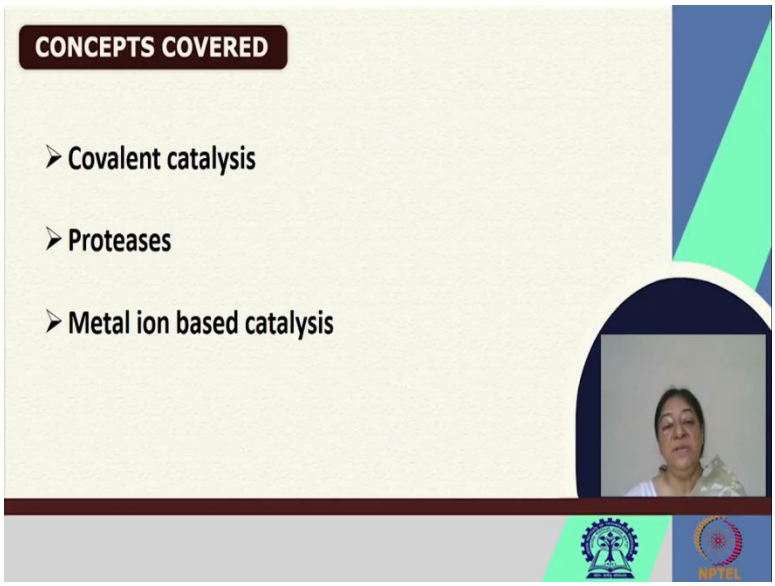
Fundamentals of Protein Chemistry
Prof. Swagata Dasgupta
Department of Chemistry
Indian Institute of Technology, Kharagpur

Module - 06
Enzymes and Enzyme Mechanisms
Lecture - 30
Enzyme Mechanisms - III

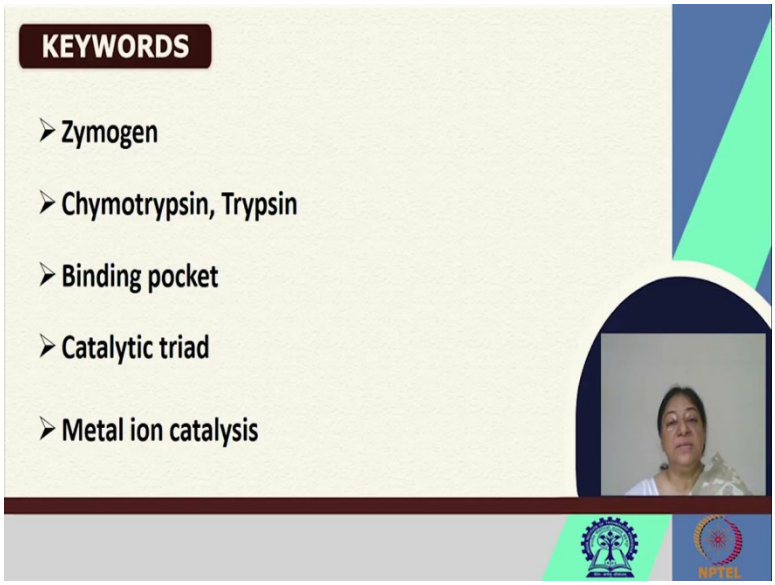
In the last class of this module on enzymes and enzyme mechanisms, we will be looking at specific enzyme mechanisms in terms of the types of catalysis possible.

(Refer Slide Time: 00:26)



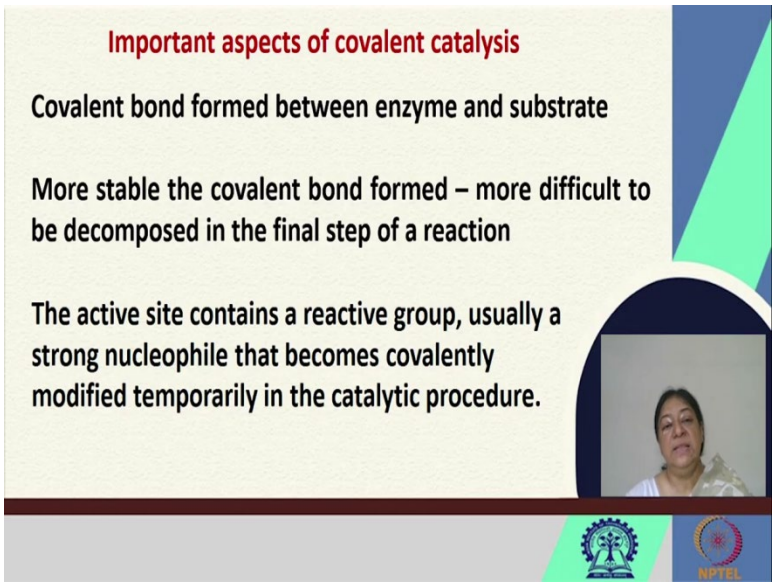
In our previous lecture we looked at acid base catalysis and we also looked at the several enzyme classes and the specific reactions that they catalyze. In this lecture we will focus on covalent catalysis in the example of proteases and metal ion-based catalysis.

(Refer Slide Time: 00:49)



Here we will look at specific proteins like zymogen, trypsin, chymotrypsin, their binding pockets, what we mean by a catalytic triad and metal ion catalysis.

(Refer Slide Time: 01:04)



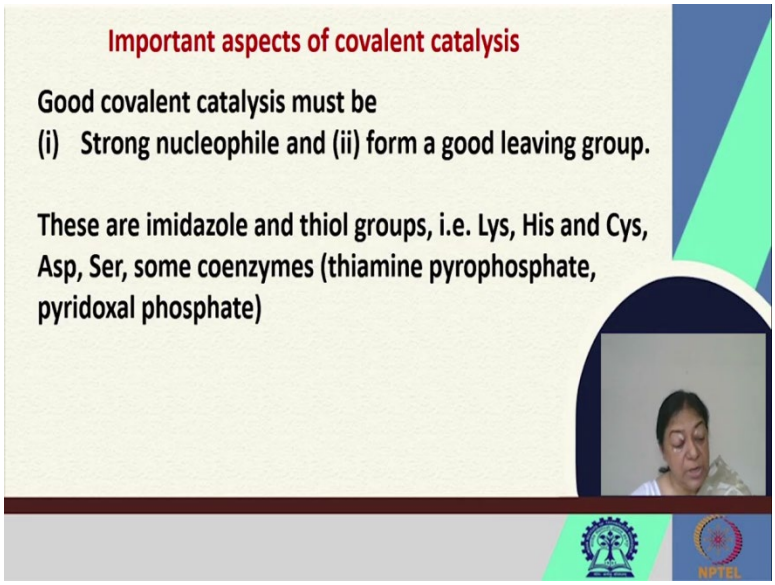
The important aspect of covalent catalysis indicates that there is a covalent bond formed between the enzyme and the substrate as the name implies. The presence of a covalent bond indicates a very strong bond as opposed to the other non covalent interaction types, in terms of where we are looking at acid base catalysis in the sense of the formation of a hydrogen bond or a proton donation or a proton acceptance.

A proton-donor acceptor system or an acid-based system, where we have the histidines, as we saw in the previous lecture, where we do not have any covalent bond formation. So we

understand that the presence of a covalent bond means there is a strong connection between our enzyme and substrate.

The process has to be such that this bond has to be cleaved for the enzyme to restore its original conformation for the active site, so that it can then bind another substrate. The active site in this case is in the enzymes of the examples that undergo covalent catalysis. They have a reactive group that is usually a strong nucleophile, that temporarily becomes covalently modified in the catalytic procedure.

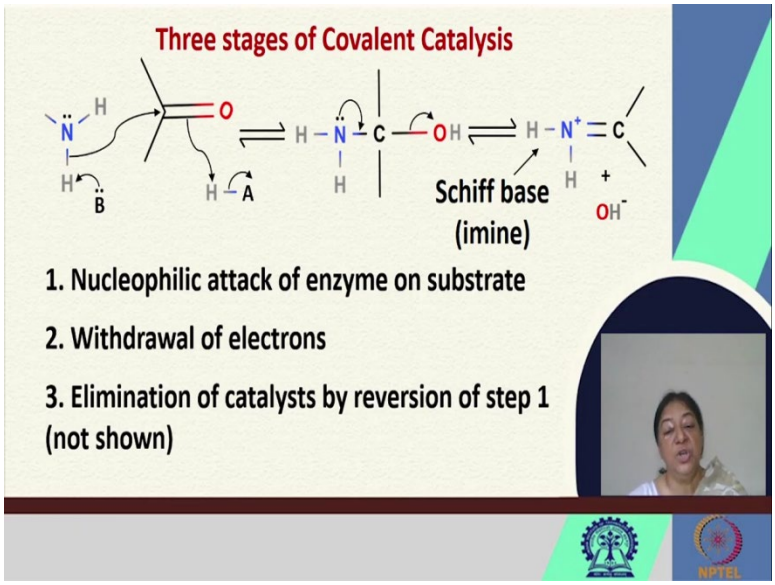
(Refer Slide Time: 02:46)



The important aspects of covalent catalysis, indicate that there has to be a strong nucleophile and it also has to form a good leaving group, because we do not want the bond to be too strong so that it cannot be broken in the catalytic procedure.

