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

Module - 06
Enzymes and Enzyme Mechanisms
Lecture - 29
Enzyme Mechanisms - II

In our previous lecture we looked at enzyme mechanisms in terms of the stability of the enzyme substrate complex, the importance of the transition state and how the energetics are important in the formation of the product.

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In this lecture, we are going to look at the different enzyme classes, specific catalytic mechanisms to see how the amino acid residues are involved in the process and how they can act in acid base catalysis, as proton donors and acceptors. How the enzyme restore its structure, so that it is able to bind another substrate in a subsequent reaction.

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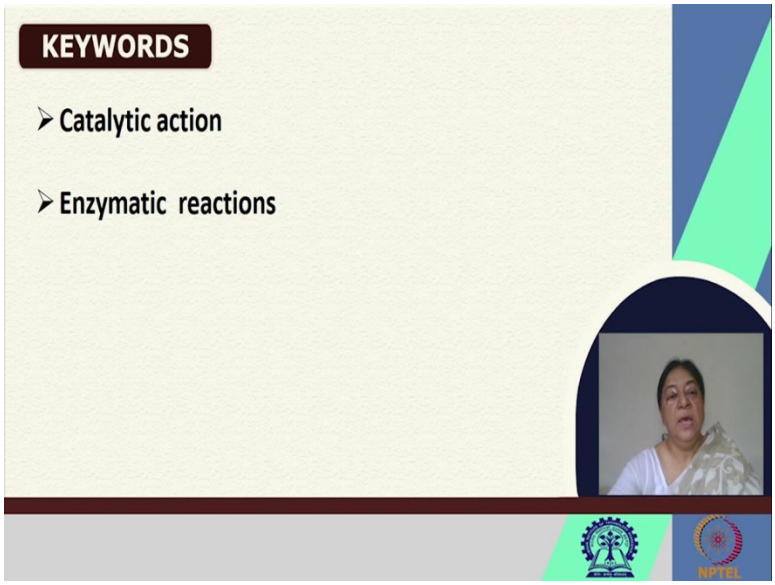

We will look at catalytic actions and enzymatic reactions.

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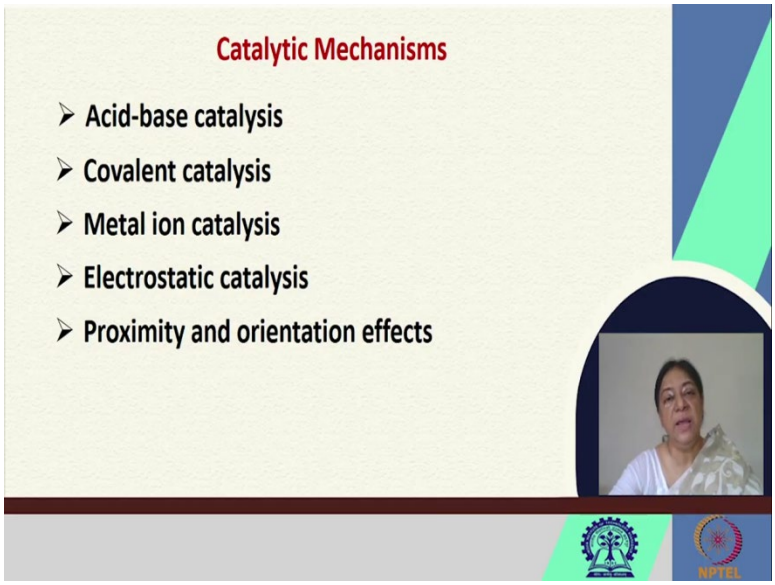

To understand this, we need to know what kind of catalytic mechanisms are possible. These are acid base catalysis, covalent catalysis, metal ion based catalysis, electrostatic interactions and the proximity and orientation effects, which we had visited in protein ligand binding.

We know that enzyme substrate binding is a subset of protein ligand binding and the involvement of the ligand, in this case the substrate to be recognized by the enzyme, to form the enzyme substrate complex, is an important event in the process of catalysis.

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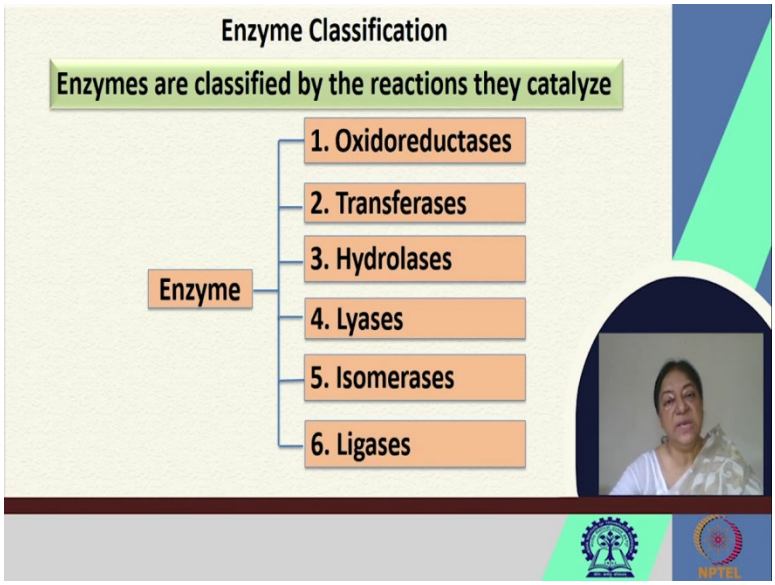

In the enzyme classifications that we learned in the first lecture of this module, we know that they are classified according to the reactions that they catalyze. For example, we have oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. We will visit each of these types, looking at specific examples to see how they conduct their catalytic operation.

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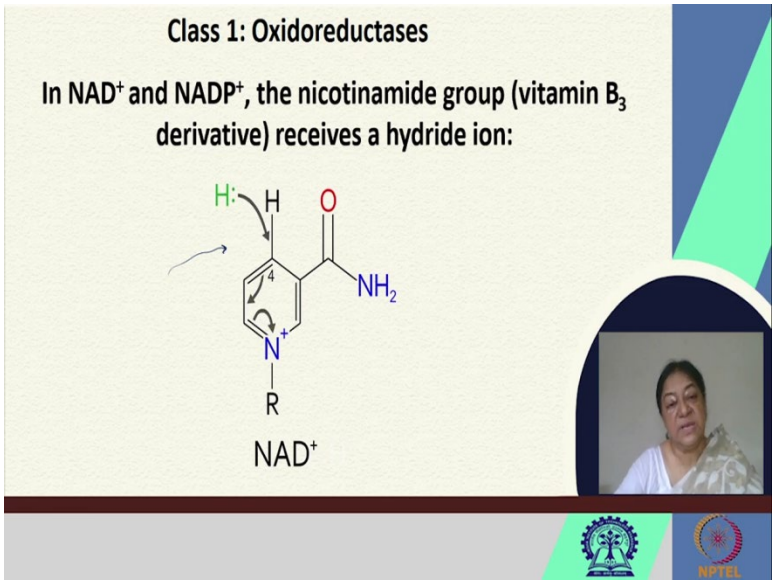

For oxidoreductases, we will be looking at NAD^+ and $NADP^+$, the nicotinamide group, a vitamin B_3 derivative; that [refer to slide] receives a hydride ion.

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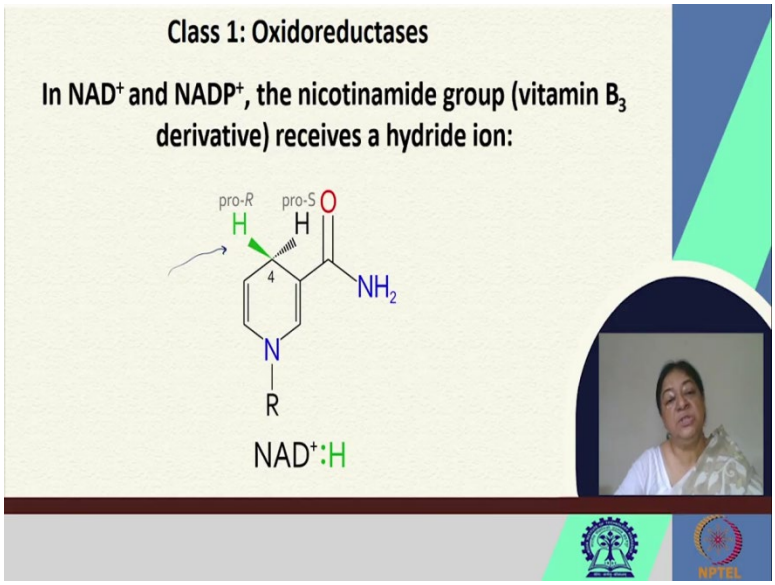

And we learnt in the previous class, the importance of the pro R and the pro S for the chirality of the specific molecule.

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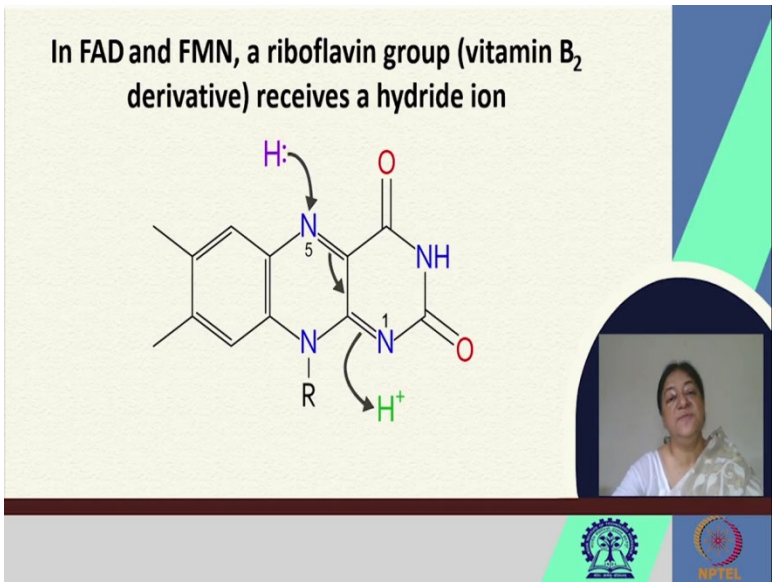

Similarly if we look at another example, where we have FAD and FMN.

