Biochemistry - I

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Lecture –10
Enzymes Mechanisms –I

Welcome, we will continue our discussion on Enzymes. Last time we have considered Enzyme Kinetics and how we could actually considered is what would happen if we had specific inhibitors to the Enzymes. (Refer Slide Time 1:00 min). In the category of inhibitors to the enzymes we considered Competitive inhibitors, Noncompetitive inhibitors and Uncompetitive inhibitors and how they interacted with either the enzyme or the enzyme-substrate complex.

Today, what we are going to do is for two classes we will be considering Enzyme Mechanisms. And for this what I have done is I have chosen three types of Enzymes where we will be considering the Enzyme Mechanisms of Ribonuclease A, Lysozyme and Proteases. Of the proteases, we will be doing only one type of Serine Protease that is called a Chymotrypsin.

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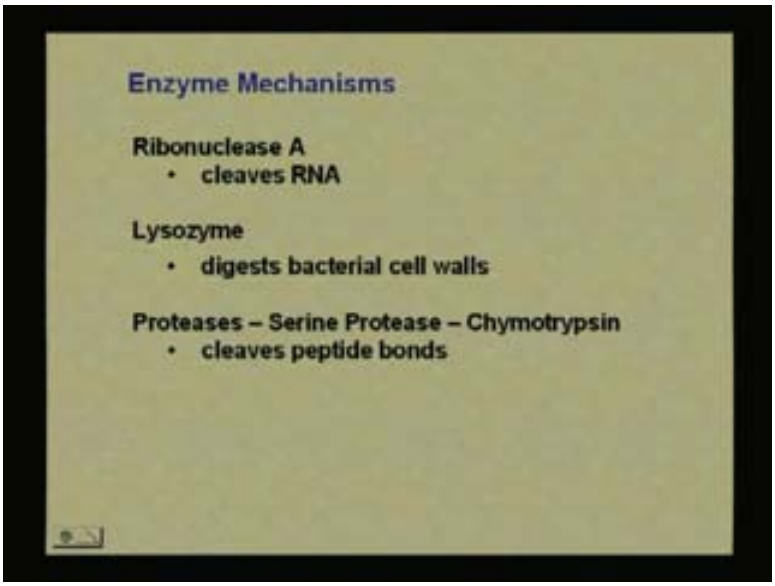

Now, essentially what three different enzyme do is that Ribonuclease A cleaves RNA where RNA is ribonucleic acid? When we speak about an ease we know for example a Protease would cleave a Protein or rather it cleaves a peptide bond. And what we are going to understand is when we consider an Enzyme we know that since it is a biological catalyst the way that is going to work is we are going to have a specific residues that are

going to bring about a certain reaction and after this reaction the enzyme has to get back from where it started because it has to go and catalyze another substrate molecule. so that is exactly what we will see for each of these three cases as to how Ribonuclease A gets back to its original RNA ease structure after cleaving RNA where it can go and cleave another RNA. (Refer Slide Time 2:48 min)

Similarly we will see how the Lysozyme works and also Chymotrypsin which cleaves peptide bonds. Now, when we look at the Amino acid residues that can actually involved in all this mechanistic procedure that go on.

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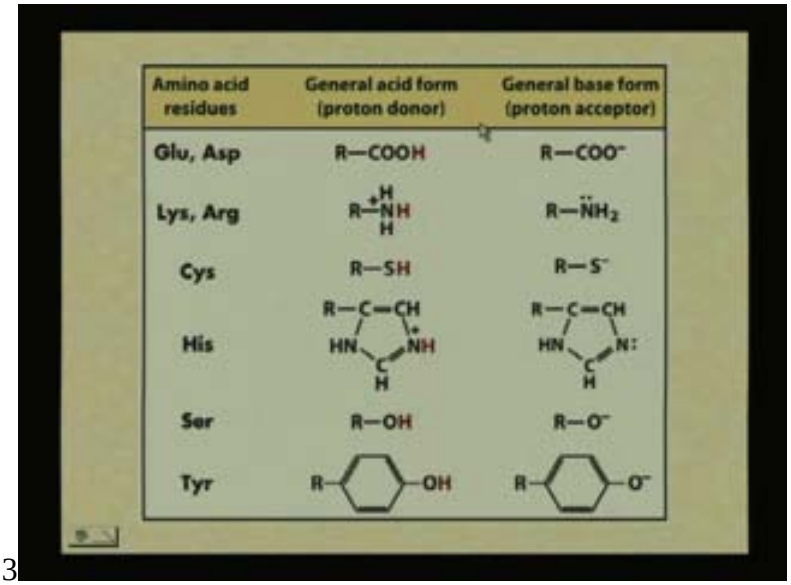

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But just to emphasize how each of these particular Amino acid residues can assist in the catalytic activity or in the catalytic mechanism of each of these enzymes is extremely important. For example, for Glutamic acid and Aspartic acid we know that it can act as a proton donor and the anion form can act as a proton acceptor. For Lysine and Arginine it can as a proton donor as well as a proton acceptor. For Cysteine, Histidine, Serine and Tyrosine we will see how most of these are involved in an Enzymatic Mechanisms that we are going to study today. (Refer Slide Time 3:58 min)

For example, the one that we will be considering definitely is Histidine and we will also see how Serine and Aspartic acid and even Lysine is involved in these activities. So, the first one that we are going to consider is the Ribonuclease mechanism. What an 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 generally an acid-base catalysis that occurs. In acid-base catalysis what you have is you have a Ribosugar molecule, to the Ribosugar molecule we have different attachments but the basic attachment or important thing that we have to see here is the phosphate which is attached to this three prime bondage.

