Inorganic Chemistry of Life Principles & Properties

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Lecture – 41

Role of Zinc in life – General perspectives including oxidoreductases & hydrolases

Welcome you to the next class on the Inorganic Chemistry of Life Principles and Properties. In the previous class, previous 3 classes, we have looked at all the copper kind of enzymes and we have seen variety of copper enzymes like type 1, type 2, type 3, type 4 which is nothing but a type 1 plus type 2 plus type 3 enzyme, then, we will also look at superoxide dismutase. So, we have gone through all of these.

Now, in this class let us begin with the, what is the kind of a role of zinc in biological inorganic chemistry. The role of the zinc in biological inorganic cxhemistry is quite important and interesting.

It is important why? There are more than 200 enzymes of zinc are associated in our body doing variety of reactions. In a while, we will see what are the variety of reactions. It is interesting. Why is it interesting? Interesting because you know zinc is present in the body as what zinc 2 plus. Can zinc 2 plus undergo any redox? No, zinc 2 plus is always zinc 2 plus not like iron 2 plus going to iron 3 plus and back; not like copper 2 plus going to copper 2 and back etcetera. Not that kind of thing the manganese can undergo a lot of redox reactions. So, unlike all these, the zinc is the only one case among the transition metal and 2 plus and 2 plus is the detente system ok.

So, therefore, zinc 2 plus enzymes, there is a kind of a catch. It is not easy to get good spectra because there is no d-d transitions; no magnetism because there is no unpaired electrons in d etcetera. So, therefore, you can call the zinc as a mute as a silent kind of an enzyme.

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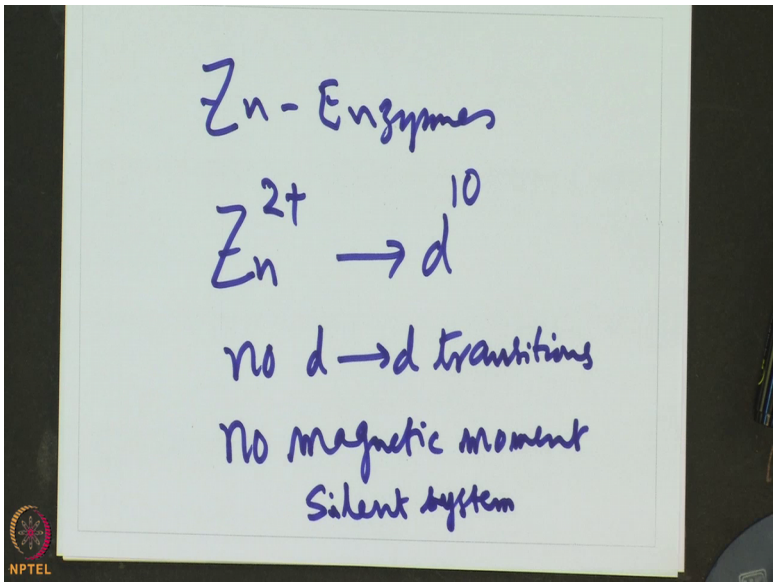

So in zinc Enzymes so you have zinc 2 plus and this is d 10, no d-d transitions and no magnetic properties; no paramagnetic.

So, therefore, it is a silent system. Do we need silent system? Do we need a system which is which speaks to us; we need a system which speaks to us. So, for a silent system, how will you understand?

So, you have to make, you have to break its silence. How do you break its silence, when somebody is silent; you how do you make him to start speaking. You just crack a joke and then, the guy will suddenly start speaking out of that or smiling, laughing etcetera.

So, similarly in the zinc enzyme, zinc enzyme being silent, we need to make it weak wake it up with a some kind of activity. How will you do that? You can do that by putting any other metal ion like cobalt, like manganese, like nickel etcetera.

This is precisely is done in many of the biological inorganic chemistry of zinc enzyme case, when you want to study their spectral properties, when you want to study their structural properties etcetera.

So, you can replace this and luckily this zinc 2 plus can be replaced by many of these divalent transition ions. So, this is a boon in disguise. So, you can handle the zinc silence by replacing the zinc by other ions.

So, that is why where as I said the interesting and also not so simple and easy ok.