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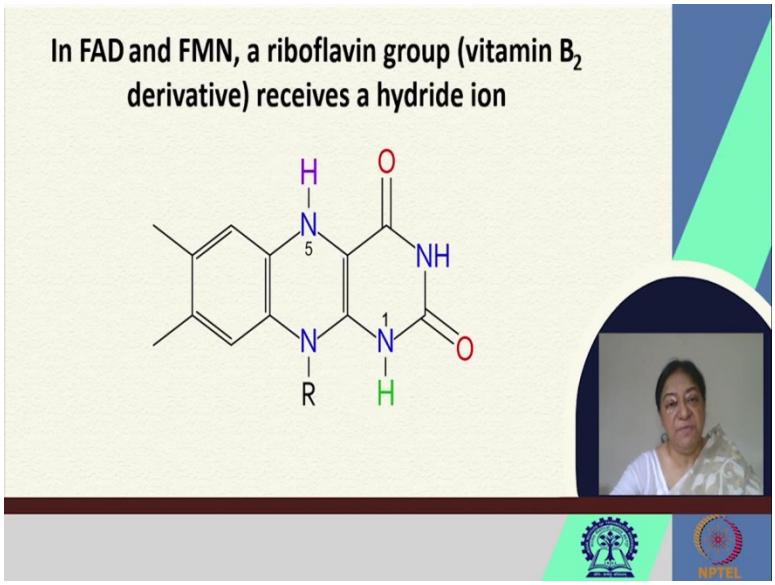

They are in the riboflavin group, which is the vitamin B_2 derivative. This also receives a hydride ion in an oxidoreductase example.

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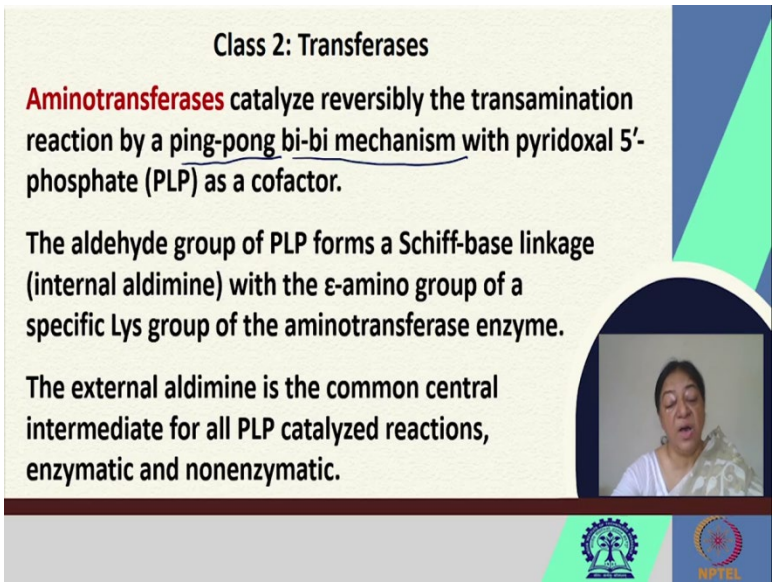

For the transferases, we will be looking at aminotransferases. Aminotransferases catalyze in a reversible fashion, the transamination reaction by what is called a ping-pong bi-bi mechanism. When we look at enzyme kinetics, we will see what we mean by this bi-bi mechanism or ping pong mechanism, in terms of enzymes substrate complex formation.

There are reactions which involve two substrates, these would be called bi substrate mechanisms and they will react with each other in the formation of an intermediate product going on to form a final product. So there may be two substrates and two products formations, which we will see later on in the kinetic mechanisms that we will explore.

In this case we have the aldehyde group of the pyridoxal 5'-phosphate, PLP which is the cofactor. This forms what is called a shift base linkage between the C = O and the NH_2. And the NH_2 in this case is obtained from the epsilon amino group of a specific lysine residue, that is present on the aminotransferase enzyme.

We will look at a diagrammatic representation, where the lysine has not been shown to be tagged along with an enzyme or a large group; but this lysine is part of the aminotransferase enzyme. Then there are several processes, several reactions involved, that are also base catalyzed and we have a hydrolysis as well, in the formation of our product.

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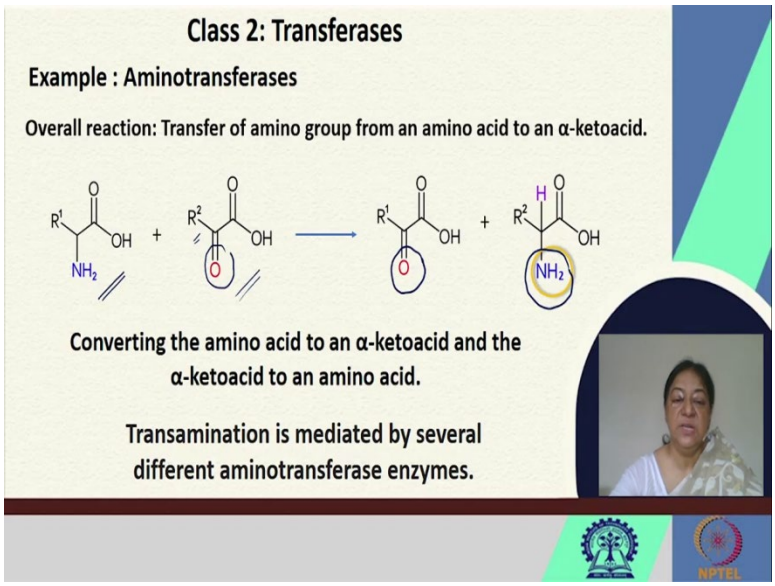

The overall reaction involves the transfer of an amino group from an amino acid to a ketoacid. {Refer to slide] here is our amino acid, this is going to be transferred to an α-ketoacid. This is the ketone group and this is the ketoacid; this is the amino group, the amino acid and this amine group we will see is transferred now to R^2, that originally had the ketoacid, whereas this R^1 now becomes the ketoacid. So, the transfer involves the amino group being shifted in a reaction that the aminotransferase enzyme does, that is part of the transferase class of enzymes.

Transamination, the process is mediated by several different aminotransferase enzymes and as we saw, it is converting the amino acid to an α-ketoacid and the α-ketoacid to an amino acid.

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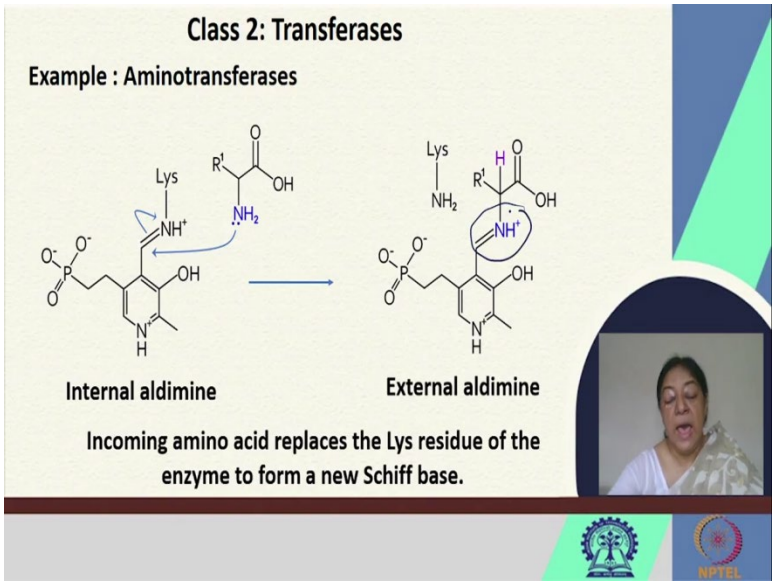

In the step that we have here [refer to slide], there is an incoming amino acid that replaces the lysine residue of the enzyme to form what is called a new Schiff base.

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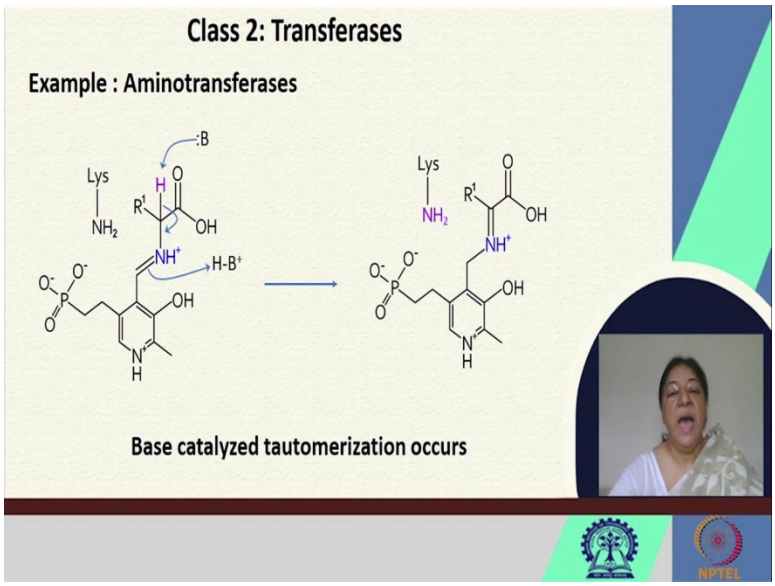

Then from the new Schiff base, we have a base catalyzed tautomerization, which involves an amine and imine group.