In this case also we have imidazole and thiol groups, and specific coenzymes like thiamine pyrophosphate and pyridoxal phosphate.

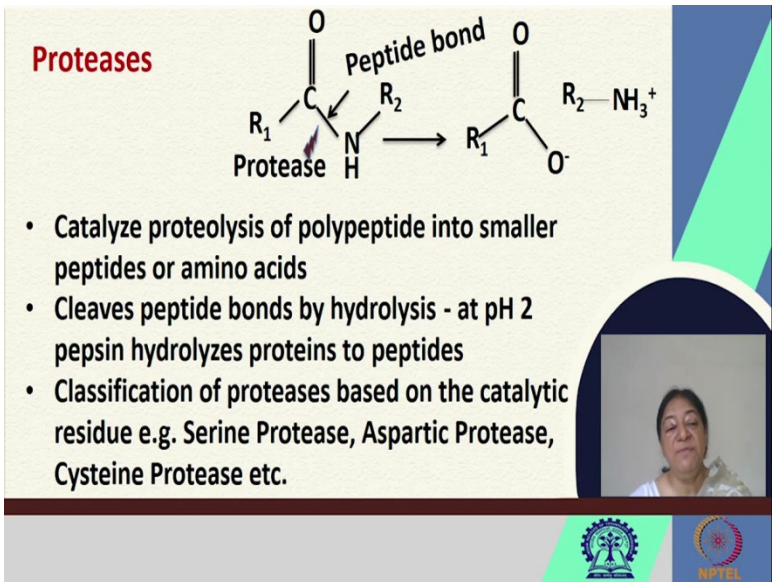
(Refer Slide Time: 03:18)



There are three stages of covalent catalysis. In the first stage [refer to slide] we have a linkage and then we have a covalent formation, then subsequently we have to have a release. There is a nucleophilic attack of the enzyme on the substrate that is going to result in a covalent bond formation. And there is going to be elimination of catalysis by a reversion of step one, which is not shown in this particular indication.

The process here is that we have a covalent bond formation, followed by which there is a catalytic reaction. And there is going to be a cleavage of the covalent bond that was initially formed to bring back our enzyme to a position, where it is able to bind another substrate.

(Refer Slide Time: 04:16)



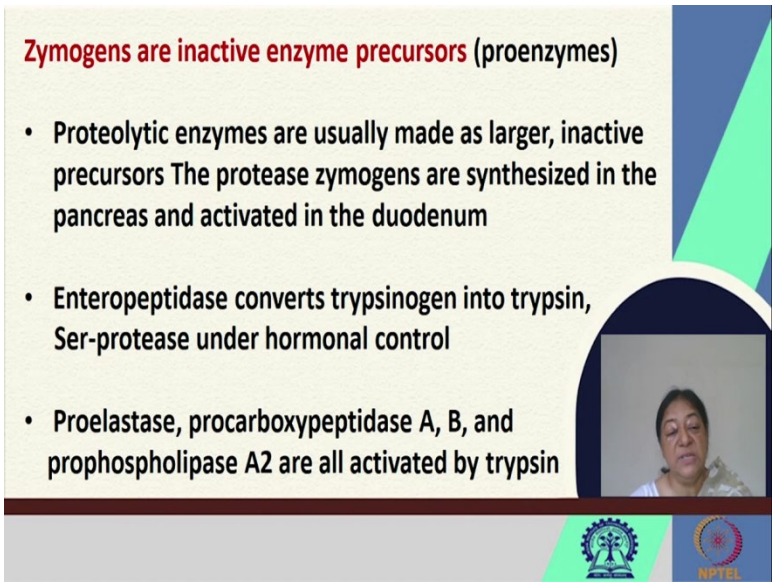
The examples of covalent catalysis are mostly shown in proteases. Now proteases as the name implies, catalyze the proteolysis that is the cleavage of polypeptides into smaller peptides or

amino acids. This indicates the cleavage of the peptide bond. This cleavage is possible through several enzymes.

For example at pH 2, pepsin is able to hydrolyze proteins to peptides. Then the classification of these proteases comes from the specific catalytic residue that is involved in the covalent catalysis.

In a specific example of proteases, we have the cleavage of the proteolysis of the peptide bond.

(Refer Slide Time: 05:18)



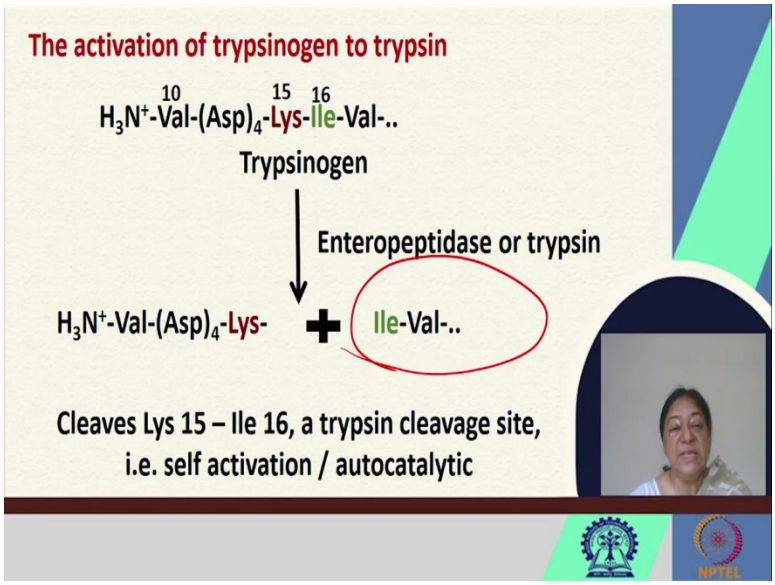
We realize that if there are such proteins that have the capability to break a peptide bond, they are actually detrimental in their active form, because of the numerous proteins that are there. Which would mean that these would go about their job of proteolysis.

Nature has devised a method for this, where there are zymogens that are inactive enzyme precursors called proenzymes. That is an actually inactive form of the specific enzyme that has a proteolytic nature or a protease. The proteolytic enzymes are therefore made as larger inactive precursors.

We realize it would not be a good idea to have them as they were, without being inactive. If they were active, they would break the peptide bond. So, the protease zymogens are synthesized in the pancreas and they are activated in the duodenum.

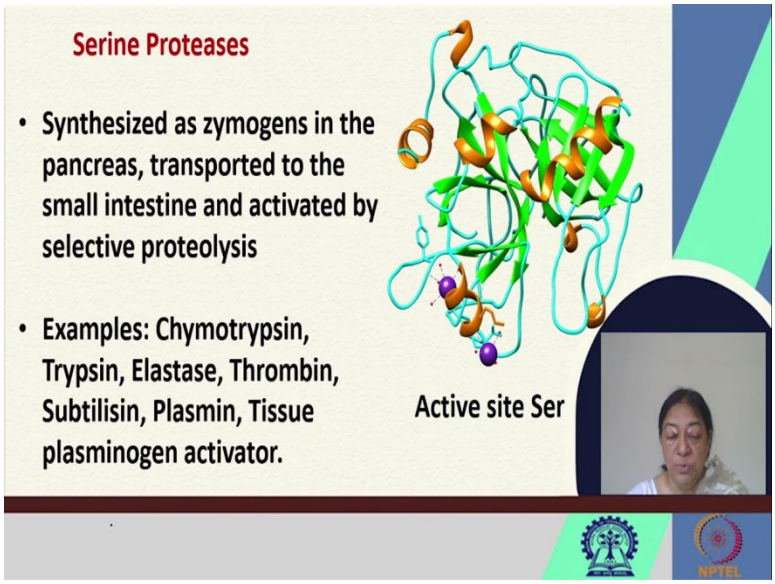
For example enteropeptidase is one such protein that converts the trypsinogen into trypsin. That is the serine protease under hormonal control. Then we have proelastase, procarboxypeptidase, prophospholipase, all of these are proenzymes.

(Refer Slide Time: 06:50)



And the way they act has been shown [refer to slide] as an example here in the activation of trypsinogen to trypsin. So we have trypsinogen. There is a cleavage about a specific bond, that will result in the formation of another enzyme that is going to be important for our catalytic process. This can be a self activation or an auto catalytic process.

