Biochemistry
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Lecture - 11
Enzyme Mechanisms I

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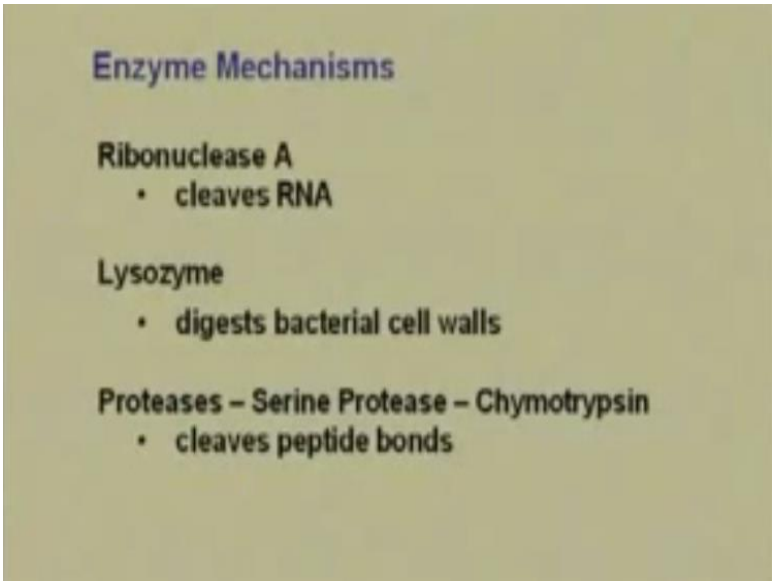

Okay, we continue our discussion on enzymes, last time we considered enzyme kinetics and how we could actually consider what would happen if we had specific inhibitors to the enzymes. Inhibitors to the enzymes, we consider competitive inhibitors, non-competitive inhibitors and uncompetitive inhibitors and how they interacted with either the enzyme or the enzyme substrate complex.

Today, what we are going to today is for two classes will be considering enzyme mechanisms and for this what I have done, I have chosen three types of enzymes where will we considering the enzyme mechanisms of Ribonuclease A, Lysozyme and Proteases, of the Proteases which we will see what they mean, we will be doing only one type of Serine Protease, that is called a Chymotrypsin.

Now essentially what these three different enzymes do is that Ribonuclease A cleaves RNA, RNA is ribonucleic acid. When we say or we speak about an A's, we know for example, a protease would cleave a protein or rather it cleaves a peptide bond.

What we are going to understand is when we consider an enzyme, we know that since, it is a biological catalyst, the way that it is going to work is that we are going to have specific residues that are going to bring about a certain reaction and after this reaction is through the enzyme has to get back to where it started from, because it has to go and catalyse another substrate molecule.

Okay, so that is exactly what we will see for each of these three cases as to how Ribonuclease A after cleaving RNA gets back to its original RNase A structure where it can go and cleave another RNA. The Lysozyme similarly, we will see how that works and also Chymotrypsin which cleaves peptide bonds.

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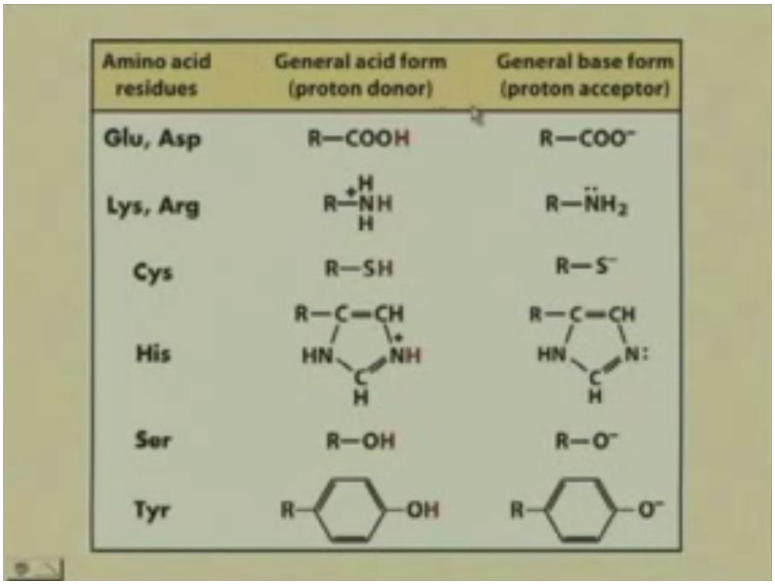

Now when we look at the amino acid residues that can actually are involved in all these mechanistic procedures that go on. This is a slide that I showed you before, but just to emphasise how each of these particular amino acid residues can assist in the catalytic activity or in the catalytic mechanism of each these enzymes are extremely important.

For example, for glutamic acid and an aspartic acid, we know we have, it can act as a proton donor and the (()) (03:37) can act as a proton acceptor. For Lysine and Arginine, it can act as a proton donor as well as a proton acceptor, so for Cystadane, Histidine, Serine and Tyrosine and we will see how most of these are involved in the enzymatic mechanism that we are going to study today.

For example, the one that we will be considering definitely is histidine. We will also see how serine and aspartic acid and even lysine is involved in these activities.

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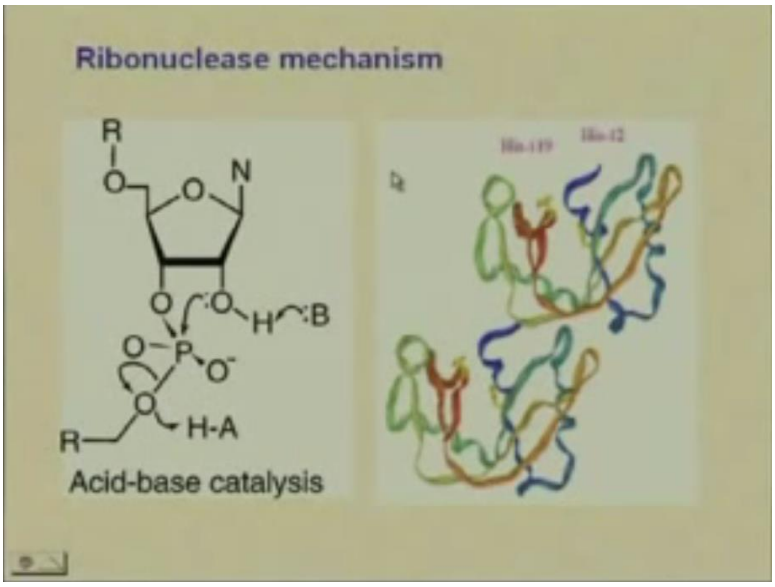

So the first one that, we are going to consider is the ribonuclease mechanism. Now what ribonuclease actually does is, we will be studying the structures of the nucleic acids later on, but for now what we have to consider is this is generally an acid base catalysis that occurs. Now in an acid base catalysis what you have, is you have, this is called a ribose sugar molecule.

To the ribose sugar molecule, we have different attachments but, the basic attachment of the important thing that we have to see here is the phosphate that is attached to this 3' bond as it is called. This N group happens to be a base when we consider the RNA molecule or the DNA molecules, which we will be studying as we go along. Now what happens is there is an abstraction of the OH of the hydrogen by a particular base okay.

This is going to be general acid base catalysis, but we will see how ribonuclease A in its structure will have the capacity or the capability to abstract this hydrogen from the ribonucleic acid molecule. So if we just consider the overall structure, there are two histidines, histidine 12 and histidine 119. These are the two histidines that are the catalytic residues for ribonuclease A.

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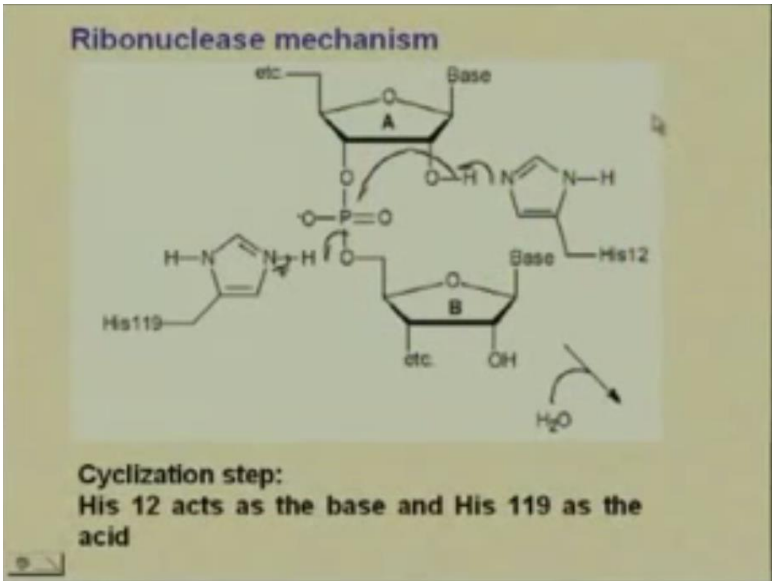

Now in the mechanism that we will study, we will see how we have the histidines act as proton donors and as proton acceptors. For example, in the first step, now let us go through the slide very carefully. What we find here is in the middle, we have the RNA molecule okay, what is the RNA molecule, you see there are two rings marked A and B. This is part of the RNA molecule, which is linked by what is called a phosphodiester okay.