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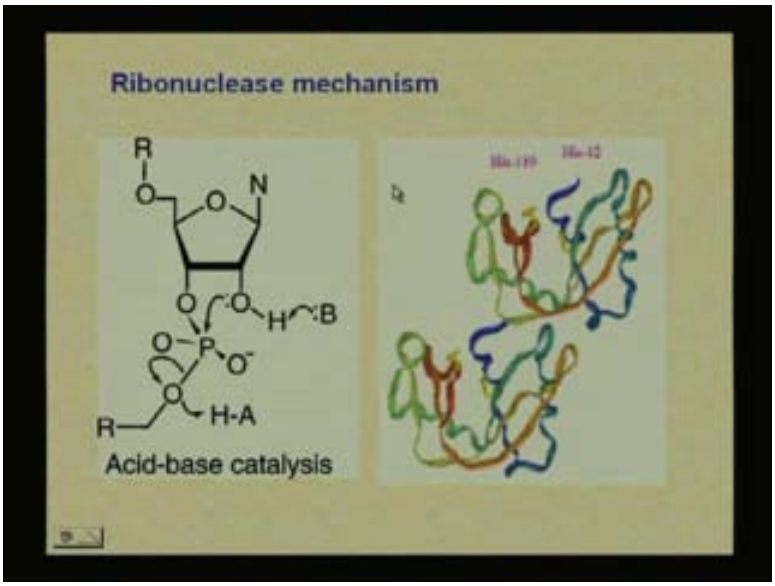

This N group happens to be a base when we consider the RNA molecule or the DNA molecules which will be studying as we go along. Now what happens is there is an abstraction of the OH of the hydrogen by a particular base. This is going to general acid-base catalysis. But we will see how Ribonuclease A 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 which are Histidine 12 and Histidine 119. These two Histidines are the catalytic residues for Ribonuclease A.

In the mechanism that we will study we will see how the Histidines can act as proton donors and can act as proton acceptors. For example, in the first step what we find here is in the middle we have the RNA molecule. What is the RNA molecule is you see there are two rings marked A and B, this is part of the RNA molecule which is linked by a phosphodiester. So this is the phosphodiester link and where we have etc and etc written is the RNA chain. (Refer Slide Time 6:30 min)

This RNA forms a chain and we have two good Ribosugars in this case which are marked as A and B and we have Base basis during the Purines or Pyrimidines that are these basis which we will study later on. And when we study the structure of RNA later on it will become more apparent as to what exactly this basis could be or how the chain is linked. But for now what we need to know is Ribonuclease the name itself means that it is going to cleave Ribonucleic acid and it means this is going to be broken into two different parts where A is going to separate it from B for this phosphodiester linkage.

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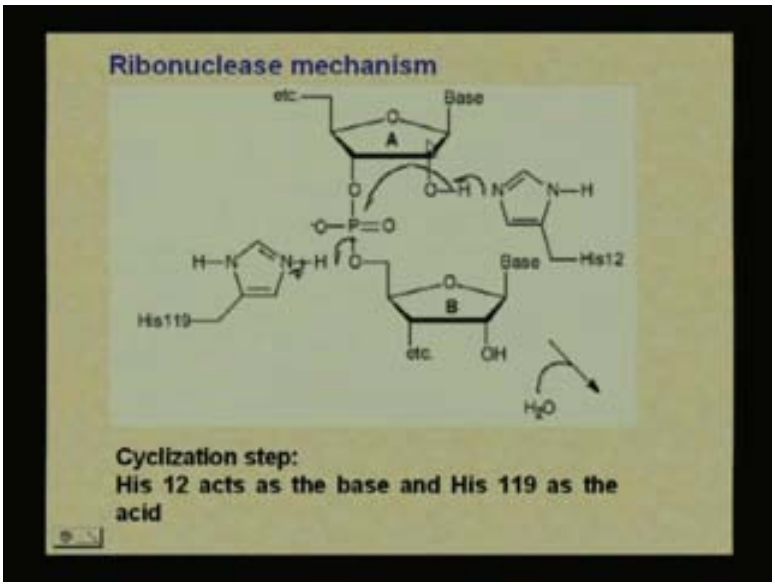

So, it is linked by the phosphodiester linkage and it is going to be cleaved right here by Ribonuclease A. Now we will come to the protein molecules. We know that the protein molecules are long polypeptide chains where we have our Histidine 12 and this Histidine 12 is on right hand side and we have Histidine 119 is on the left hand side. The first thing that is going to happen is you can see this OH this hydrogen is going to abstracted by Histidine 12 but not Histidine 119. It is going to be abstracted by Histidine 12 so this can act as on electron pair donor. So it is acting as a base. So Histidine 12 is acting as a base in abstracting the proton from the two prime OH of the A ring of the Ribosugar of the Ribonucleic acid.