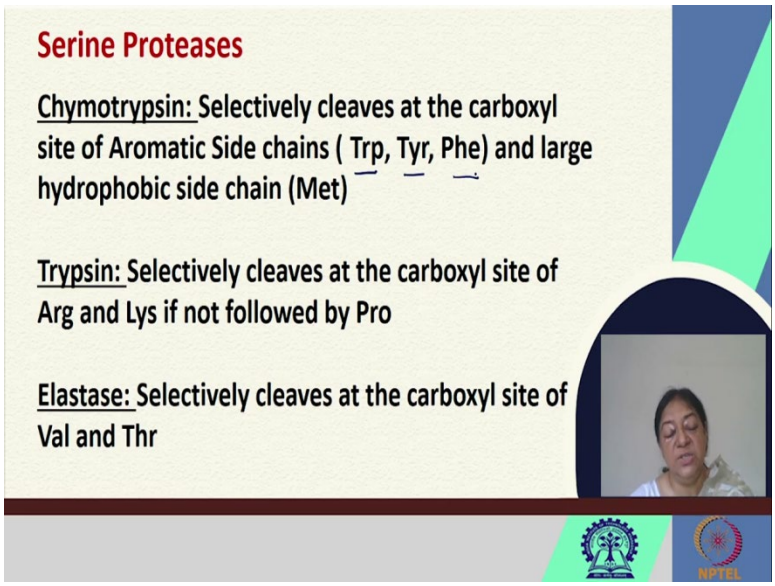
(Refer Slide Time: 07:24)



We look at serine proteases where the active site is serine. As we mentioned the terminology of the proteases is based on the type of covalent bond formation that occurs.

So this [refer to slide] is an example of a serine protease, the chymotrypsin molecule they are synthesized as zymogens in the pancreas, then they are transported to the small intestine and activated by selective proteolysis that is going to give rise to the enzyme of action, as we had seen before in the zymogen action.

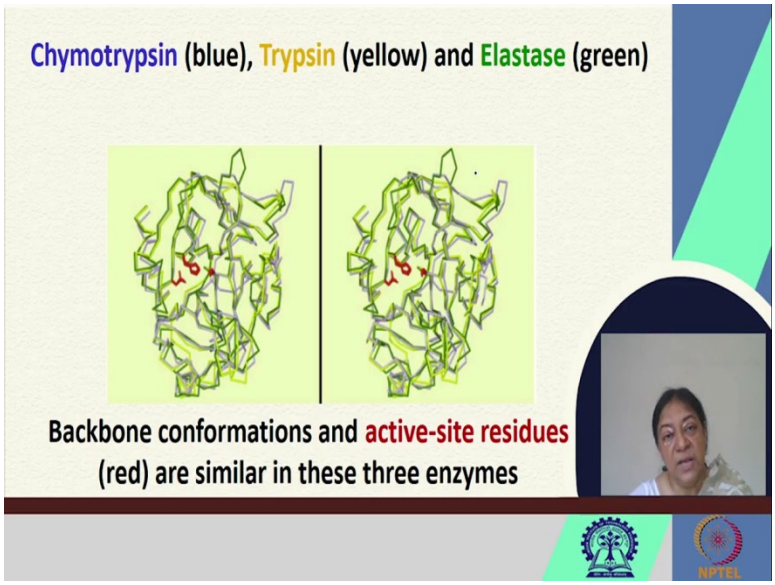
(Refer Slide Time: 08:15)



Now the serine proteases, that is the chymotrypsin, as like any other enzyme has substrate specificity. The substrate specificity in terms of chymotrypsin, is that it cleaves selectively at the carboxyl site of aromatic side chains. We have tryptophan, tyrosine and phenylalanine. Chymotrypsin has the capability of cleavage at the carboxyl end of the aromatic residues.

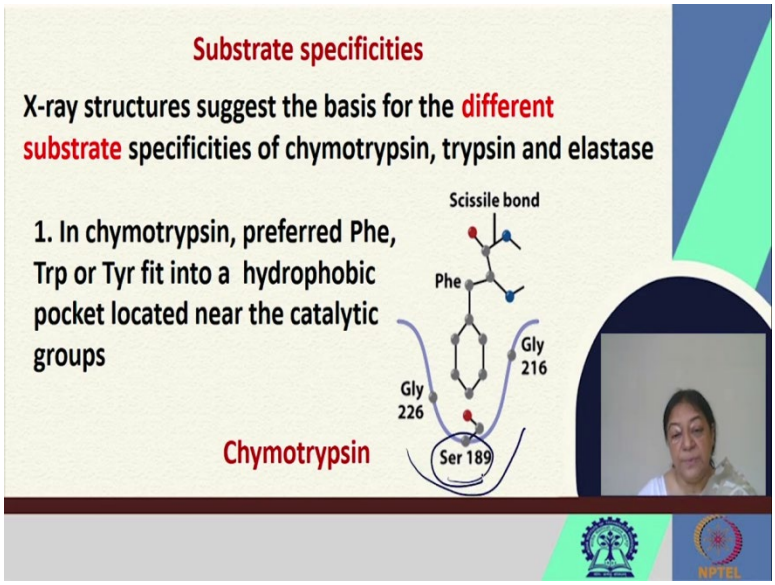
Trypsin selectively cleaves at the carboxyl site of basic amino acid residues; like arginine and lysine, provided it is not followed by a proline because we know that proline is an imino acid and the orientation of the backbone may be in a manner that cannot be bound to the active site of trypsin. Similarly, elastase selectively cleaves at the carboxyl site of small residues. In this case valine and threonine.

(Refer Slide Time: 09:31)



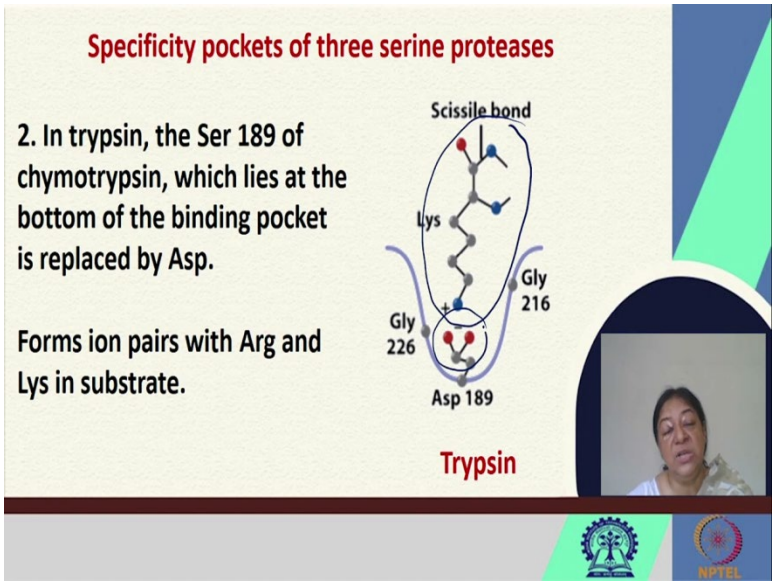
If we look [refer to slide] at the backbone conformations and the active site residues of these three enzymes, we will see chymotrypsin shown in blue, trypsin in yellow and elastase in green and the active site residues in red. As we can see the disposition of the active site residues is the same because their functionality is the same in the cleavage of the peptide bond.

(Refer Slide Time: 10:00)



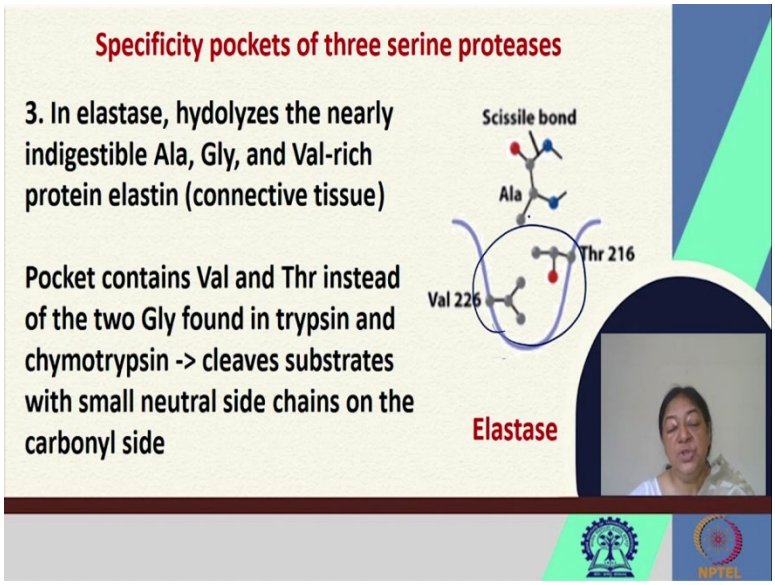
Now to look at substrate specificities. This was established by x-ray structures that established the specificities of the three enzymes that we spoke about. Where we would have chymotrypsin which had a capability of cleavage after the aromatic side chains, which would indicate that it would fit into a specific hydrophobic pocket that is located near the catalytic groups here [refer to slide]. Here is our serine.