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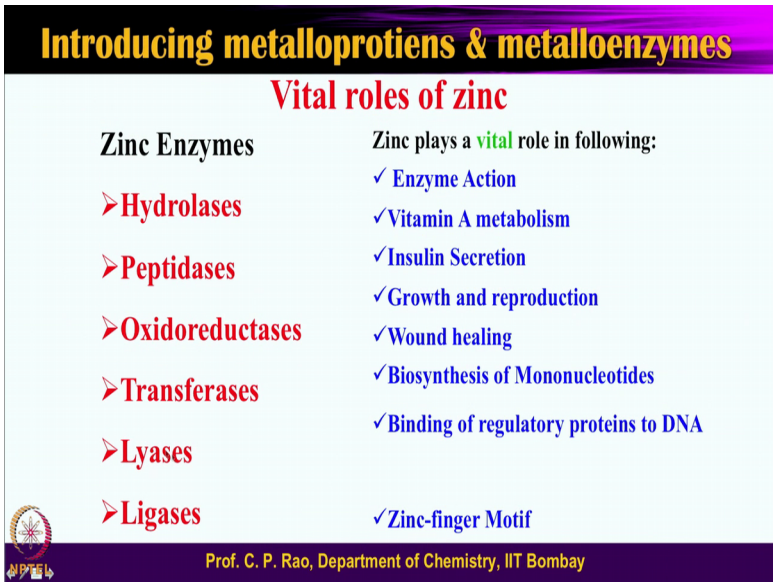

Here is a slide made I have shown all different kinds of a functions that the zinc Enzyme show shown more than about half a dozen. Just initially look at on the left side these are the main functions; that they can function like a Hydrolysis, they can function like a peptidases. The hydrolysis means adding water.

Generally, you can add water to condense bond. Peptides, you can add water in to break the peptide bond into carboxylic and amine function because the peptide is formed from the condensation airy carboxylic acid and the amino group by the dehydration reaction. So, it is a reverse to the dehydration reaction.

So, peptidase is a reverse reaction to that of the peptide bond formation. And you can see Lyases, where you can add water to the carbon dioxide.

Then, you have Oxidoreductases and where, you can take the substrate from oxidized to reduce and reduce to the oxidized. Wait a minute here and I said in the beginning that the zinc is not involved in any redox from 2 plus. It does not go to 1 plus, it does not go to 3 plus.

But, now I am saying oxidoreductase so oxidizing the substrate, reducing substrate. So, which means it is not the zinc directly doing in this redox, it is something else which is

called the Co-factor. So, when I come to the, this particular example alcohol dehydrogenize, I will explain you details of this in a while.

So, for the time being you can take it as granted even though the zinc 2 plus is redox silent. The zinc 2 plus enzymes are not the redox silent. They are redox active because of the presence of the some co-factor and the transferring one group to the other etcetera, from one to the other that also we will see for example, and Ligases, this is CO 2, additional Lyases oxen water addition to the la CO 2 and Ligases and the 2 moieties are joined together. So, the 2 moieties are joining together. So, through formation of a bond and that is Ligases.

So, we will roughly look at an example of each of this case as we keep going next couple of a classes ok. So, this is a zinc can show a variety of reactions as I said more than 200 active enzymes, this are there in Zinc. And one more thing which I have a forgotten to mention is that, the in the zinc enzymes, the zinc is catalytic; in some zinc enzymes, a part of the zinc could also be structural to so that means, zinc can play both catalytic role as well a structural role.

In the just the previous example that we saw in the copper zinc superoxide dismutase, what did we see? We saw that the copper is a catalytic center, zinc is a structural center. And so, similarly in the zinc enzymes, zinc is of course, is the catalytic center in some of the zinc enzymes, zinc is also a structural center too.

Now, let us look at the various the zinc plays a vital roles (Refer Time: 07:46) variety of enzymatic actions, that is what we refer to enzyme action, variety of enzymatic action.

So, all these are different types of enzymatic actions. Vitamin A metabolism, you can see in the process of the insulin secretion, the zinc is associated growth and the production, the zinc is very essential.

We have seen in the early stage the zinc deficiency can bring a problem of growth, problem of a production; we have already seen that. So, therefore, minimum levels of zinc are always required and zinc is one of the important nutrient in the body and in parallel with iron and copper.

And zinc is also involving wound healing in some kind of a form like zinc histidine kind of a complexes and this also involved in the Biosynthesis of nucleotides Mononucleotides.

So, it also involved the interaction of proteins DNA or DNA with proteins. So protein DNA interactions a lot of this and Zinc-finger Motifs; so there are a lot of different kinds of a the functions that you can see where, the zinc is playing a role ok. So, these are the zinc enzyme actions ok.

