Biological Inorganic Chemistry
Professor Debashis Ray
Department of Chemistry
Indian Institute of Technology, Kharagpur
Lecture 43
Carboxypeptidase and Metalloproteinasess

Hello, good morning and welcome to the class where we will be talking today on carboxypeptidases and metalloproteinases. Just look at the spellings and all these things are very important, because when you are trying to write all these big names, why these components are there, and what we should discuss in this particular class.

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So, we take the simple example of the zinc ions, obviously the bivalent zinc ion. And we will be talking simply about the zinc ion dependent hydrolytic reactions. And if we talk about the different bonds like amide and ester bonds, we will talk about the natural process. These are all naturally occurring molecules, so basically is the natural product chemistry, sometimes it is called, in terms of the organic chemistry but these also can be considered as some naturally occurring thing what we are trying to explore, or identify the function of the zinc ions.

So, two important molecules, again a very old molecules have been identified during 1953 or so. These are carboxypeptidase A and B. And the name itself will tell what does it mean basically. Try to understand the name first, then what does it mean basically because the peptide is there and ase that means the peptide hydrolytic agent.

Then matrix metalloproteinases so when we talk in terms of the corresponding proteinases, the protein you have, then proteinases, then you bring metal as the metalloproteinases, and what sort of thing can be considered as the matrix, because this can be useful for the

degradation of the collagen molecules and all these than hydrolytic cleavage of the collagen, which is extracellular in nature. So, that extracellular collagen cleavage can be taken care of by these sort of molecules.

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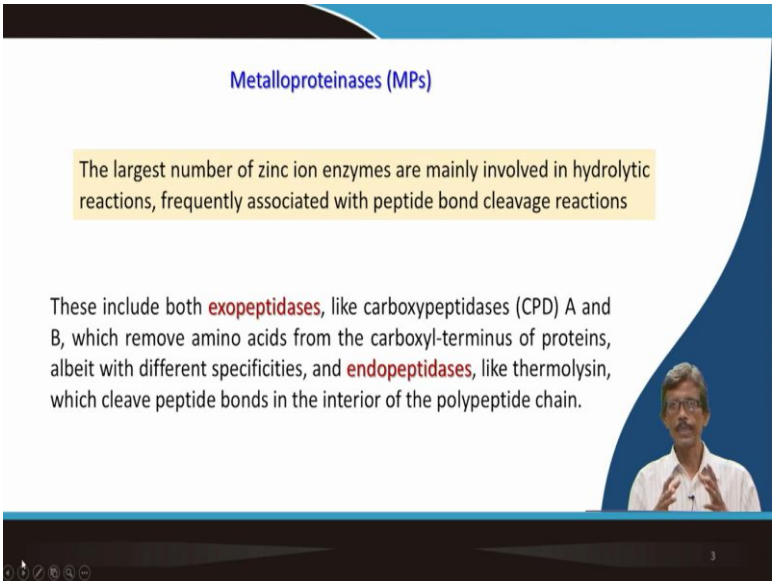

So, we will start with a very simple thing, simple nomenclature, these are MPs, though you bring the metallion for the hydrolysis of the main important bond which is your peptide bond CO NH bond, or CO NH function because in our earlier classes we have seen that how the zinc center is important to break the CO NH bond, or CO OR bond for the esters.

So, it is basically the largest family, and also we have studied these for the last 70 years or So, because they are very useful one also, even for our body. And they basically, most of the time, they will be involved in picking or cutting the CO NH bond. So, we will have two types now since we are having the peptidedases, so one can be exo type that means towards the end, and one is endo type. So, what does it mean basically?

So, the typical textbook definitions you should understand, you should remember, and you should recall back also quickly, what does it mean, basically. You have heard the name but what is that CPD? So, CPD A and CPD B. So, that will be used to cut the one particular part that means the end amino acid part, which if it is carboxy terminal because when you have the polypeptide chain, we know the amino acid one it is your amine end, and another is neurocarboxy end.

So, if you bring the second amino acid, the NH two end to this particular point, so what will you do get? The COOH, and NH two function will give you the corresponding amide link.

So, this is your amide linkage. And these are the two ends. So, we started with this particular one, in case of your NH two CH two COO is like glycine, and we are linking or attaching these again in the same orientation, NC, NC, NC, NC. So, what will you find if you have a dipeptide, if you have a tripeptide or even if you have a tetrapeptide?

You will have, again, one end is NH 2 and another is your carboxy end. So, that will be very much similar to that of our amino acid. So, try to remember in that particular fashion that whenever you have a peptide, long peptide chain, one end will be carboxy end, another will be your amine end. So, that carboxyl terminus, you will have, and the amine terminus will have.