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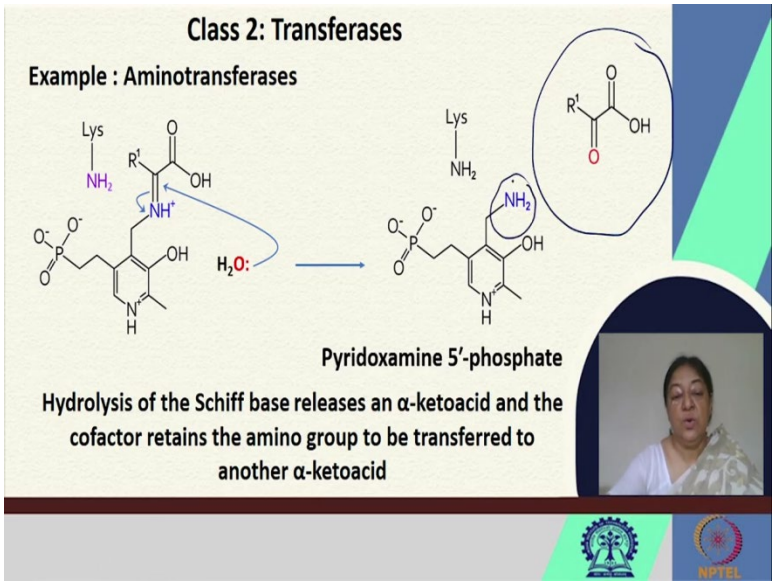

And following this, we will have what is called a hydrolysis. This hydrolysis of the Schiff base, as you can see [refer to slide], is releasing the ketoacid that is one of the products in this reaction. So we have the substrate bound and one of the products, the ketoacid. The amino part has been taken up by the cofactor and this cofactor now has to release this to the other group that was originally the α-ketoacid to have the actual transfer of the amino group.

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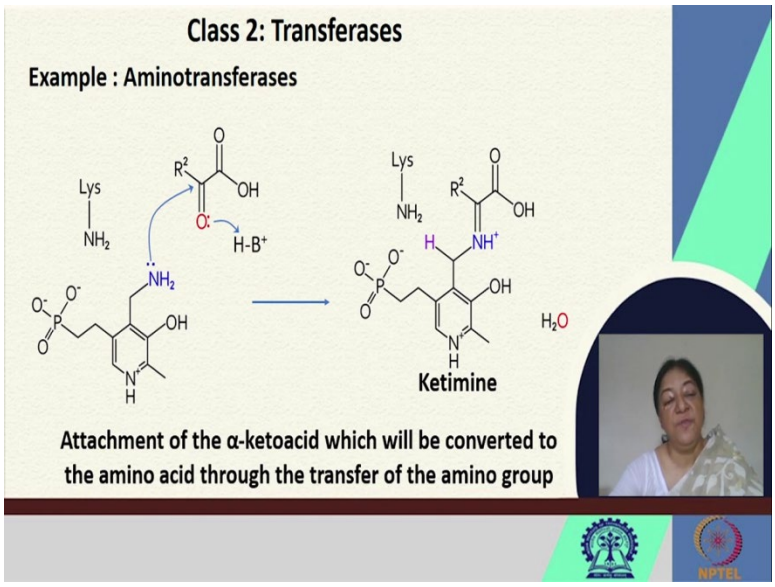

So now that we have the NH_2, we have to transfer this NH_2 to the other R^2 group containing α-ketoacid and the attachment of the ketoacid to the periodoxl phosphate, will now take it to the transfer of the amino acid, that is going to ultimately lead to the product formation.

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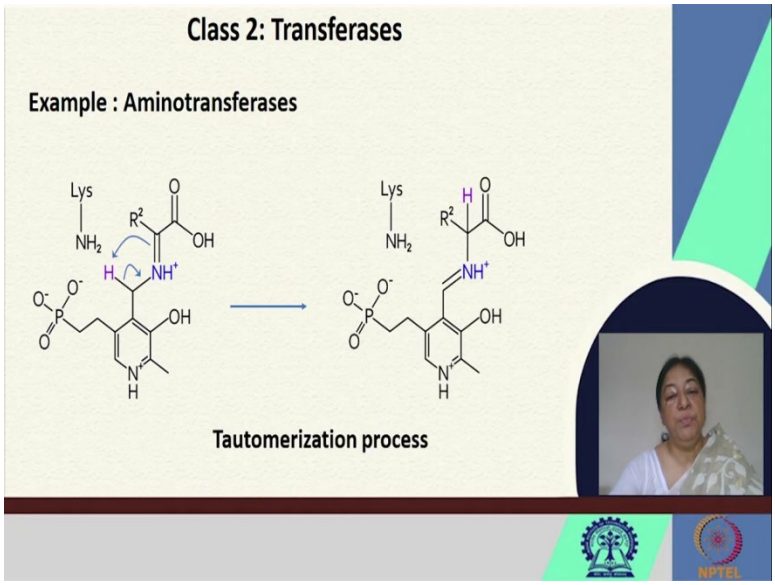

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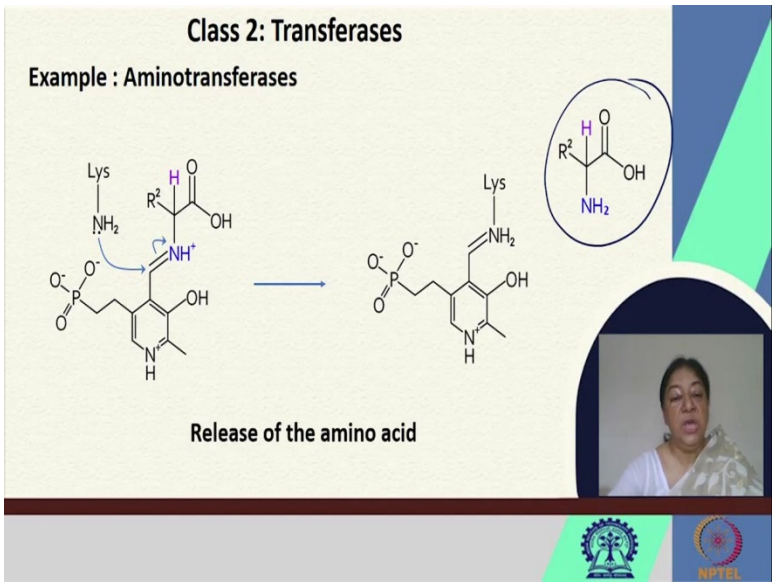

Following a tautomerization, as we were shown in the previous slide, we will now have the amine transfer to the R^2 group and this will release the amino acid.

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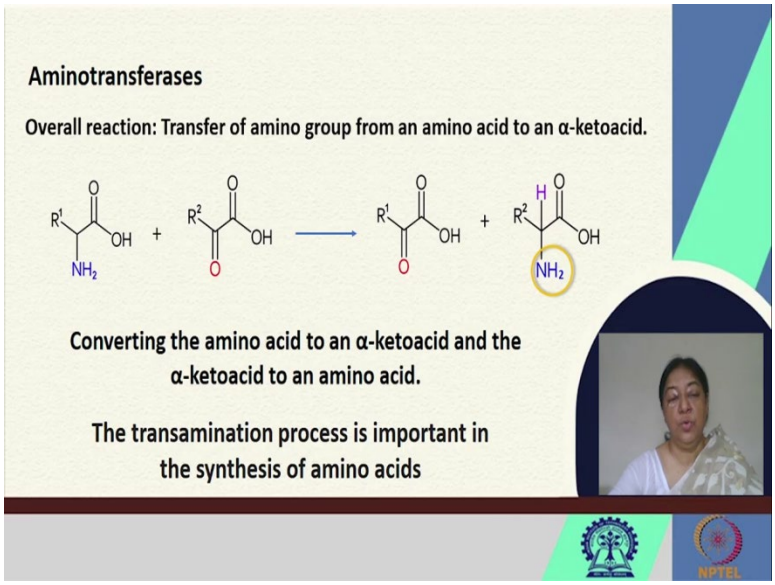

The overall reaction has been the transfer of the amino group from an amino acid to an α-ketoacid. And this [refer to slide] is the overall reaction and the process is extremely important in the synthesis of amino acid, which is understandably so, considering that we have a transfer of an amine group.

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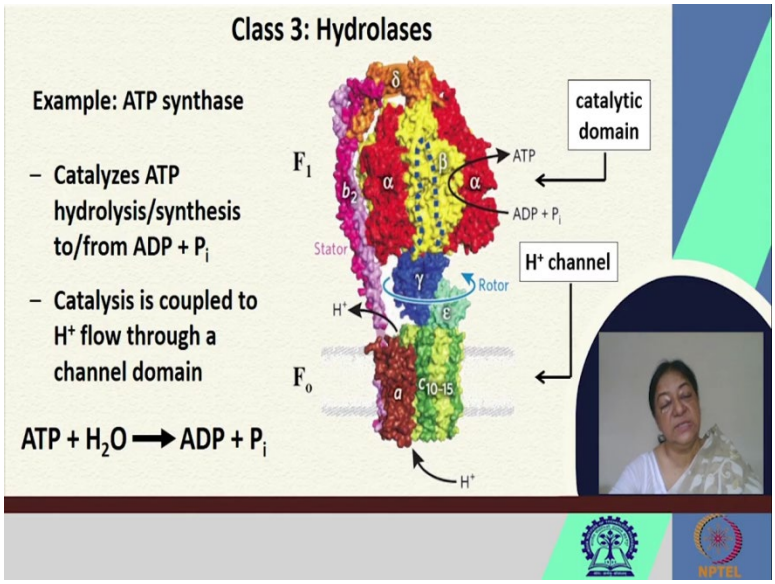

In the example of hydrolases, we will be looking at another very important enzyme. This is called ATP synthase and as the name implies, it synthesizes ATP. This is a beautiful enzyme that catalyzes ATP hydrolysis and the synthesis to/from ADP + P_i.

Now, this catalysis is coupled to a proton flow through a channel. We will visit this enzyme in further detail when we look at both motor proteins and membrane brown proteins, to see how the

catalytic domain and the proton channel work, to bring about the overall ATP hydrolysis in this example of hydrolases.

