoxylase, cis-trans isomerase, acetyl Co-A synthase, ok.

So, look at these reactions I just wanted to draw your attention to one of the aspect in each of these reaction. So, each of these reaction if you see very carefully you would see some kind of a gaseous molecule either produced or utilized in the reaction of this. And you can see here the ammonia, you can see here hydrogen, you can see here CO, CO 2, you can see here methane, you can see here O 2 and the only you case that you do not see any gas is this particular one which is glyoxylase and cis-trans isomerase etcetera.

So, therefore, we can generally classify that the enzymes of nickel are primarily associated with either the utilization of the gas, molecules or generating the gas molecules in this. So, having said that now let us go through these reactions one by one. Urease reaction, urease is urea is means breaking, so breaking the urea or hydrolyzing the urea. So, that is you can see that urea NH 2 CONH 2 with 2 equivalence of water giving NH 2 ammonia and the H 2 CO 3, ok.

The hydrogenase is a kind of a reversible you have that is protons are reduced to hydrogen gas and the hydrogen gas is oxidized to protons. So, the both means that we have this kind of a enzymes functioning. So, the reduced enzyme will function to a reduce further and the oxidized enzyme functions to oxidize further. So, therefore, H 2 gas should be oxidized to H plus 2 electrons and the H plus to be reduced to H plus plus 2 electrons to due to H 2. Now, we also have another one carbon monoxide dehydrogenase. So, dehydrogenase means removing the hydrogen removing them hydrogen is nothing, but oxidation. So, CO dehydrogenase is nothing, but an oxidative kind of a reaction oxidative.

So, so how do you make out that? CO on the left side whereas, you have CO 2 on the right side. So, the carbon is attached with one oxygen on the left side carbon is attached to two oxygens. So, what is the formal oxidation state of the carbon monoxide c? So, you know oxygen is taken as two minus then you will get the correspondingly 2 plus. On the other hand if you take CO 2 again neutral there are 2 oxygens, so 2 into 2 minus 4 minus. So, the carbon should be 4 plus.

So, what is it happened? Carbon monoxide carbon center which is 2 plus goes to carbon dioxide with the carbon 4 plus, ok. So, therefore, it is basically oxidation. So, in other words carbon monoxide dehydrogenase is nothing but an oxidative kind of an enzyme. So, you take the other enzyme methyl coenzyme M reductase, this is the coenzyme M I

am not showing the structure over here little later stage I will show that, and you have another center of the coenzyme these two reacting together give the methane gas and you have a oxygen oxidized form of those two and that will be re reduced by another set of enzymes. So, we are not going into the details of that, but let us take it as gadget that the methyl coenzyme is converted to the its oxidized form with the methane gas, ok.

Superoxide dismutase nickel based why I wrote here nickel because we have already seen in the past superoxide dismutase. What are the other metal ions that we have seen? I am sure you will be able to recollect manganese case we have looked in, iron case we have looked at too. So, we have quite a few cases where superoxide dismutase is functioning and we will be see in nickel now we will also see in case of copper which is bit differently functionally. Therefore, we are familiar with the reaction of the superoxide dismutase is superoxide is converted to hydrogen peroxide plus oxygen in this, ok. ah

Go to the next enzyme here glyoxylase. So, it is converting this kind of a structure methyl glyoxyl to the pyrowink, ok. So, therefore, you have two structures over there. So, you have the oxidative kind of a reaction going on as you can see that.

Cis-trans isomerase. So, its title itself says it is an isomerization and it is a cis to trans. So, you can see here the two acetyl moiety is the competence of oriented in the same direction, if this is the right side the carbonyl moieties are oriented in different directions. So, that you have a cis you or here and the trans over there is giving a different kind of a structure. So, this also is carried out by nickel kind of an enzyme.

So, the other reaction I have brought on this slide is that the acetyl coenzyme A synthase, that is A is the type A cluster les not worry too much about it. So, this shifted from CH 3 CFe SP using the other part of the coenzyme in presence of coenzyme this it will go to the CH 3 this. So, therefore, we have now seen a host of enzymes based on the nickel where different kinds of reactions are happened, one of the reaction is hydrolysis reaction, other reaction is a redox reaction of the H 2 to H plus kind of reaction, and then another oxidative reaction carbon monoxide to carbon dioxide all these several kinds of things that we have in the nickel.