So this is a phosphodiester bond and where we have ETC and ETC (()) (06:26) RNA forms a chain and we have the two ribose sugars in this case marked A and B and we have bases Purines or Pyrimidines that are these bases that are going (()) (06:47) when we study the structure of RNA later on, it will become more apparent as to what exactly these bases could be or how the chain is linked.

But for now, what we need to know is ribonuclease, the name means that it is going to cleave ribonucleic acid and it cleaves it means this is going to be broken, broken into two different parts were A is going to be separated from B via this phosphodiester linkage, so it is linked by this phosphodiester linkage and it is going to be cleaved right here by ribonuclease A. Now we come to the protein molecular.

Certainly, the protein molecule we know now is a long polypeptide chain where we have our histidine 12, that is this histidine on the right-hand side and we have histidine 119, that is on the left-hand side. Now the first thing that is going to happen is you see this OH, right. This hydrogen is going to be abstracted by histidine 12, not histidine 119. It is going to be abstracted by histidine 12, so this acts as an electron pair donor, so it is acting as a base.

So histidine 12 is acting as a base in abstracting the proton from what is called the 2'-OH of the A ring of the ribose sugar of the ribonucleic acid. Now what is going to happen as soon this proton is abstracted, there is a cyclic formation forming a cyclic phosphate. You see what is going to happen is, this or minus is going to attack this phosphate, once it attacks the phosphate, this is going to be cleaved.

Once this is cleaved, then this O is going to take up the hydrogen from histidine 119, so histidine 119 acts as an acid okay So let us go through this once more. We have the ribonucleic acid chain along the middle and we have to cleave (()) (09:18) the ribonuclease A is the enzyme that is cleaving RNA, the linkage is a phosphodiester linkage here and the molecule is going to be cleaved by the protein. The two catalytic residues are histidine 12 and histidine 119.

In the first step, histidine 12 acts as the base; it abstracts the proton from the 2'-OH of the ribose sugar ring of ring A that is linked to ring B by the phosphodiester linkage. In an event what happens that there is a cyclic phosphate intermediate formed and this H, the O that forms here this H, is abstracted from histidine 119, in what is called a cyclisation step and histidine 119 acts as the acid where histidine 12 has acted as the base.

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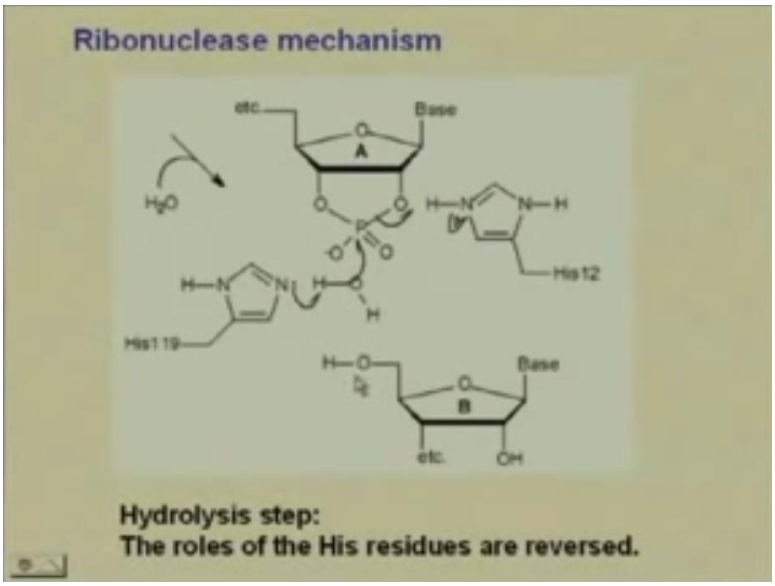

In the next step, so this is the cyclic part that is formed, you have to remember that this OH, this H came from the histidine 119. In the previous slide what did we see, this H was picked

up from histidine 119 because histidine 119 acted as the acid. Now histidine 12 had acted as the base and what had they done, it had accepted the H from the O.

Now when we have this, what do we have, we have a cyclic intermediate. Now this cyclic intermediate was formed in the previous step where we have now cleaved A and B okay, so RNase A has cleaved A and B. We now have a water molecule coming here, which is situated here, so we have HOH. Now what happens here now is histidine 119 now acts as the base, it abstracts a proton from water, creating OH minus that is going to attack the phosphate.

This phosphate then opens up here and picks up the hydrogen that was attached to histidine 12. In event what happens, histidine 12 now has provided the hydrogen, so it is an acid, so the roles of the histidine residues are reversed.

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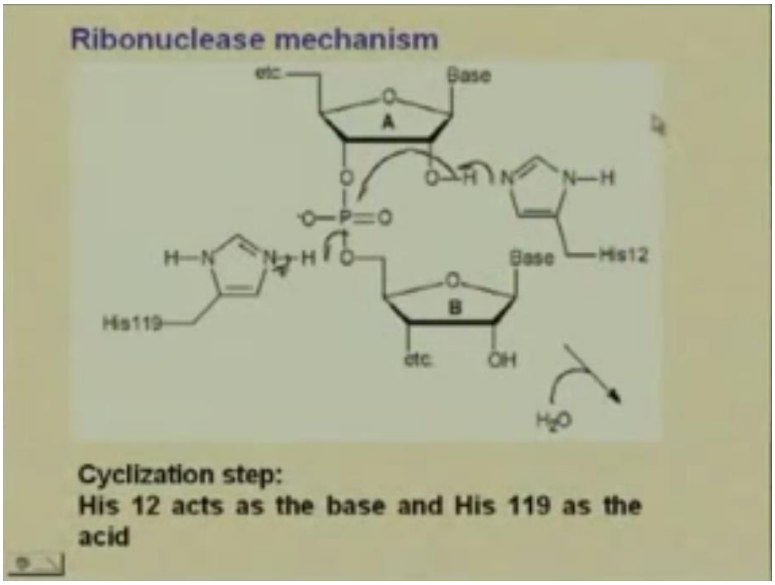

In the previous step, let us go back one slide. In the previous step, this histidine 12 acted as the base. It abstracted the H from the 2'-OH. Now after it abstracts the H, there are a cyclic phosphate formed and this cyclic phosphate results in the cleavage of the phosphodiester bond here releasing the rest of the RNA molecule. As this is released, this takes up the proton from histidine 119.

So histidine 119 behaves as the acid and histidine 12 behaves as the base in the first step that is called the cyclisation step.

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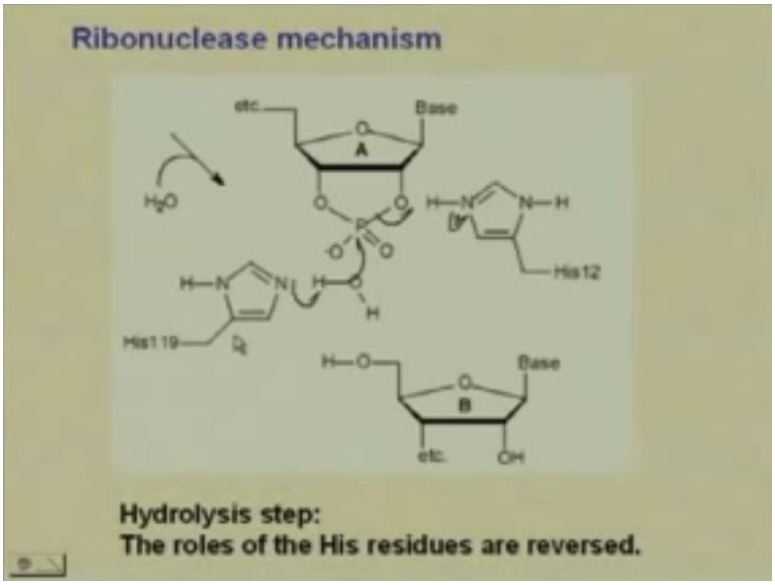

In the next step, so what do we have, we have now our cyclic phosphate intermediate. From the cyclic phosphate intermediate, we now have to get back the ribonuclease A protein where histidine can act as a base once more. So, what we are looking at now is we are looking at how histidine 119 in this case acts as a base and what is it do, it abstracts an H from water thereby becoming an H again here.