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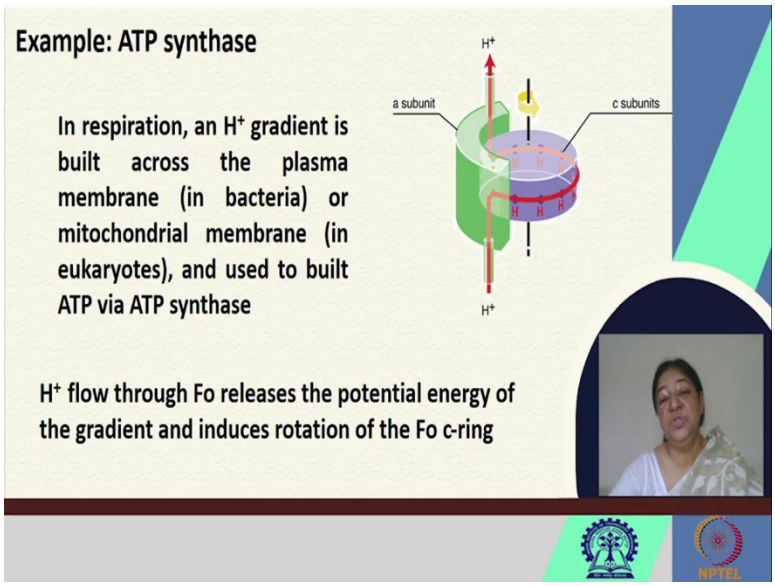

There are several subunits associated with this and as we know ATP is called the currency of energy. The energy released in the hydrolysis of ATP is used for several reactions. The proton flow is important as it reduces a rotation in the ring, which we will look at when we go to motor proteins to see how this motor actually acts and see how this is embedded in the protein to bring about that proton gradient.

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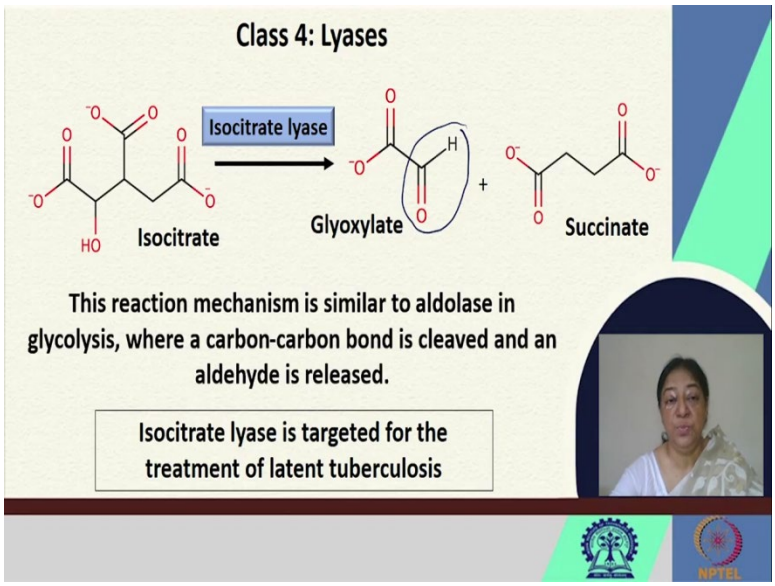

In our class 4 enzymes the lyases, we are going to look at the example of isocitrate lyase. As the name implies, it involves the cleavage of a bond, where we cleave a bond in isocitrate to produce glyoxylate and succinate.

This reaction mechanism is similar to aldolase in glycolysis, where a carbon-carbon bond is cleaved and an aldehyde is released. Here [refer to slide] is the release of the aldehyde in this case, in the term of the glyoxylate ion and we have the succinate ion, both of which are derived from the isocitrate. The importance of this enzyme is this isocitrate lyase is the target for the treatment of latent tuberculosis. So inhibit design is important in this particular enzyme.

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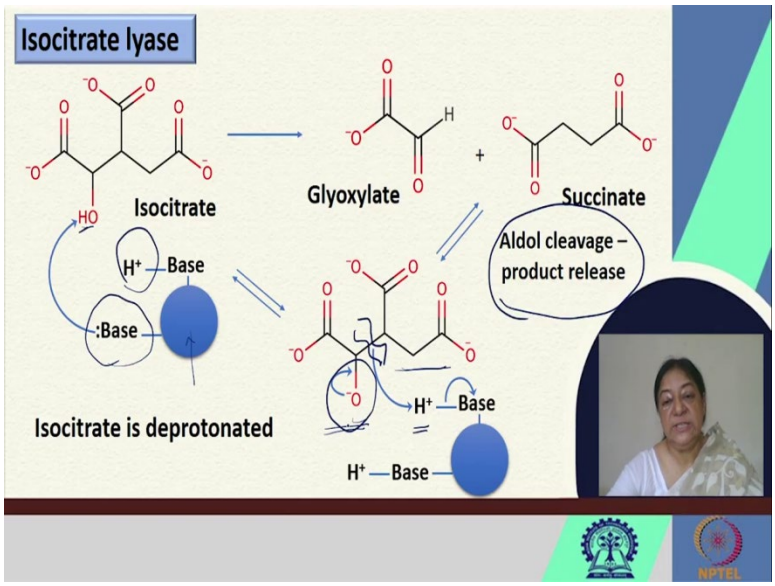

In isocitrate the first step involves the deprotonation of isocitrate, that is brought about by an imidazole; which can act as a proton donor and a proton acceptor. This blue sphere that we see here [refer to slide] is part of the enzyme that we are interested in, that provides the histidine residue to act as a base in the deprotonation of isocitrate.

The deprotonated form, has a transfer with another acceptance of a proton, where we will see that this bond is being cleaved and we will have our aldol cleavage to give us our product, where we can see this is part of the succinate part and this is part of our glyoxylate.

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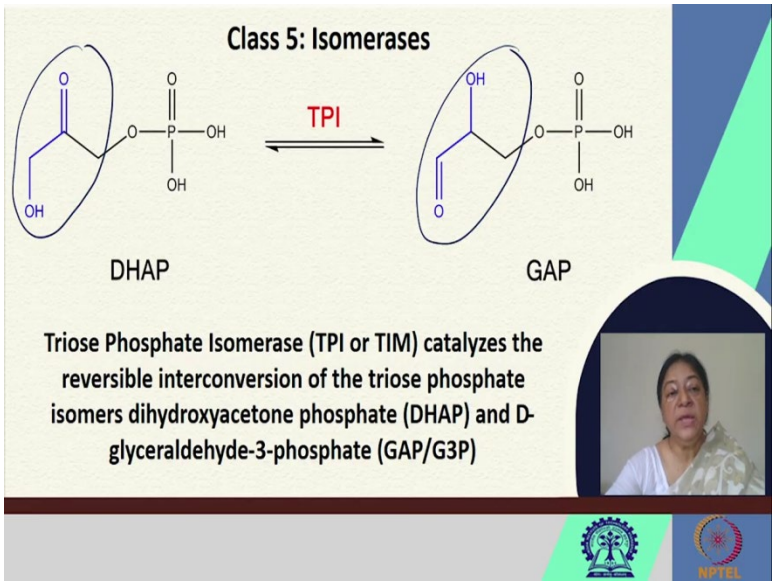

In the 5th class of enzymes we will be looking at the isomerases. In this particular example, we will be looking at triose phosphate isomerase, that catalyzes the interconversion of the triose phosphate isomers. As we can see [refer to slide] we have dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate. This is where we see the variations of DHAP and GAP.

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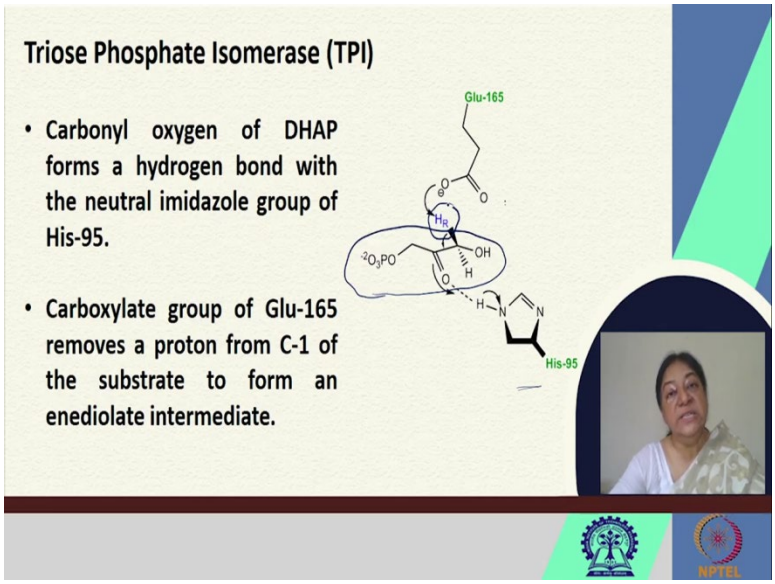

The working of the enzyme is such that, the carbonyl oxygen of DHAP, forms a hydrogen bond with the imidazole group of His-95 of the enzyme.

The carboxylate group of Glu-165, removes the proton from the substrate that forms what is called an enediolate intermediate.

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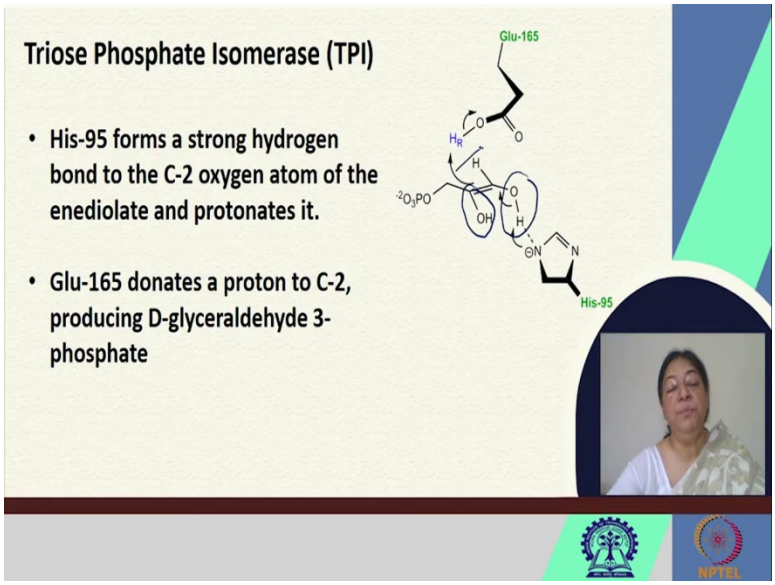

Enediolate intermediate is formed. So, here [refer to slide] we have an OH group, here we have another OH group that is the diol and the double bond that we see there is the ene. So this is our enediolate intermediate.

In this process His-95 forms a strong hydrogen bond to the C-2 oxygen atom and protonates it. Then Glu-165 donates a proton to C-2, producing the product.