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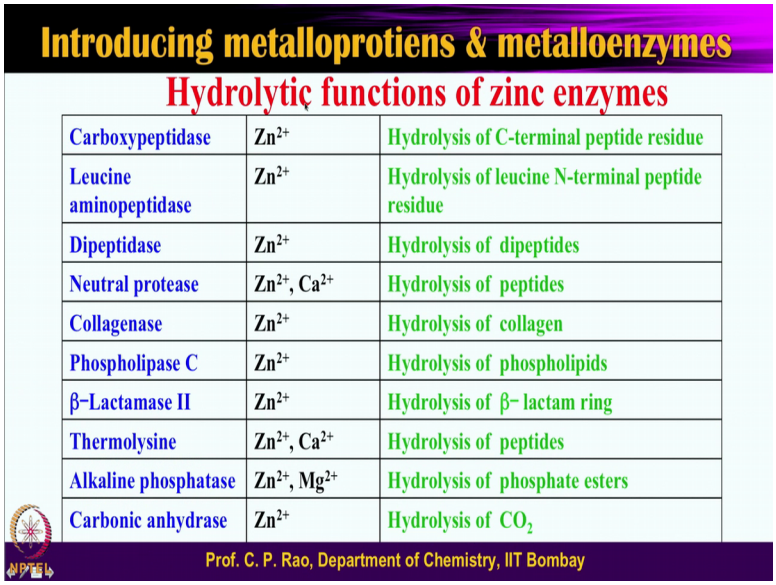

Even if you look at just the hydrolysis, we may think very simple hydrolysis. This entire table talks about the zinc enzymes and the reaction is only hydrolytic in nature and nothing else.

Let us look at one by one just to understand a little bit better, a little bit deeper. Carboxypeptidase. Carboxy I will come back to you later, it talks about carboxylic terminus peptidase, peptide breaking and this is by the zinc, the hydrolysis of C-terminal peptide residues.

Leucine aminopeptidase the so, this is the hydrolysis of leucine, leucine N-terminal peptide residues. Dipeptidase, this will hydrolyze the dipeptides. The Neutral protease, this will hydrolyze the neutral peptides. Collagenase, it will hydrolyze the collagen.

Phospholipase, it will be hydrolyzing the phospholipids. So, you will get the phosphate and the lipid part.

Lactamase, Beta-Lactamase an Hydrolysis of lactam rings. Thermolysine, this is Hydrolysis of peptides again alkaline phosphatase; hydrolysis of phosphate esters carbonic anhydrase; hydrolysis of CO 2. So, we cannot take it as granted that the hydrolysis is a very simple kind of a function. It looks like is a very simple function, you see variety of these and that is why you have 200 plus enzymes where, the zinc is working and this is only for the hydrolysis kind of a action ok.

Just to add to this now, we have graduated to a stage where we can see what kind of a coordination sphere that one would expect in the zinc enzymes; you can see the coordination cores here.

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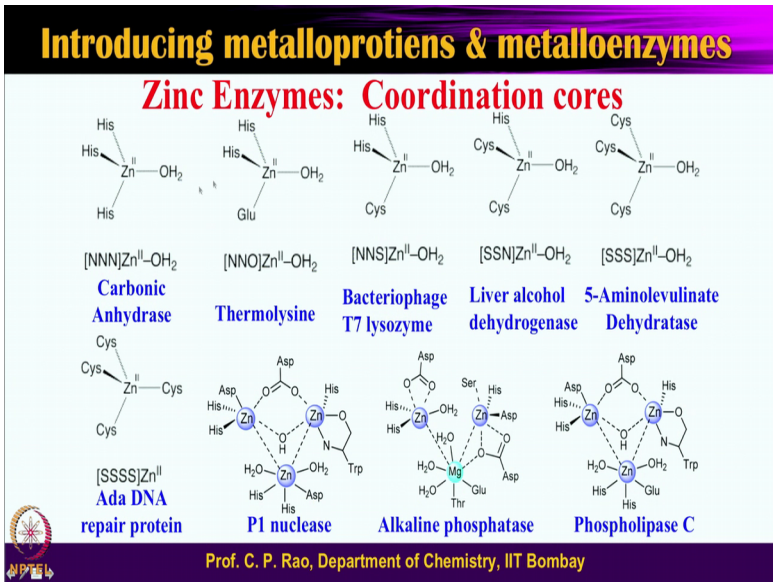

So, you can see the coordination cores in this, out of these 200 enzymes, few are picked up and then, shown over there. Of course, the 200 enzymes are not 200 categories of enzymes, the classes wise there are about half a dozen, half a dozen plus categories of enzymes. So but different enzymes to take care of the different kinds of substrates or to take care of different kinds of reactions in all this.