This OH minus now attacks the phosphate, this PO bond is cleaved, this or then takes up the H from histidine 12, so histidine 12 acts as the acid providing the H. So, the roles are reversed in what is called the hydrolysis step. So, we have a cyclisation step followed by a hydrolysis step. In the cyclisation step, histidine 12 is the base and histidine 119 is the acid. In the hydrolysis step, the roles are reversed. Histidine 119 is the base and histidine 12 is the acid.

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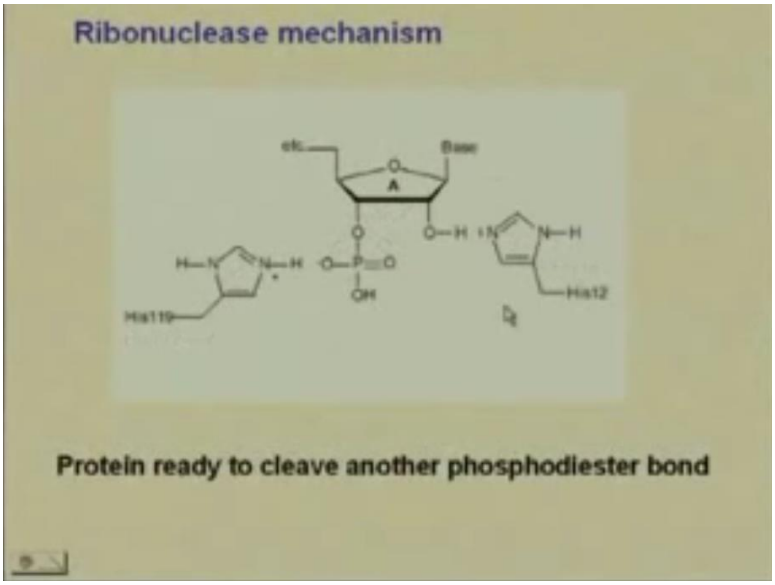

So whatever you left with now, we are back to our ribonuclease A where we have now histidine 119 that is now ready to provide another proton to behave as an acid and this histidine 12 has now lost the proton that it had and it is now back with the electron pair that it can do what, it can donate to another RNA molecule or another RNA part of the RNA chain where it will be able to cleave another RNA phosphodiester linkage.

Is that clear that is the way, so we have to remember now since each of these enzymes are catalytic. What is a catalyst mean, it has to be reusable? So, if it is reusable, what we have is we have this histidine 12, now what can it act as, it can act as a base again and the histidine 119 can act as an acid again. So, what can happen now, we can have another cleavage right. So, we can get back, so there are two steps, the cyclisation and the hydrolysis that will get us back to histidine 12.

But we have to remember you have to have these two steps. If you just had the first step, histidine 12 would have remained protonated and it would not be able to act as it should be acting when it is forming a catalyst or when it is acting as a catalyst. So that is basically the ribonuclease mechanism.

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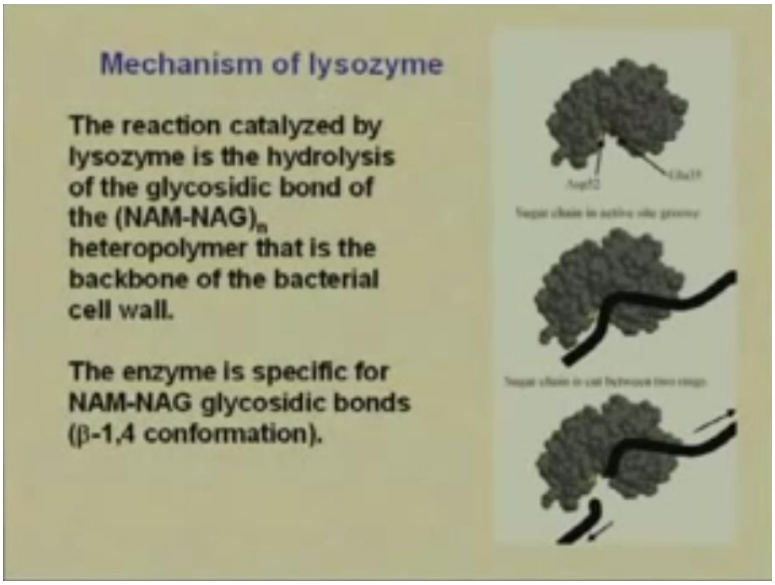

Now we will consider the mechanism of lysozyme. Now what lysozyme actually does is it cleaves a polymer that forms the backbone of a bacterial cell wall okay, let us understand this first. Now what you have is when you link sugars together, you have glycosidic linkages okay. When you link sugar molecules together, we will study in more detail when we do carbohydrates.

But for now we just suffice for us to know that we have a glycosidic linkage that is linking the two sugar molecules together. Now what are these sugar molecules, there are two sugar molecules here, one is called NAM and one is called NAG. We will see what these mean in a moment, but essentially let us look at just this schematic that we have on the right-hand side. We have two residues that are important in this particular enzyme.

Lysozyme important residues are aspartic acid 52 and glutamic acid 35 okay. What happens is, it has what is the site called, the site is called the active site where our substrate which is the sugar chain in this case will fit in the groove and then what will happen, there will be an enzymatic reaction and then there will be cleavage of the sugar chain okay. This is part of the bacterial cell wall and this enzyme is specific for what are called beta 1,4 glycosidic linkages okay.

We will study what these mean later on in more detail when we do carbohydrates, but now since just considering mechanisms, we will see how these two acidic groups actually work on the sugar chain.

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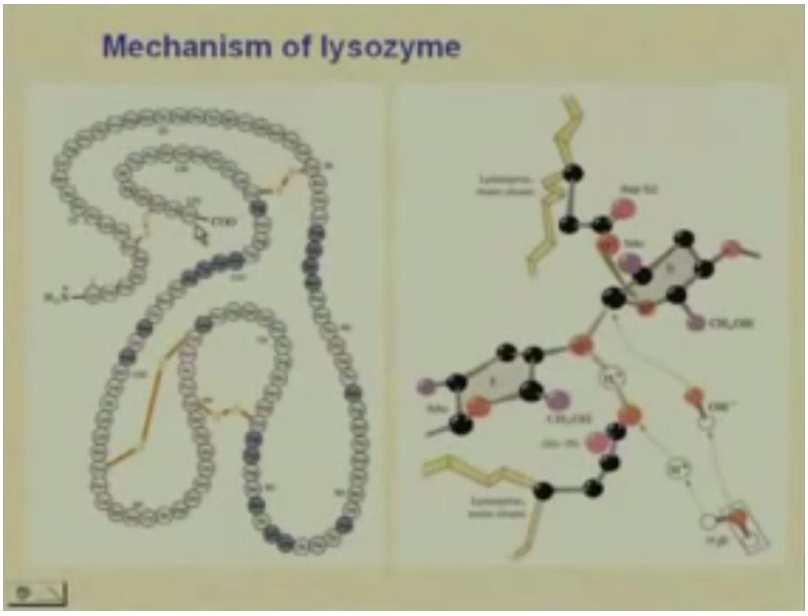

So this is essentially are lysozyme, it has a 129 amino acid residues and the important residues here are aspartic acid 52 and glutamic acid 35. So this yellow part that we see here is the main chain of the protein. The yellow (()) (18:56) that you see here is also a part of the main chain of the protein. When we look at the aspartic acid, we know we should have two oxygen atoms linked to these black atoms, which are the carbons.

So we have the C-alpha, we have another C and then COO, that is aspartic acid with glutamic acid, we have one extra CH2. So, we have our CH2CH2COO. Now what happens here is we have a D ring and an E ring of the sugar chain. Now we will see what that is in the next slide.