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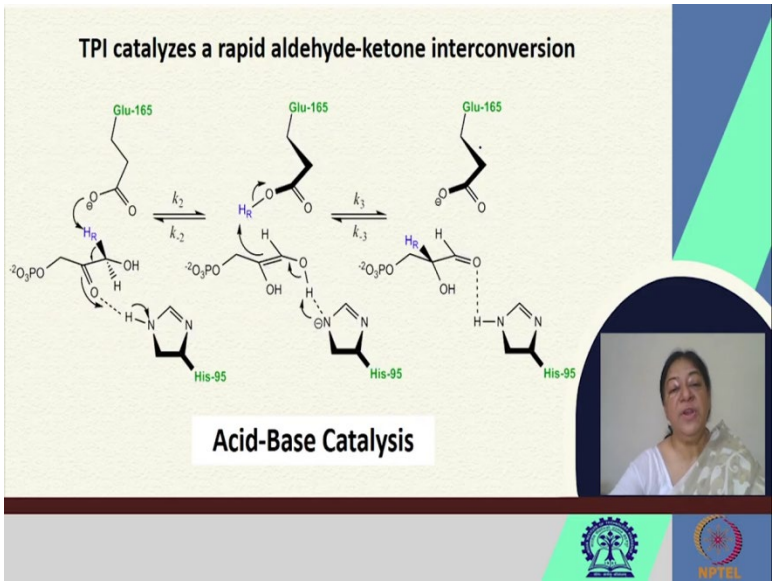

So, the overall reaction is as we can see an acid base reaction; an acid base catalysis example involving an isomerase.

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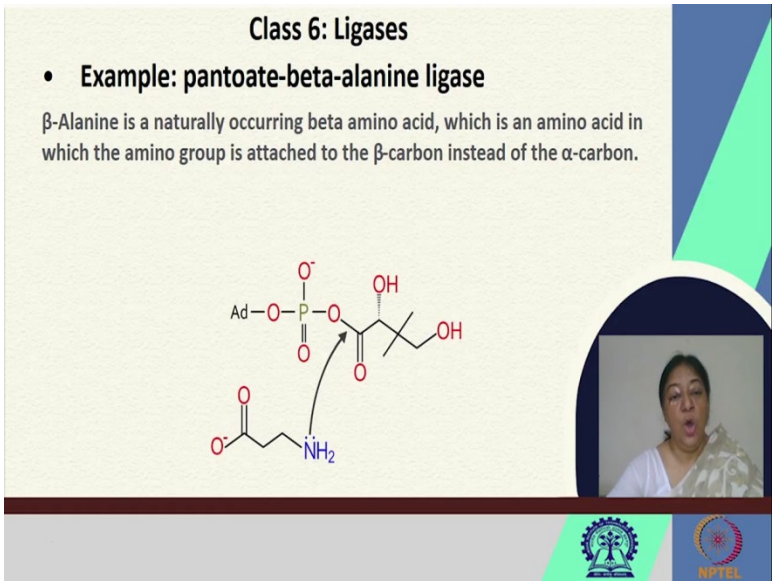

In our last class of enzymes the ligases, we will be looking at a specific example of pantoate β alanine ligase; as the name implies, this is the connection between pantoate and β-alanine. β-alanine is a naturally occurring β-amino acid, which is an amino acid in which the amine group, instead of being attached to the α-carbon, is now attached to the β-carbon.

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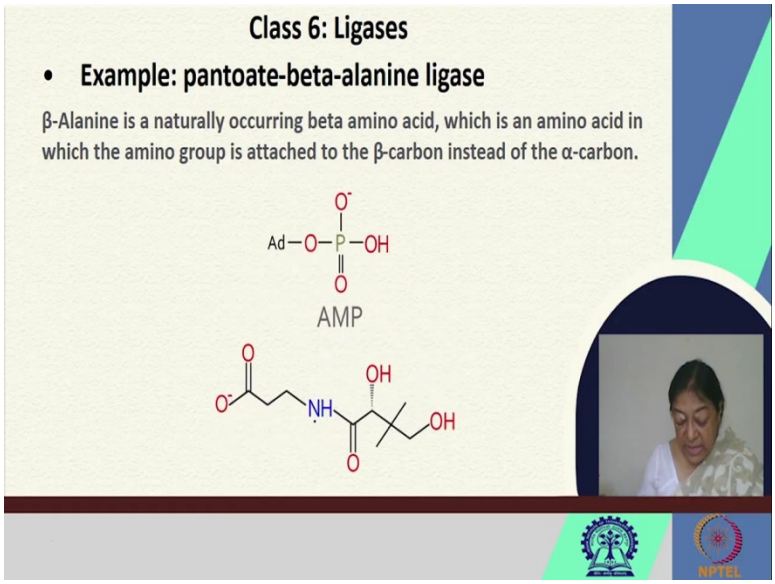

In the overall reaction what happens is, we have the formation of pantothenic. [Refer to the series of slides given].

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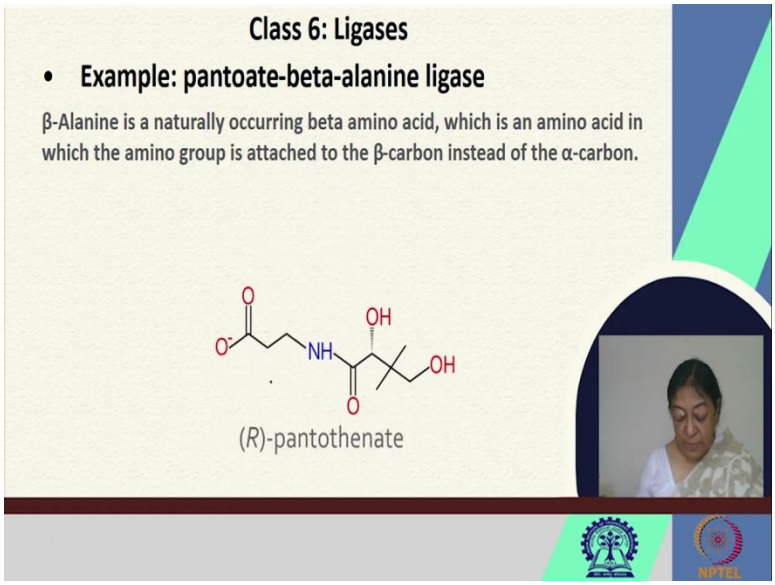

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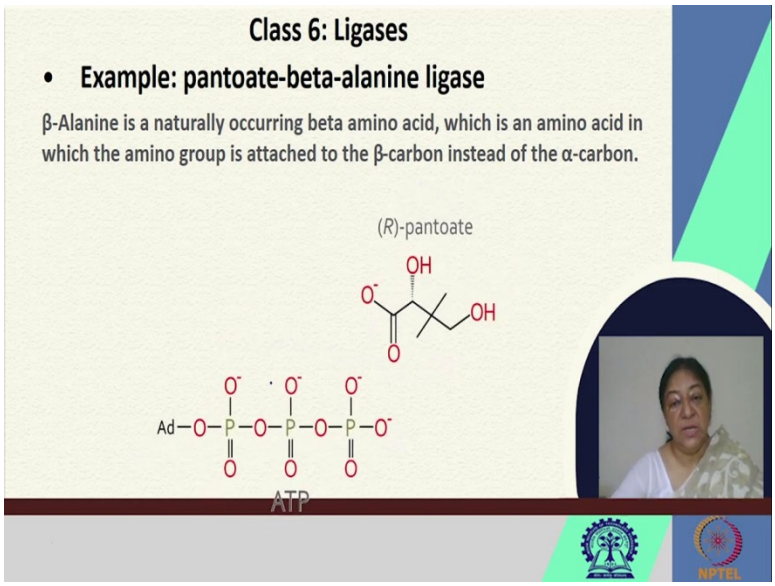

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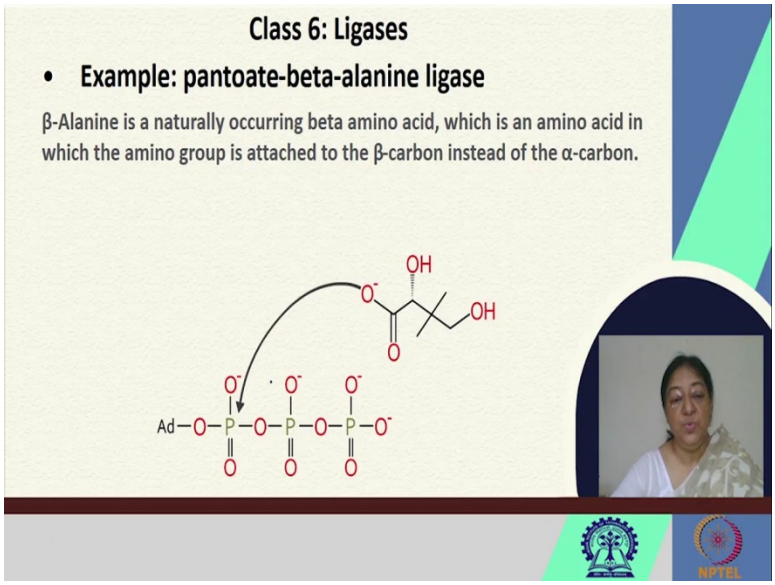

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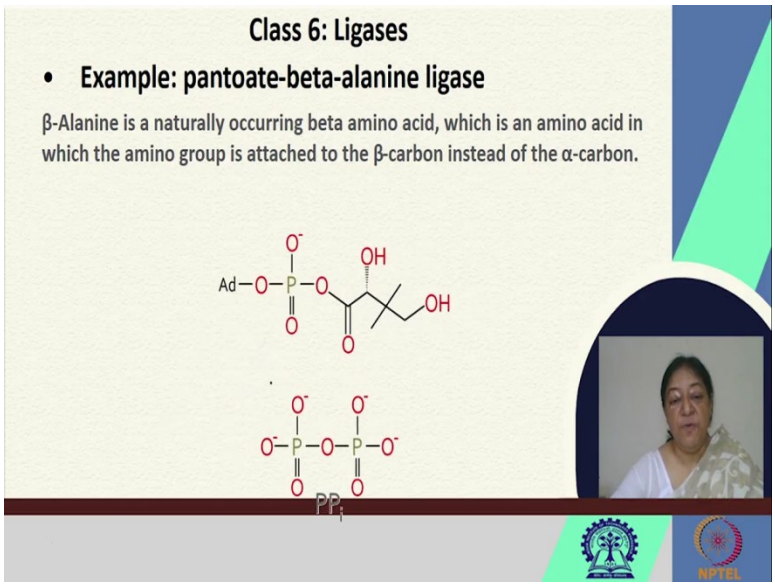