(Refer Slide Time: 10:33)



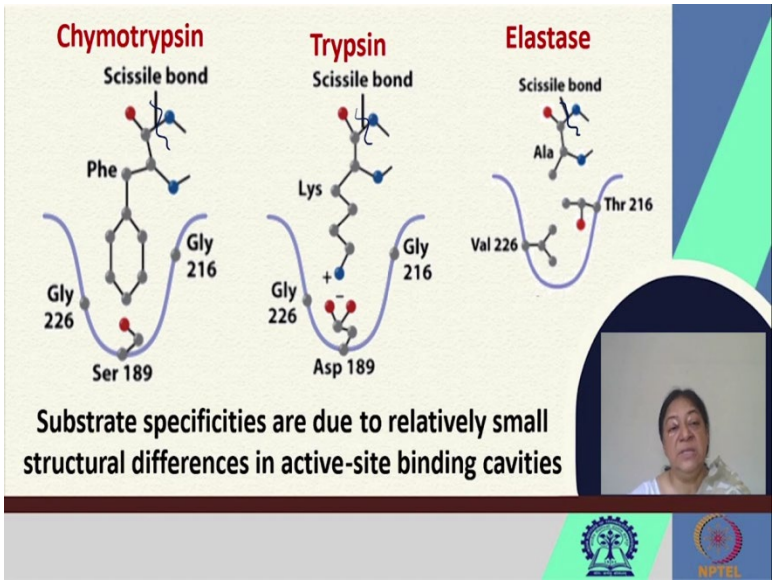
In the same way we look at the other chymotrypsin case, where we have the serine replaced by an aspartic acid here. And this trypsin molecule is preferable to basic amino acid residues in the cleavage, after the carboxyl site of the basic amino acid residues. And it has a specific binding pocket understandably an acid, so that there is an electrostatic interaction in the recognition of the substrate.

(Refer Slide Time: 11:07)



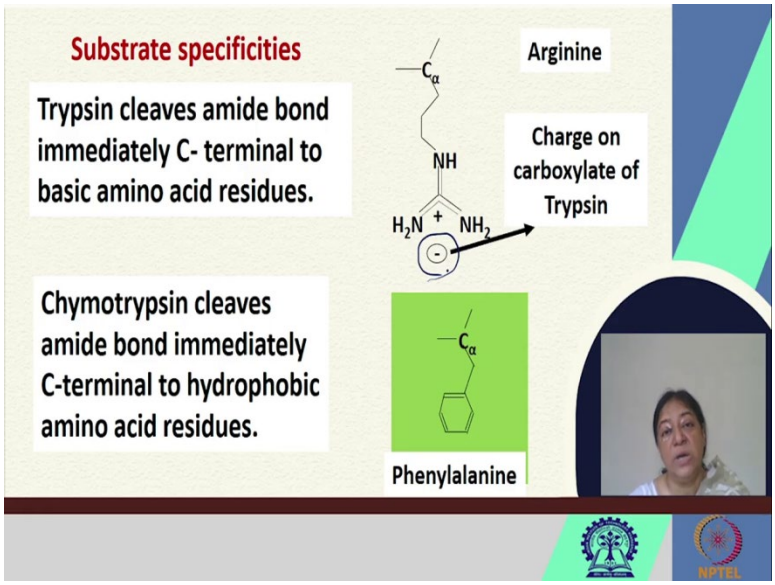
In the elastase, there are small hydrophobic molecules that align the active site, that help in the recognition or the binding of the small amino acid residues which are going to be cleaved. So this cleaves substrates with small neutral side amino acids again on the carboxyl.

(Refer Slide Time: 11:32)



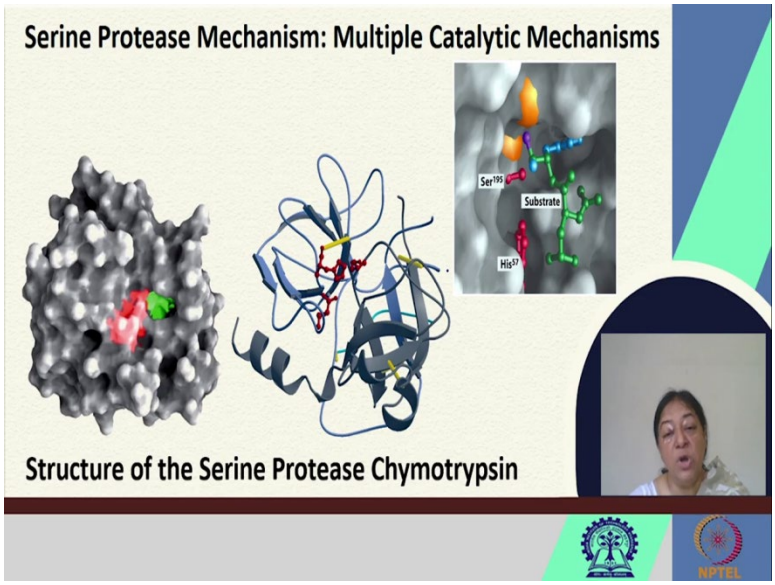
We have the specific scissile bonds indicate that these [refer to slide] are the high peptide bonds that are going to be broken in these three types of enzymes; the chymotrypsin, the trypsin and the elastase. And the substrate specificities are due to relatively small structural differences as we can see in the active site binding cavities. And we realize the importance of the recognition based on the residues that are present in the active site.

(Refer Slide Time: 12:09)



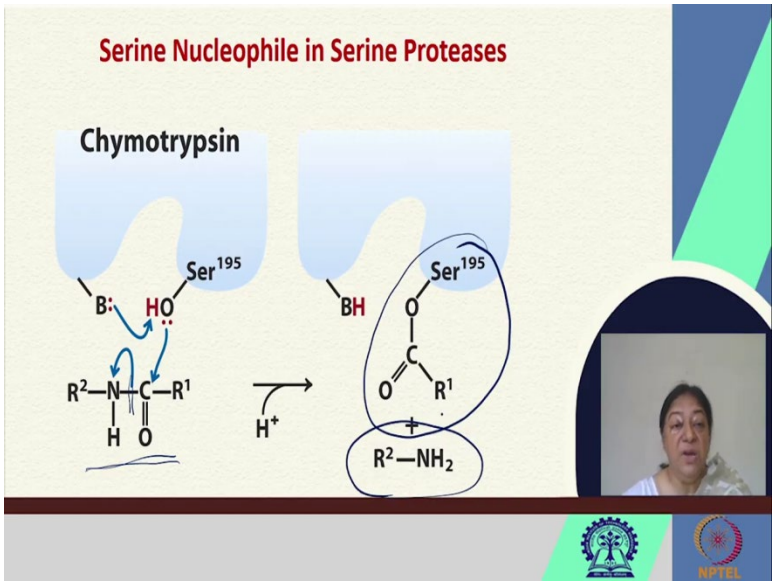
We have trypsin that cleaves at the C-terminal of the basic amino acids. And we know that this charge on the carboxylate comes from the aspartic acid in trypsin and chymotrypsin cleaves the amide bond following hydrophobic amino acids, where we have a hydrophobic pocket that is going to accommodate the substrate.

(Refer Slide Time: 12:37)



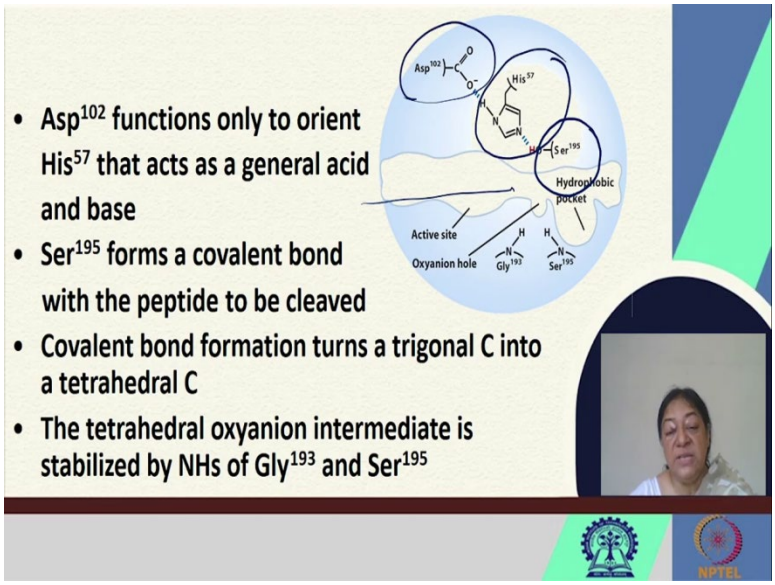
We will just be briefly looking at the serine protease mechanism, where we have the active site structures as we looked at and we know that we are going to have a covalent linkage that is going to be brought about by the serine.

(Refer Slide Time: 12:54)



Ser^{195} is the nucleophile and we have a covalent linkage formed which is important in the breakage. So, here [refer to slide] is our peptide bond that is going to be cleaved and here we have the R^2 being lost initially, the R^1 still connected which will subsequently be released.

