Inorganic Chemistry of Life Principles & Properties
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Lecture - 42
Role of Zinc in life - Carbonic anhydrase and carboxypeptidase

Good morning welcome you all to the next class on Inorganic Chemistry of Life Principles and Perspectives. Let us look back into what we have done in the previous class. Just in the previous class, I have started regarding the zinc enzymes and explained to you; there are at least 200 zinc enzymes are functioning in human body having more than half a dozen different kinds of functions.

And I have brought the different types of functions to be notice and some of these you can see once again here.

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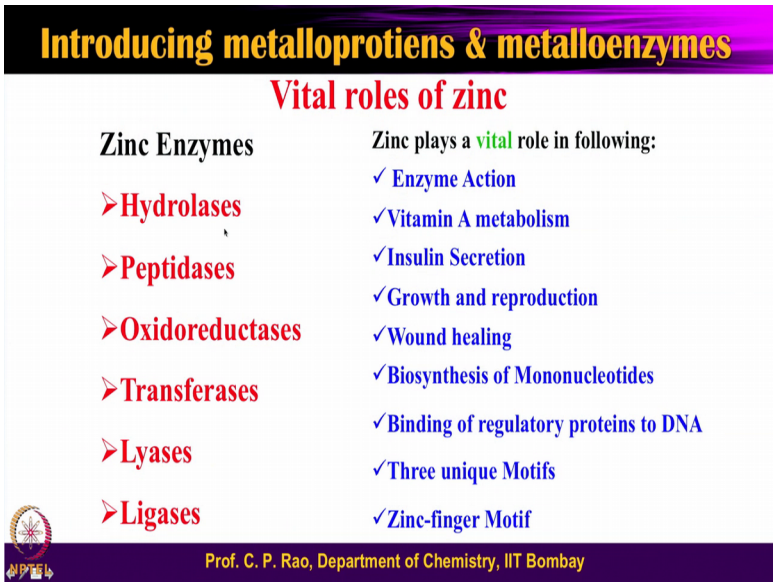

Hydrolases peptidases lyases, then you have oxidoreductase transferase and ligase, I mentioned to you in spite that the zinc does not undergo redox, zinc containing enzymes have got activity of the oxidoreductases, we I have also explained to you in the previous class that the is cofactor is involved in doing such kind of a such kind of a redox mechanism.

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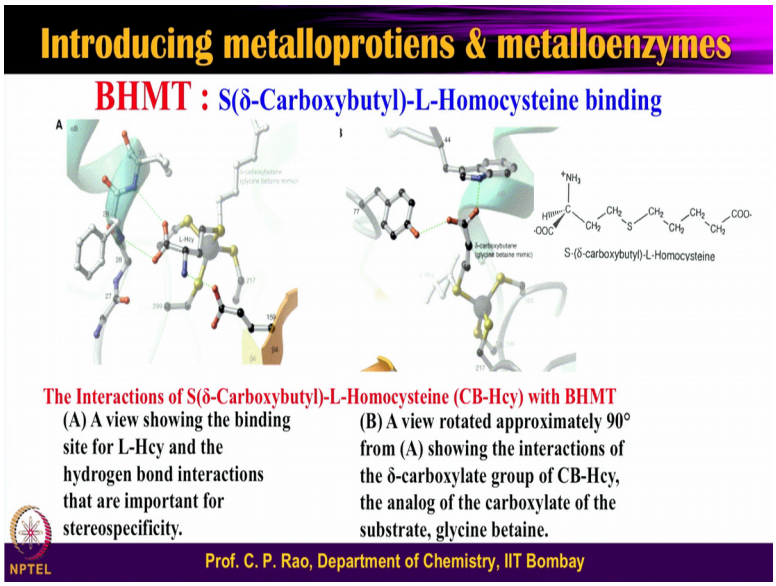

And this has been explained very well by the example of a example that is using the alcohol dehydrogenase, alcohol dehydrogenase is the enzyme which takes this you know alcohol to aldehyde and also its reversible kind of thing from aldehyde to alcohol.

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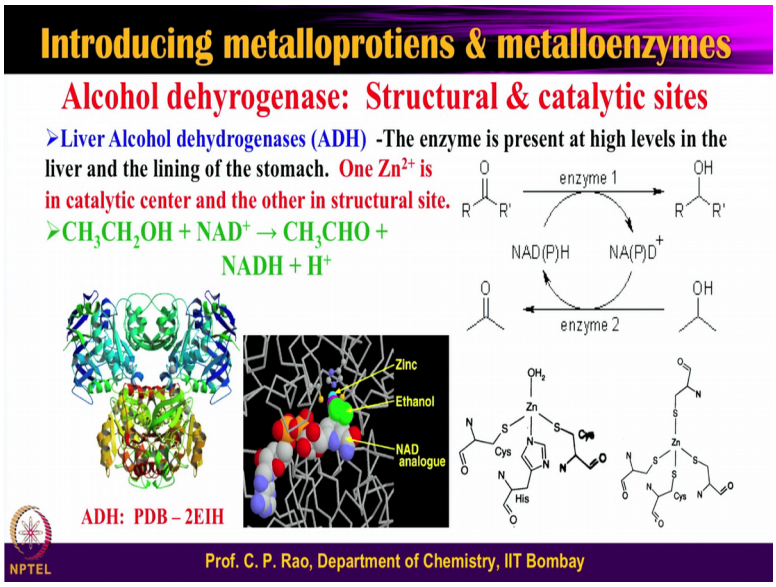

But instead of zinc undergoing redox, it is the NADH NAD plus is the couple. So, as you can see here, NADH NAD plus these are the things that happen. So, in stuff, the zinc undergoing redox and the cofactor additional cofactor which is NAD plus NADH so; that means, in the zinc vicinity the NADH NAD plus should be poised in such a way.