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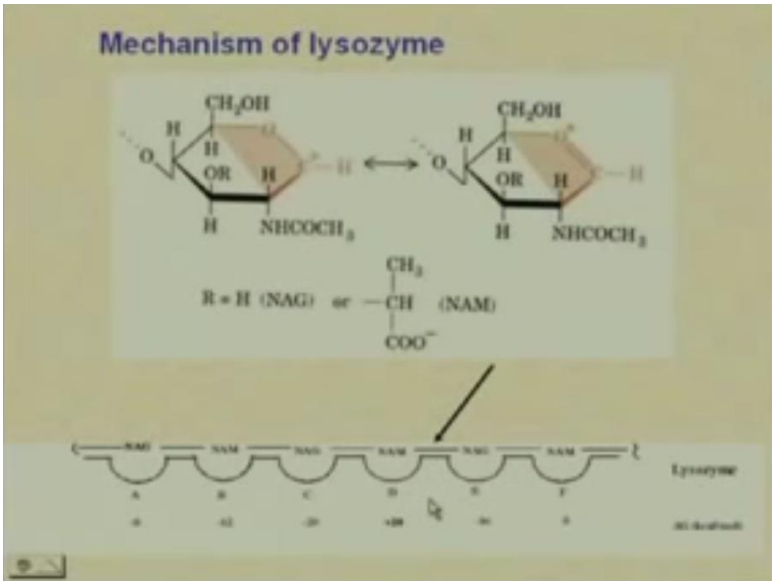

Now this is what is NAG and NAM okay. What are these, these are sugar molecules okay. You all studied sugar molecules in your organic chemistry class. Now what we have here is we have an R group. When this R group is hydrogen, it is called NAG (N-Acetylglucosamine). When this R group is (()) (20:21), it is called N-Acetylmuramate. So it is NAG and NAM and the N-Acetyl is because we have an NHCOCH3 attached here.

So, the basic structure of this is an NHCOCH3 that accounts for what is called the N-Acetyl in both cases and when we have this is just a resonance structure of the two sugar moieties that can happen and if the R is H, we have NAG and if the R is CH3CHCO minus it is NAM. Now what we have is you have alternating, NAG-NAM-NAG-NAM-NAG-NAM residues in the bacterial cell wall okay.

So, in the bacterial cell wall, we have there are six such sugar moieties in the bacterial cell wall part that fits in the active site groove of lysozyme okay. So, the active site groove of lysozyme that we saw in the initial schematic that I showed you can accommodate just six of these okay, it accommodates six of these and the alternate. So, we have an N-Acetylglucosamine and N-Acetylmuramate, NAG-NAM-NAG-NAM-NAG-NAM and so far.

Now the cleavage occurs this what I have below here are the delta G values. One of them is plus 20 okay, and this is where we have the cleavage, it cleaves between rings or other sugar moieties or other rings D and E. So the active site of the lysozyme fits these six sugar moieties and after it fits these six sugar moieties in the acid its active site groove, it cleaves between rings D and E, which is basically what we saw in the previous slide.

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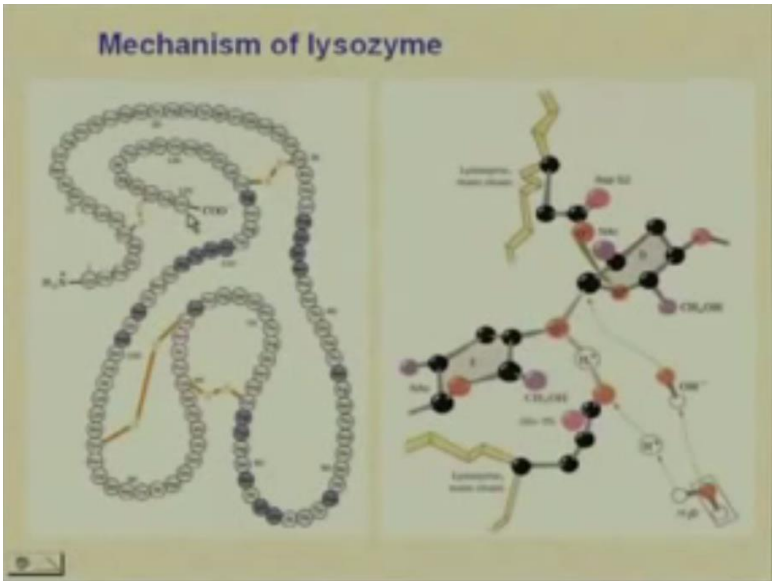

So we have rings D and E and they are linked by what is called a beta 1,4 glycosidic linkage. So we have a beta 1,4 glycosidic linkage that links the NAG and NAM residues together and the lysozyme chain or the lysozyme active site can accommodate six NAG-NAM parts of the bacterial cell wall and it will cleave between moieties D and E okay.

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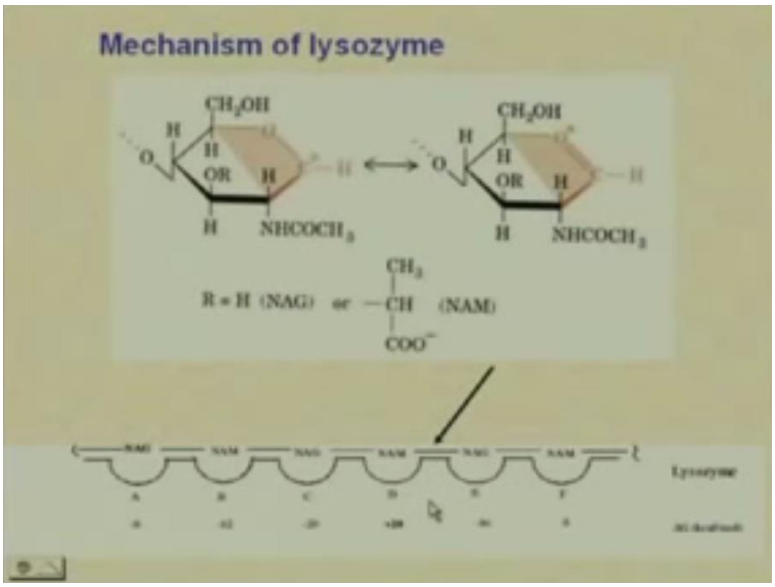

So we have our NAG, we have our NAM, we have a NAG-NAM set of six such units, which fit into the active site and the cleavage is going to occur between the D and the E. So this is what we have okay.

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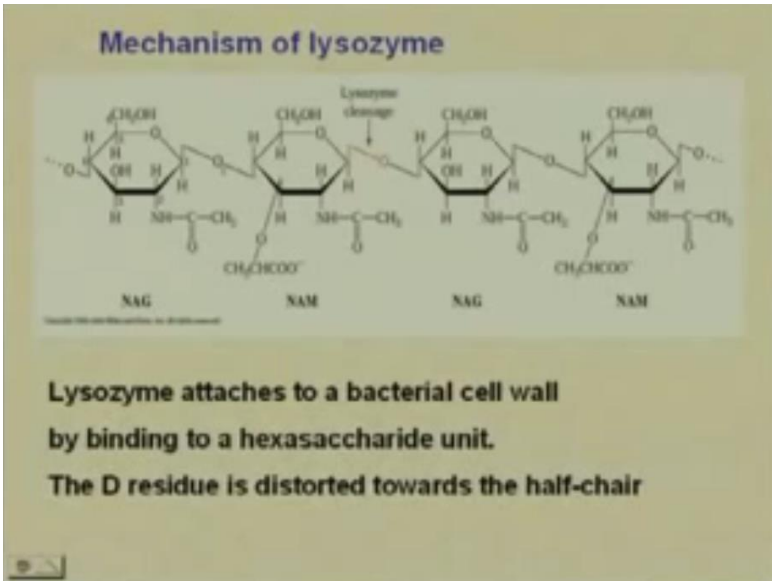

We have our NAG, what is the R for the NAG is H, that is this H as we see here. What is NAM, when we have CH3CHCOO minus okay, so that is NAM. Then we have the lysozyme that acts exclusively on this bond, it does cleave this bond, it is that specific okay. So it will only cleave the beta 1,4 glycosidic linkages okay. So what does it do, the lysozyme attaches to a bacterial cell wall by binding to a hexasaccharide unit.

What is this hexasaccharide unit, it is the ABCDEF alternating NAG-NAM residues or NAG-NAM units that we saw in the previous slide. The D residue where it is going to cleave, what do these type of six member chains form, six number cycling they can have a boat conformation or a chair conformation okay, so the stable conformation is the chair conformation.

Now what you have is, you have the D residue distorts towards the half-chair okay. When the NAG-NAM hexasaccharide unit fits into the active site groove of lysozyme, what happens is the D residue is distorted towards the half-chair formation.