So, therefore, thus nickel is a very specialized special kind of an enzyme how is it able to, how is it able to do that? We have already seen that except the glyoxylase, you have some kind of a gaseous molecule either utilized or generated in the reaction that we have

seen. Other thing which is not seen here clearly which you will see later on when we look at the mechanism is that the nickel in these enzymes undergo redox very facially from nickel to either to oxidize from nickel 3 or the reduced form nickel 1. So that means, nickel 1, nickel 2 and nickel 3 these are the 3 kinds of a things which are essential nickel case nickel plus 1, nickel plus 2 and nickel plus 3. So, these are the 3 kinds of oxidative oxidation states, ok.

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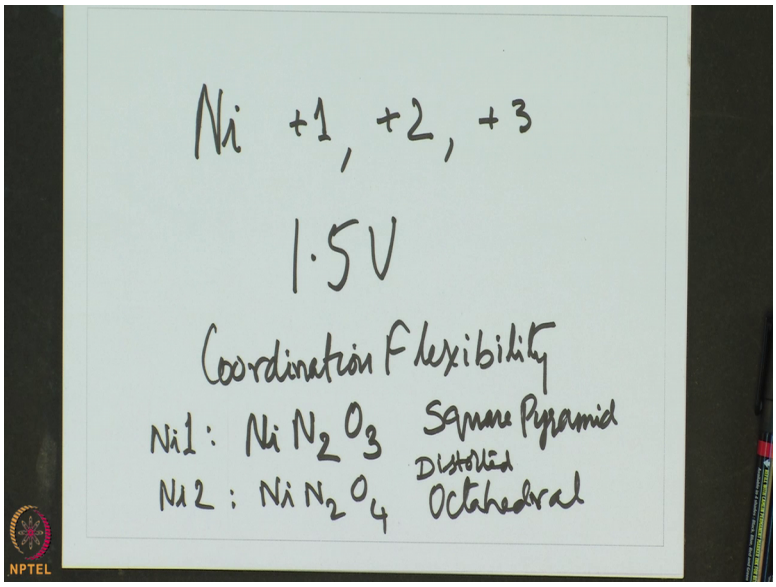

And that is what is basically favoring all these kinds of reactions. And all this can be achieved within about 1.5 volts.

So, the total thing can be achieved within 1.5 volts of the potential of these redox processes. So, that is very interesting. So, whatever I mentioned to you earlier that you can see the same thing on this particular one that is except the glyoxylase you have all other enzymes are either producing the gas or utilized in the gas. I also mentioned to you there is a flexibility for the redox between plus 1 plus 2 plus 3 for nickel, also there is a flexibility of the coordination. So, coordinating to different kinds of a ligating sectors as well as different number the coordination numbers as well.

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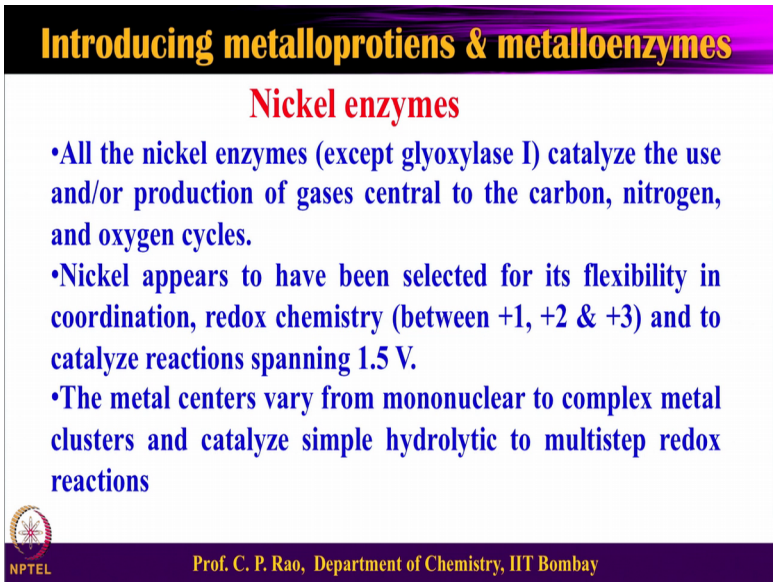

So, the coordination flexibility is the additional parameter that helps in this is coordination flexibility, and all this is possible within by changing the potential within about 1.5. So, and the other thing which is not clear from the previous slide is that all these enzymes do they have 1 nickel do they have more than 1 nickel. In fact, we have enzymes with 1 nickel we also have enzymes with more than 1 nickel too which you can see from the next slide.