Now what is going to happen is as soon as this proton is abstracted there is a cyclic formation forming a cyclic phosphate. (Refer Slide Time 8:43 min). You will see that what is going to happen is this O^- is going to attack this phosphate. Once is attacks the phosphate this is going to be cleaved, once this is cleaved then this O will take up the hydrogen from Histidine 119. So Histidine 119 acts as an acid.

Let us go trough this once more, we have the Ribonucleic acid chain along the middle and we have to cleave means the Ribonuclease A enzyme is cleaving RNA which the cleave the linkages of 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 and it abstracts the proton from the two prime OH of Ribosugar ring of ring A which is linked to B by the phosphodiester linkage. (Refer Slide Time 9:55 min)

In an event what happens is there is a cyclic phosphate intermediate formed and the O that forms here and this H is abstracted from Histidine 119 in the cyclization step and Histidine 119 acts as acid where Histidine 12 as acted as the base.

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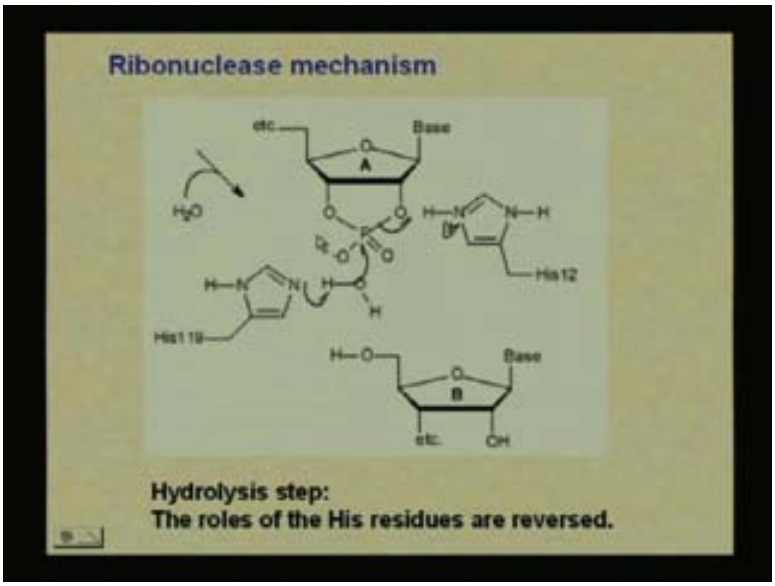

In the next step, so this is the cyclic part that is formed and you have to remember that in this OH this H came from the Histidine 119. In the previous slide what did we see is this H was picked from Histidine 119 because Histidine 119 was acted as the acid. Now Histidine 12 has acted as the base and it had accepted the H from the O. When we have this? What do we have? Here we have a cyclic intermediate and this cyclic intermediate was formed in the previous step where we have cleaved A and B. So RNA has cleaved as A and B. now we have a water molecule coming from here which is situated here so we have H-O-H. (Refer Slide Time 11:29 min)

Now here what will happen is Histidine 119 can acts as the base, it abstracts the proton from water creating OH^- that is going to attack the phosphate. Then this phosphate will opens up here and picks up the hydrogen that was attached to the Histidine 12. In this case what happens is Histidine 12 has provided the hydrogen so it is an acid. So the roles of the Histidine residues are reversed. In the previous step this Histidine 12 acted as base and it abstracted H from the two prime OH. There is a cyclic phosphate formed after it abstracting the H and here this cyclic phosphate results in the cleavage of the phosphodiester bond releasing the rest of the RNA molecule. As this is released this takes up the proton from Histidine 119.

So the Histidine 119 behaves as the acid and Histidine 12 behaves as the base in the first step that is called the cyclization step. (Refer Slide Time 13:29 min). In the next step, we have now a cyclic phosphate intermediate. Now from the cyclic phosphate intermediate we have to get back the Ribonuclease A protein where once more Histidine can act as a base. So now what we are looking at is how the Histidine 119 acts as a base in this case.

And what is it do is it abstracts an H from water there by becoming NH again, this OH^- is now attacks the phosphate, this P-O bond is cleaved and this O will then takes up the H form Histidine 12. So, Histidine 12 acts as the acid providing the H.

So the roles are reversed in the hydrolysis step. So we have a cyclization step followed by a hydrolysis step. In the cyclization 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. (Refer Slide Time 14:49 min). so now what ever we left is we are back to our Ribonuclease A where have Histidine 119 that is now ready to provide another proton to behave as an acid and this Histidine 12 has lost the proton that it had and now it back with the electron pair it can donate to an other RNA molecule or an other RNA part of a RNA chain where it will be able to cleave an other phosphodiester linkage.