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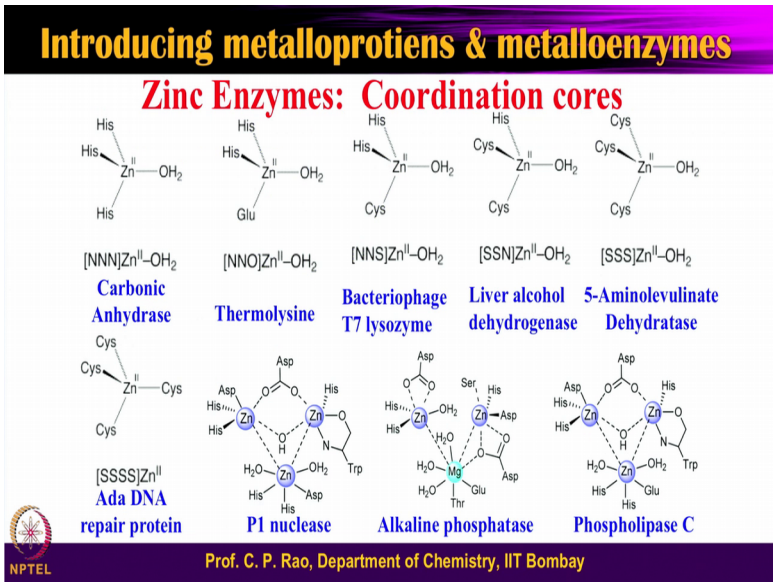

Then the reaction triggers and that is what I explained to you in the previous class also explained to you; there are zinc centers which having 1 zinc metal ion, there are zinc centers which are having zinc enzymes which are having 2 or more; for example, here you can see that there are 2 zinc and there are 3 zinc; there are 3 zinc, etcetera.

So, so, we have a range of zinc containing enzymes; so, taking you once again through the alcohol dehydrogenase that you can see the aldehyde to alcohol; alcohol to aldehyde; so, oxidase form requires to reduce this 1 NADH goes to NAD plus. So, and then the other way is NAD plus goes to the NAD.

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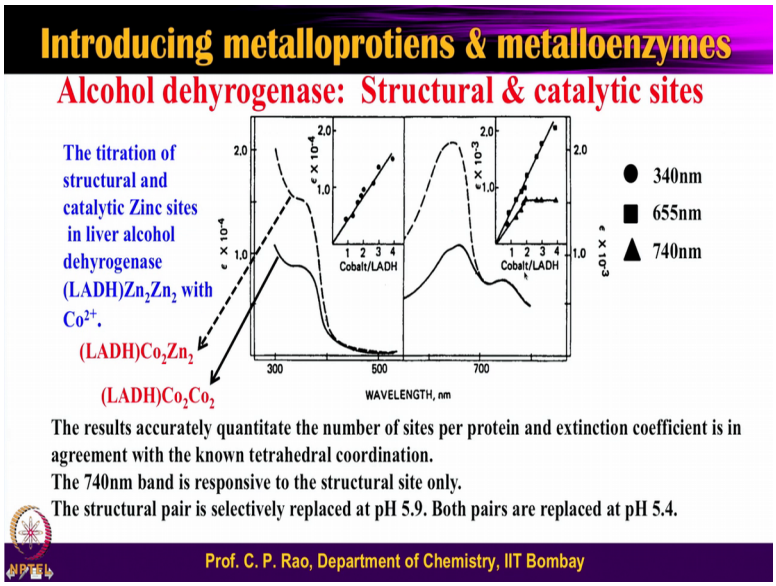

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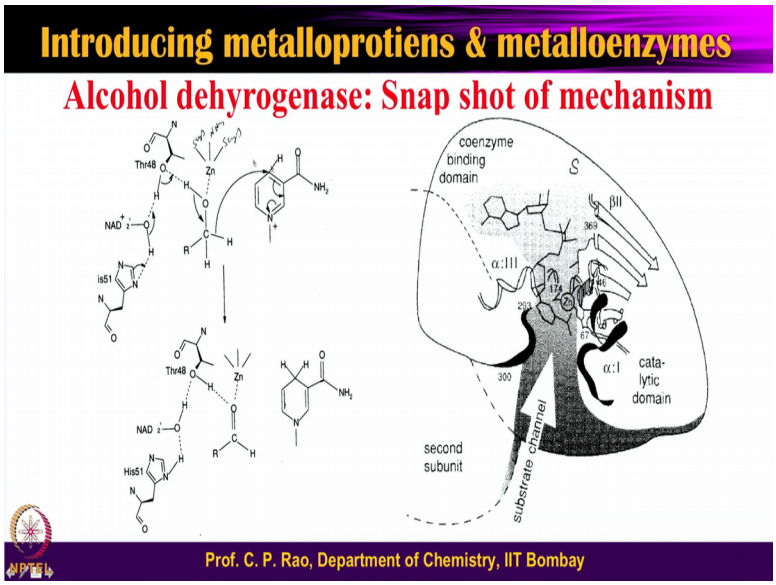

So, I am explaining that the mechanism where the cofactor lies in close proximity to the zinc center and zinc center does not undergo redox, but the redox will be happening at NAD plus NADH as shown here, the way that the orientation of this I have also explained to you, there are a 2 subunits the large and the smaller one where you have the of course, in this case, you have a 2 zinc ions, 1 zinc center is catalytic which you see here. Other zinc center is with the tetra cysteine one which is a structural one also, you can see the coenzyme binding domain and then you have your substrate entering into the thing.