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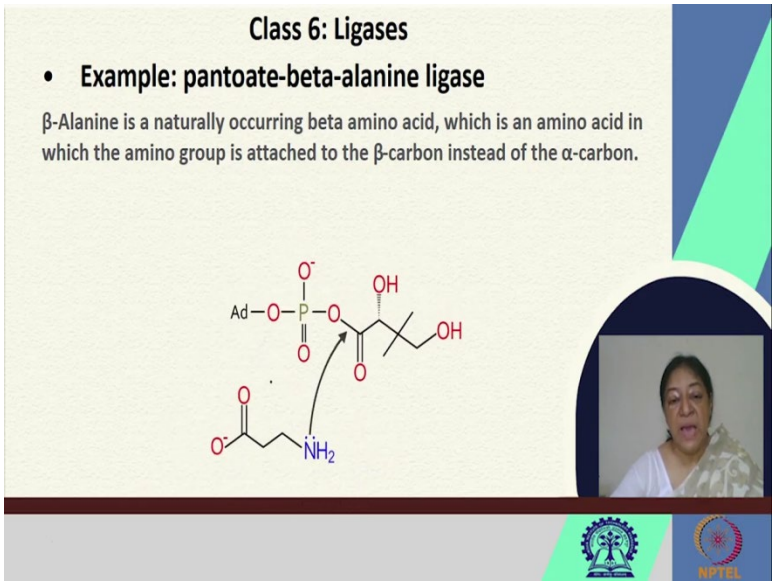

We have pantoate, we have ATP forming the AMP and the PP_i .

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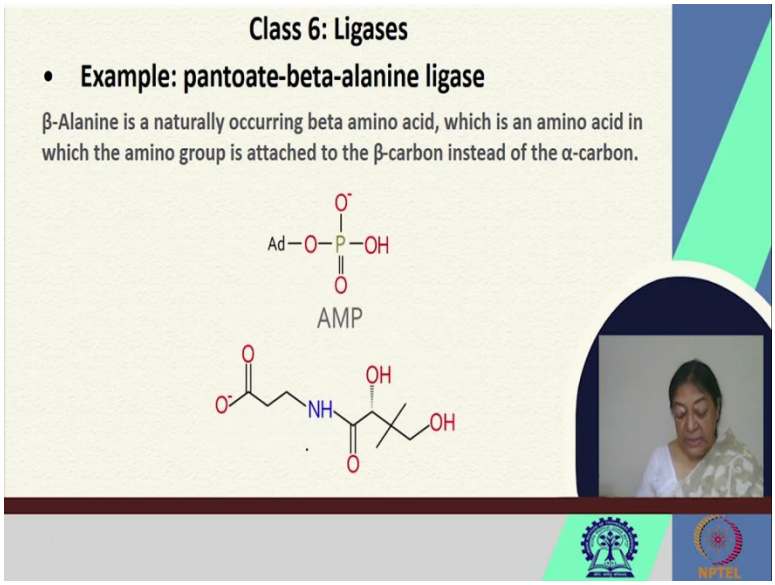

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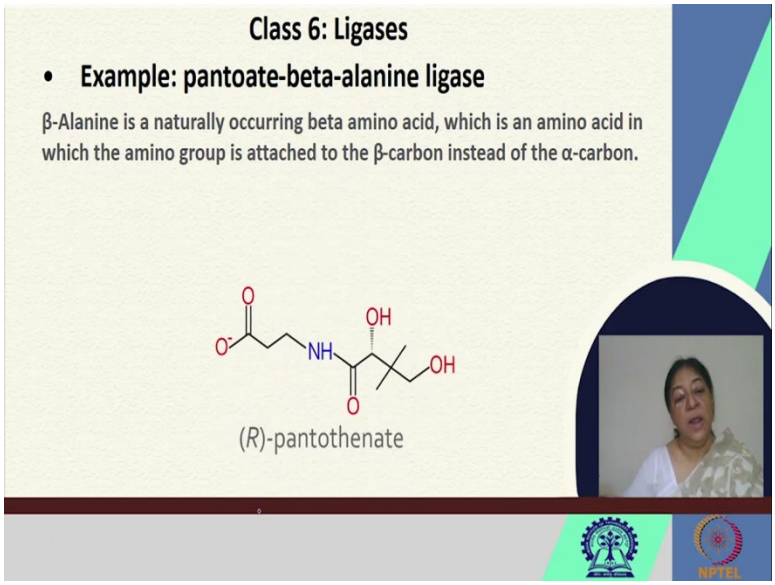

The β-alanie come into the picture to form what we call (R)-pantothenate.

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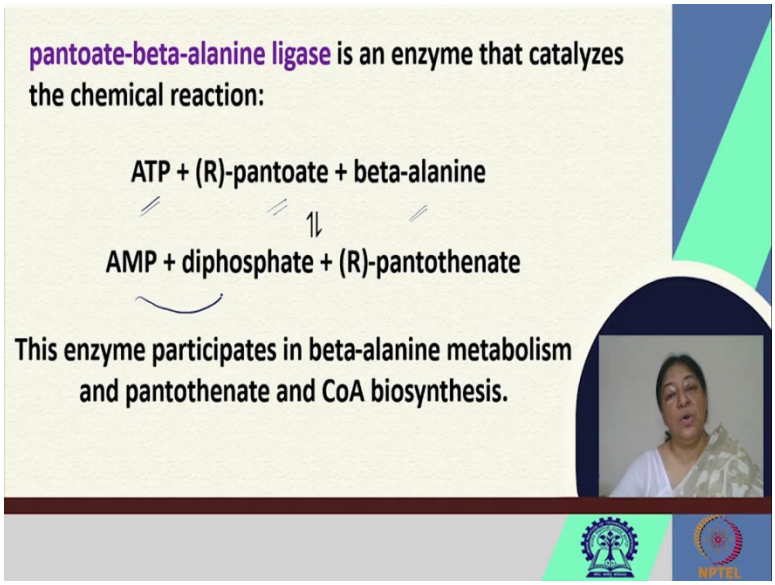

This pantoate-β-alanie ligase is an enzyme that catalyzes the overall chemical reaction, where we have ATP, (R)-pantoate, and β-alanine. This being a ligase enzyme, will connect pantoate and β-alanine to form the pantothenate. In the process, the energy from the cleavage of the ATP is used to bring about the ligation. This enzyme participates in β-alanine metabolism, pantothenic and CoA biosynthesis, both of which are important in several biochemical processes and we will see them in several biochemical cycles as well.

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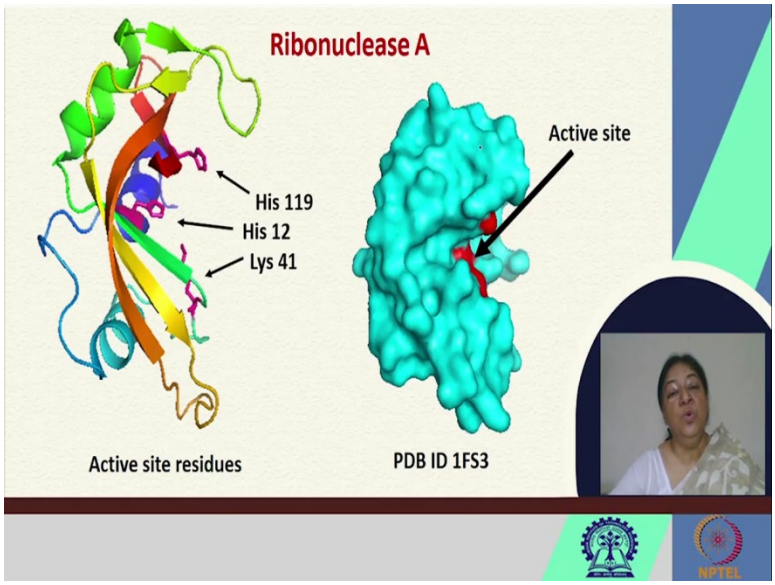

In another protein ribonuclease A, we will be looking at specific active site residues. This [refer to slide] is a hydrolase enzyme, but nevertheless being a very important protein; we will just look at it's mechanism of action and see how beautifully the active site is arranged in a manner that can recognize the substrate. In this case, the name ribonuclease A indicates that our substrate is RNA and ribonuclease A is involved in the cleavage of RNA.

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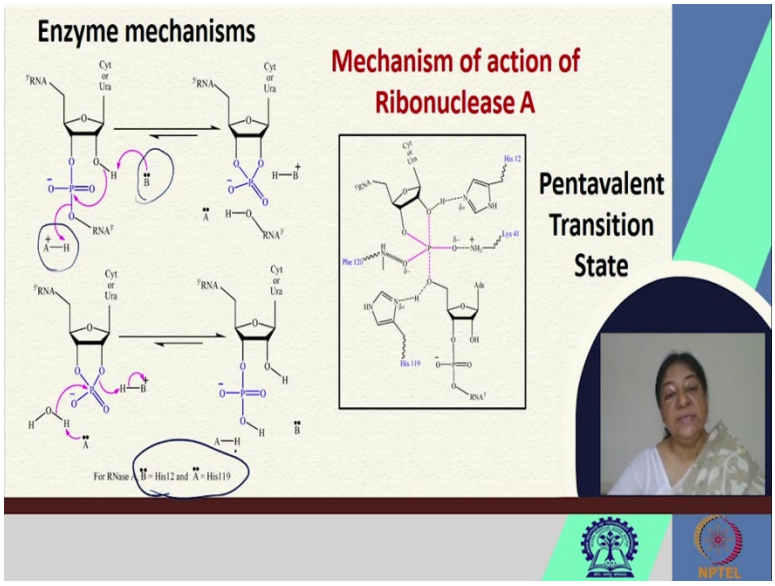

In the enzyme mechanisms known, the mechanism of action of ribonuclease A; there is an involvement of a base and of an acid. We have a proton donor and we have a proton acceptor. The beauty about the proton donor and the proton acceptor in ribonuclease A is, both are histidine residues.