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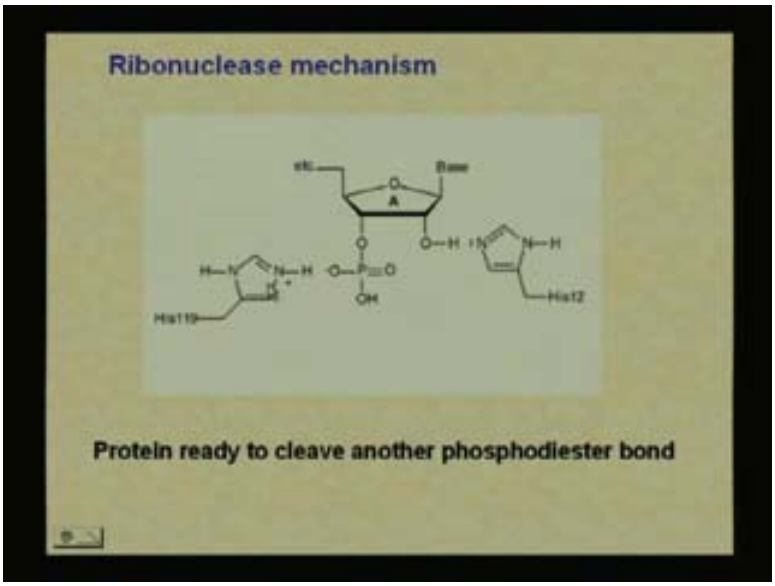

You have to remember since each of these enzymes is catalytic in nature means it has to be reusable. So if it is reusable then what we have is we have this Histidine 12 and now again it can act as a base and again Histidine 119 can act as a acid. So now what can happen is we can have another cleavage so we can get back. So there are two steps that are the cyclization and the hydrolysis that will get us back to Histidine. But you have to remember that you should have these two steps. If you just have the first step Histidine 12 would have remain protonated. And it would not be able 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. (Refer Slide Time 16:33 min)

Now we will consider the Mechanism of Lysozyme. What Lysozyme actually does is it cleaves a polymer that forms the backbone of a bacterial cell wall. Now what do you have is when you link sugars together then you have glycosidic linkages. We will study the linking sugar molecules together in more detail when we do carbohydrates.

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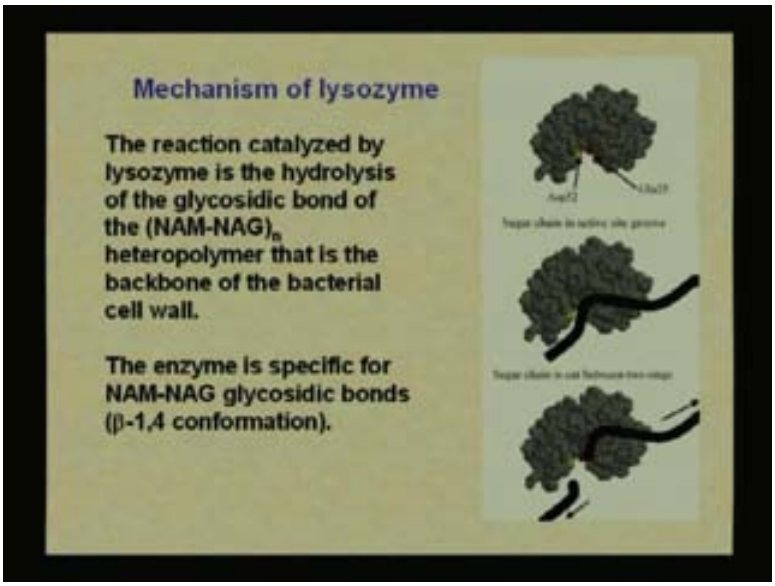

But for now you just this suffices for us to know that we have a glycosidic linkage that is linking the two sugar molecules together. What are these sugar molecules? Here there are two sugar molecules one is called NAM and other is called NAG. Essentially let us look at this schematic that we have on 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.

What happens is it has the active site where our substrate which is the sugar chain in this case will fit in the groove and there will be an enzymatic reaction and then there will be cleavage of the sugar chain. This is part of the bacterial cell wall and this enzyme is specific for what are called beta β- 1, 4 glycosidic linkages. We will study later on what these mean in more detail when we do carbohydrates but now since we just considering mechanisms. We will see how these acidic groups are actually worked on the sugar chain. (Refer Slide Time 18:37 min)

So this is essentially our Lysozyme which has hundred and twenty nine amino acid residues and here the important residues are Aspartic acid 52 and Glutamic acid 35. So the yellow part that we see here is the main chain of the protein it is this yellow strand.