But there are other peptidases, that is why it is endo, so exo is that you can cut selectively the amine end of the polypeptide chain or can cut selectively, again the carboxy end of the peptide chain that means the adjacent one, where you have the free carboxy end. The adjacent peptide bond, you can cut it such that you can take out through this hydrolytic reaction one amino acid from that particular chain. But what is endo peptidases?

The endo peptidases not quite often we write it in this particular form, but it has some other name because these names are coined first time when people are working on it, they are given the name, and having mainly the Greek origin or the Latin origin by terminal by the wordings also.

So, occasionally we will term it as the thermolysin, so you should know it, the thermo is the thermal thing is there, so it is the historical nomenclature or the traditional name of all these things. So, basically these are endopeptidases. So, try to remember that the thermosylin thermolysin molecules are nothing but your endopeptidases.

So, you have the two ends. These are exo ends, and the central parts, all, will be your endo ends of the peptide bonds. So, they basically will be able to clip that internal peptide chain or internal peptide bond. So, it is basically, sometime, it can be dangerous one also that if you cut a huge polypeptide chain at the central part of these using the thermolysin. So, that will also have some effect.

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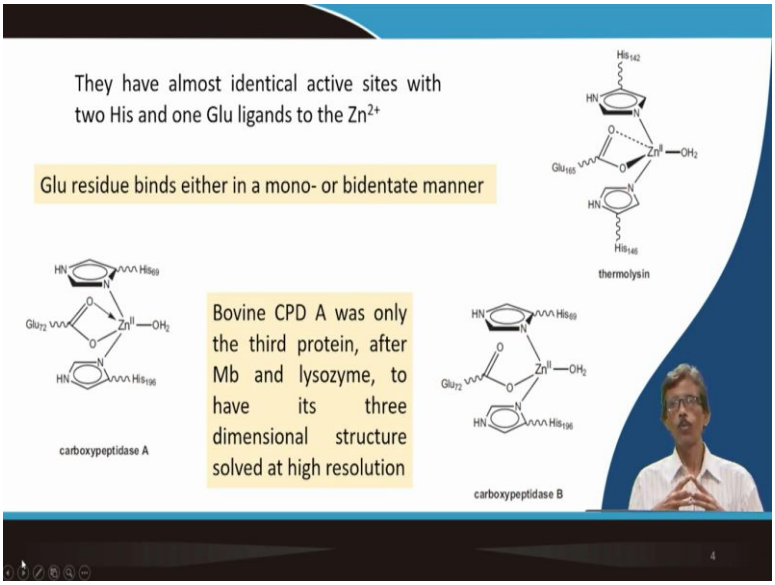

So, what we basically get from there that these almost have identical sites. All we know now that how we can get, or how can manipulate a particular metal ion center bringing again the facial coordinations. Every day almost I am telling this thing. So, you should be now very much master about the thing, only the diversity in the molecules are there. If you simply recall back what I told you regarding your hemocyanin molecule.

So, hemocyanin is there, which is a oxygen carrying molecule in crabs, in lobsters and all arthropods also. There you have the three histadine residues only, but the copper is binding. Now, you are having the different metal ion, which is the 3D 10 metal ions chain. Now, you change one of the histadine residue by a carboxy end of the glutamic acid or the glutamate anion.

So, glutamate is your charged one, you have the COO minus, the carboxylate ion. And that carboxylate ion will basically replace one of the histadine residue around that particular metal ion center which is now your zinc. So, you will have now N2O coordinates in environment around these zinc center, again in a facial mode.

So, you have the face, you have the bowl, and on it you have the zinc center, which can come and bind. So, if you look at like this, from this particular site, or from this particular site, so you have these three. So, these tips basically. So, these tips basically when they are coming, so one is your histadine and at the top. Another is your glutamate at the center and again, the histadine.

So, these are the three orientation one by one because is a in plane projection or the planar projection. It is not a three-dimensional structure you can have all these rotations possible. So, you have the numbering also, not only you have to remember the histadine as the amino acid and you have to draw the corresponding imidazole residue. When it is the glutamate, it is the carboxy end and when, again, it is histadine, but the numbering is also important.

So, histadine 69 and glutamate 72, these two are adjacent. That is why you can have always try to consider that these are simply a little bit longer bidentate motif of N and O. So, is NO bidentate part? Then, again, it is connected, but is at the longer part. So, you will reach around 196 is histadine amino acid residues.

So, when it is trapping, you have that thing and we have the water coordinates. So, the problem will also come when you have the carboxyl at end, whether it is coordinating in a monodented fashion or a bidented fashion, or (something) sometimes weakly coordinating one.

So, this thing can happen because you see if you consider carboxylate coordination for the different metallion salts, we know, like the carboxylate coordination in your nikel acetate, as well as copper acetate, as well as iron acetate, or ferric acetate. So, go back to your textbook, what you have studied so far, and try to open up those pages where you have read all these metallion salts at the corresponding carboxylate salts.