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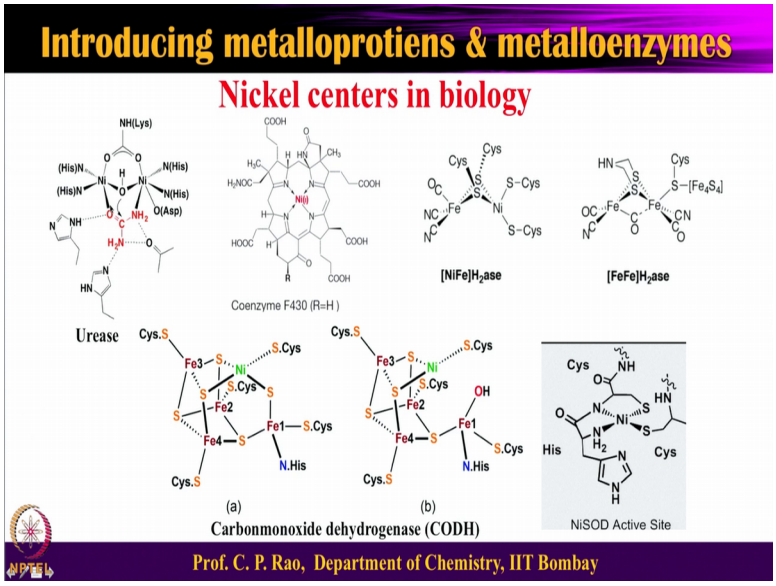

As you can see start from the top left structure which is in dinickel center go to the right is 1 nickle here, go to the further right this is 1 nickel, 1 iron is a combined one and this is another one iron iron which is not a nickel, but comes under the hydrogenase class and you have a core structure with the iron and nickel being together form specialized structure, you see that nickel over there, you see that nickel over there.

So, now and you have a mono nickel center here. So, you have a basically mono nickel center, but it is a cluster with iron and this is a mono nickel center this is a dinickel center, this is again mono nickel center with some kind of a urease which looks like a prosperin, but which is not a prosperin which is called the F430 factor and then you have 1 nickel 1 iron come out of things.

So, now from this slide what do we understand? We understand that the nickel enzymes have either 1 nickel 2 nickels or even nickel with some other kind of a ions like ion and even in a cluster with a cluster. So, this kind of a cluster is reminiscent of what you have seen in the past. Where did you see such kind of an cluster? I assure you can recollect that when I talk to you about the electron transfer by iron which is called iron sulfur proteins not by cytochromes, but iron sulfur proteins there you have seen with 1 iron 1 sulfur, 2 iron 2 sulfur, 3 iron 3 sulfur, 3 iron 4 sulfur, 4 iron 4 sulfur. So, all these kind of clusters you already seen and you see one very similar with one of the additional unit is the nickel over here.

So, now, let us get back to this structures this dinickel center on the left top is the urease which we have seen the reaction earlier. The second one is factor 4 thirty which is found present in the coenzyme M reductase and these two or the hydrogenases will be discussing only this hydrogenase, but not this one is completely iron hydrogenase and this is these two units are present in the enzyme called carbon monoxide dehydrogenase. And this is in an site centre which is present in the nickel superoxide this dismutase.

So, we will see details of quite a few of these systems in terms of their mechanistic aspects as we keep moving across that. Let us look at the first case, the first case is case on the nickel urease the nickel containing urease, ok. So, this can be seen from here nickel containing urease is that where you have the urea and net up with 1 ammonia with carbamate and then another ammonia and the H 2 O, H 2 CO 3 carbonic acid.