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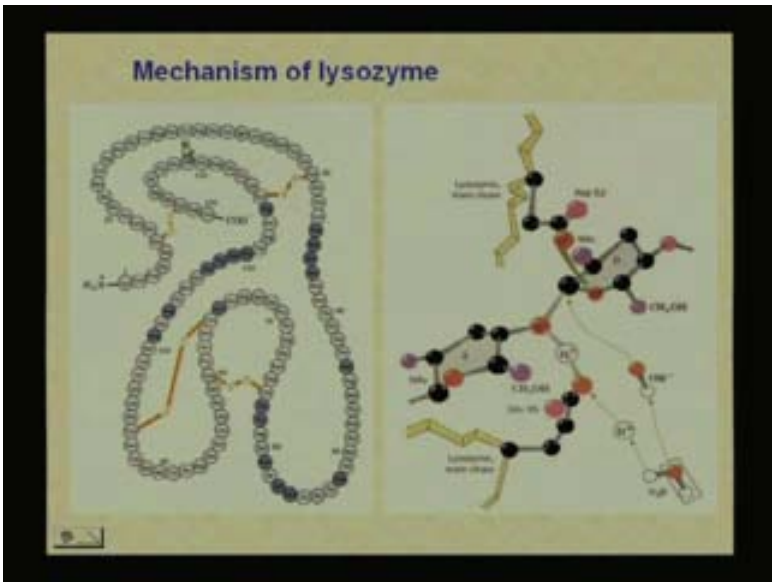

The yellow strand that you see here is also a part of the main chain of the protein. When we look at Aspartic acid we know that we should have two oxygen atoms linked to these black atoms which are the carbons. So we have the C_α, we have an other C and then C O O that is Aspartic acid. We have one extra CH_2 with Glutamic acid. So we have our CH_2 -CH_2 -C (O) O. (Refer Slide Time 19:34 min). Now here what happens is we have a D ring and an E ring of the sugar chain. This is what is NAM and NAG. What are these? These are sugar molecules. You all are 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 then it is called NAG which is N Acetyl Glucosamine. When this R group is this moiety then it is called N Acetyl Muramate. So it is NAG and NAM and the N Acetyl is because we have a $NHCOCH_3$ attached here. So the basic structure of this is an $NHCOCH_3$ that accounts for the N Acetyl in both cases and when we have this which is just a resonant structure of the two sugar moieties that can happen and if the R is H we have the NAG and if the R is CH_3CHCOO^- then we have NAM.

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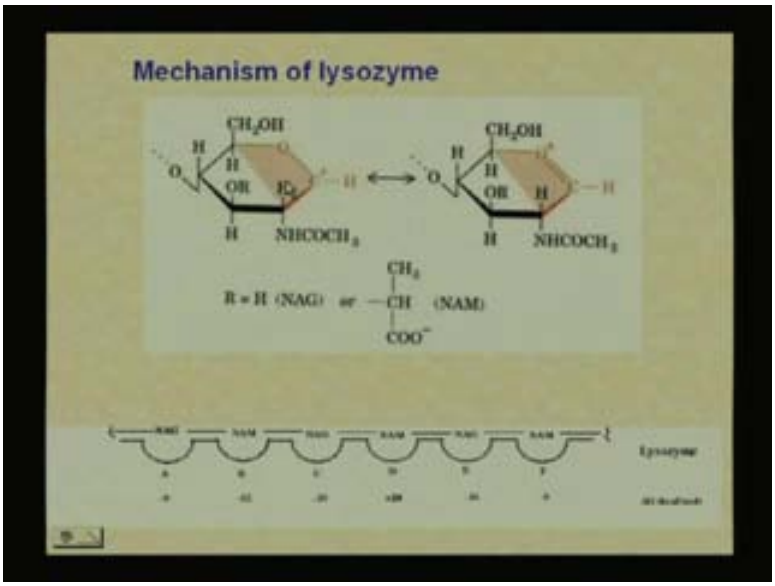

Now what we have is we have alternating NAG NAM NAG NAM NAG NAM residues in the bacterial cell wall. So we have six such sugar moieties in the bacterial cell wall part that fits in the active site groove of Lysozyme. The active site groove of Lysozyme that we saw in the initial schematic that I showed you can accommodate just six of these. It accommodates six of these and they alternate. So we have an N Acetyl Glucosamine N Acetyl Muramate NAG NAM NAG NAM NAG NAM and so on and so forth.

The cleavage occurs. What I have below here are the ΔG values, one of them is +20 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 can fits these six sugar moieties and after it fits these six sugar moieties in the acid of its active site group it cleaves between rings D and E which is basically what we saw in the previous slide. (Refer Slide Time 22:54 min)

So we have rings D and E and they are linked by a beta β- 1, 4 glycosidic linkage. So we have β- 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. So we have R NAG, we have R 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 E. So this is what we have. So we have our NAG and the R for the NAG is H that is this H which has been seen here.