That is why the studies on these carboxylate metallion salts are very important. We use in for the different synthesis of the metallion complexes, coordination compounds, organic chemists also use all these things, because these have some different property compared to your typical inorganic acid-based anions like sulfate from sulfuric acid, nitrate from nitric acid, and chloride from hydrochloric acid, or phosphates from the phosphoric acids.

So, since these two are coming, so one tip is your oxygen tip, another tip is your oxygen tip, which is coming, and your zinc center is sitting over there. But thing like that that you have a four member ring, zinc is a bigger one, a little bit bigger compared to your copper and other that if the space is available, your that carboxylate end can come and bind in a bidented fashion forming a four membered ring.

But what it happens for the other? So, you can have the possibilities that you can have the monodented, as well as the bidented coordination from the carboxylate end of the glutamate

residue. So, if it is a monodented one, so we always write that is the COO minus, when you write as that other part. So, CO is a pendant one.

So, only the charged oxygen, O minus can coordinate to your zinc that means you do not have any enough space for that particular part. You get another molecule. So, the change in the coordination only. Everything is same. All the amino acid residues are similar, numbers are similar, everything is same, but due to the variation in the coordination motif basically, if you consider the left-hand chain, it is basically two two one.

And it is five coordinate. This is penta coordinated geometry. But if you look at these, the right hand side, the carboxypeptidase, it is four coordinated. So, these are the two extreme positions, what we can consider only for the CPD A and CPD B. But what about the other one?

So, first, we have isolated all these things structurally characterised and all in a nicer way that the bovine blood CPD A was the third protein that is why it is studied for the long time after myoglobin and lysozyme having a three dimensional excess structure in hand of high resolution that means the way we do for the small molecule excess structure, not for the protein crystal copy, sometimes the resolution is bad.

It is only 2.5 armstrong, 3.3 armstrong, or something like that. So, precisely, you cannot compare some bond distances like these two bonds which are having some doubt that whether the second oxygen is directly coordinating to that particular zinc center or not, or it is at some longer distance. That means it is weak link. Neither a bond, but it is occupying the position.

So, that is the case for the third example for thermolysin. So, if you look at these three figures, and if you know the coordination environment, then you can only comment that you see how much important is your the coordination environment, the coordination number, as well as the geometry around the zinc center for the three different types of reactivities.

So, how you can change or modulate the reactivity pattern from carboxypeptidase A to carboxypeptidase B to thermolysin two are your exopeptidases, and the third one is your endopeptidase. So, the selectivity in terms of your coordination environment can gives all these informations, and if you consider these two are extremes for the carboxypeptidase A and B, one having a coordination number of 5, another having a coordination number of 4. And what about your then thermolysin?

It is neither 4 nor 5, but we are showing some dotted interactions, that means the distance is long. So, if you have a dotted interaction, you can consider a coordination number, which is the fractional one. So, you have not read it in your book, but your ideas would always be there, how you can remember it, how you can understand it. So, consider the thermolysin coordination environment around zinc is 4.5 then.

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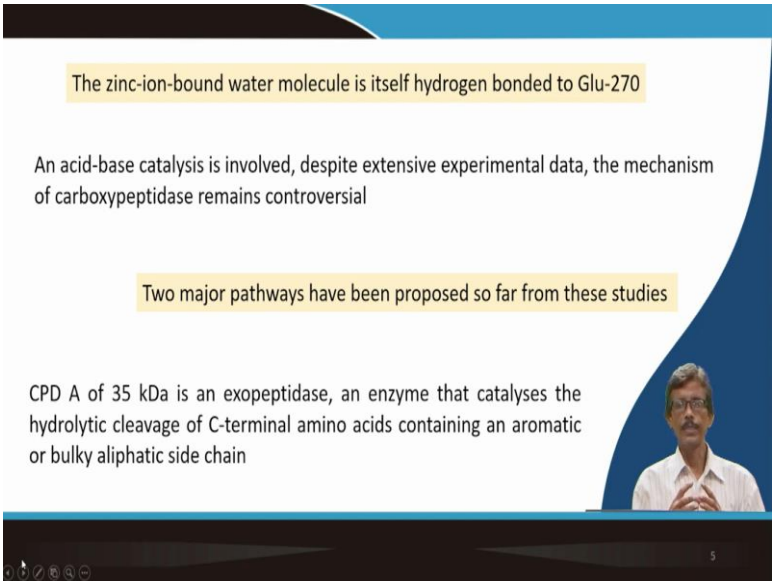

So, what we find then that you have a zinc center, and you have bound water molecule, and that water molecule has to be stabilized for that through secondary coordination interactions with the glutamate 270 which is further apart. And we will be looking for, as we have seen earlier, that either you can have the lewis acid base reactivity or the bronsted acid base reactivity with respect to that of your bound water molecule or hydroxide ion or the zinc center.