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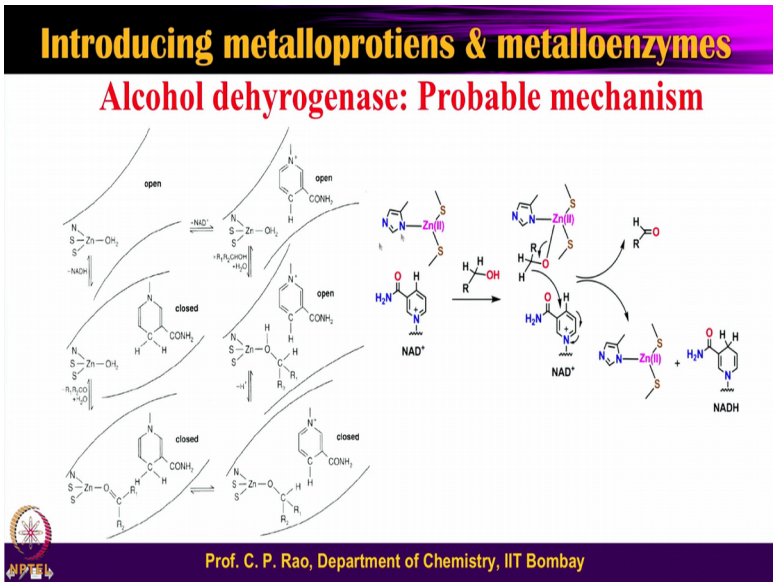

So, these are all we have looked at and we go through the all this mechanism.

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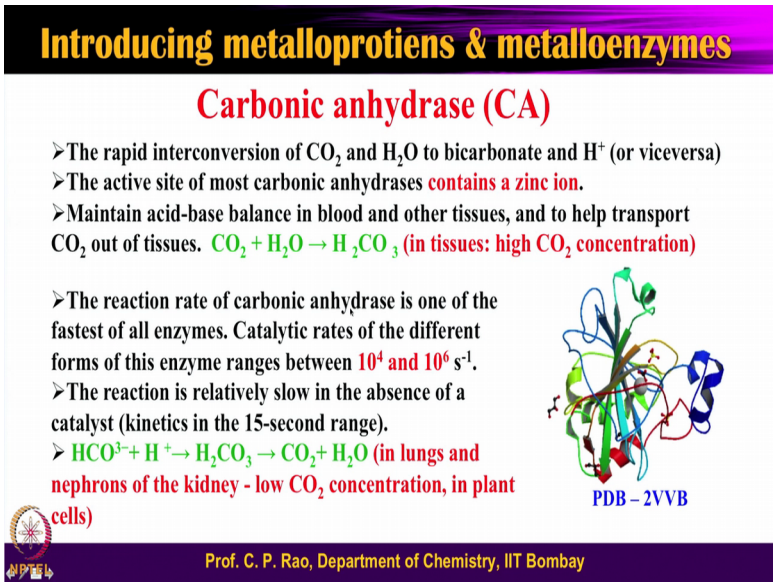

So, let us look at with a newer enzyme and this is an enzyme which is nothing, but hydration of carbon dioxide; you know in the tissues body cells tissues, etcetera you always have certain CO 2 being present there so, in order to maintain acid base balance in the body in the tissue in the blood.

So, one needs to sort of remove the co two by as a bicarbonate and the vice versa. So, therefore, you can clear the the tissues from all these species. So, the enzyme which does

is the carbonic anhydrase. So, if you take the carbonic anhydrase, it requires a zinc ion, it is a must without the zinc, it does not show if you take the carbon dioxide in water, you would see the conversion, but very very very slow, but when you put this enzyme, the reaction goes to the 10 power 4 to 10 power 6 per second.

So, which is the reaction is expedited by this enzyme; that means, enzyme is involved in converting the CO 2 to H 2 CO 3. So, this is how the acid base make a balance is being maintained in the body in lungs and in nephrons of the kidney, etcetera, low CO 2 concentration and in plant cells also. So, in all the things, the CO 2 needs to be balanced by this ok.

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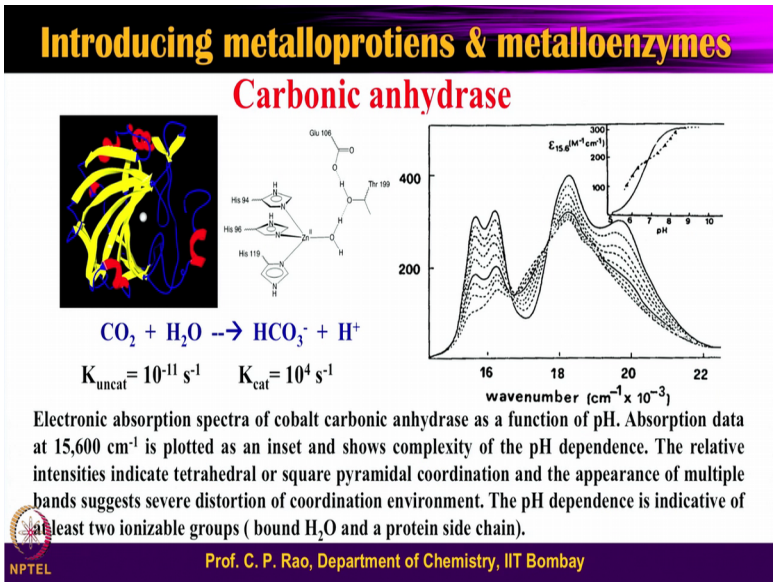

Let us look at the spectral properties of the carbonic anhydrase; can we get good spectral properties of carbonic anhydrase when zinc is present? No, because there is what is the reason? The reason is zinc is a zinc 2 plus zinc 2 plus is detain; detain does not give much of a magnetic kind of the spectrum.