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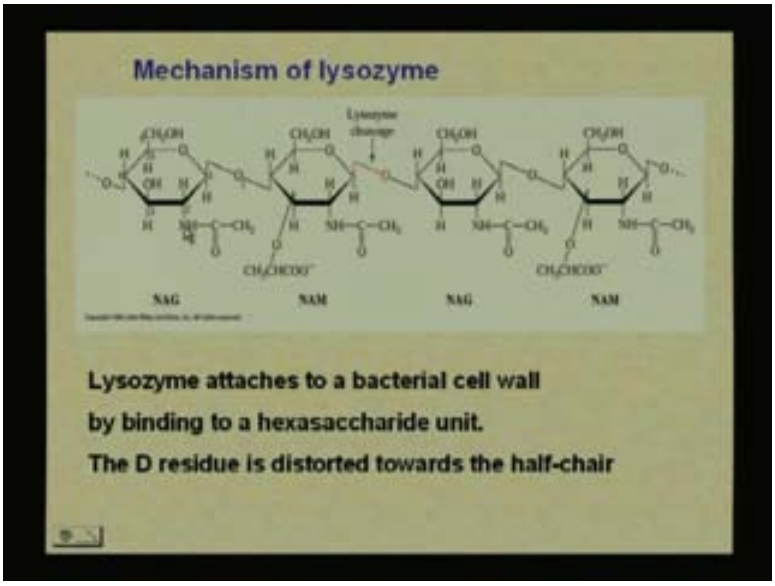

Then what is NAM? When the R is CH_3CHCOO^- then that is NAM. Then we have the Lysozyme acts exclusively on this bond it does not cleave this bond it is that specific. So it will only cleave the β- 1, 4 glycosidic linkages. So what does it do is the Lysozyme attaches to a bacterial cell wall by binding to a hexasaccharide unit. What is this hexasaccharide unit? It is the ABCDEF alternating NAG NAM residues or NAG NAM units which we have seen earlier.

What do these types of six member chains forms six member cyclic is they can have a boat confirmation or a chair confirmation. So the stable confirmation is the chair confirmation. Now what you have is you have the D residue distorts towards the half chair. 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. So have a NAM and a NAG. These are the sugar units. It is originally in the chair form because a chair form is stable.

We have the two residues Glutamic acid 35 and Aspartic acid 52 that are catalytic residues for Lysozyme. Now what is this OH, where as this come from? It is come from Glutamic acid and it can act as an acid because it is basically an acid. So here what happens is this OH cleaves the linkage between the NAG and the NAM. (Refer Slide Time 26:21 min). So we have our N Acetyl Muramate and our N Acetyl Glucosamine that is cleaved. Then what happens it Glutamic acid 35 transfers its proton to the O1 of the D ring this is the O1 of the D ring. So what happens is the C1- O1 bond is cleaved generating an oxonium ion.

So what was happened is now we have transferred a proton from Glutamic acid 35 which has now becomes COO^- and Aspartic acid is as it is yet.

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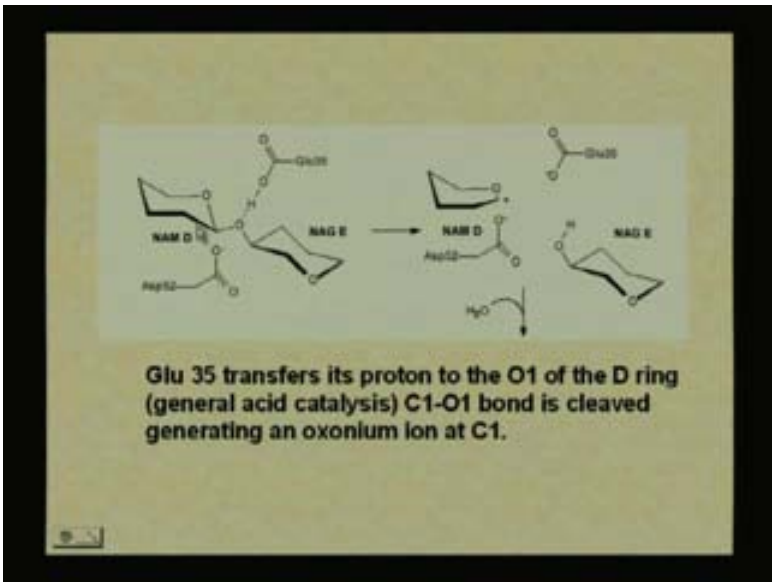

Now what is going to happen is the Lysozyme molecule at its present state cannot cleave an other NAM NAG because Glutamic acid does not have its proton to donate. So for Lysozyme to act as a catalyst once more the Glutamic acid has to get back to OH. So what happens is the role of the Aspartic acid 52 is to stabilize the oxonium ion. Where is this oxonium ion formed, how is it formed? It is formed from the cleavage of the C1-O1. (Refer Slide Time 28:11 min)

If we go back to the previous slide what is happening here is we have a C1-O1 bond cleavage, this bond is being cleaved this bond. So what do we have? We have this plus charge here. Now this plus charge is stabilized by the O^- of Aspartic acid. So we have Glutamic acid 35 that has donated its proton into the cleavage of this NAG NAM NAG set and we have an oxonium ion that has been formed with a plus charge and that is stabilized by the O^- of Aspartic acid 52 charge-charge interaction.