So, these acid base catalysis is well known, structural, it is also prove the environment, and what we are getting out of that reactions that also we know. But the detail mechanism, the stepwise mechanism is still it is controversial. Many people propose one type, and the other people also discard that and propose a new type.

So, basically during all these years, the two major pathways have been established so far. But now the theoretical justification, the theoretical calculations, all these things basically support only one type. So, here we will only discuss that particular type of mechanism where you can have certain type of all these things, the activities and all, but it is a very small molecule. You see, only 35 kilo dalton molecular weight of this variety of exopeptidase and basically responsible for the C terminal amino acid.

That is why it is carboxypeptidase CPD A. C, immediately you can think of as the C terminal amino acid containing aromatic or bulky aliphatic sidechain. If you have a aromatic, so selectivity you are further increasing with respect to the nature of the substrate molecule. If your substrate molecule does not have the bulky aliphatic chain, or bulky aliphatic groups like the tertiary butyl group, or the aromatic ring like phenyl ring, there enzyme will not be so much effective or rate of the reaction will be less.

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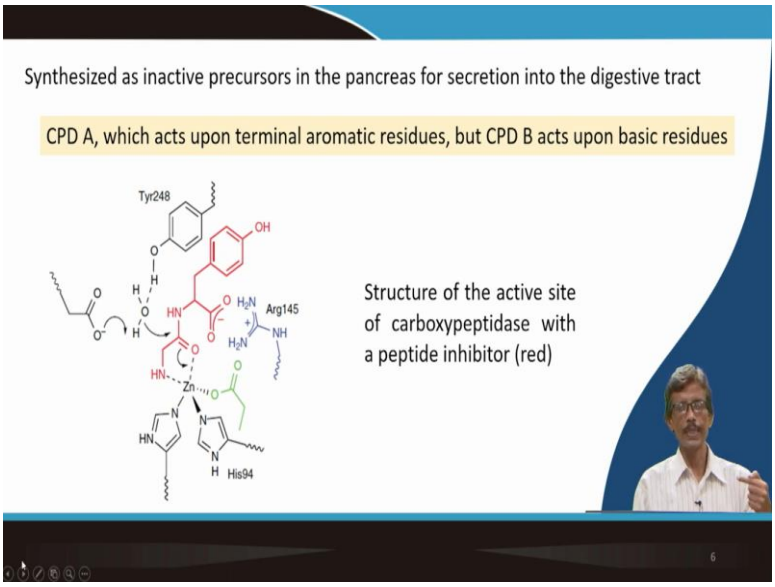

So, is basically where we get this, we are very much dependent on it. If there is any problem, we are having trouble with this because they are the inactive precursors, as when they are synthesized for the first time in the pancreas for secretion in the digestive tract. So, when it moved from pancreas to the digestive tract, when it is coming in contact with all these protein chain molecules or the peptide molecules for our digestion of the food molecules, they immediately convert it to either CPD A or CPD B.

So, CPD A is basically working on aromatic residues, but CPD B is acting on the basic residues. So, these are the differences, basically, because both of them are the carboxy end controlling peptidase molecule. So, the basic thing what we have seen so far that environment around zinc.

Now, if you try to elaborate a little bit from that particular point, that if you go from there that what you get in terms of your corresponding structure, you will find that, but you remove the water molecule, and sometime the inhibitors are there and try to grab the inhibitor there and have the structure because it is sometimes is very easy to get the corresponding crystallization and the structure.

Now, you see the red molecule which is your peptide inhibitor, so which is either sitting, inhibiting the reaction, or sometimes, if the reaction goes with that, the water molecule which is now further apart from that particular bound form, and that will allow a nucleophile and activated nucleophile, and that will attack your CO function of your amide bond.

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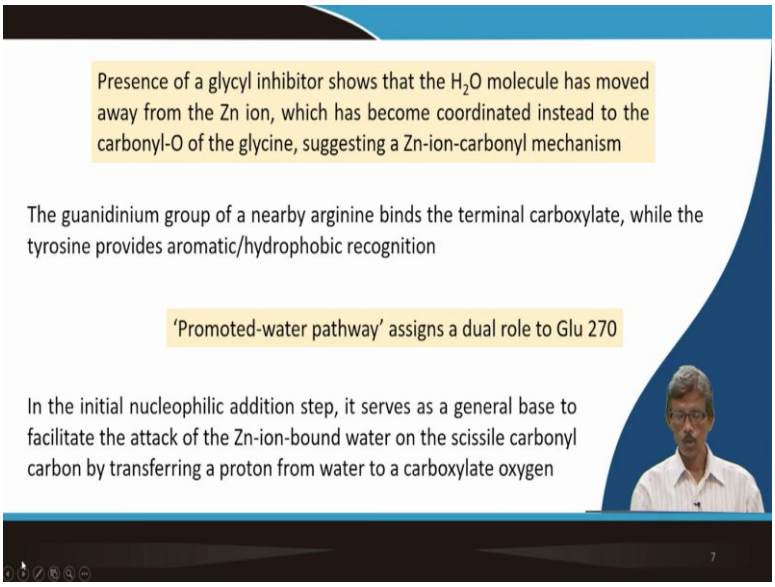