Now, let us look at from left to the right; let us see many of these have got 1 zinc this is also one thing. But many of these lost the have got 2 this has got 3 zincs, 2 zincs and 1

magnesium and 3 zincs. What this conveys? In the zinc Enzymes there are mono zinc containing center as well as multi zinc containing centers as well. In some enzymes, in some enzymes there could be more than 1 mono zinc enzyme and zinc center; one of the zinc center could be catalytic, other zinc center could be the structure.

For example, Liver alcohol dehydrogenase has got 2 zinc centers; one zinc center has got 2 cysteines, 1 histidine, 1 water; other zinc center has got all a 4 cystines and this is also found in the DNA repair protein.

So, here also you have 2 zinc centers; 1 zinc center is a catalytic having 2 cysteines, 1 histidine 1 water the other zinc has got all 4 cysteines and that is the structural nature. So, you can have the zinc enzymes mono-zinc, di-zinc or tri-zinc etcetera.

Now, let us go back one by one this is carbonic Anhydrase, Thermolysine we have seen in the previous slide, the Bacteriophage lysozyme, a Liver alcohol dehydrogenase, Aminolevulinate Degydratase and DNA repair protein.

This can also be a part of the part of this Liver alcohol dehydrogenase with a zinc being in the structural form; then, you have nuclease enzyme, you have Alkaline phosphatase enzyme; where the 2 zinc centers will help one to bind, one to attack etcetera we have already seen a similar kind of thing with the acid phosphatase kind of story there with the iron and alkaline phosphatase with a zinc and then, you have Phospholipase. So, we will be looking at several of these centers as we keep going go across.

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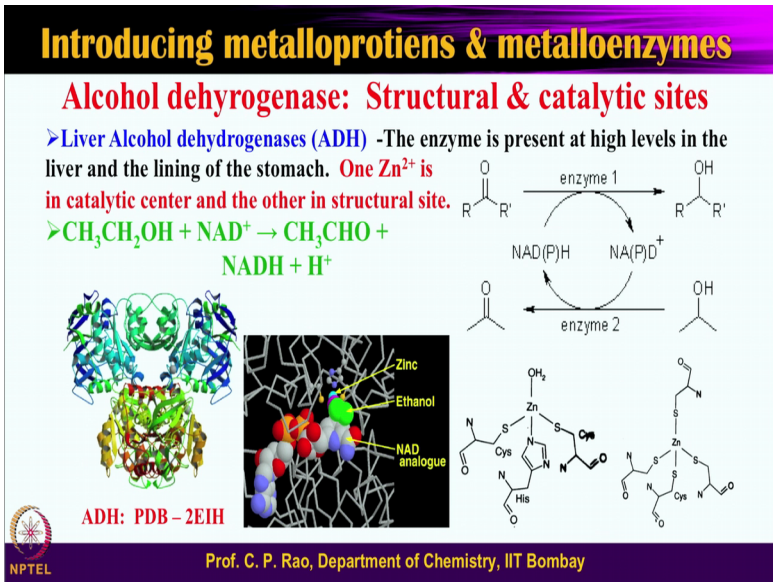

So, let us start with the first enzyme. The first enzyme is Alcohol dehydrogenase. What is this alcohol dehydrogenase and where is this present? This is present in the liver, alcohol dehydrogenase. So, this is as simple as understanding that when you consume alcohol, then you go into the other state.

But if there is no enzyme, you will not be written back to the normal state at all and it is this enzyme will convert your ethanol what you consumed into the corresponding aldehyde and thereby, your you know that that feel of the alcohol consumed is brought back to the normal.

So, this enzyme is essential, if you were to consume the alcohol besides the legislations. So, legislations are something for social factor. This enzyme is a biological factor. If you are deficient of this enzyme, you are not supposed to take the alcohol even though the legislation says that you are older than 18 and you can take and that is not the way it is ok.

So, this has this enzyme as you can see has this kind of a different subunits of all this. It has a catalytic center and one of them is a catalytic center; other is the structural type as I told you the reaction is CH 3 CH 2 OH in presence of a oxidized co-factor.