So, what you need you need to replace this by the cobalt. So, initially you take the carbonic anhydrase, you remove the zinc by using certain kind of a and then you can create a apo enzyme. So, this one can be titrated with cobalt. So, you can get cobalt you know embedded. So, you can see the protein over there and this is the zinc position, this position the zinc can be removed and replaced by the cobalt. So, that you have a cobalt containing carbonic anhydrase.

Now, on this side, you have a number of spectra as you can see whose intensities are vary and these spectra have come because of what; by varying the pH of a cobalt containing and carbonic anhydrase. So, you take the cobalt containing carbonic anhydrase and look at the absorption spectra as a function of pH and you see something like the spectrum the intensity is going up. The intensity is going down as a result of this, you have a crossing here you have a crossing here. So, what is this crossing known as? Isopiestic point; the when do one find such isopiestic point? When there is a kind of a transition occurring for one species to other or when there is a binding of a metal ion on dissociation on that. So, events that are taking place so; that means, there is a real interactions going on.

So, now if you look at this particular band as a function of pH and try to plot its intensity or absorbance, either way is epsilon or pH intensity as a function of pH, you see that the curve that is going. So, this one is the one where you have more than one sigmoidal; that means, there are more than one possible ionization diionizations are possible and this is as you can see; there are two ionizable groups the water and the protein side chain and that is why you are finding one here and there one more here.

So, therefore, from this, your pH titration; you can get definitely the pka values and the information as I mentioned earlier. So, what is the reaction? We know very well CO 2 plus H 2 O HCO 3 minus plus H plus and this reaction is if there is no catalyst; that means, no enzyme the reaction is 10 power minus 11 per second and if there is a catalyst or an enzyme, it is 10 power 4; see the kind of exploitation.

So, what is the real rate to enhancement? Real rate enhancement is from minus 11 to plus 4 which is nothing, but 15; 10 to the power of 15. So, that is a huge level of increase in the reaction rate ok. So, as you can see that the zinc with the three histidines and the water and this is being sort of coupled by a side chains of tyrosine, etcetera and glutamate in a sequential way.

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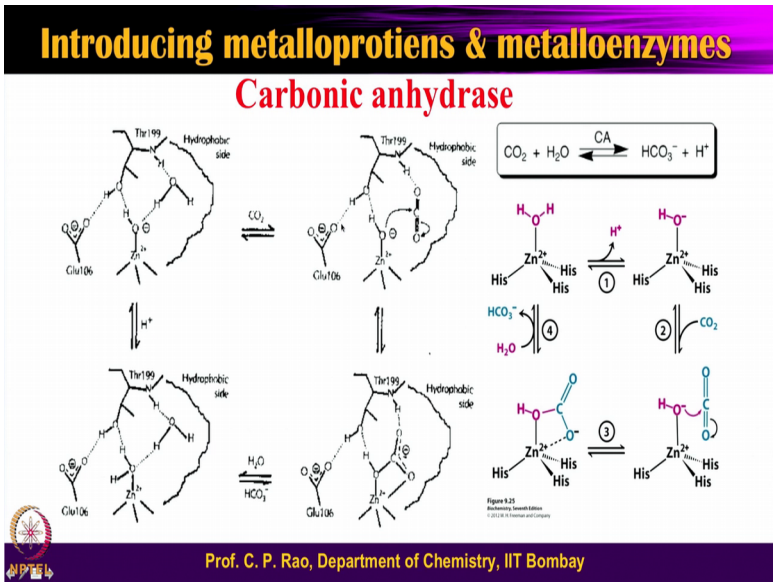

Now, let us look at the details of the reaction mechanism of this, but the details of the reaction mechanism this. So, you have zinc center as we have seen already and you have a protein here. So, you take this you know curved line kind of thing as the protein you have certain groups which are protruding here for example, thionine and this is being coupled by a glutamic. So, the glutamate acts as a general base. So, this base can pull out the proton over here and that can trigger the reaction over there and that leads to the further reaction to take place.

Now, in these resting enzyme, what you have? You have a water which is collected through this NH where hydrogen bond and which is also forming a hydrogen bond with this a zinc hydroxyl, now when you when the CO 2 comes in, actually, there is a conformational change comes in and it will knock out the water out and binds takes the position of the water and thereby binds in this way.

Now, what is the step is called the recognition. So, in this fashion the CO 2 is recognized by the enzyme through this kind of a hydrogen bond and this enters in as a result of replacement of the water that brings appropriate conformational change and this is the things that you look at here and now as a result of the replacement of the water and attaching the CO 2 through a hydrogen bond will place the carbon dioxide in strategically in such a way it is placed in the strategic manner such that the zinc hydroxide oxygen can have a nucleophilic attack.

Let us look at for a while; how you get the zinc hydroxide. So, if initially you have a water and the water is being activated by this group neighboring group and that basically it was the glutamate which will remove and make the hydroxide and you know that the hydroxide is a base and if when it bound to the zinc center will be the equivalent a as strands bases oh minus you take sodium hydroxide put it in water you know, it is a very strong base.