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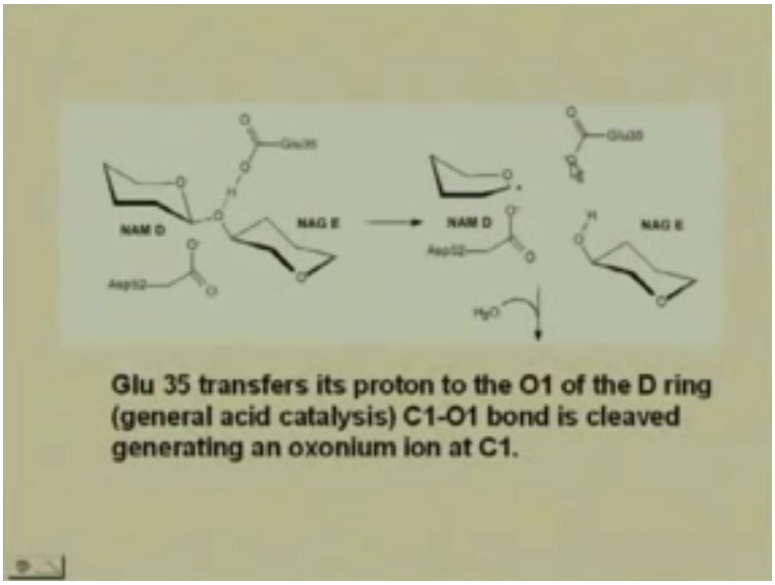

So what do we have, we have a NAM and a NAG. These are what, these are the sugar units okay, it is originally in the chair form. Why because the chair form is stable, now we have the two residues glutamic acid 35 and aspartic acid 52 that are the catalytic residues for lysozyme. Now what happens here is, this OH, what is this OH where has this come from glutamic acid, it can act as an acid because it basically is an acid.

So what happen is it cleaves then the linkage between the NAG and the NAM. So we have our N-Acetylmuramate and our N-Acetylglucosamine that is cleaved, what happens is glutamic acid 35 transfers its proton to the O1 of the D ring. This is the O1 of the D, so what happens is the C1-O1 bond is cleaved generating what is called an oxonium ion okay.

So what has happened now, we have now transferred a proton from glutamic acid 35, which has now become COO minus, aspartic acid is as it is yet. Now what is going to happen is now the lysozyme molecule at its present state cannot cleave another NAM NAG, why because glutamic acid does not have its proton to donate. So for lysozyme to act as a catalyst once more, the glutamic acid has to get back to OH, so what happens.

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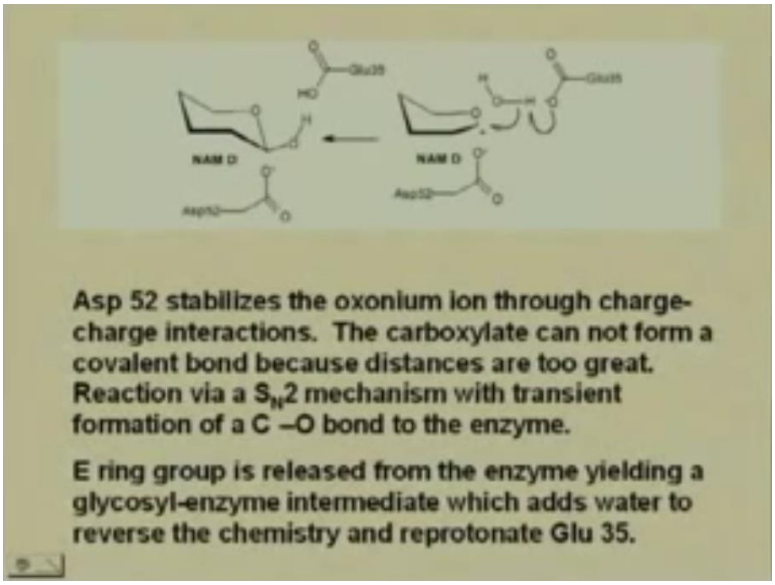

The aspartic acid 52, the role of this is to stabilise the oxonium ion, where is this oxonium from, how is it from, it is from the cleavage of the C1-O1 right.

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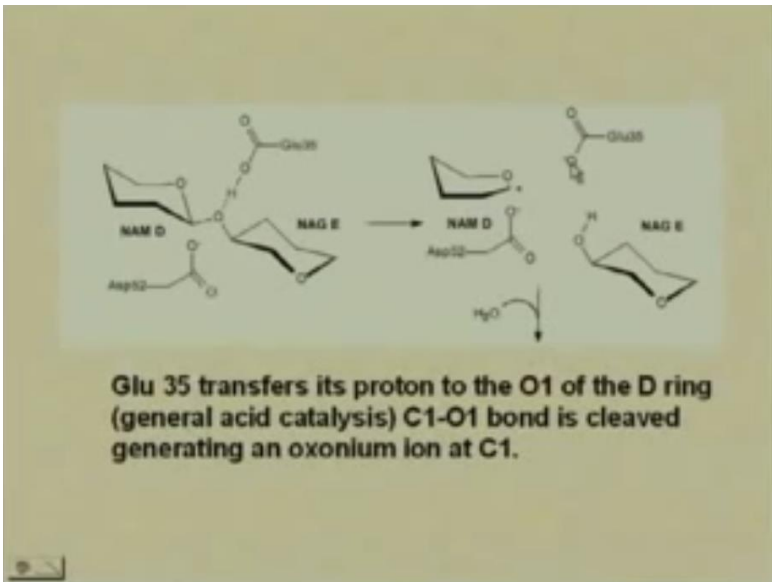

If you go back to the previous slide what is happening here, we have a C1-O1 bond cleavage. This bond has been cleaved, this bond. So what do we have, we have this plus charge here, now this plus charge is stabilised by the O minus of aspartic acid okay. So now, we have glutamic acid 35 that has donated its proton into the cleavage of this NAG-NAM-NAG set and we now have an oxonium ion that has been formed with a plus charge that is stabilised by the aspartic acid 52 O minus by charge-charge interaction.

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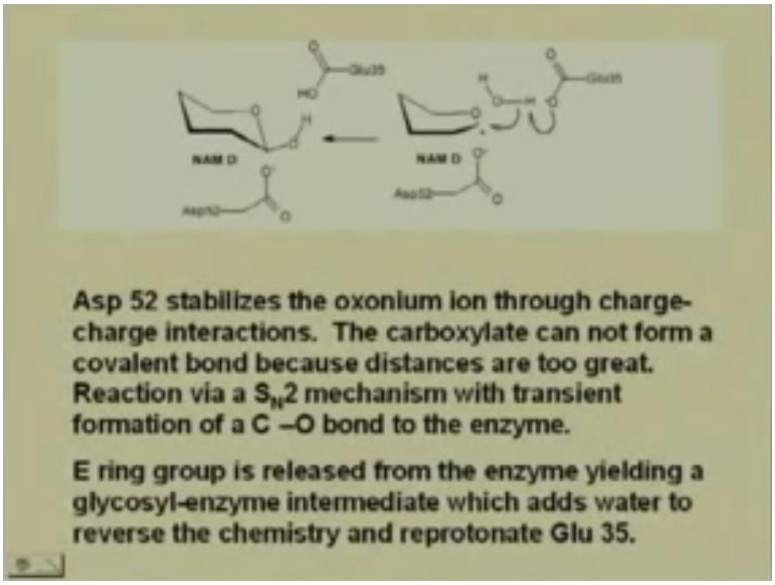

What happens now is, it does not form a covalent bond because the distances are too great, but it is enough to stabilise the oxonium ion. Now what happens is, there is a certain S N 2 mechanism with a transient formation of a C-O bond to the enzyme. What that means is that you have, for now what we need to know basically is that, first of all what is the first step is this H is abstracted from glutamic acid 35, this H okay.

This H is taken up and it is not as close, but the proximity is enough to stabilise it for a transient period and we have a water molecule come in and what happens to this water molecule, you have to remember that this O minus was formed due to what, when the H was taken up right for the cleavage. So now we have the O minus of glutamic acid 35 abstract and H from water.

Then what do we have, we have OH minus, what does OH minus do, it goes to the oxonium, the plus charge that is here and forms the sugar ring again. So what do we have, we have a cleaved ring okay. We have a cleaved basically glycosidic linkage and what do we have back, we have our glutamic acid back to the OH form, so now what can it do, it can go cleave another NAM-NAG set okay. So this is basically the way the catalysis would work okay.