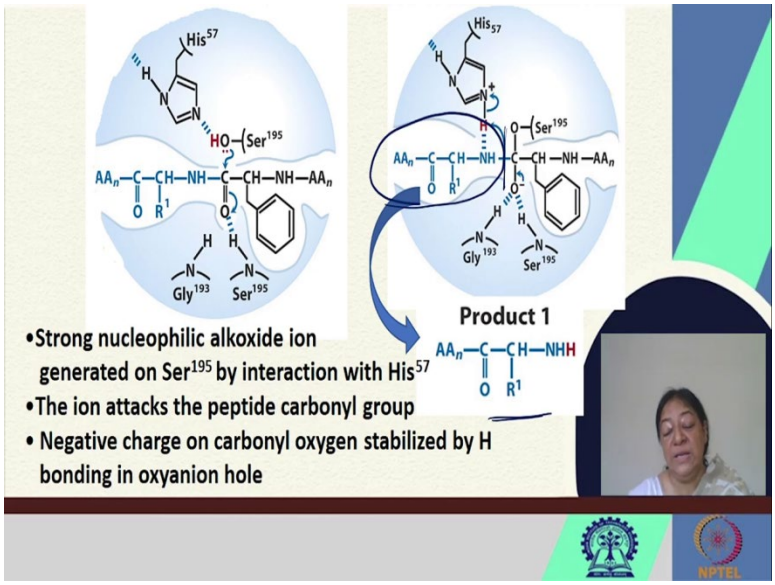
(Refer Slide Time: 13:22)



We have conditions here, where we have the catalytic triad, where the Asp^{102} acts to orient the His^{57}, that acts as a general acid and base.

The Ser^{195} is the one that forms the covalent linkage and in the formation of the covalent linkage, we have the active site and we have these orientations of the active site residues possible, where we have a stabilization of our molecule.

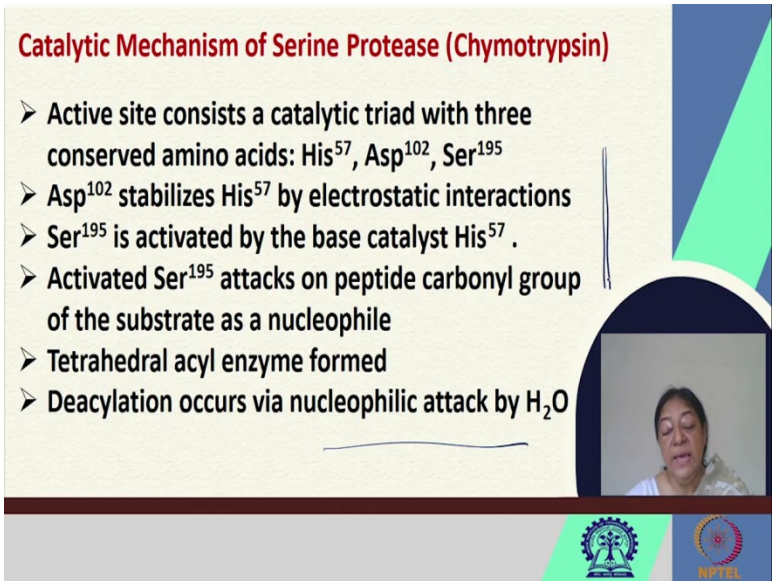
(Refer Slide Time: 14:11)



Without going into too much detail, but just to understand the beauty of the breakage and the recognition of how the molecule is able to enter the active site. There is a covalent bond formation and then we have the cleavage of our peptide bonds. So we have an initial product form, this [refer to slide] is our product form where we have the cleavage around this.

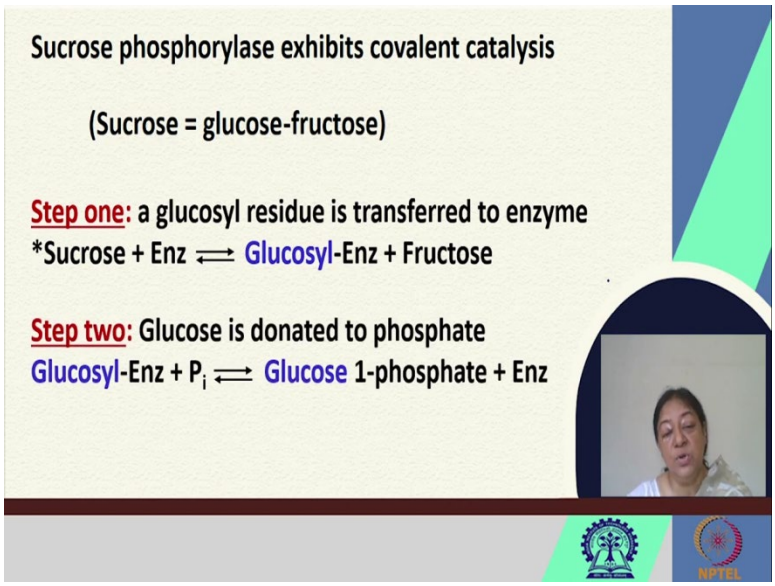
We realize that there is a catalytic triad. The catalytic triad, the importance of orientation, the importance of connection and the importance of acid base catalysis. And in this case of covalent catalysis a formation of a covalent linkage, which subsequently breaks to bring back our original enzyme, which is usually brought about by a hydrolysis or an attack by water.

(Refer Slide Time: 15:10)



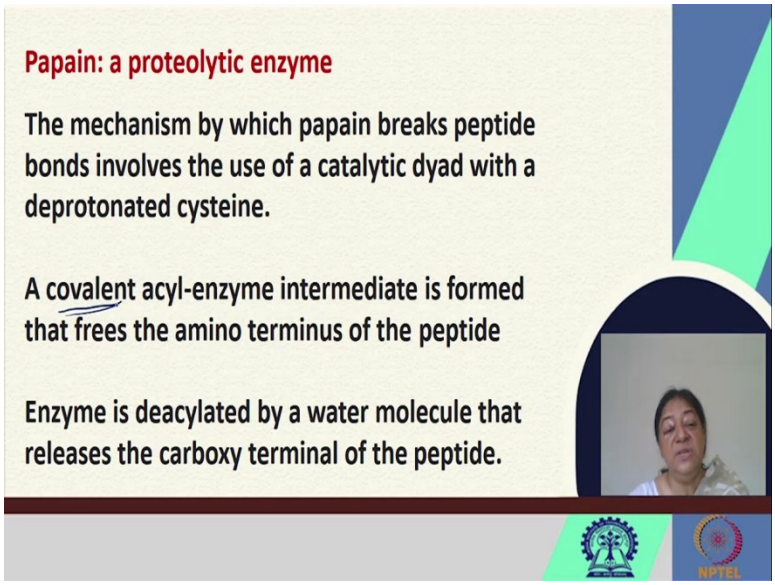
The active site in this example of a serine protease chymotrypsin; The catalytic triad has His^{57} Asp^{102} and Ser^{195}, each with their own roles to play in this catalytic reaction.

(Refer Slide Time: 15:45)



There are other examples of covalent catalysis, where we have sucrose that is a combination of glucose and fructose, where we have a glucosyl residue that is transferred to the enzyme, followed by the glucose donated to the phosphate and the formation of the product.

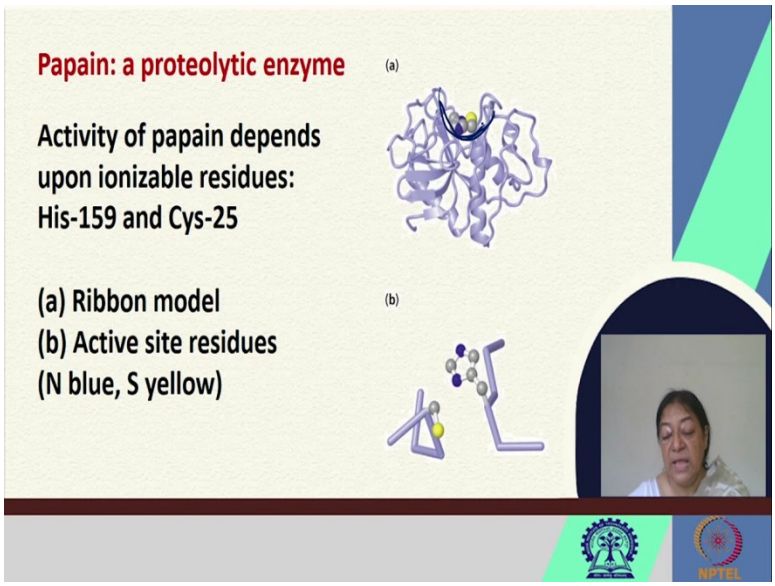
(Refer Slide Time: 16:05)



Another example is papain. This is also a proteolytic enzyme and here it uses a catalytic dyad with a deprotonated cysteine. In this case also, as in a covalent catalysis, we have a covalent acyl-enzyme intermediate formed, that frees the amino terminus. We have to realize that in a proteolytic system, we are cleaving the peptide bond; these are proteases, proteolytic enzymes that are cleaving the peptide bond.