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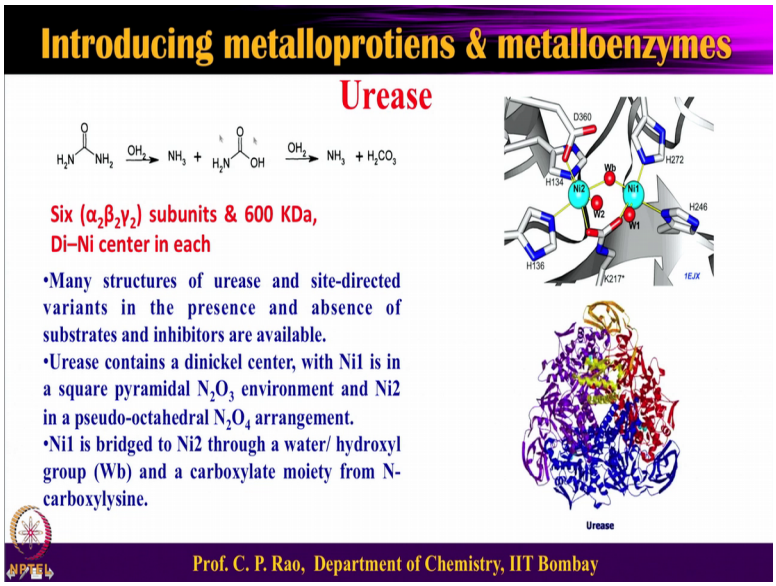

So, if you combine these two what will happen? The urea combines its two moles of water giving rise to two equivalents of ammonia and H 2 CO 3, this is the total reaction. Why was this written in this way this is written in this way? Because this reaction by the urease goes by two step process in the first step one ammonia is released and the second step one more ammonia is released and then the enzyme is regenerated back, ok.

So, that is the precisely the reason why the reaction is shown over here, but if you want you can write the total reaction as well; no problem urea plus 2 water molecules giving two ammonia molecules plus H 2 CO 3 absolutely fine perfect. By writing this you get into yourself to understand the system much better

Now, let us look at the enzyme urease enzyme urease is not a small enzyme it is a very huge enzyme how do we say is a huge enzyme number one you can see it has a 6 subunits, the alpha 2, beta 2, gamma 2. So, that is a is a alpha beta gamma twice so that means, it is dimer of a tri subunit, ok, so trimeric subunit dimer of that. So, this has a huge molecular weight of about 600 kilo Dalton and of course, there is a dinickel center in each of these. So, there are 6 subunits and there are 6 dinuclear centers; that means, the enzyme can function from each of its subunit. Now, if the one urea enters into one subunit you will get 2 ammonia, one more urea enters into another subunit you will get 2 more ammonias like that. So, you have 6 subunits can consume 6 urea molecules and

produce 12 ammonia molecules. So, you understand that, there are 6 subunits and each of this has got a dinuclear center in each of this.

So, now, let us the this whole protein is shown over here, as you can see from the colors. So, there is one part, there is another part there is another part 3. So, it is the 3 subunits and you see above that above this, above this, so 3 more. So each one is its it is a dimer of a trimer., so dimer of a trimer, so the trimer is alpha beta gamma the dimer is twice. So, therefore, totally you have all this, ok. So, this is the enzyme. So, this is a very complex enzyme where you can have 6 moles of urea reacting and giving rise to 12 moles of ammonia. Now, if you look at as I said that each subunit has as a dinuclear nickel center then you can see that one here on the right side on the top. So, you would see this one nickel here you will see another nickel here.

So, one of these nickel has got N 2 here nitrogen nitrogen binding as one oxygen another oxygen from carbonyl other by water. So, what is this one? Nickel 1. So, what is the nickel 1 coordination? Nickel 1 coordination is 5. So, what are the 5 coordination geometries? 5 coordination geometries are generally trigonal bipyramidal or square pyramid. In this particular case this is more of a square pyramid rather than of a trigonal bipyramid, ok. So, this gives a N 2 O 3 kind of a structure. So, this you have the this structure is nickel N 2 O 3, this is square pyramid, ok.