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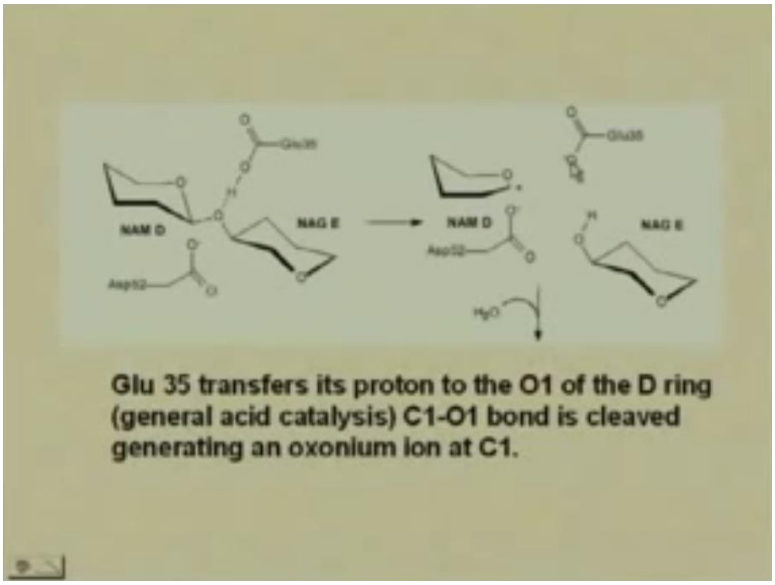

So what is our first step, let us just go back once more. So we have a beta 1,4 glycosidic linkage. In a beta 1,4 glycosidic linkage, what happens in the first step we know we are going to have a cleavage of between hexasaccharide, so the hexasaccharide unit fits into the active site groove of lysozyme. Once its fits into the active site groove of lysozyme, there is going to be a cleavage of this bond.

You have to remember it is a he cleavage of this bond, not this bond okay. The cleavage is between the NAG, the D, NAM rather the NAM, D, C1 and O1. What happens is glutamic acid 35 donates its proton and in the event it becomes COO minus, what happens is the cleavage of the C1-O1 of NAM occurs and it forms an oxonium ion that due to the proximity of the aspartic acid 52 is stabilised transiently by charge-charge interactions.

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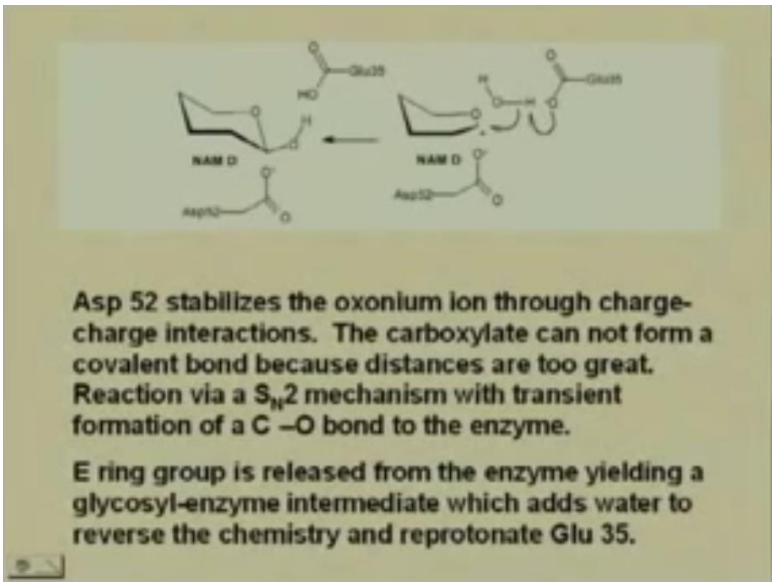

Once we have the stabilised form of the charge-charge interactions, it has enough time to get a water molecule in here. Then what happens to this water molecule, this glutamic acid 35 that is O minus abstracts an H from water, which forms OH minus, This OH minus then links with the distorted NAM ring that had been formed with the oxonium ion and forms the cleaved sugar ring here.

And we have aspartic acid, it is just the aspartic acid you see the role of aspartic acid is to stabilise the oxonium ion. It is the glutamic acid that is actually donating its proton then taking up a proton again okay. The role of aspartic acid here is to just stabilise the oxonium ion, but you understand that is extremely necessary for this to occur, so the glutamic acid carboxylate an ion as the OH of the water come close by and O minus can abstract the proton from the water.

So that this OH minus can then attack the oxonium ion or link with the oxonium ion to form the NAM okay. That is the way this would form, so we have a reversal in the chemistry that has occurred and we re-protonate glutamic acid 35.

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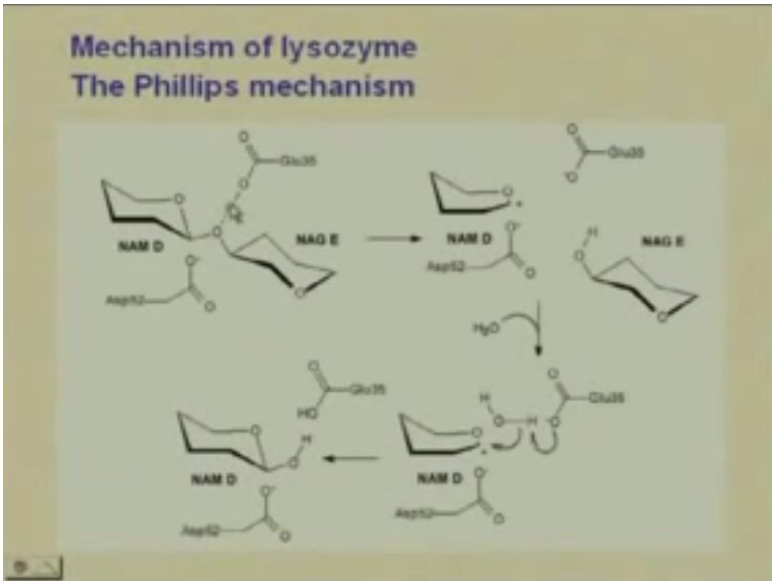

This is basically just a summary of what we just said, it is called by the way The Phillips mechanism and we have here therefore in the first step, the glutamic 35 produce or provide the proton. It provides this proton to this NAM-NAG set. We have the oxonium ion produce and the glutamic acid 35 form the CO minus.

Then we have a water molecule come in, what happens to the water molecule, the hydrogen is abstracted by the glutamic acid 35, which is now COO minus into becoming COOH once more and the OH minus attacks this oxonium ion. So we have now the NAG has already come off with an OH, the NAM also comes off with an OH. So we have a cleaved hexasaccharide unit between rings D and E when lysozyme attaches or the lysozyme catalyses the bacterial cell wall.

And we have this glutamic acid 35 ready to provide its proton to another NAG-NAM set okay. So you understand how these catalytic mechanisms are such, it is easy to look at these catalytic mechanisms because you have to remember that the protein has to get back to where it started right, because it has to cleave another substrate molecule and it can only cleave another substrate molecule, for example in this case, only if it can provide the proton.

In the previous case for ribonuclease A what did we see, we know that the histidine 12 can act as a base only if it has the electron pair to donate. So there has to be a step that is going to be a reversal in the chemistry in each case, which will get back to where you wanted it okay.

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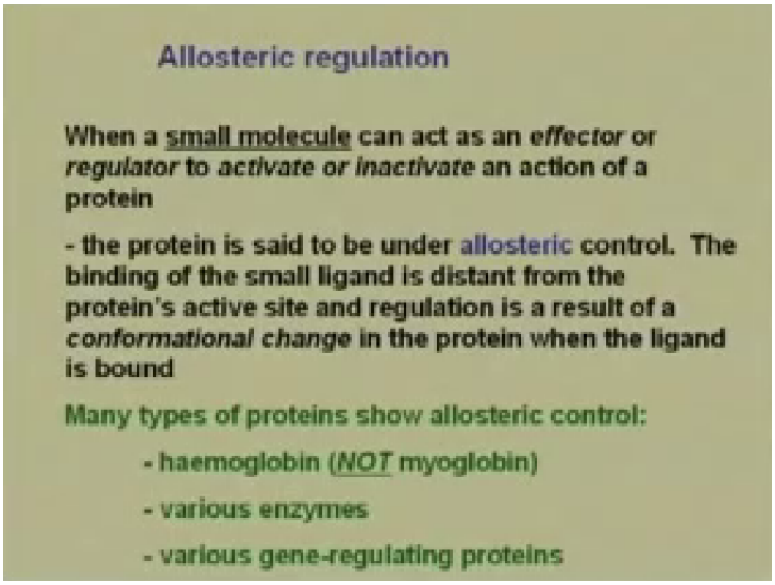

Now there are other things that we have to consider, which we will be later on where we consider what is called allostery because when we are studying enzymes, we need to know what allostery actually is. Now we have certain molecules that we have looked at inhibition of enzymes, activation of enzymes okay.