Now, you put the OH on top of zinc 2 plus which one is a stronger base. So, the stronger base is free hydroxide not the zinc hydroxide, but; however, this genzyme prefers zinc hydroxide not the free, why the reason any idea about this reason is that because this hydroxide whichever is formed has to have an attack or reaction or nucleophilic reactivity at the carbon center of the CO 2 and nowhere else, on the other hand, if you have a free OH, the free OH can start deprotonating many groups in the in the protein.

So, therefore, free OH is not favored by the enzyme and the zinc bound hydroxide is because this cannot go and take out any proton that is required because it is far away. So, it can only give a nucleophilic attack on the carbon center of the CO 2 because CO 2 is bound in such a strategic manner at this position through a hydrogen bond this is what is an important aspect of it, now this whole thing; you can call it as a CO 2 recognition by enzyme.

Now, once you have the CO 2 placed in a proximity to the zinc center, proximity to the zinc hydroxide; zinc hydroxide will take the advantage of having a nucleophilic attack and that nucleophilic attack will give basically a kind of a intermediate like this. So, this here and this minus it will go to plus. So, you will get some kind of a metal loss cycle. So, this is the carboxylic group with a metal loss cycle and this is the basically an intermediate one

So, this intermediate is broken in presence of the water, you can see that and that will leave out the HCO 3 minus and then this upon the protonation will give the next step back to the normal one. So, therefore, enzyme catalysis is complete. So, enzyme catalysis is complete. So, simply you can say. So, zinc 3 histidines in water and this is replaced by some general base from the proteins side, chain will knock out the proton and create the zinc hydroxide zinc hydroxide will attack on the CO 2 not in a free

system, but in enzyme system because it binds to that and that will give an intermediate and this intermediate upon hydration will give HCO 3 minus.

So, what is the role of the protein role of the protein is to provide the position recognition for the CO 2 and the position with respect to zinc hydroxide. So, that the zinc hydroxide can have a mechanism of nucleophilic attack on this, hope you understand. Now this whole system in enzymes always enzyme has to notice has to identify has to recognize the substrate here the substrate is CO 2, CO 2 is recognized through the hydrogen bonding interactions of this ok.

And places in such a way, then this is close by and the rest of the thing is quite well understood of this now.

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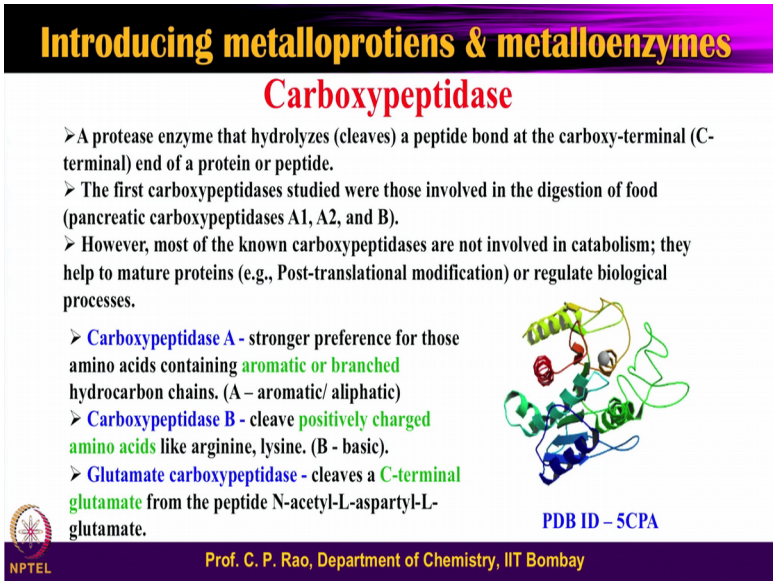

So, that takes care of the how carbon dioxide is converted the H 2 CO 3, etcetera and then maintain or balance the acid base balancing in the tissues by the carbonic anhydrase, let us look at another enzyme, we know very well a large number of peptidases are there which are based on zinc and they are all involved in the breaking the protein into individual either peptides smaller peptides or even amino acids and that is how you get the amino acid pool to synthesize the protein ok.

So, in that you have a peptidases which are carboxypeptidase also there are peptidase which are amino maintains; what is this nomenclature from carboxypeptidase means the

enzyme is active in the C terminal or carboxylic terminal of the protein, this is active in the C terminal of the protein and amino peptidase in the amino peptidase, it is basically active in the amine terminal of this, but in this particular course we will be studying only a carboxypeptidase not the iron peptidase. So, now, you got one thing.

So, what we understand? We understand that the carboxypeptidase can cleave the peptide bonds and the C terminal; what kind what kind is dependent on the type of the enzyme; if you have a neighbor which is aromatic, if you have neighbor which is cationic, if you have a neighbor amino acid which is anionic, there are different kinds of enzymes carboxypeptidase A where the neighbor amino acid is the aromatic or branched and if you have a positively charged amino acids like lysine arginine, etcetera in the neighbor amino acid to the peptide with respect to the peptide which you are cleaving and then, you this will be carboxypeptidase B and if you have a negatively charged kind of a species in the in the neighbor then it will be glutamate carboxypeptidase. So, it is also called peptidase carboxypeptidase C, carboxypeptidase A, carboxypeptidase B, carboxypeptidase C, let me explain you a little more understandable way.