Now, look at the nickel this is the nickel one center and the other one you have on this side is the nickel 2, the nickel 2 center. So, this is the nickel 2, this is the nickel 2. The nickel two has got 6 connected atoms of which again 2 or the nitrogens coming from the imidazoles or the histidines and the remaining are coming from the oxygens either the bridged oxygen or a water or a carboxylate, this carboxylate all kinds of things. So, you have, so therefore, the nickel two center is a is Ni N 2 O 4 it is a octahedral. Of course, this is not a perfect octahedral and this is a distorted octahedral. So, and that is how it is, so therefore, now you can see that the coordination flexibility in this itself one of the nickel is the nickel with the 5 coordination, other nickel is with the nickel 6 coordination under in these things you see that, ok. So, these are kinds of the things.

Then what other things you can see from here? There is one other interesting feature the two nickels are not separated, but connected by a bridge. So, the bridging group over there could be water hydroxyl etcetera. So, this is a connected bridge from these between

these two. So, therefore, we have two distinct coordinatively two distinct nickels. So, therefore, we can think each one of these could be useful one different kind of a function that we will learn in just in a while very soon now in the next couple of slides we will try to understand this, ok. So, you have the oxygens coming from carboxylic groups, nitrogen is coming from imidazoles, oxygen coming from water all these kinds of things and the bridging. Let us look at the this idea about this particular urease mechanistic aspects.

Next, 3 to 4 slides we will try to understand how this urea is finally, cleaved to ammonia and H 2 is CO 3.

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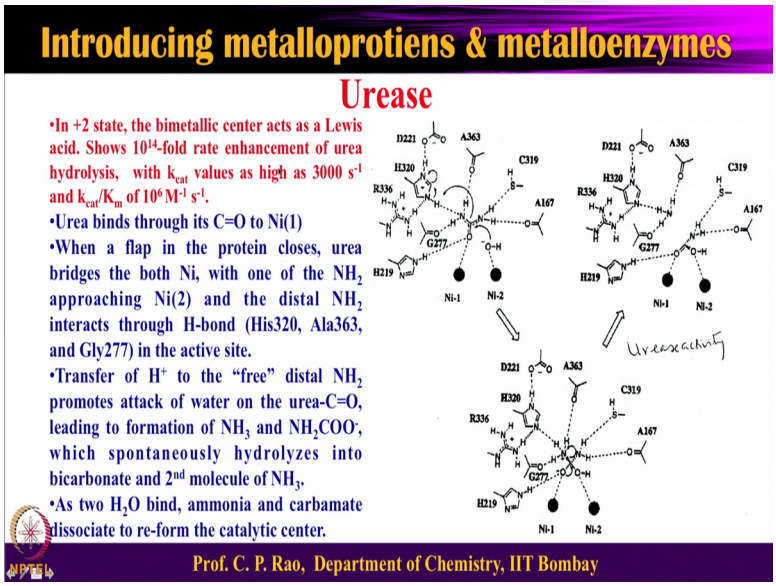

Before going to that let us look at this is an enzyme enzyme with a nickel 2 plus and in this enzyme there is no redox, none of the nickel undergoes any reduction or oxidation. That means, both the nickels stay in plus 2 state all through point number 1. Number 2 if you see urea if you put in water there will be some amount hydrolysis that will be very very very very slow kind of thing.

But in presence of the urease enzyme if you put urease in that the reaction goes very fast, ok. So, it is the k catalysis is around 3000 second inverse, and the k cat by K m is 10 power 6 mol inverse second inverse. So, therefore, the enzyme gives a very high fold of enhancement which is about 10 power 14 fold reaction rate as compared to the reaction

of urease without enzyme. You just take the urea put into the water you take the you take the urea in water, but put urease enzyme. So, these are the two differences that it tells.

So, this particular sentence explains you that the reaction of the hydrolysis, reaction of the urea is expedited by 10 power 14, you know 10 power 14 is a huge number 10 power 6 is million and 10 powers 9 is billion is almost like billion millions kind of thing. So, huge that is what is the reaction rates that you have looking at.

Now, let us get into the our self into the reaction aspects of it. How does it do the thing? Now, for this I have shown some structures on this side I will draw your attention as in when is required and we can try to understand this one, ok. So, we take these two dots parts as the nickel 1 and nickel 2 center and they when the urea approaches the nickel these enzyme it is directed towards the this particular region is called the catalytic site where the nickel centers are there and that is directed towards this, because the some of the protein moieties which are now you can see is covering they are opening they are in a opening form.