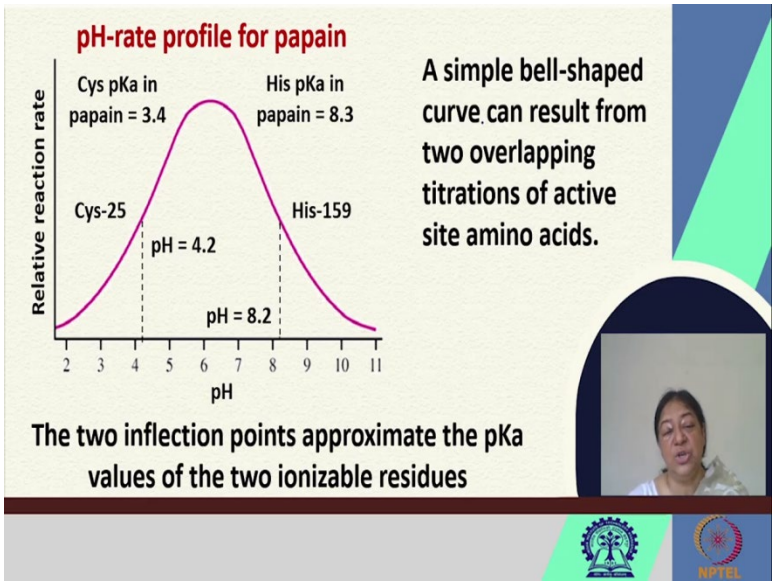
So, there are the generation of a new amino terminus for the cleaved peptide and a new carboxyl terminus. So, the enzyme is then deacetylated by a water molecule that releases the carboxy terminal of the peptide.

(Refer Slide Time: 17:00)



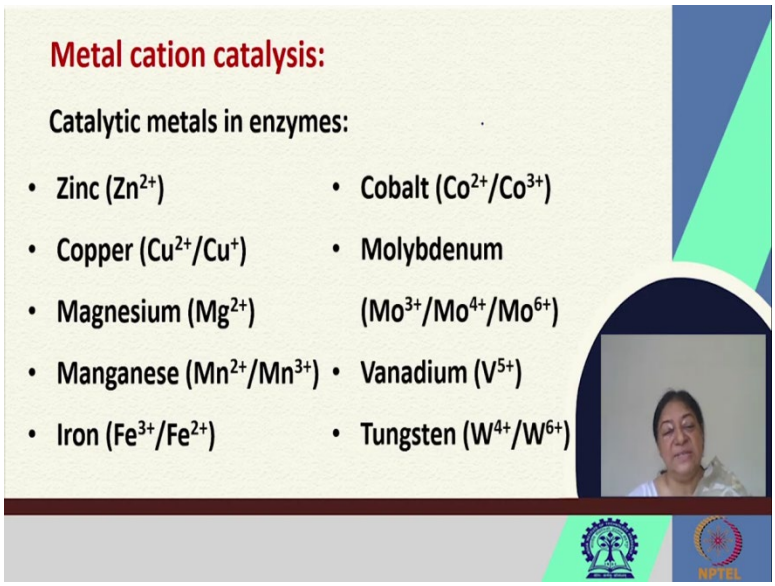
If we look at this again, we have ionizable residues histidine and cysteine. There is a specific picture [refer to slide] as in the orientation, where we have an active site cleft that can bind the enzyme and the substrate.

(Refer Slide Time: 17:20)



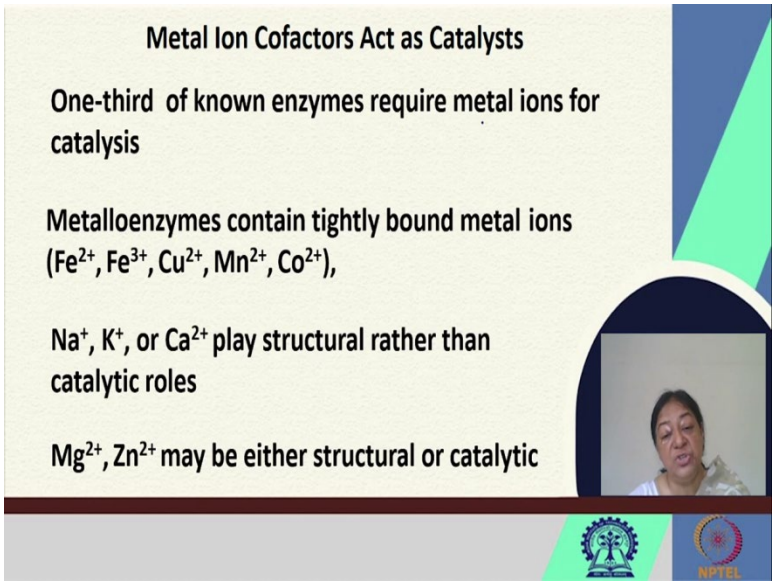
If we look at the titrations of these curves of the two overlapping titrations of the pH rate profile for papain, it is interesting to note that we have two Cys^{25} and His^{159} and the pKa changes to such a degree that allows the specific catalytic reaction tell us that these two ionizable residues are involved in this particular catalytic process, the catalytic reaction of this protein.

(Refer Slide Time: 18:04)



In our next examples we will be looking at metal cation catalysis. In the catalytic metals in enzymes there are zinc, copper, magnesium, manganese, iron and a whole list of such metals are present.

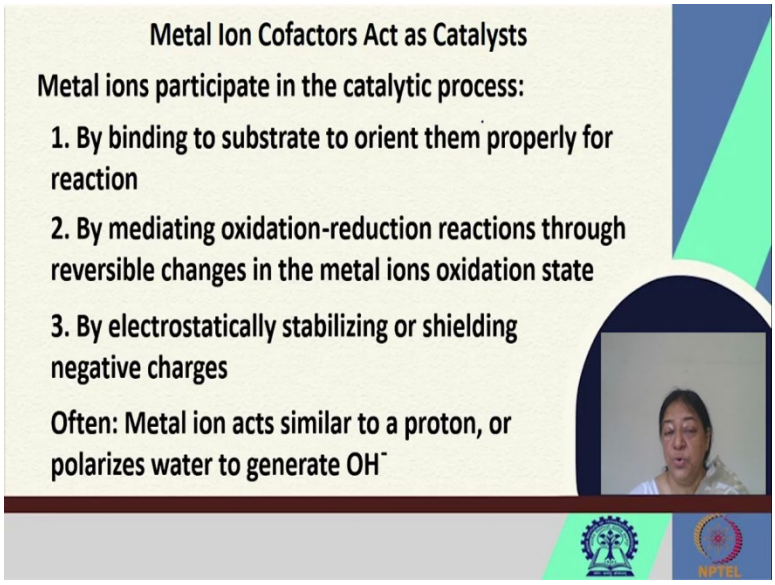
(Refer Slide Time: 18:26)



These metal ion cofactors act as catalysts. They have more than one third of known enzymes require metal ions for catalysis.

The metalloenzymes contain tightly bound metal ions. And the smaller ones where we have sodium, potassium and calcium, they play structural roles rather than catalytic roles. And we have Mg^{2+} and Zn^{2+} that may be structural or catalytic in the roles that they play.

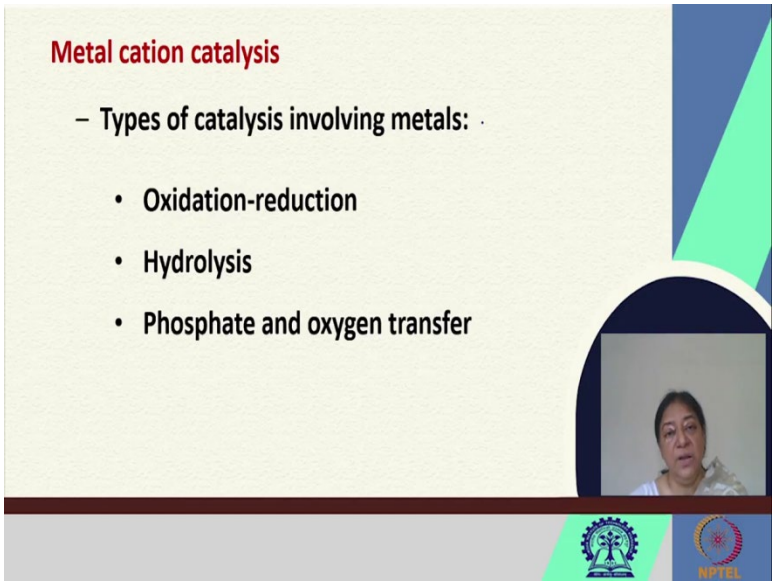
(Refer Slide Time: 19:07)



The way they participate in the catalytic process is, they can bind to the substrate in order to orient them properly, so that they can participate in the catalytic reaction or they can mediate the oxidation-reduction reactions, through reversible changes in the metal ion oxidation states. As there is iron there can be a reversible change in the oxidation states, that will facilitate the specific oxidation reduction reactions.