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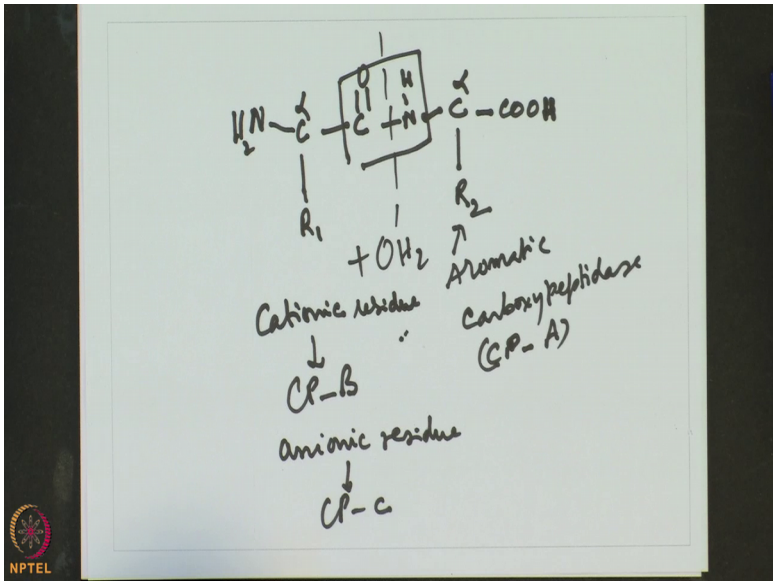

Now, you have you know that you have a carboxylic terminal or an amine terminal, then you have a C alpha and then you have CO and then you have NH, then you have C alpha and then you have COOH. So, you are looking at the time. Now, you need to look at this R 1, you need to look at this R 2. So, therefore, if you have at the end, this if you have A;

this as aromatic, etcetera, than you then this will be carboxypeptidase A carboxy peptidase. So, this is we refer it as CA CP A and if you have the cationic residue as a neighbor, then this is CP B and if you have a anionic residue then it is the CP C.

So, carboxypeptidase which can cleave C terminal if there is aromatic, it is called carboxypeptidase A and if you have a cationic residue in the amino terminal as sorry, carboxyl terminal, then it will be carboxypeptidase B and if it can cleave in a peptide bond where you have a I mean anionic residue towards the in the neighbor of this particular peptide bond.

So, this then it is carboxypeptidase C. So, basically what you are doing is your hydrolyzing this. So, you are adding water here that is what is the peptidase. So, what you will get? You will get the corresponding COOH is corresponding NH 2 that is what you will get. So, now, you understand, there are three different types of the peptidases; carboxypeptidase A, carboxypeptidase B, carboxypeptidase C, all of these will hydrolyze the peptide bonds of the carboxylic terminal, but depending upon what the neighbor residues are if the neighbor residues are aromatic or branched aliphatic than carboxypeptidase B where positively charged species as a side chains in the neighbor, then it is carboxypeptidase B on the other hand, if you have a anionic kind of a species and the carboxylic terminal then it will be carboxypeptidase C.

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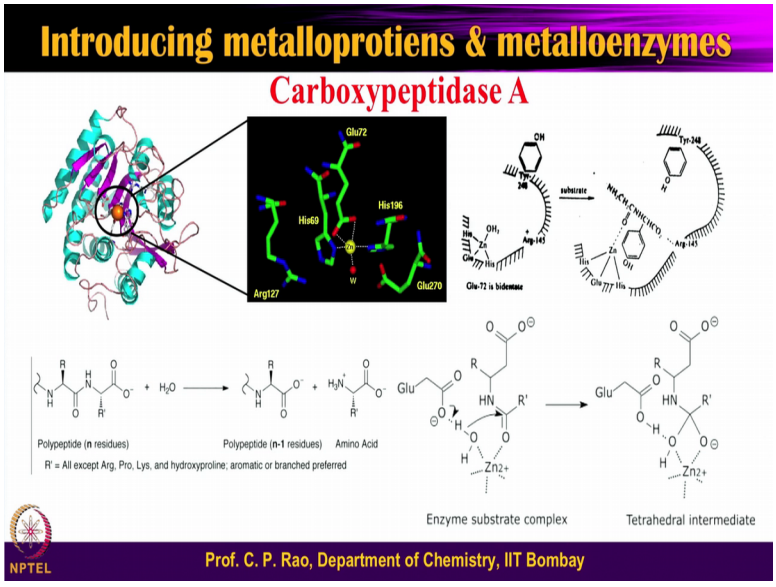

Now having got that; let us look at the enzyme code. So, this is the enzyme and this is the carboxy the sorry, zinc ion this bound. So, if you expand this region you can see, it is the zinc ion, there is one carboxylic glutamic carboxylic group. So, number is not very important, it is center in this glutamate 270 days. Its binds like a bidentate and there is one histidine over here, there is one more histidine over here and there is a water molecule and there are some additional residues far away and this will be you can refer it as a secondary coordination sphere. These will form the secondary coordination sphere. They are helping the process of primary coordination sphere whatever happens in this, what is the reaction; as I have shown you on the earlier by writing that you have the peptide bond you add a water, then you will get a carboxylate will get a amine, that is what basically reaction is and this is what you refer it is a cleavage peptidase.

In other words, you can also say hydration of peptide bond hydration of peptide bond is same as peptidase. This case; now you can see in one of the case a probably, it is a carboxypeptidase kind of thing, then a glysyl tyrosine here. So, this is a to glysyl tyrosine. So, this is glysyl tyrosine.