So, when they are away is called a flap open, the urea can enter in and then bind through once it comes in binds through the carbon almighty. So, once it binds to the carbon almighty these residues which were open earlier this residue this residue, these residues, these are all open which were earlier in an enzyme before substrate exposure they are turned they twist their common else towards this particular thing and then flap is closed.

So, now urea has come, urea initially interacts to the carbonyl to the one of the nickel center, and now this particular urea is now held like kind of a prisoner inside this. So, you can see this is kind of a prison chamber which is count. So, earlier all these residues are opened up and when the urea comes and binds they are they are closed. So, therefore, this kind of a closing will make the recognition of the urea of by the enzyme 2.

So, the how does this recognition works? This recognition works when it flap closes these hydrogens you can see that the NH, the NH etcetera etcetera all of these NHs are stabilized by the hydrogen bonding, ok. So, you can see there is a hydrogen bonding stabilization coming from all of these. And you can also see there is an NH from the histidine, this proton can transfer when this C N bond is ready to cleave, when the C N bond is ready to cleave then you can basically talk about the protonation in other words the proton push towards this will expedite this particular nucleophilic attack.

So, that is we are look at one kind of a nickel center. Then on the nickel neighbor nickel center you can see that the water which is activated, the water which is activated and becomes the OH and you know nickel bound OH is a base is a nucleophile can give an attack at the CO which is an electrophilic center which is present in the urea. So, that attack will form an intermediate of this kind you see that. So, now, you can see the urea molecule once binds the CO is recognized by all these hydrogen bonds I will tell the details in the later slides, but now take it as granted that there is a lot of hydrogen bonding interactions are going through this and this attack of the oh nickel bond oh of the second nickel would give you an intermediate to this.

And this when the proton is transferred from this imidazole to the nitrogen center that will favor the cleavage of the NC bond and will come to the one side will come as ammonia other side will come as the NH 2 H CO 2 or NH 2 C O OH, ok. So, this will be now comes into the picture of NH 2 C O O.

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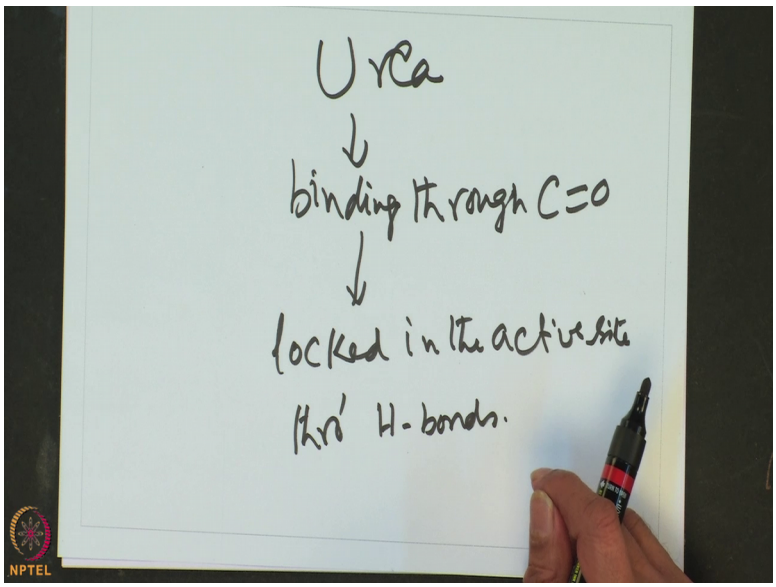

So, now, you have a urea molecule, urea binding through CO, binding through CO group and then locked in the active site through hydrogen bonds. So, that is what all you have seen.

And then you have also seen that the proton from the from the nearby histidine can facilitate the reaction of the cleavage of this.