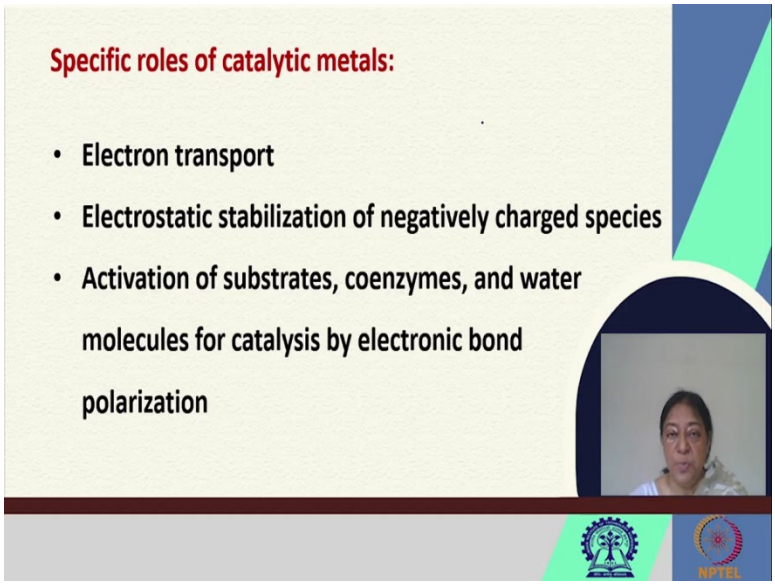
It can also electrostatically stabilize or shield the negative charges, being positive in nature and often the metal ion can also act like a proton that polarizes the water to generate OH^-.

(Refer Slide Time: 19:59)



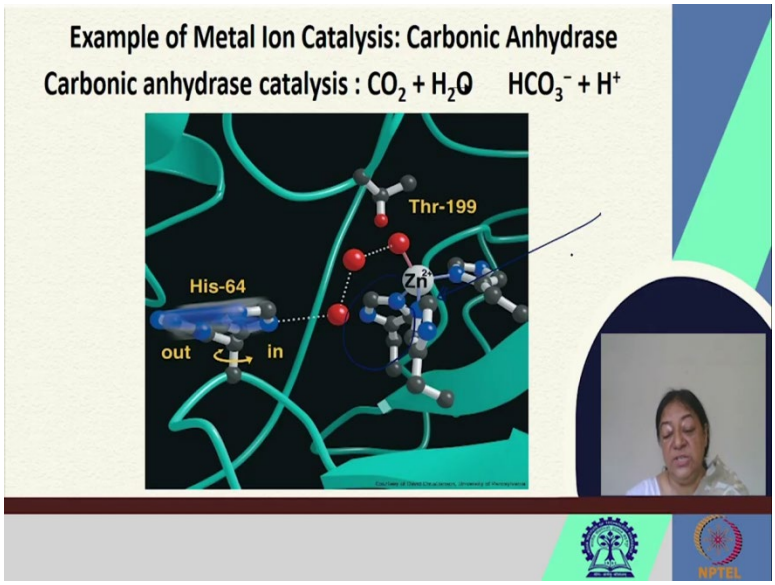
We will be looking at some examples. So the types of catalysis that involve metals are oxidation-reduction reactions, hydrolysis and phosphate and oxygen transfer.

(Refer Slide Time: 20:13)



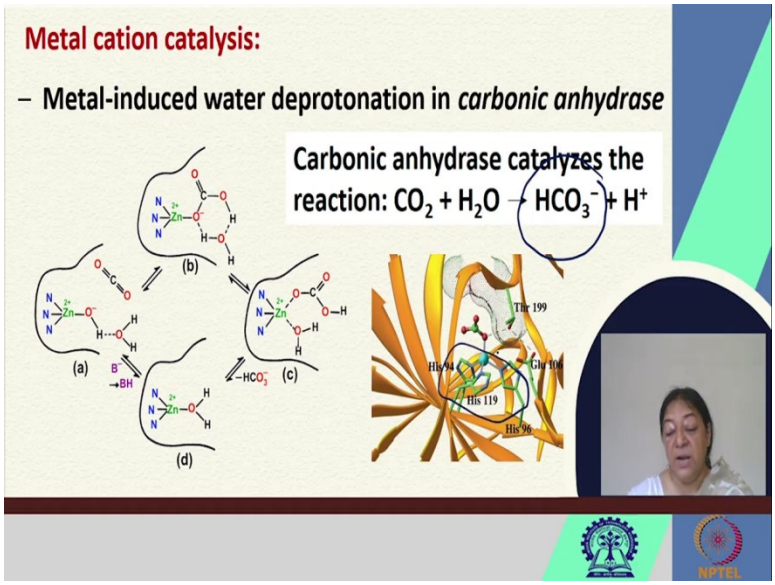
In the examples we have, electronic transport possible, electrostatic stabilization, activation of the substrates, coenzymes and water molecules for a reaction; a catalytic reaction that is going to involve a metal.

(Refer Slide Time: 20:31)



The example that we will be looking at is carbonic anhydrase, where a catalysis occurs in the formation of the bicarbonate ion. This [refer to slide] is the catalytic site of the enzyme active site, where we see the zinc ion in a specific location because of the role that it has to play and the coordination for the zinc ion, is with histidine residues; which we will see in a moment.

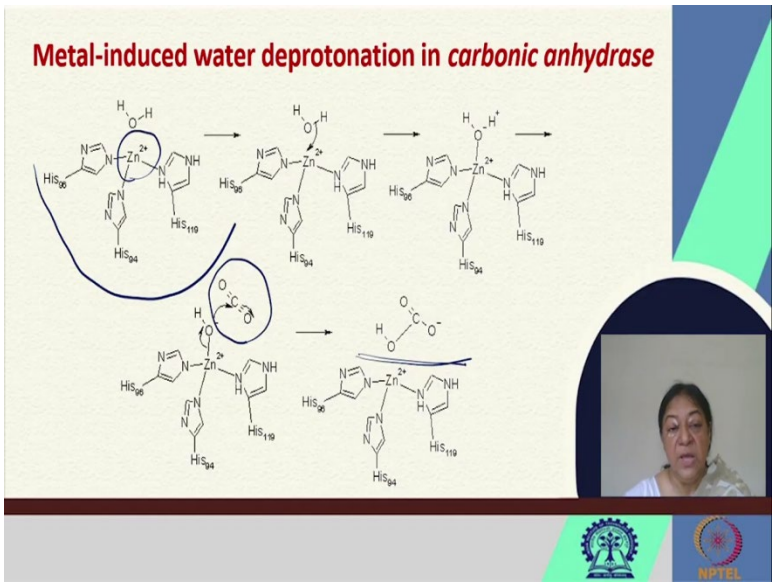
(Refer Slide Time: 21:03)



In the metal ion catalysis therefore, that occurs in the overall reaction in the catalysis, where we have the formation of the bicarbonate ion from the carbon dioxide; involves a metal induced water deprotonation using the zinc, which is the metal that is present in the active site of carbonic anhydrase.

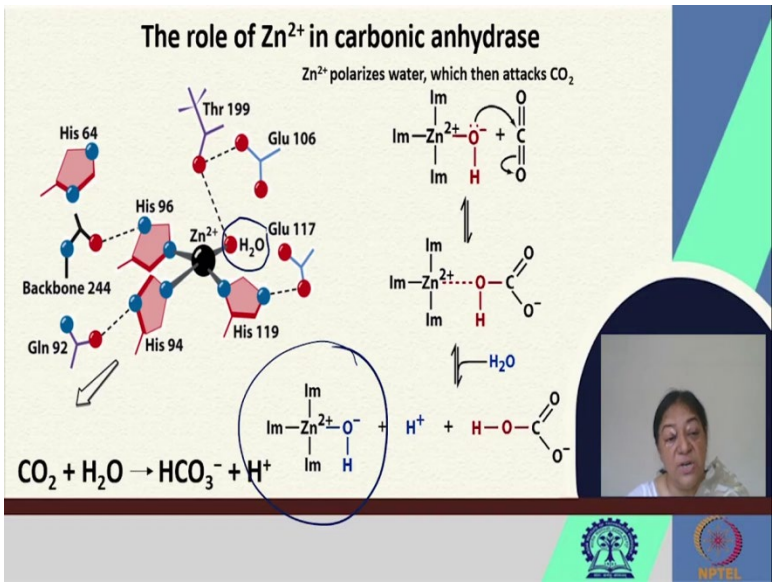
This [refer to slide] is the location of the zinc in the carbonic anhydrase and these are the histidine residues that are involved in its catalytic activity.

(Refer Slide Time: 21:38)



So, this [refer to slide] is the overall example of the catalytic cycle. These are the histidine residues that are involved in the coordination of the zinc. We have this zinc activated, we have the carbon dioxide come into the picture and the bicarbonate being released.