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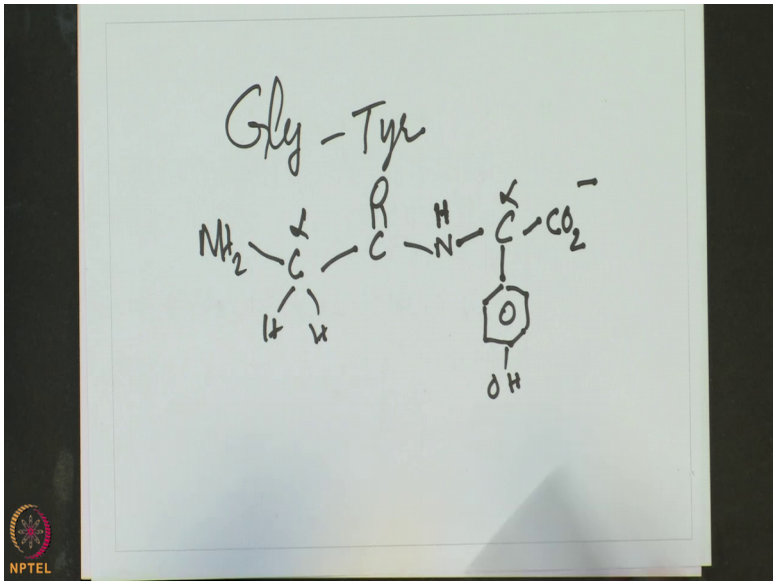

So, this is NH 2 C alpha. So, since it is glysine, it will be CH and CO, then NH and what you have C alpha here, what do you have is the if it is a tyrosinyl, you will have OH and you will have the next group.

So, which is the CO 2 minus; so, this is the amine terminal, this is a carboxyl terminal, this is the glysyl tyrosine. Now, you can see now in this particular the slide and this is the center where the zinc is bound to the 1 glutamate and 2 histidine with the water and this the peptide when you bind of course, it can bind over here and this has been checked by a crystal structures and this is actually poison because this particular tyrosine in when it binds, there is a conformational change you see the tyrosine is which is looking outside in a in a resting enzyme when the substrate comes in, this is the substrate and this turns into this side; that means, there is a purpose.

So, how do you know; you look at the simple resting protein enzyme structure and the enzyme structure in presence of the substrate can you ever crystallize the presence of substrate; no. So, you use something very mimicking to that of the substrate and, but it will act as an inhibitor. So, inhibitors can be easily crystallized and studied and that is what is studied over here. So, that is because this hydroxyl group is in close proximity to that particular pertain moiety; that means, this is basically required.

And another way of understanding this is that the zinc with the water can be converted into the easily by the glutamate neighboring here into hydroxide this hydroxide can attack at the at the peptide bond and then by form a kind of a intermediate which is the 4 member intermediate just like the one which we have seen in the previous case for the hydration of carbon dioxide ok.

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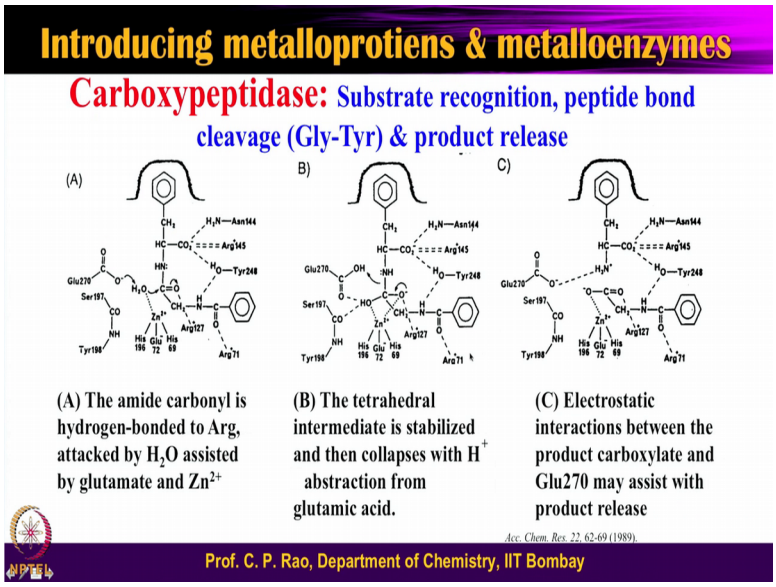

So, therefore, the carboxypeptidase; there is a said in the previous case which is the case of the carbonic anhydrase, always there should be a recognition the right substrate to be recognized and then there should be a reaction occurring. So, therefore, in this case, let us let us break the steps into the form of three possible steps one is substrate recognition. So, then next is a peptide bond cleavage and that is not sufficient, then the product has to release otherwise it will bound.

So, the whole reaction can be treated in terms of the three possible steps a step of substrate binding recognition a step of cleaving the peptide bond a step of releasing the product. Now look at the A and the B and the C here. So, the A as you can see here very nicely, this is the kind of the thing where the this is the this is a different dipeptide which is the real dipeptide on which the enzyme works and this is nothing, but this is nothing, but your. So, this is nothing, but your NH glysyl NH glysyl.