Now what happens is we have these small molecules, small inhibitors or effectors or

substrates act on the enzyme, what they essentially do is this small molecule that you have can actually act as an effecter or a regulator to activate or inactivate an action of a protein, now what do we mean by that. When this small molecule, for example we will be doing haemoglobin later on, for example, we know that haemoglobin binds oxygen.

Oxygen is a small molecule that binds to haemoglobin. Now haemoglobin when oxygen is bound to it, it acts as an effecter or regulator into activating haemoglobin. What is that mean, the protein is set to be under allosteric control, this means that for example, if I just show you what it means here, suppose we have our haemoglobin molecule, we have our haemoglobin molecule that is actually comprised of four different subunits.

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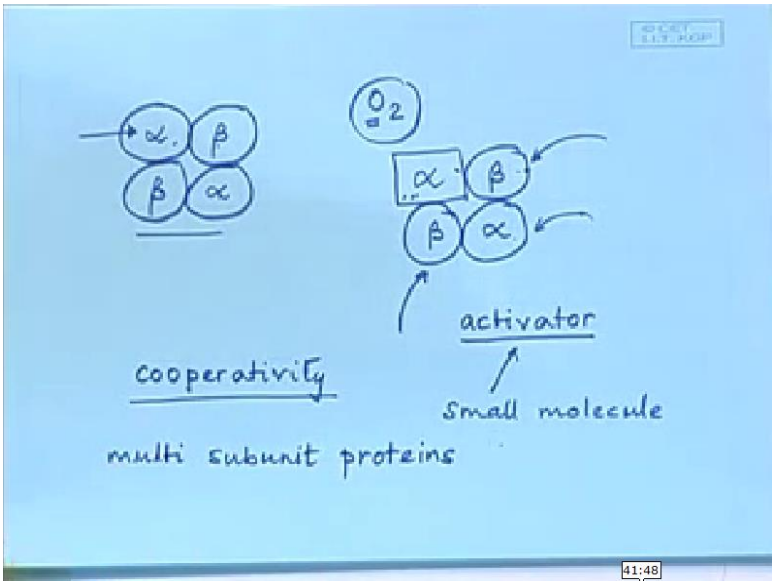

Now in the four different subunits, this is something that we did in one of the earlier classes, we have alpha subunits and two beta subunits okay. Now what happens is each of these bind an oxygen molecule. An oxygen molecule is a small molecule. Now when it binds to say, it binds to this alpha. If it binds to this alpha, there are some mechanisms that say that the conformation of the alpha changes slightly into becoming like this.

So then we know that it is that alpha that has bound the oxygen to it okay, because it has a slight conformational change. Now due to this oxygen being bound to this alpha, this will assist another oxygen being bound to the other subunits of haemoglobin. So it acts as what it is called an activator. What is this activator, the activator acts in such a fashion that if one of these happens to bind to one of the subunits.

Then it is going to help or facilitate the binding of oxygen to the other subunits. This is what is called cooperativity. What is cooperativity then, it once this oxygen is bound, it helps the binding of the other oxygen molecules because four oxygens can bind, there are four oxygen molecules actually bind, so now in this is case the affinity for the oxygen for each case is the same, but as soon as we have one of the oxygens bind.

Then what happens is the affinity for oxygen increases in what is called a cooperative effect okay. So essentially what we have is we then call the small molecule that we have here, what is a small molecule in this case, it is oxygen. The small molecule becomes our activator right or it is an effecter. We can also have an inhibitor in this case. Like for example, if carbon monoxide binds to haemoglobin.

But what we mean by cooperativity then when we have the facilitation of binding of the other ligand molecule**s** that is assisted by the binding of the first okay, so that is what is called cooperativity. There are many proteins actually that show cooperativity and the ones that show cooperativity are usually multi-subunit proteins.

What is that mean because you have these subunits together, if you have one of them activated or one of them bound to the small molecule, then it activates the binding of the other subunits to the ligand as well, so these are usually for multi-subunit proteins okay.

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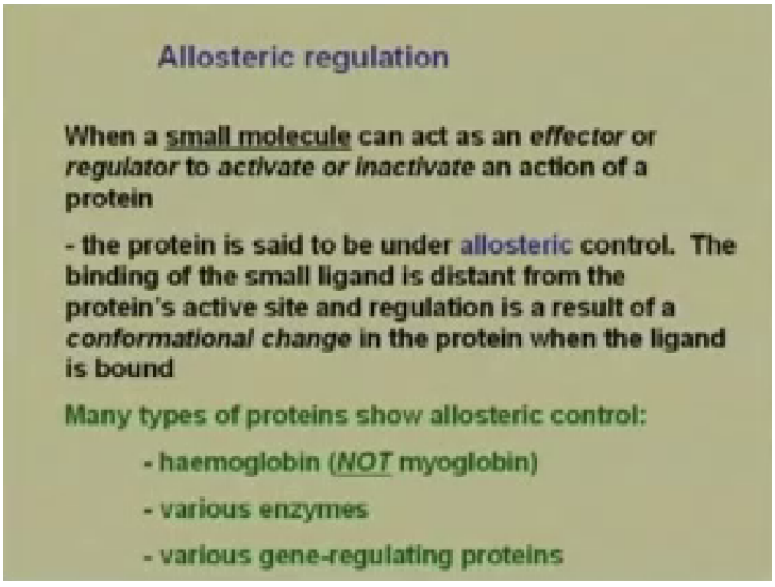

So now what do we have here therefore, we have a small molecule that can act as an effecter or regulator to activate or inactivate an action of the protein, so what is the action of

haemoglobin to bind oxygen. What is it doing, when the small molecule is bound, it activates the binding of other oxygen molecules to the other subunits; then we say the protein is under allosteric control okay.

This is allosteric regulation basically, so the binding of the small molecule is distant from the proteins active site and regulation is a result of a conformational change in the protein when the ligand is bound okay. There are many types of proteins that show allosteric control, for example haemoglobin, not myoglobin. Myoglobin is a single-chain protein that also binds oxygen, but it does not show any allosteric control primarily.

Because it is not multi-subunit, and just has a single polypeptide chain. There are other various enzymes that show allosteric control and there are gene regulating proteins that also can show allosteric control.

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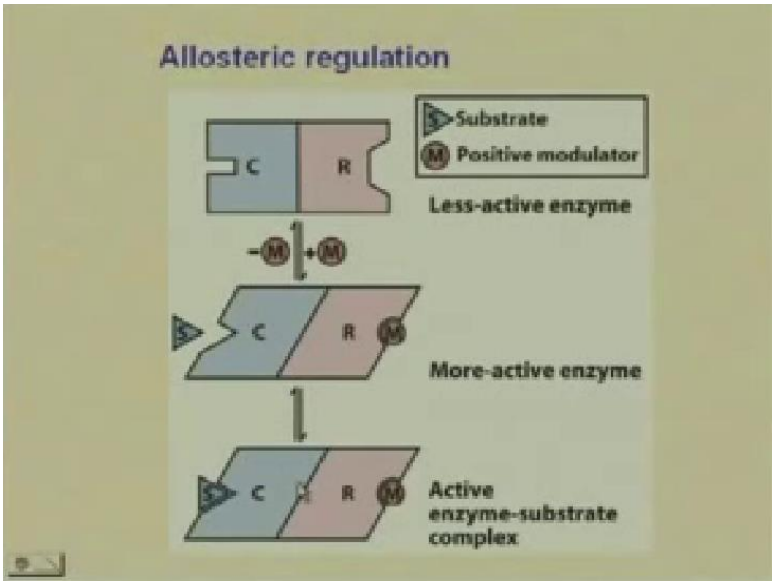

So example, let us have this, we have an allosteric regulation, a less activate enzyme okay. These are two subunits of the enzyme; we have a positive modulator and the substrate. Now if you look, we can consider say a lock and key mechanism for this and we say basically what happens is if we look just at lock and key mechanism, this substrate cannot fit anywhere okay, it does not fit in the active site and this is basically a less active enzyme.

As soon as we have a modulator fit into this, so there is an induced fit of this particular subunit that will link to the modulator. Once this linkage occurs, we have a conformational change here, what happens to the conformational change then, this can now accommodate the

substrate easier, so we have initially we had a less active enzyme, we have a modulator that make some more active enzyme and then we have the enzyme substrate complex okay.