What happens now? The carboxylate can not form a covalent bond because the distances are too great but it is enough to stabilize the oxonium ion. Now what happens is there is a certain S_N2 mechanism with a transient formation of a C-O bond to the enzyme. for now what we need to know basically is the first step is this H is abstracted from Glutamic acid 35 this H, this H is taken up and it is not as close but approximately is enough to stabilize it for a transient period and we have a water molecule coming. And what happens to this water molecule is you have to remember that this O^- was formed due to the H was taken up for the cleavage.

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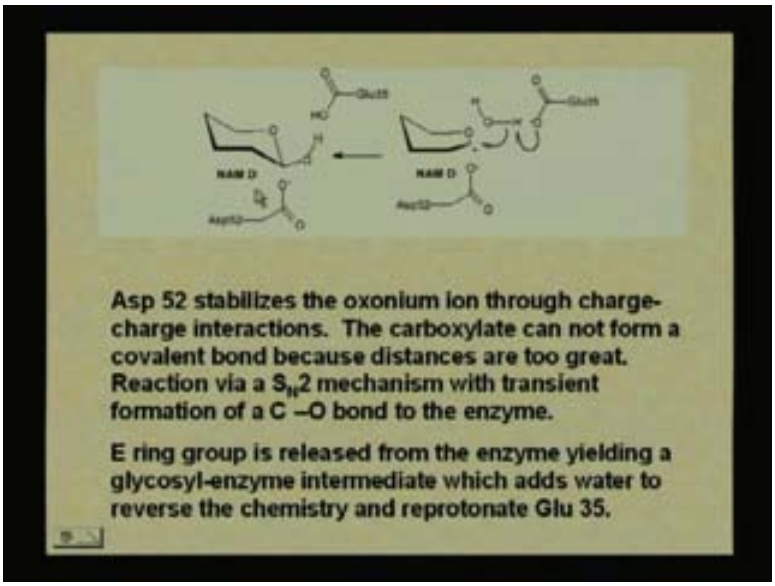

So we have the O^- of Glutamic acid 35 abstract an H from water then we have OH^-. What does OH^- do? It goes to the oxonium the plus charge that is here and forms the sugar ring again. So we have a cleaved ring we have cleaved basically glycosidic linkage. And what do we have back? We have our Glutamic acid back to the OH form. So now what can it do is it can go and cleave another NAM NAG set. So this is basically the way the catalysis would work.

So what is our first step? So let us just go back once more. (Refer Slide Time 25:32) We have β- 1, 4 glycosidic linkages, what happens in a β- 1, 4 glycosidic linkage? In the first step we know we are going to have a cleavage of between the hexasaccharide units. So the hexasaccharide unit fixed into the active site groove of Lysozyme once it fits into the active site groove of Lysozyme then there is only a cleavage of this bond. We have to remember the cleavage of this bond not this bond, the cleavage is between the NAG the D NAM rather the NAM D C1and O1. (Refer Slide Time 32:01 min)

What happens is Glutamic acid 35 donates its proton and in the event it becomes COO^-. So what happens is the cleavage of C1- O1 of NAM occurs and it forms an oxonium ion that is due to the proximity of the Aspartic acid 52 is stabilized transiently by charge-charge interactions. And once we have the stabilize form of the charge-charge interactions It has enough time to get a water molecule in here. Then what happens to this water molecule? The Glutamic acid 35 O^- abstracts an H from water which forms OH^-. This OH^- links with the distorted NAM ring that has been formed with the oxonium ion and forms the cleaved sugar ring here. and we have Aspartic acid it is just the Aspartic acid, the role of Aspartic acid is to stabilize the oxonium ion. It is the Glutamic acid that

is actually donating its proton then taking up a proton again. (Refer Slide Time 33:25 min)

The role of aspartic acid here is to just stabilize the oxonium ion. but you understand that it is extremely necessary for this to occur so that the Glutamic acid carboxylate anion has the OH of the water come close by and O^- can abstract the proton from the water. So this OH^- can then attack the oxonium ion, link with the oxonium ion to form the NAM. So that is the way this would form. So we have a reversal in the chemistry that has occurred and we reprotonate Glutamic acid 35. (Refer Slide Time 34:05 min)

This is basically just summary of what we just said, it is called by the way of Philips mechanism. And we have in the first step the Glutamic acid 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 forms the COO^- 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^- into becoming COOH once more and the OH^- attaches this oxonium ion.