So, the glycine inhibitor basically shows that you are moving the water molecule from the zinc ion, and it is now, instead coordinated to the carbonyl O of the glycine or any other peptide molecule so the carbonyl coordination is therefore important, and the water molecule which is taken away from the zinc center will now be your good nucleophile and definitely it will be activated.

Then, from the origin inside you have the guanidinium growth, and which can also be useful for tracking the carboxylate end of the molecule, which we are going to clip. So, out of these two, one is the nucleophilic path, one is the promoted water pathway, and can have the dual role for your glutamate 270 molecule and the glutamate 270 molecule is important. It is functioning as a gel base because the glutamate is the negatively charged species like your hydroxide ion.

So, definitely it would be a base, and facilitate that will facilitate basically the zinc ion bond water which is your nucleophile and on the carbonyl carbon by transferring a proton from the water to the carboxylate oxygen. So, your proton from the water, whether it is bound to your zinc center or not that can be taken up by your glutamate 270, and your glutamate 270 will be protonated, but you will get a better nucleophile out of your water molecule.

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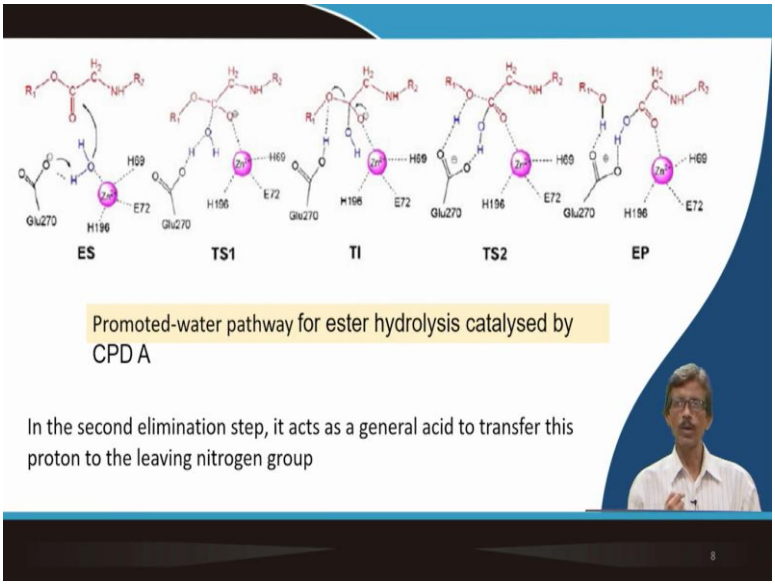

So, water molecule will be producing the hydroxide ion, and that hydroxide ion will now give all these intermediates for the products of this reaction. So, ES at the left and EP at the right. So, what is that? What is that ES and EP that we see now? ES is your enzyme bound to your substrate and EP is your enzyme bound to your product.

So, between these two, we can have some good idea from the extra structure determinations, or any other idea that we know what is ES, and we know what is EP, but how to coordinate? How we can move from this point to that point, is basically travel a journey from one point to the other, but you have the corresponding heel also, in terms of your energy profile, you have to move to that particular hill to reach that corresponding catalytic product through that of your transition state.

So, you have to excite the system, that ES you have to excite, go to the transition state, and you have the transition state one, then you can consider Ti, which is a transition level, intermediate, then another transition state which is three. So, all these three intermediates will have some transient lifetime.

People can measure it from the first spectroscopy techniques and all these things. People have measured it, people have theoretically justified these all nicely in such a fashion that you can propose these three intermediates when you move from ES to EP. So, very small changes are there, sometimes its the proton tunneling, or the proton movement is basically what we are looking here only the attack of the CO function and movement of the protons only. There is no electron transfer in all these cases.

But still, the situation can be complicated if many such other functions are coming. So, now you can think of the participation of glutamate 270, why this glutamate 270 is important, and the coordinated water molecule and the corresponding available the red molecule which we are going to cut. And at the point, you can have the intermediate that is your TI, the transient state intermediate what you can have.

So, that carbone, that carbonyl function what you can have already so that carbonyl function when you attack it by the water molecule or the hydroxide ion, that carbonyl center, which is a planar SP two hybridized molecule carbonyl C double bond O and two other bond. So, is a planar molecule. Like your carbon dioxide molecule, we are hitting that carbon dioxide molecule when you have the bond vibrations.