So, oxidized co-factor is NAD plus is oxidized co-factor that will convert into aldehyde NADH reduced form of phase. And this is what how the enzyme. In fact, this enzyme

can do both ways; aldehyde to alcohol, alcohol to aldehyde too depending upon whether the NAD is in the NADH form, NAD plus form.

So, if it is NAD plus form, it will oxidize the ethanol to aldehyde; if it is an NADH form, it will reduce the aldehyde to the alcohol. So, these are both of these. You can see the same thing here, you have in alcohol and you can make it a aldehyde; you have an aldehyde or ketone you can go to that.

So, in this case NAD plus will go to NADH. In this case NADH will go to NAD plus, that is what exactly I told you this term. I was talking to you there are 2 centers you can see the 2 centers over here. One is zinc with a cysteine and another cysteines, histidine and water; this is indeed is the catalytic center. How the catalysis takes place, we will see in the next couple of slides.

But, let us now look at only the center. The second one is this one where, the 4 cysteines are gone and this is a structural one ok. So, you can see this, the NAD in fact, there are 2 parts of the enzyme domains and one of the domain has a catalytic one and the other domain has got the reducing co-factor or oxidizing co-factor which is NADH, NAD plus, but these 2 are in proximity; these 2 are in proximity and they work in tandem they work together and that is what is important.

So, though they are in 2 different domains, these 2 are close to each other where the reaction takes place, where the zinc is bound ok.

So, hope this is a clear and this is enzyme. zinc does not undergo redox, but it is the NAD going to NAD plus versus NADH and that is what happens. So, therefore, reduced from the enzyme can reduce for it can reduce the aldehyde to alcohol; the oxidized form of enzyme can oxidize alcohol to alcohol to ketone or acid or aldehyde. So, understand this ok.

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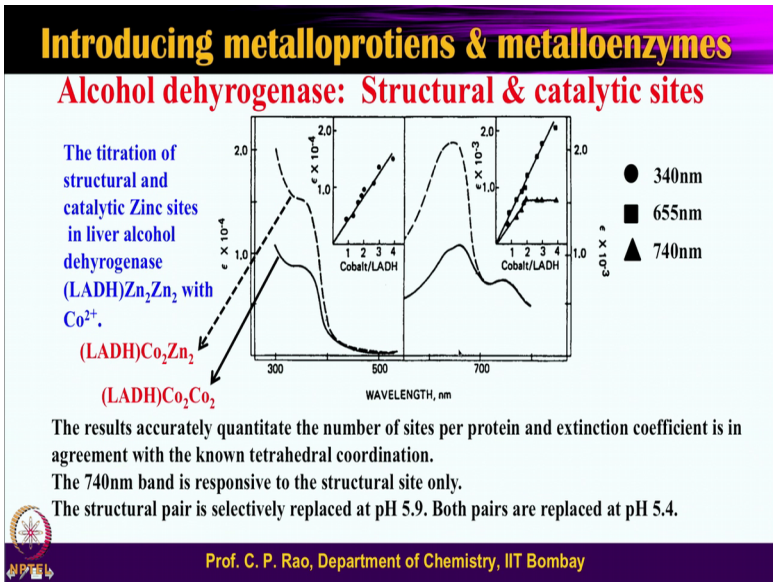

Let us look at I will talk to you in the beginning of this zinc story that zinc is a silent enzyme. As I told you the reason for silence is d 10 system. The reason for silence is no unpaired electrons. So, therefore, these are all silent factors. So, I also told you the remedy for this.

The remedy for this is you replace the zinc by another divalent ion like cobalt O-O or copper or manganese or something other kind you can do. So, once you do that you have made activity center and that activity center can give either a d-d transition O it can also give some paramagnetic. So, all of these are possible. So, that will be very interesting to look at this.

So, now if you take alcohol liver; L refers to liver alcohol dehydrogenase LADH. LADH is liver alcohol dehydrogenase and this is a dimmer; each dimmer has a domain which is a large domain and small domain. To a large domain contains a catalytic active center zinc and smaller domain contains the structure as well as the in the interface the NAD is bound.

So, therefore, it is a dimeric center. So, therefore, you have Zn Zn Zn Zn twice. So Zn 2 Zn 2. So, one of the set, you can take is active; another set you can take it as a structural catalytic in structure.

Now, you can try to replace these ones by one of the centers by copper cobalt 2 or both of them. So, you can see very nice things. So, when you increase the cobalt 2 cobalt both the cobalt etcetera you can follow.