So we have what is called a pentavalent transition state, which we learned in our previous class as to the importance of the transition state and how the enzyme will form a complex with the transition state. The stability of this transition state is important.

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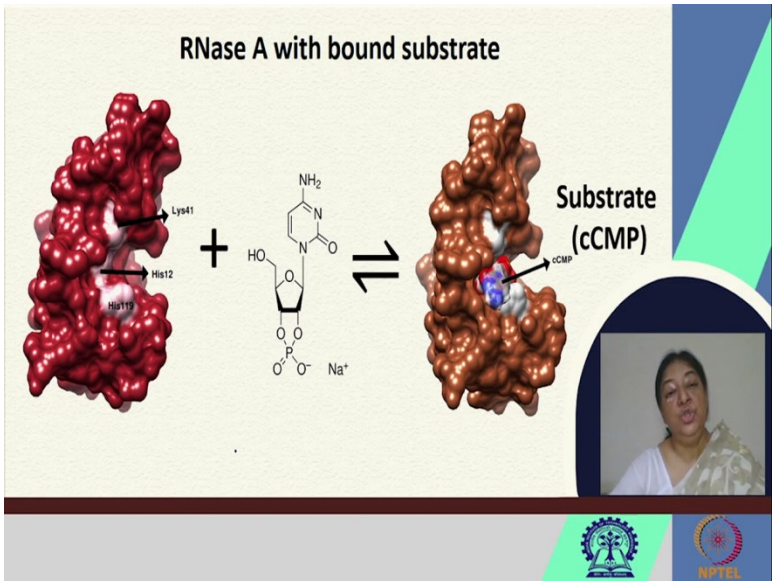

If we look [refer to slide] at the ribonuclease A with the bound substrate, we have a specific mechanistic detail that occurs with the histidine 12 and the histidine 119, in what is called a trans phosphorylation reaction followed by a hydrolysis. But the understanding that the importance of the residues comes from their recognition of the substrate to the active site, which in this case involves a transition state formation; involves 2 histidines that act as a base and an acid. And in the second step their roles are reversed.

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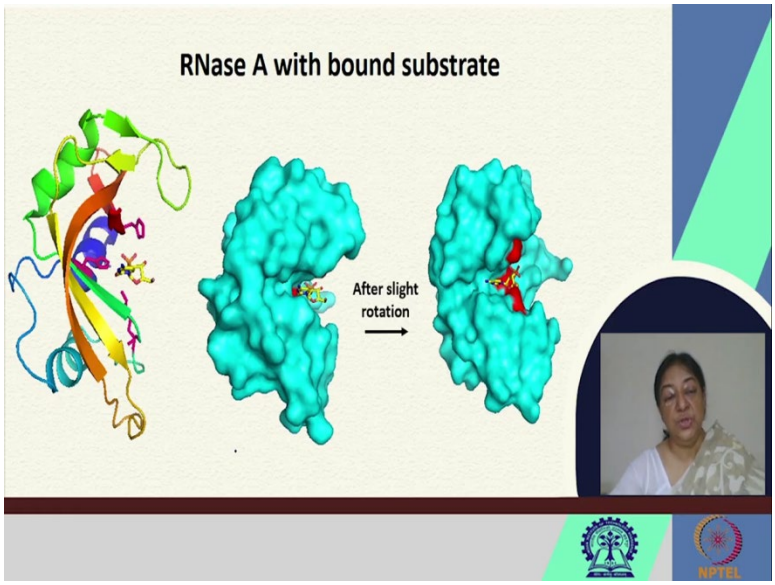

So, if we look [refer to slide] at a picture of RNase A with the bound substrate, we can see how beautifully it fits into the cleft.

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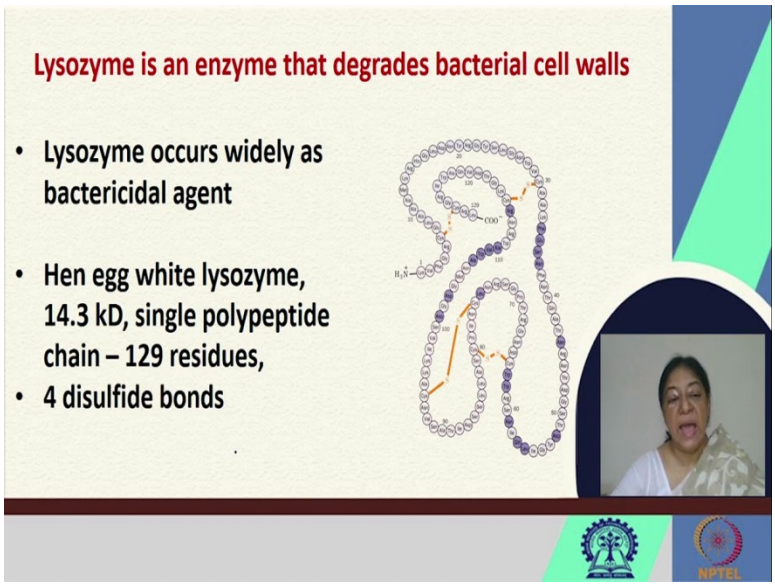

The last example that we are going to do in this lecture, will be lysozyme. Lysozyme is another very important protein that degrades bacterial cell walls. It occurs widely as a bactericidal agent, indicating that it is killing the bacteria; because it degrades the bacterial cell walls.

The hen egg white lysozyme, which is one of the very well studied proteins is a 14.3 kD, single polypeptide chain with a 129 amino acid residues and has 4 disulfide bonds.

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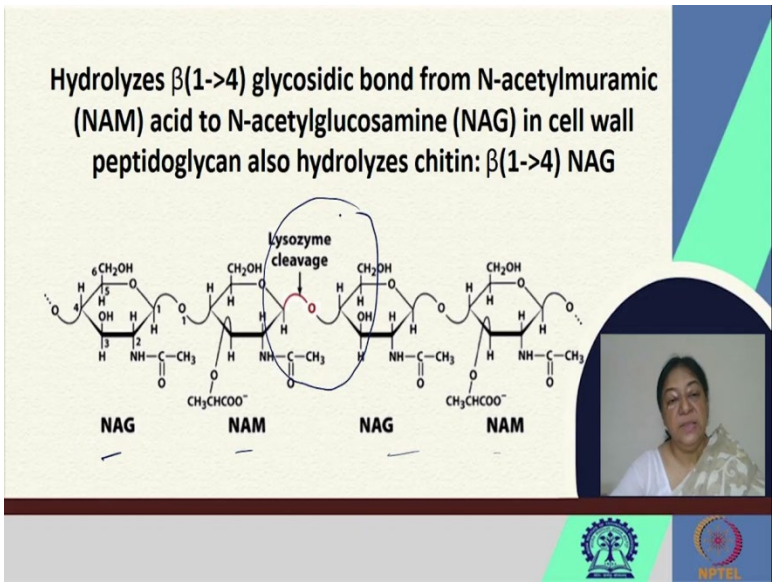

This hydrolyzes the glycosidic bond from the NAM and NAG conditions of the cell wall. So here [refer to slide] we have the NAG, NAM, NAG, NAM system. The lysozyme cleavage occurs at this specific position, which indicates that the binding site or the active site of lysozyme, should have specific recognition for this particular bond, the β(1->4) glycosidic linkage, that will be cleaved by the enzyme.

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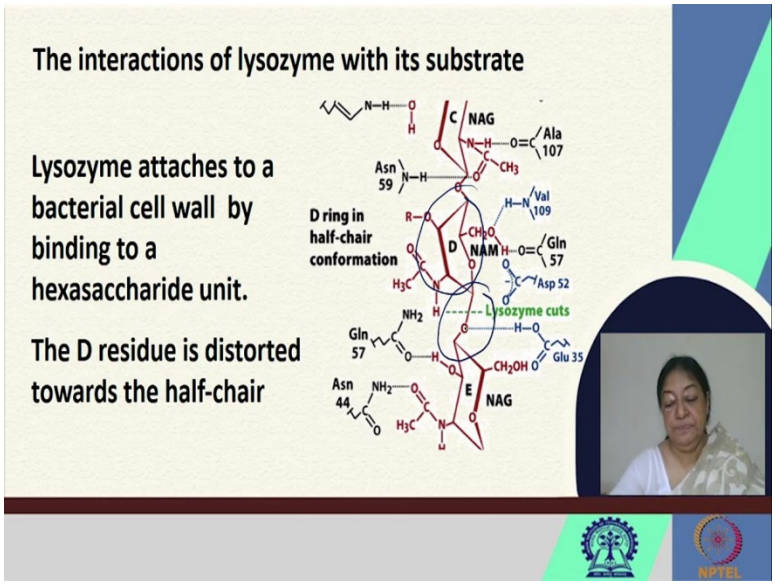

The interactions of the enzyme indicate, that lysozyme attaches to the bacterial cell wall by binding a hexasaccharide unit. That is it binds 6 of these [refer to slide] units together and it breaks the bond between this specific unit here. This means that there is a specific recognition as we will see by 2 acidic residues, aspartic acid and glutamic acid.

We have the C, D and the E linkages; these are the glycosidic; these are the hexasaccharide units and it breaks it between the D and the E units.

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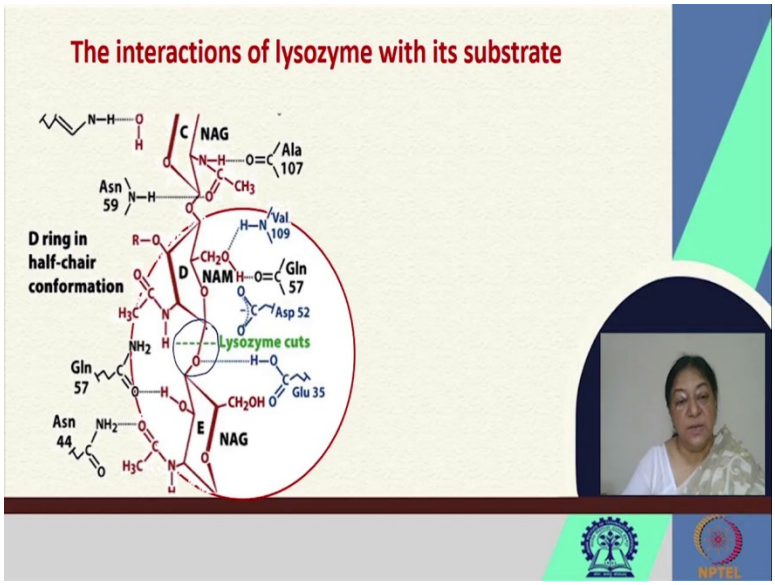

Look at this [refer to slide] picture, where lysozyme is going to cleave at this position.