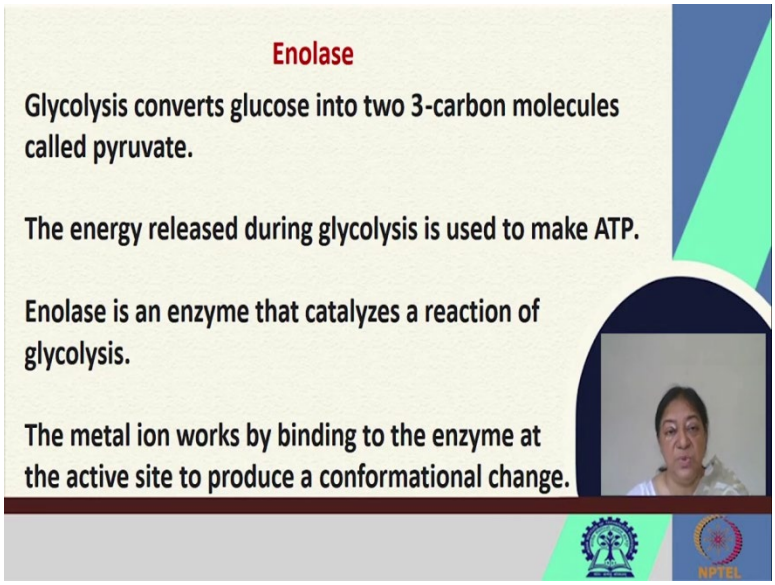
(Refer Slide Time: 22:02)



If we look [refer to slide] at the enzyme action; the role zinc plays in carbonic anhydrase. The three histidine molecules that hold it together in the linkage and it polarizes the water molecule.

The water molecule that is attached here is polarized by the zinc, that then attacks the carbon dioxide, which subsequently is linked to the Zn^{2+} in a manner, which is then hydrolyzed to form the bicarbonate ion and we have our imidazole connected to Zn^{2+}. So, this is the overall reaction that occurs.

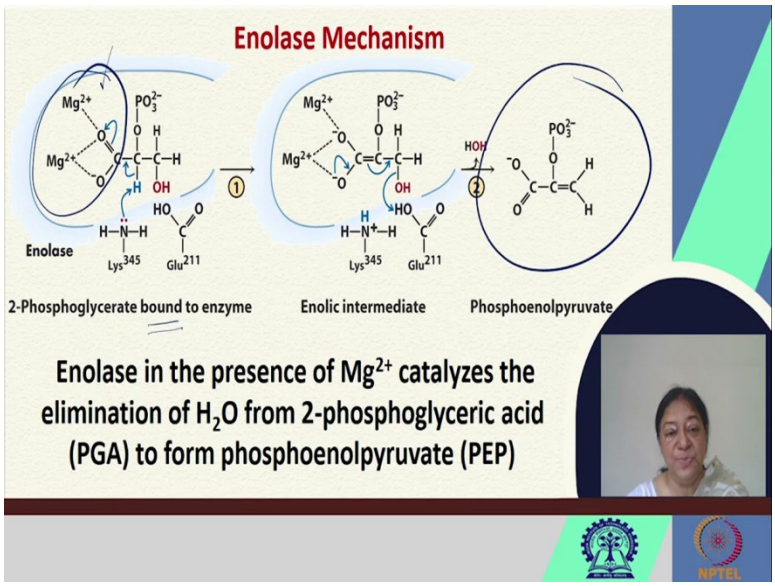
(Refer Slide Time: 22:53)



Another example that we will look at is enolase. We know glycolysis converts glucose into two 3-carbon molecules that are called pyruvate and the energy released during glycolysis is used to make ATP.

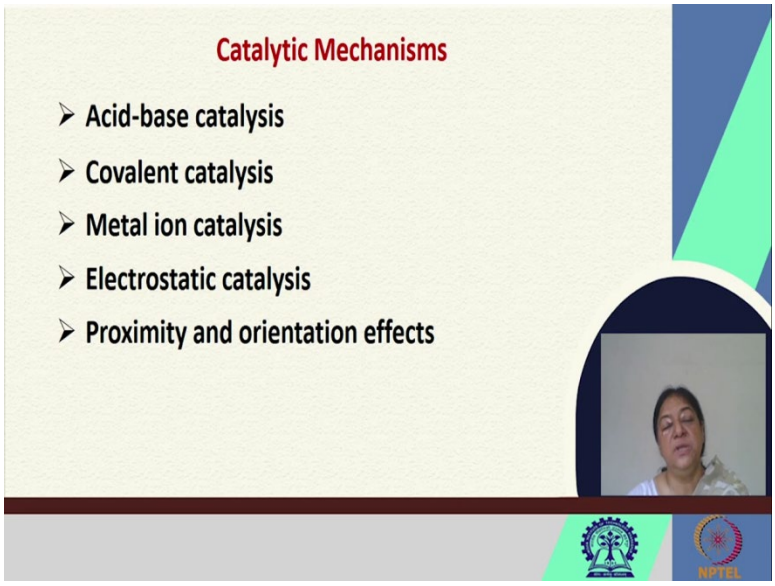
Enolase is an enzyme that catalyzes a specific reaction of glycolysis and the metal ion works by binding to the enzyme at the active site, as we would expect in a metal catalysis to produce a specific conformational change.

(Refer Slide Time: 23:28)



What happens in this case, is the enolate in the presence of magnesium now, catalyzes the elimination of water from 2-phosphoglyceric acid molecules. So, we have 2-phosphoglyceric acid molecules bound to the enzyme. In an example where we have the metal, in this case Mg^{2+}, that catalyzes the elimination of the water, in the event forming phosphoenolpyruvate which we know is important in our glycolysis reaction.

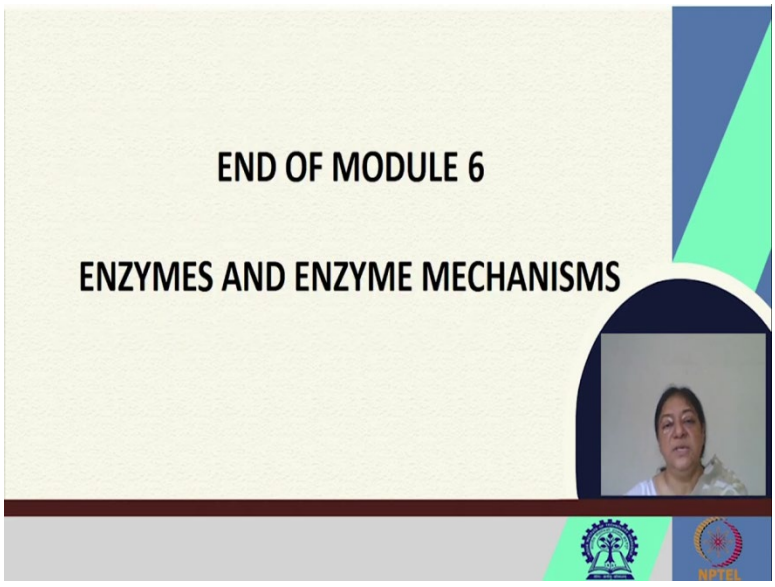
(Refer Slide Time: 24:09)



We have looked at acid-base catalysis, we have looked at covalent catalysis in the formation of a covalent bond and subsequent cleavage in our proteases in the proteolytic enzymes, that involve the cleavage of the peptide bond.

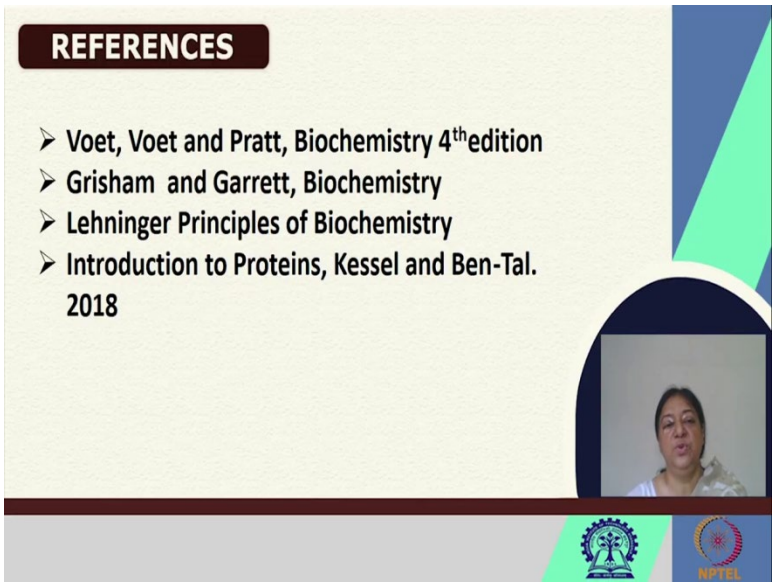
Then the metal ion catalysis, where we looked at several examples and had an idea of which metals are actually involved in the catalysis and the roles that they can play. The electrostatic catalysis that involves the electrostatic interactions, in mostly all the cases where we have an interaction between the specific residues involved in catalytic reactions and the type of residues. We also looked at the proximity and the orientation effects.

(Refer Slide Time: 25:03)



This is the end of module 6, enzymes and enzyme mechanisms

(Refer Slide Time: 25:09)



The books that we have followed have been specified in the specific lectures.