Now, why is this essential, when you have a biological activity, you do not want the enzyme substrate complex to always be formed, because you probably do not need the product in the first place okay. So you have to have it regulated, so that when the product is needed, there were will be a small molecule, a positive modulator, they are usually called cofactors or you have metals even metals irons that act like this, where only in the presence of the metal iron or in the presence of a particular.

Let say small group you will have the enzyme active, because you do not want the enzyme to be active all the time, that might lead to a like if you have say haemoglobin active all the time, it is going to keep on adding oxygen, keep on adding oxygen, but you wanted to de-oxify also okay, so it has to be deoxygenated at time, so we have to have regulation okay.

This regulation is brought about by these modulators or effectors, so what we have is the positive modulator that is going to act on a less active enzyme to give you or conformational change. The conformational change is going to amount to what; it is going to amount to a fit of the substrate to the enzyme now, which was not previous earlier. It was not possible earlier previously, which is now possible and you form an active substrate complex that can then do what, it can then go onto form the product okay.

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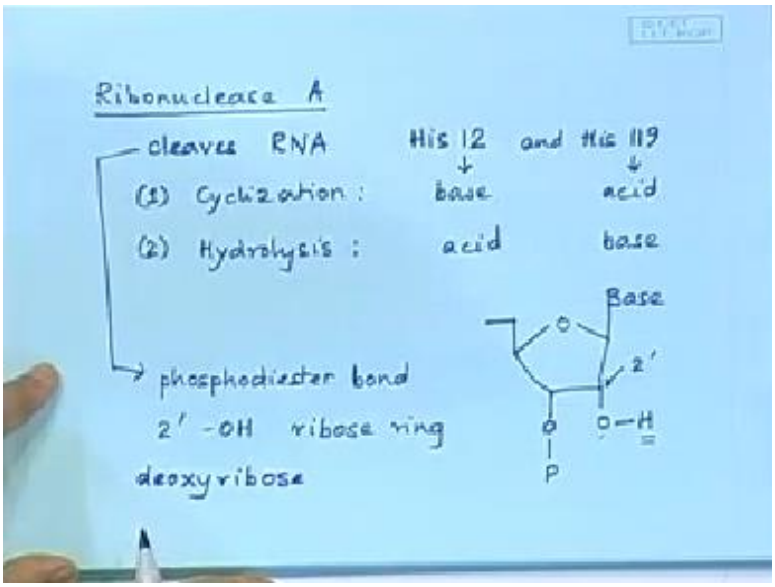

So now let just summarises to what we did today, so we looked at the mechanism of

ribonuclease A, which cleaves RNA. The two residues that are important in this case are histidine 12 and histidine 119. We have two steps; the first step is a cyclisation step. In the cyclisation step, histidine 12 acts as the base and histidine 119 acts as the acid okay. Then in the second step, in the cleavage what has been cleaved here.

We have the cleavage of a phosphodiester bond. Now in the next step, we have what is called hydrolysis. In hydrolysis, we have a reversal of roles, so histidine 119 now acts as the base and histidine 12 acts as the acid. Now when we have this phosphodiester bond and we have the cyclisation, we still have not studied DNA and RNA structure, but we are looking at ribonuclease A. Now if you look at the basic form of the sugar, this is where we have a base attached.

A base is either a Pyrimidine or a Purine. What did we have here, we had the O and we had the P right, what did we have here the O and the H right that is what we had initially. Now what happen to this H, this H was taken up by the histidine in the first step right and we had the formation of a cyclic, so this then attached here and we had a cyclisation basically, it is called a cyclic phosphate ring that is formed here and then we have the hydrolysis.

Now if this particular carbon is called a 2' right, so we have the 2'-OH of the ribose ring where is H is abstracted. Now if I have what is called DNA okay, if I want to know whether ribonuclease A is going to cleave DNA, what DNA is, DNA is deoxyribose okay, so what is deoxyribose.

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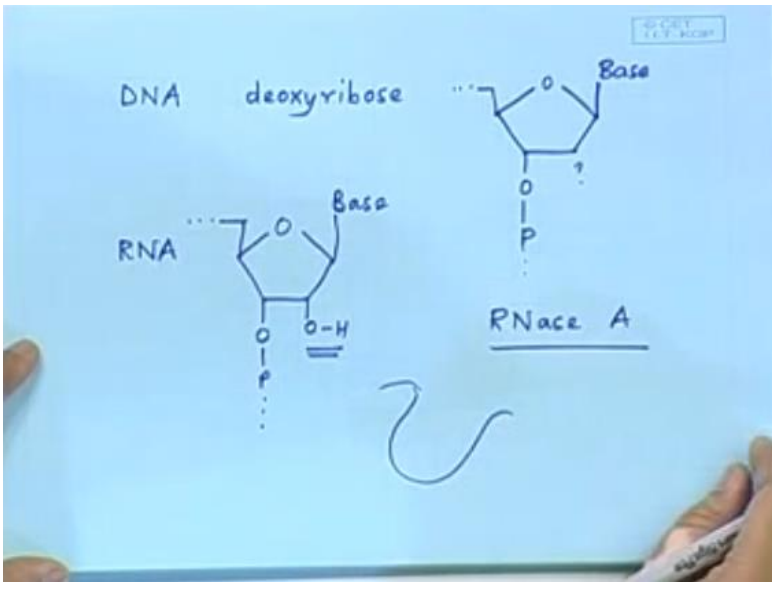

If we have DNA, we have deoxyribose, it is essentially the same, (()) (49:33) this is missing, it is de-oxy at the 2 position, not (()) (49:50). What did I have for RNA; I had this O, P and the base. Now therefore, if I have ribonuclease A, which is written in short-form like this RNase A act on these two. It will not be able to cleave DNA, why because there is nothing here right.

It cannot abstract the proton here, it cannot form a cyclisation step or anything here, so what can this be, this can act as a sort of an inhibitor to ribonuclease A, because it looks very similar, but ribonuclease A will not be able to cleave DNA right, but there will be other parts are similar, so when it fits into the active site, say we have an active site here, when it fits into the active site, it may so happen that it probably will fit.

But it will not cleave why because it cannot form the cyclised phosphate intermediate just because of the absence of this OH. So you see how extremely specific this particular protein is in cleaving just RNA okay. So this is the way, we have the enzymatic mechanisms extremely specific for whatever particular enzymatic reaction they are supposed to catalyse okay.

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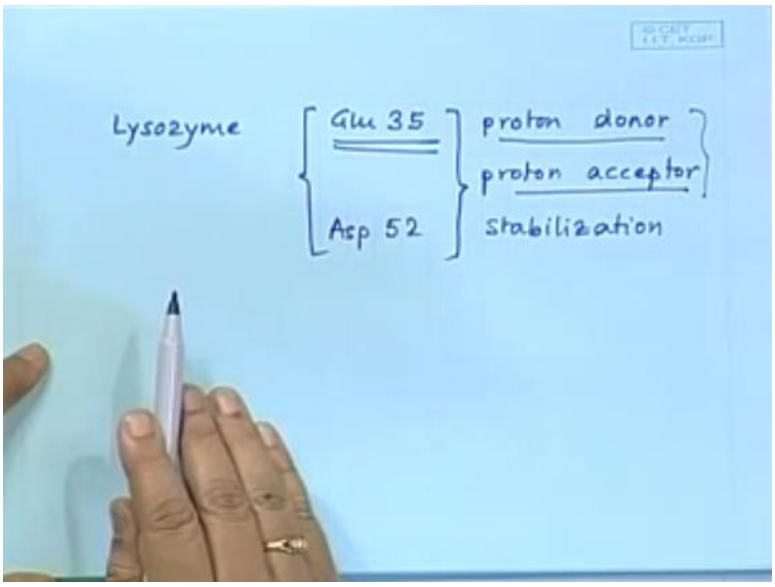

So what we did next was we consider the mechanism of lysozyme where what we saw was glutamic acid act as what did it do initially in the first step act as a proton donor and then a proton acceptor. So it is basically only the glutamic acid that is taking part and the aspartic 52 was there for just for stabilisation right that is the role of the aspartic 52. Now so can you tell me now at what PH this would be active.

The PH at which lysozyme would be active would be lower than RNase definitely, why because you have to have a proton donation and a proton acceptor activity of glutamic acid. When is that possible, because you know the pKa of glutamic acid is low, so the pH has to be low for the particular protonation, deprotonation to occur, but for ribonuclease A it is histidine, so it is going to occur around 6, 7 in that range. So we will stop this lesson and we will go on to the next one later on. Thank you.