Similarly, this carbonyl stretching is that the bond stretching and all these things. So, you have the bending also. And at one point when it is energetically excited, that means the thermal energy is giving you the vibration, you will be able to attack that particular carbon by your nucleophile. So, it is forming that extra bond.

Now, carbon can have four bonds. So, a tetrahedral intermediate that is why is Ti. So, is the more stable tetrahedral intermediate what you can have on that particular carbon, where you are going for that particular cleavage. So, then you have the corresponding again, the product of TH two, which are having the weak interactions but already you got the clipped product that means you have transferred the OH or the oxygen atom to that particular part where you have the cleavage.

But basically, from our school days what we have learned that you have a bond, the ester bond or the amide bond, how you can hydrolyze it? You bring the water molecule, and we put the water molecule, and you add up. So, addition and subtraction reaction with that of your water molecule, molecule molecule addition, like your arithmetic addition and subtraction. So, you add the water molecule, then you go for the subtraction.

So, that water molecule, this is the function that how it attack the center, and you go for a reaction where you have the water promoted, we call it as the promoted water pathway is basically the water promoted reaction. Water is useful, but you have the control from the zinc coordination. You have the control from glutamate 270, and all these things are making that water molecule so good reagent that it can attack your peptide chain and go for the cleavage reaction.

So, in the second where you can get the elimination, so after you have still the thing is bound that means E plus product, enzyme plus the product, still it is there, but you have to release the product from there. So, how you can release that particular product from that is the thing, what is written over there in language also that during the second elimination step, it acts, also as general acid to transfer this proton to the living nitrogen atom.

So, you have the living group, so it is shown for the ester basically is a showing as the ester hydrolysis. So, instead of ester, make it amide, the same thing, or the same pathway can be followed, only instead of your OH function on the right, what you have this is OH. So, this OH function what you get there, so that OH function will be your NH function or NH2 function, that amine function what you can get on the nitrogen group.

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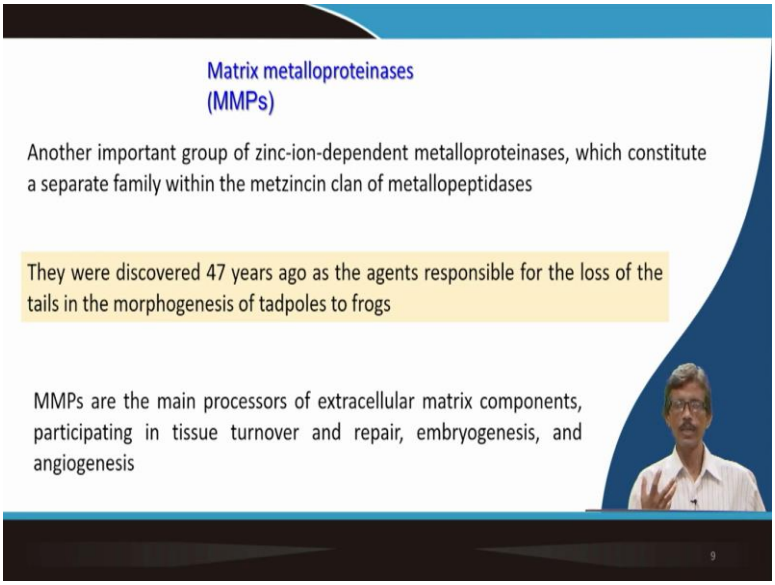

Then we come to a very important class of molecules. So, already we have seen the detailed thing, or the detail characteristics of all these molecules, where we see that sometimes these metalloproteinases can do some extra role, which we can call as your matrix metalloproteinases. So, these matrix metalloproteinases are something different.

What we can see now that is another important group of zinc ion dependent metalloproteinases, is not your carboxypeptidase A or B type, or the other type what we have discussed just now, but is a separate family within the met gin cin clan of the metallopeptidases. So, it is the clan, it is a family of that particular molecule.

Metallion is a zinc, and is that met zincsin that is why there is some nomenclature people used it, and it has also discovered 47 years back, that how we can use these for thing, and

how it was discovered that is historically very important. Informations people identified these as the loss of the tails of the tadpoles of two frogs. When we move from tadpole stage to the frog stage, we know that they are losing the tails.

So, this particular morphogenesis is catalyzed by these metalloproteinases. So, you see that visibily we can see, we can follow that the tail, we are losing, but the thing which is catalyzing this thing is your MMP, one of the MMP is important. So, it is also important for doing many other reactions, like that of your extracellular matrix component is not within cell, outside the cell also.

So, the concentration of these MMPs will be very high outside the cell. And within these matrix components, when we have that issue, we have the collagen and all these things. So, it can be useful also for participating in tissue turnover and repair. Not only it is clipping or cutting the collagen molecule, but it can also go for the growth or new collagen formation, that means the repair work because in our body also we can have some injury, we can have some accident, but we need to repair those things.