So; that means, you can follow the enzyme reaction by following the absorption spectroscopy by titrating the original species by the original enzyme by the cobalt ion slowly and then, replacing the Zinc. You can in fact, do selectively you can remove the zinc and then you can add the cobalt 2. So, those details of course is not so much important.

So, using this kind of a titration you can very accurately quantitate the number of sites per protein and the extension coefficients or in agreement with the known in this case tetrahedral. You see that the values are much higher and the higher values are only obtained from tetrahedral or the square pyramidal or trigonal bipyramidal, but not octahedral.

When it goes to the octahedral then, the values will be absolutely will be very low. So, from that you can say whether there is any change in the geometric center or not that can be identified understood too.

Now, here you can see that the 740 band ok. So, these intensities of these bands are plotted over here, you will see that the triangles. It goes this way and takes a flat region and this conversion is a 2; that means, cobalt by LADH is 2; that means, two of the cobalt ions are incorporated kind of thing. So, you can follow the absorption spectra and then get quantification ok.

So, and this 740 is the one which is coming from the structural part of it. So, therefore, structural part is being replaced. So, you have a structural part, you have a catalytic part and therefore, one can definitely identify difference identify quantification you quantify the ion.

So, either you will be replacing the structure or you will be replacing the catalytic or you will be replacing both that can be identified from the absorption spectra when you do the absorption spectra as a function of the addition of the cobalt ion and that is what is exactly done.

So, you can quantify the things.

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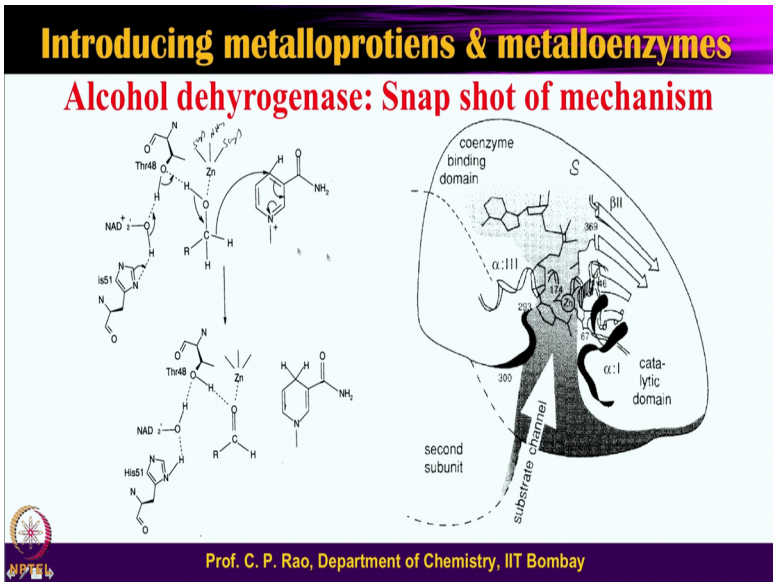

Now, just look at a snapshot of the mechanism in this case. As I told you, you have a zinc center catalytic, you have a structure which is not shown over here and closed by you have a NAD plus because the enzyme is NAD plus; this can oxidize ok, oxidize the alcohol to aldehyde and self get reduced to NADH ok.

So, therefore, the snap shot here is the zinc center acts as a center for binding to the substrate. Here, the substrate is ethanol and general formula I have shown over here or CH 2 OH and the closed by NAD plus is involved in redox process and you can see there is a kind of a protonation or deprotonation of this, deprotonation of this and redox of this.

So, its combining the protonation, deprotonation with the redox process. So, redox is occurring by the NAD plus, not by zinc. So, zinc is staying as zinc 2 plus. This is a snapshot; that means, you have a zinc bound, ethanol close in the proximity NAD plus and then NAD plus can be involved in the reaction. So, that will lead to aldehyde and the reduced form of NAD plus which is called NADH.

So, how will how is this happening? You can see here, you can see very much here the co-enzyme binding domain which is nothing but NAD, NAD plus NADH and you have your catalytic one and you have somewhere the structure one as well and which is not clearly shown over there.

So, therefore, you have the catalytic domain and the binding domains as well as the structured part of this one. So, that is how it is and this will be for second subunit and substrate will go through this and bind at the zinc center. Substrate will go through that there is a kind of a channel that allows the substrate to go through the copper of zinc center because a substrate has to bind here.