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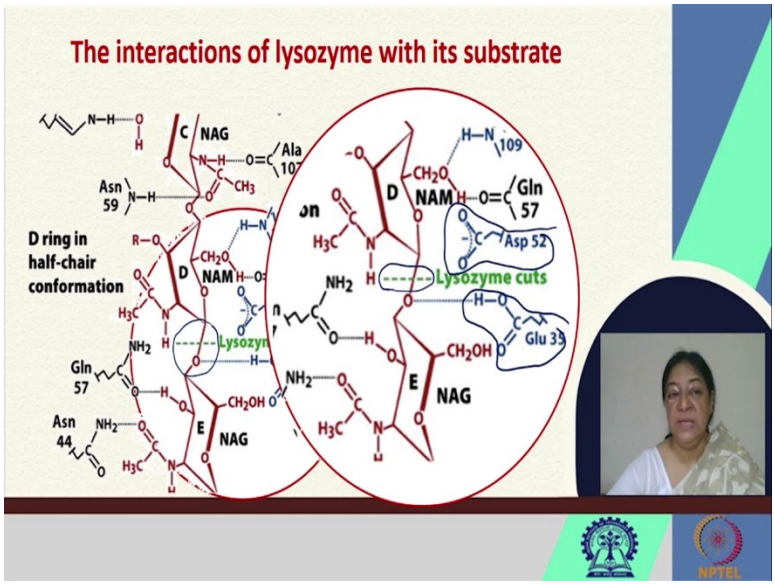

If we zoom in on this a bit, we see that we have the aspartic acid residue important here and this glutamic acid residue important, that will assist the cleavage of this particular bond, the β-glycosidic bond between the NAM and NAG of the D and E hexasaccharide unit. So, what happens?

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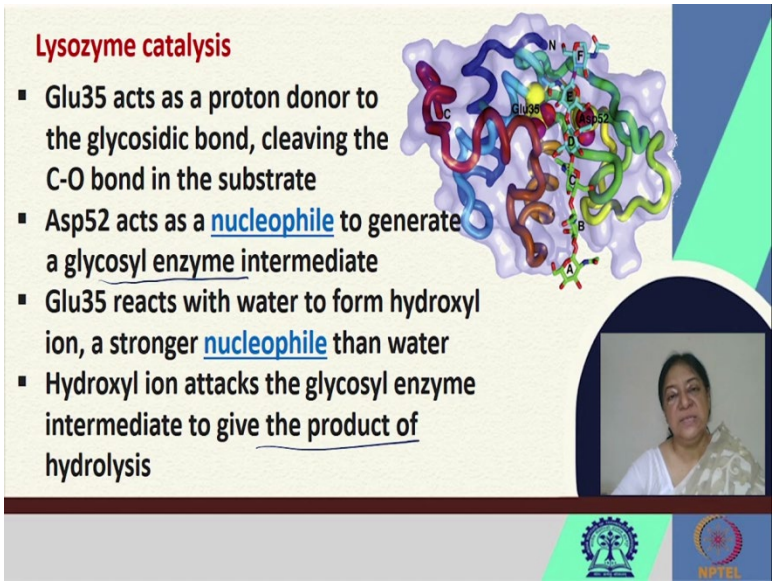

So, Glu35 acts as a proton donor to the glycosidic bond and it cleaves the CO bond in the substrate. Asp52 acts as a nucleophile that generates a glycosyl enzyme intermediate. Then there is a reaction of Glu35 with water to form a hydroxyl ion, a stronger nucleophile than water, which then attacks the glycosyl enzyme intermediate that was formed to give the product of hydrolysis.

The beauty of these reactions involves the proton, involves the hydrogen bond formation and involves proton transfer, as we have seen in a number of these acid base catalyzed reaction. This importance in understanding the acid base catalysis and what amino acids are going to be involved in the active site to bring about this acid base catalysis, is extremely important.

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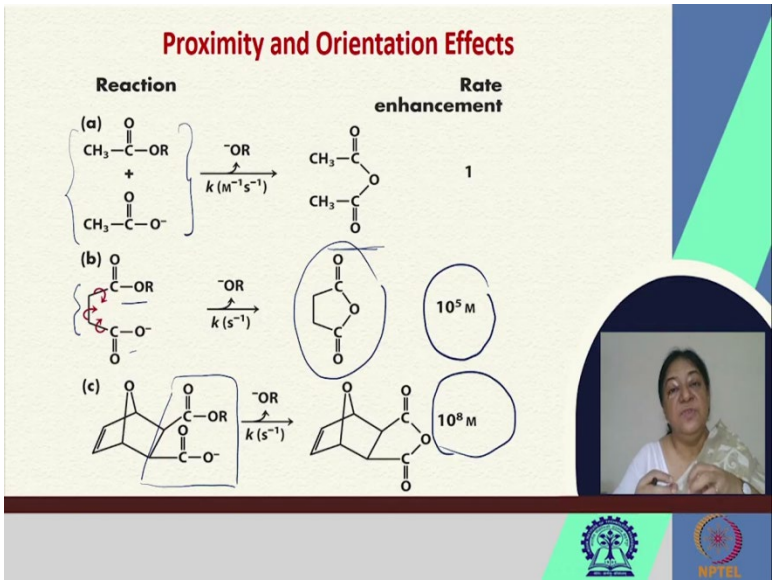

If we look now at specific methodologies, as to giving us how the proximity of the substrate to the enzyme and the orientation is important, as in protein ligand binding; we will look at the concept of rate enhancement, when we consider the rates of reactions in our enzyme kinetics lectures. However, just to understand that we are bringing about the combination of these 2 moieties, to provide us with anhydride.

Now, when we have these linked together to bring about a similar kind of product, then we see [refer to slide] the rotation about this is possible; but nevertheless because the groups are in close proximity to each other, we have a rate enhancement. However, if we bring about a further rigidity and keep the specific moieties interacting in a specific orientation, this will enhance the reaction many fold.

So, the example shows that when we have 2 moieties that are there in solution that are likely to come together; there is as we saw Brownian motion diffusion, which will not always result in a product formation. However, if we can bring them close together, in a manner that is going to give us the proper orientation and the proximity to the protein, which may be the enzyme, then we can bring about a several fold enhancement in the rates of the reactions, we will see what we mean by the rate enhancement, in our enzyme kinetics classes.

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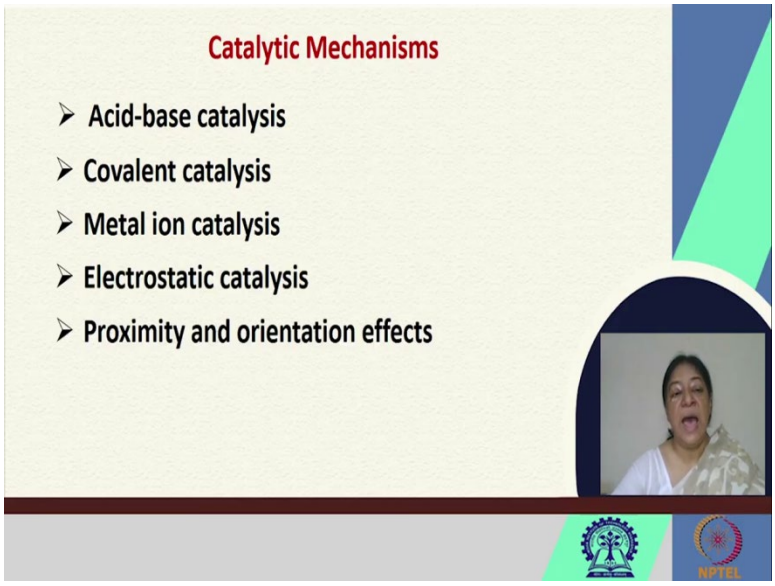

If we look at the catalytic mechanisms in general, we looked at acid base catalysis and we looked at the specific amino acid residues involved in an acid base catalysis, in the sense that we have the histidine residues, we have the aspartic acid, we had the glutamic acid involved in this. We will look at covalent catalysis and metal ion catalysis in the next lecture and overall proximity and orientation effects that we looked at.

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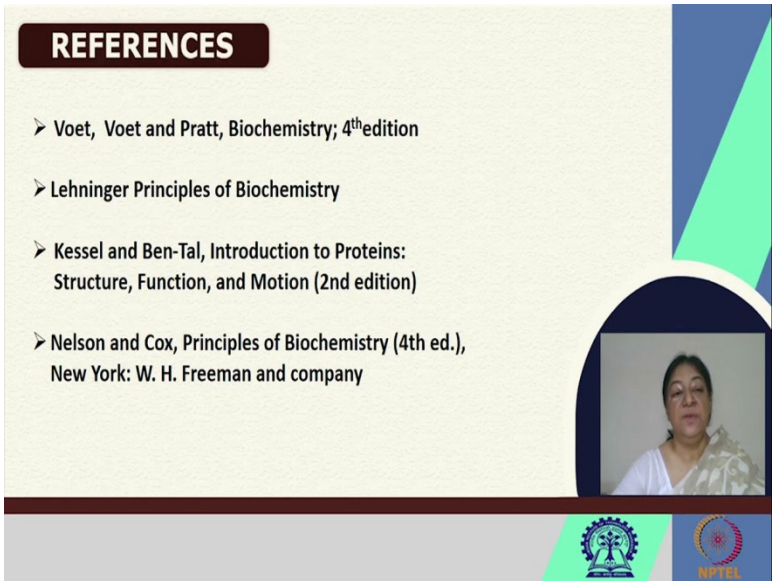

These [refer to slide] are the references.

Thank you.