So, how these things or these informations can be useful from medicinal point of view also that you require again the zinc dependent enzymes, there right concentrations for this repair type of work is maintenance because our body is a very complicated thing, and regularly we should have the maintenance, but we do not go to any workshop like your cars, what we give it to the workshop for the repair work or the maintenance work.

You have to maintain all these things yourself, but the body can take care of all these things, only certain basic principles, rules you have to follow and laws you have to follow. So, not only this turnover for these tissues and the repair work, but also embryogenesis and angiogenesis, the new blood vessel formation. The angiogenesis is the new blood vessel formation so they can also take part over there.

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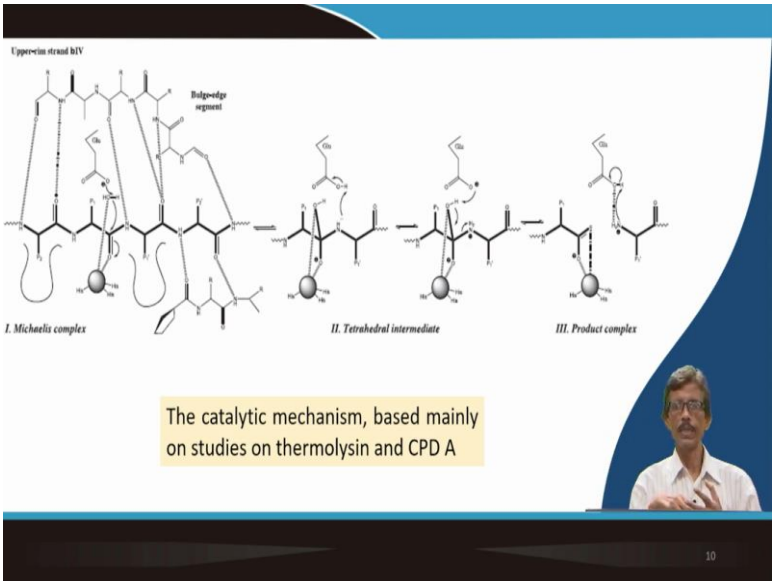

So, do not worry about the very big picture, it is taken from the Crichton's book again, but is a good example that what are the things is basically happening when you have a catalytic mechanism to write, because it is the collagen, or the extracellular matrix. So, your molecule itself should be very big, very long one. So, long chain, you can have these, and is basically whatever information we have gathered so far in terms of our understanding on carboxypeptidase A, as well as on thermolysin so the zinc environment.

So, the same zinc environment we can bring over here in the left. So, in this left figure, we find that you have the three bonds on the zinc. So, this is the bond, and you have the zinc so that you can bring from one point to the other.

Now, bring to the long chain and long chain is there, you can have certain component but which is forming a Michaelis complex. So, in terms of some scientists' name basically, the Michaelis Menten reaction we know, the equation we know, and that Michaelis complex is forming so this aggregation or the assembly from the metalloenzyme, other parts, and all these things are there.

So, you can have more number of these all peptide chains, but we are looking on some part, where we will be going for again the tetrahedral intermediate in the second step the carbon is giving four bonds, four black, dark bonds which are your tetrahedral intermediate, and then from the top basically some small group is coming again like the glutamate thing is coming. So, base is coming. So, that will control your corresponding the activity of the OH for that particular purpose.

So, OH is attaching carbon, but still it is have the control on the zinc. So, zinc is basically controlling the nucleophile. So, the control of the nucleophilic character of the zinc and it is three dimensional positioning is important. That's why you have to have the corresponding tetrahedral intermediate, and that tetrahedral intermediate you can maintain.

Finally, you can have the corresponding proton transfer. You are that corresponding controlling species, you takes up that particular proton from that particular hydroxide O function, and it is happy with that protonation, but at the same time that oxygen is being delivered to the cut molecule of your peptide chain.

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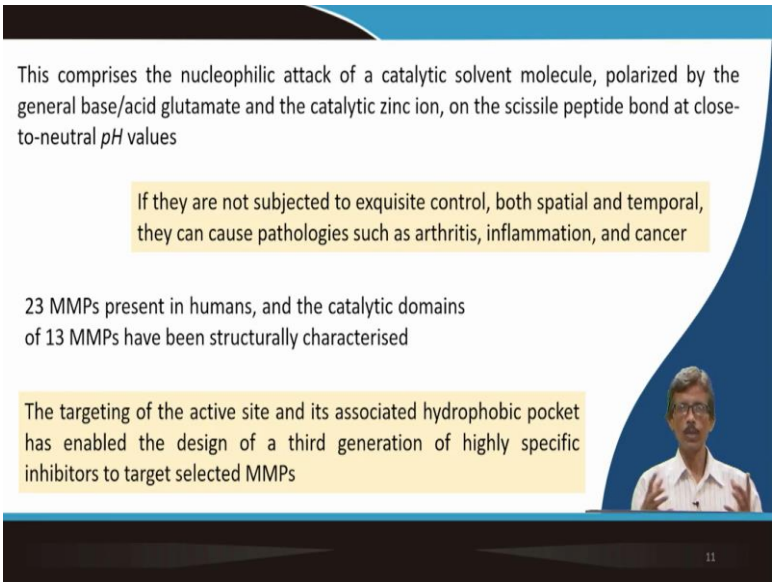