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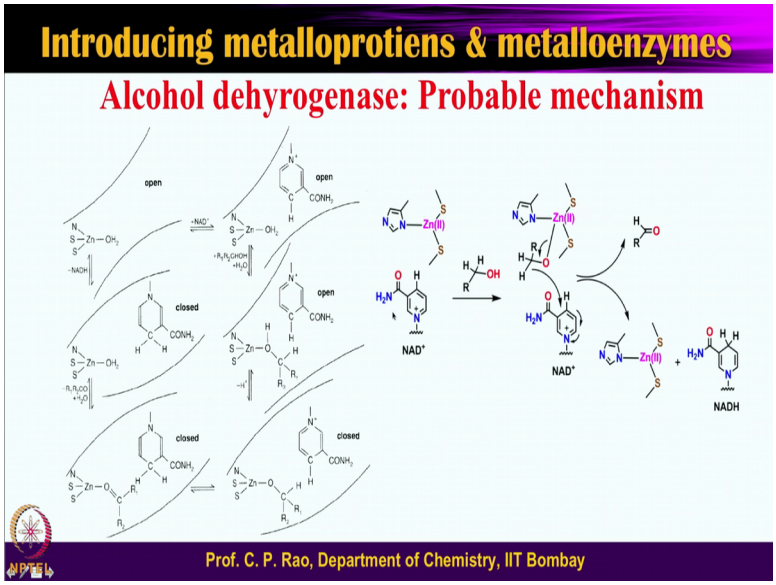

Now, we look at the detailed mechanism. First let us look at the right side. Then, we will come to the left side part of it. You can see that the zinc enzyme. So, the zinc enzyme the zinc center which can pro allow the this is not a connectivity ok. This is the cysteines, this is a cysteine and this is the histidine and there is water and that can get replaced by the alcohol.

And this co-factor is neighbor neighboring not a connected one, neighboring and very close by about 3-4 angstroms or something also and this is alcohol binding and this alcohol binding will bring the active form to these redox processes between this and that hydride kind of a transfer will make this center oxidize.

The carbon will go from alcohol to aldehyde and this NAD plus will become NADH and that is what you will see and an nothing happens to the zinc center because there is no redox. As I told you there are 2 mechanism, 2 reactions; one is going from the alcohol to the aldehyde, other is going from aldehyde to alcohol. So, the way you see here it will come out here ok.

So, and let us see that so if we move in this way, you are converting the alcohol to aldehyde and if you are going in this direction, you will be making the aldehyde in to alcohol so that is all. So, that is where the thing is that you have resting state of enzyme with the entry of the substrate, there is a conformational change that brings the NAD plus close by.

And then and it will also bind to the zinc center. So, the entry of substrate will bring a conformational change and that allows the NAD plus to take a re you know arrangement and which comes very closer to the zinc center.

And at this stage, so once the whole thing is set the substrate is bound and the NAD plus is poised in a proper way and the enzyme closes and once the enzyme process reactivity reaction redox reaction. So, the reaction-reaction because the hydrate transfer to the NAD plus and that will oxidize and then give NADH and at this stage again the conform a this enzyme throws out the product. So, therefore, this opens up and it will take a different route and go back.

So, so this is a so you are going from left starting from here go through this, it is the alcohol going to aldehyde. Starting from here and go down and go this way, then you are making the aldehyde to alcohol. So, you see that zinc and then the aldehyde portion or the ketone already in the closed form and that will make the conversion of this and then, reduce cofactor gets oxidized and then goes back to this.

So, as you can see that. So, this is the Alcohol dehydrogenase kind of a enzyme. So, therefore, this is a very interesting. So, why is interesting? zinc does not undergo any redox, but zinc brings a redox activity not by zinc itself by the kind of co-factor you have. The co-factor here we have is NAD plus and NADH.

So, NADH is the reduced form; NAD plus is oxidized form and that is what is switching over as a redox kind of a center ok. So, I hope you understand that. The next enzyme, I may just begin, but I probably I will try to give you more details in the next class, but just to have a look at Carbonic anhydrase.