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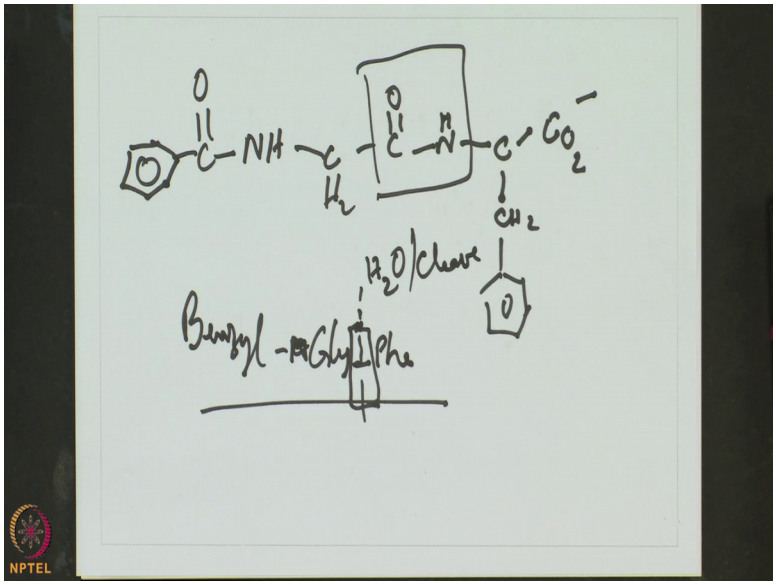

So, this is the glysyl, this is the peptide bond and this is the C alpha, here you have CH 2 and here you have phenyl. So, and then you have a CO 2 minus. So, this is the carboxyl terminal, it will recognize from this and this is blocked by some of the group here, here and ok.

Now, this is the peptide which is a cleaved. So, this is nothing, but benzyl, NH is coming here glysyl and this is what phenylalanine. So, this is the peptide which your hydrolyzing. So, it is this CO H bond which you are claiming here. So, this is where you

add water or clave. So, this is the real reaction or the on enzyme and that is what you have seen in this particular thing. So, you can see that this is when this bind this is being recognized by the CO 2 the neighbors which is there are three hydrogen bonds. So, the arginine and the other groups the tyrosine asparagine all these kind of groups will make.

And this is reason why this is being poised in very nice way close to the zinc hydroxide just like the previous case, the CO 2 is poised and here the peptide moiety is poised. So, therefore, and this is activated by this glutamate and this glutamate will make the hydroxide and then either you can have a zinc bond water which is activated by glutamate or a simple water which is activated by glutamate, but finally, the attack is on the carbonyl of the peptide and this is a this is a nucleophilic attack.

So, this is how it recognizes; it not only recognizes the C terminal, it will also recognize the N terminal by hydrogen bonding. So, the entire dipeptide sequence is recognized. So, that is why it is called carboxypeptidase the carboxyl terminal of the peptide is recognized so; that means, last two residues are being recognized hope you understand that.

So, all these small the dash bonds are recognition basically, now this one, once you have this is activate, this water is activated or zinc hydroxy is created that will create a active bond through a nucleophilic attack here and that will give the intermediate as you can see here. So, this is actually a step towards the breaking of the CO bond the CO NH bond and here you can see CO NH bond is broken, but still the thing is connected, therefore, this connected thing can be basically taken out by adding water. So, that will take out the carboxylic part of the thing amino part of the thing has two products.

So, you can see the step recognition the nucleophilic attack and the actual cleavage and the release the releases by adding the water.

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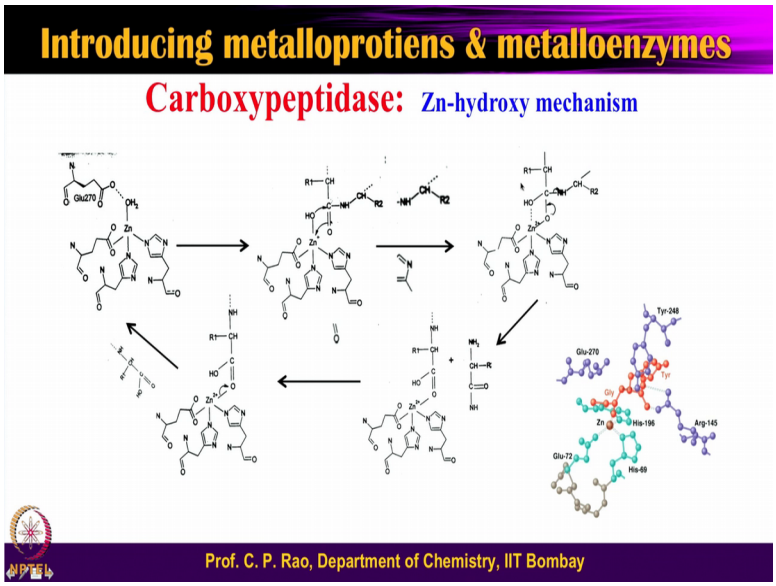

So, this whole thing whatever I mentioned can be seen in a little different way; this is nothing, but the center of the zinc with primary coordinates fear and the secondary coordination this is one of the secondary coordination.

So, these secondary coordinated groups are either useful in recognizing the substrate which is a dipeptide and are both also it is involved for some protonation, deprotonation kind of steps. Now, let us start from here; this is your free enzyme rustic enzyme and this water is activated by the glutamate and then hydroxide is formed this can be talked either the zinc bond water or the free water, it does not matter and that will attack on this peptide forms a intermediate which is the 4 member intermediate and this further undergoes the rearrangement and then breaks this particular bond and then here when you add the water and they carboxyl terminal and the amino terminal separated, then go back to this.

So, I hope you understand the kind of a mechanism. So, in this particular class, what you have seen you have seen 2 things, one is addition of the water to CO 2 forming the H 2 CO 3, a carbonate kind of an species in the second case C terminal carboxy peptidase where two of the last two residues are being recognized which of the last two carboxy terminal of the last two or recognized and these acted by the water converted into hydroxide or zinc bond water converted into hydroxide which gives a nucleophilic attack to result in a 4 4 membered intermediate which in turn rearranges to break this as a result

of the nucleophilic attack and in presence of water, this will dissociate to carboxyl terminal and the amino terminal groups or this.

So, that is the kind of thing. In the next class, we will look at other kinds of enzymes of the zinc.

Thank you very much.