So, is all will have the nucleophilic attack, and the general things what we can have for all these. So, read it nicely for all these things, the spatial orientation of all these, and the different levels. So, already we have what we have seen that already 23 MMPs have been identified in our human body also. And the catalytic domains of 13 MMPs have been structurally characterized.

So, its well known now, is a very huge area, well developed area now, and it is also pretty old. So, it is around 50 years old story for all these things such that you can have some target molecules, such that we can think about the corresponding thing where you can use the zinc. So, why we take zinc? Why we have must have the zinc?

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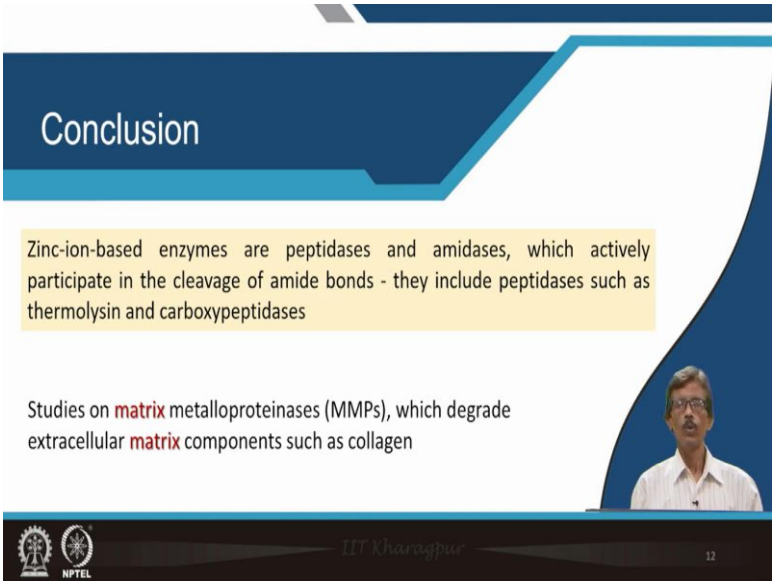

So, in our body, a 70 kilogram weight of any person can have only two grams of zinc in its or her body, but those are functioning so amazingly that you can have for all these hydrolases of the amide bonds, the peptidases, the cleavage of the all these things. And these two important molecules, or the groups of molecules thermolysin and carboxypeptidase, and in case of your MMPs what we have seen that if we have the collagen molecule, which are nothing but your matrix component and the corresponding cartilage, collagen, and all these are there for holding your bones in right position, and if there is any damage, you can repair it, all these things. But all these things, so again, you can have some zinc dependent drugs when you are under treatment.

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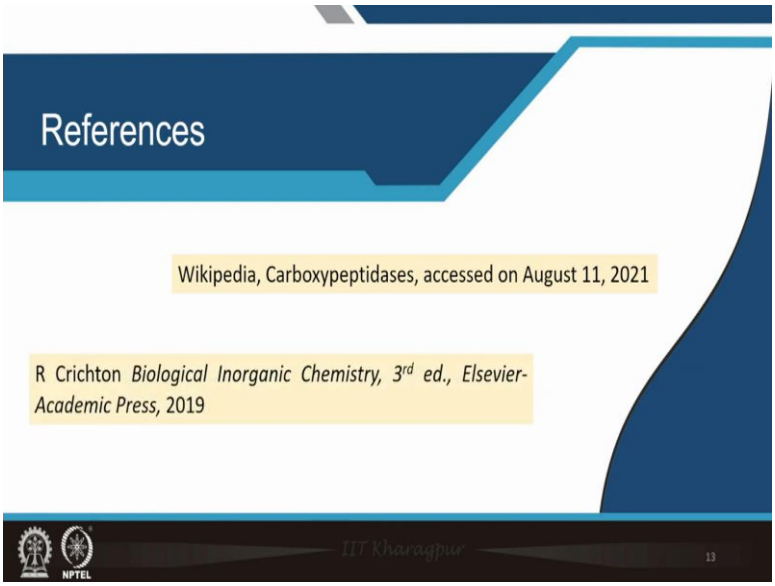

So, you just start with you read the carboxypeptidase page on the Wikipedia, and the book of Crichton. Okay, thank you very much for your kind attention.