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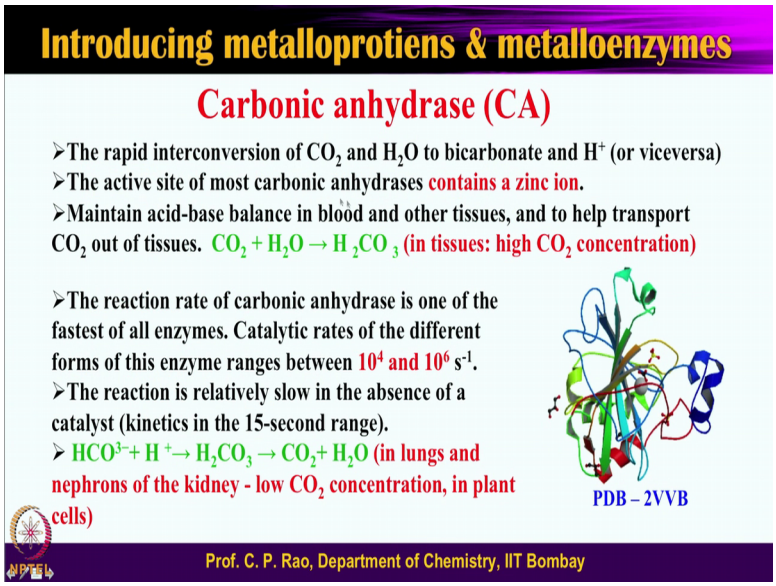

And you know that the in the human body, the blood and other tissues are contaminated with the carbon dioxide and this carbon dioxide need to be removed. So, therefore, this is converts the CO 2 into water and bicarbonate and the vice versa.

So, the active site of these enzymes Carbonic anhydrase is enzyme is thezinc ion there is no structure of zinc in this as you can see CO 2 plus H 2 O, H 2 O 3.

So, the rate of the reaction of the Carbonic anhydrase is the one which is a very fast enzymes and a variety of speed sources you have a Carbonic anhydrase, they go between 10 power 4 to 10 power 6 rates, very high rates of this and if you do not have the zinc part or the enzyme in the absence of the enzyme the reaction is very sluggish. So probably 1 per 15 seconds or something not much so in the lungs and nephrons of the kidney, this reaction and what is the thing and this is further taken out by water.

So, the low concentrations in the plant cells etcetera. So, this is very important enzyme as you can see that it has only one catalytic center and this catalytic center will basically convert this enzyme this carbon dioxide the carbon carbonate and the reversible reactions. So, these are all almost all of these as you can see the reversibility of this reaction too.

Let us look at the enzymes are what we have initially. So, what we have looked at more detailly right now is Alcohol dehydrogenase having the catalytic center, having the

structural center and in the next few classes will be look we just began Carbonic anhydrous, but we will go into more details of this. And we will also look at the peptidase kind of a things and other enzymes as we go across.

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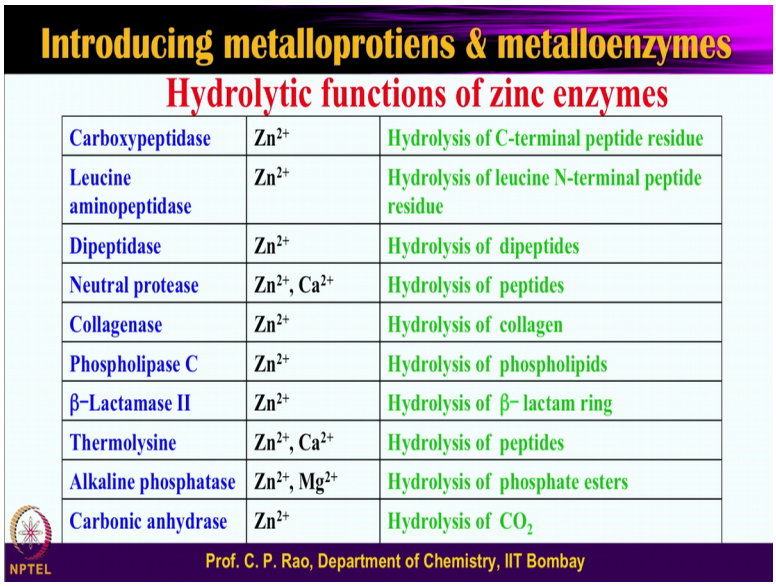

So, as you can see that the Zinc, 200 enzymes are there it does not have a redox. It is a d 10 element and there is no magnetic, but still plays a very heroic role in this particular thing this so.

So, we are going across not only does these hydrolysis, it will do the redox kind of an enzyme involved; it is also involved group transfer, it is involved in pod making process; we will look at the example of each of these as we keep going across in the next few classes or so.

Thank you very much.