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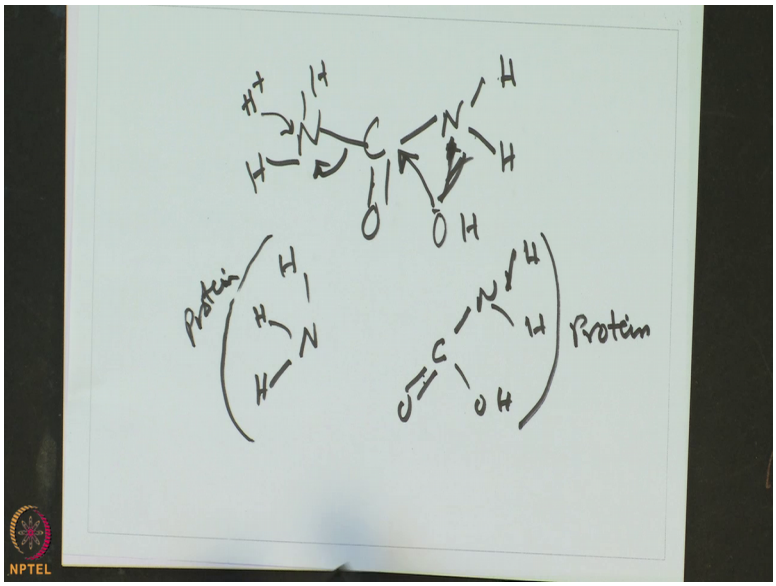

So, as a result of that so you will find. So now, urea and this is the urea you have NH 2s and NH 2s of this kind and this will get the other hydrogen, and this will have the OH from the other nickel makes this, and O sorry, now this is at the electrophilic center and that will cleave this bond. And that will give you an NH 3 and it will give C O O H and N H 2.

So, this is bound to protein, this is bound to protein and this is further. So, this is the intermediate step. Now, when this particular intermediate step is exposed to water then the further cleavage will occur which I will explain to you in the next slide on this, ok. So, this is the intermediate step that I have shown you earlier, ok.

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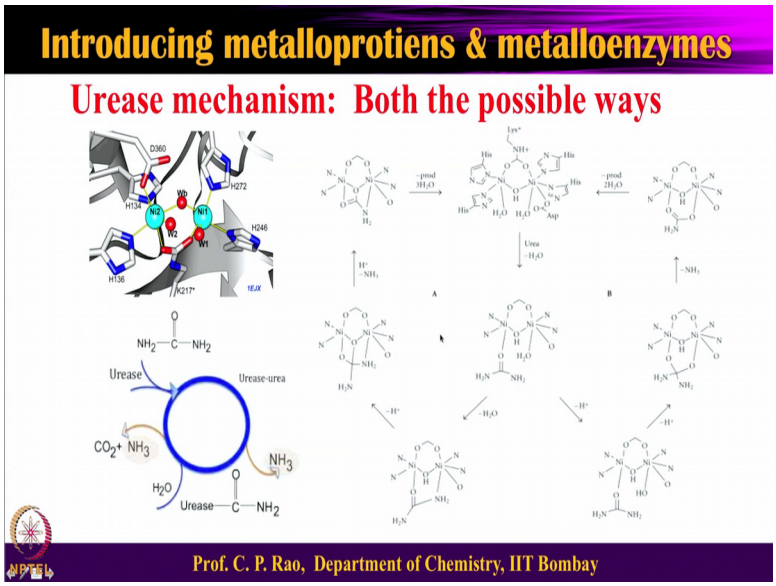

So, now, looking at the next slide, so you can see that that you the whole thing is we can we can try to recollect this the urea, urease enzyme in presence of the urea it acts on the urea and generates one mole of the ammonia and generates an intermediate of the carbamate. This upon further water reaction will again give back to ammonia and the H 2 CO 3 or in full water if its only one water molecule then you have the CO 2 is 3, and then this will reverse reverse back to that.

Now, this whole thing is explained by a mechanistic path over here in the mechanistic path over here then you can see that the urea the I am given two sets of mechanistic parts where central part you written as A in this sign and the center part is written B here. So, you start from enzyme and then you go to the next and then things this kind go back to this. So, this is the one cycle.

The other is go to this intermediate and to the next one, to the next one, and to the next one and go back to this, ok. So, I this is kind of a thing. And in the next class I will give all the details of this particular enzyme how it binds, I have already mentioned certain points of it, how it breaks by showing the structures of all these via the mechanistic aspect. And further highlight the other features there is some sulfidryl function, how that sulfidryl function also helps in the reactivity of this. So, all this I will explain you in the in the next in the next class.

Thank you very much.