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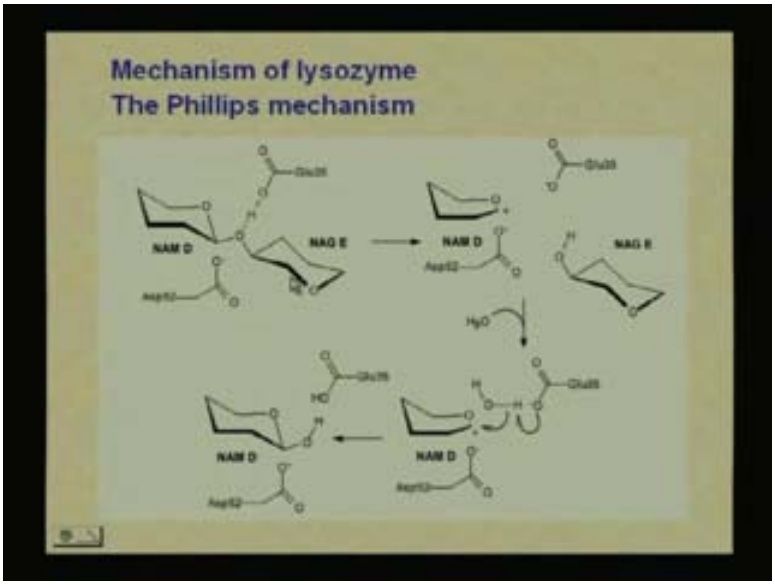

So now we have the NAG has already come up with an OH, the NAM also comes up with an OH. So we have a cleaved hexasaccharide unit between rings D and E when lysozyme attaches this or lysozyme catalyzes the bacterial cell wall. And we have this Glutamic acid 35 ready to provide its proton to another NAG NAM set. So you understand how these catalytic mechanisms are such it is easy at these catalytic mechanisms because you have to remember that the protein has to get back where it started from 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 what did we see is we know that the Histidine 12 can act as a base only if it has the electron pair to donate. So they have 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.

Now, there are two other things that we have to consider Allosteric because when we are studying enzymes we need to know what Allosetric actually is. We have certain molecules that we have looked at inhibition of enzymes, activation of enzymes.

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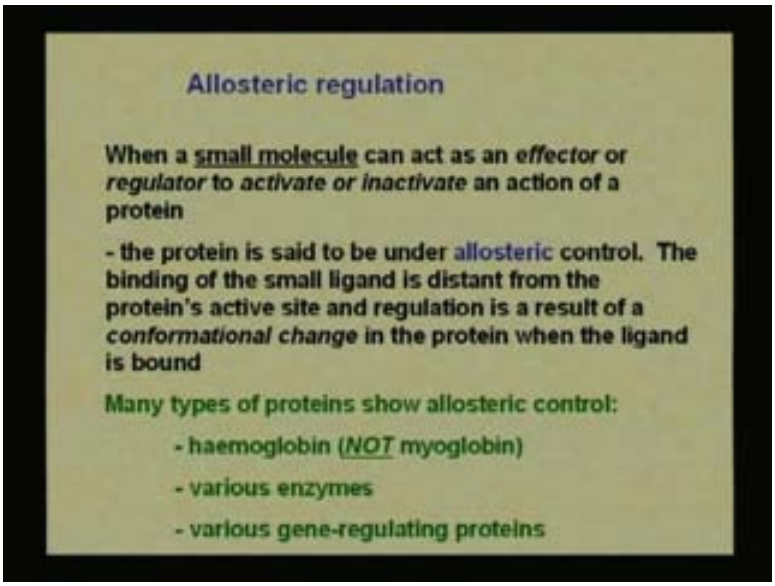

Now what happens is we have these small molecules or small inhibitors or effectors or substrates act on the enzyme so what they essentially do is this small molecule can actually act as an effector or regulator to activate or inactivate an action of a protein. When this small molecule, for example we know that hemoglobin binds oxygen because oxygen is a small molecule that binds to hemoglobin. When oxygen is bound to hemoglobin it acts as an effector or regulator into activating hemoglobin which means the protein is set to be under Allosteric control. (Refer Slide Time 37:43 min)

Suppose we have our hemoglobin molecule that is actually comprised of four different sub units. Now in the four different sub units which this is something we did in the one of the earlier class we have two α sub units and two β sub units. Now what happens is each of these bind an oxygen molecule an oxygen molecule is a small molecule. Now say it binds to this α when it binds to the α then there are some mechanisms that the conformation of the alpha changes slightly into becoming like this. (Refer Slide Time 38:32 min)

So then we know that it is that α has the bound the oxygen to it because it has a slight conformational change. Due to this oxygen being bound to this α then this will assist an other oxygen being bound to the other sub units of hemoglobin so it acts as an activator.

What is this activator? The activator acts in such a fashion that if one of these happens to binds to one of the sub units then it is going to help facilitate the binding of oxygen to the other sub units this is called a cooperativity.

Then what is cooperativity? If once this oxygen is bound then it helps the binding of the other oxygen molecules because four oxygens can bind. There are four oxygen molecules actually can bind. So the affinity for the oxygen in each case is the same but as soon as we have one of the oxygen is bind then what happens is the affinity for the oxygen increases which is what is called a cooperative effect. So essentially what we have is the small molecule which is an oxygen in this case is becomes our activator or it is an effector. We can also have an inhibitor in this case. For example carbon monoxide binds to hemoglobin. (Refer Slide Time 40:32 min)

But what do we mean by a cooperativity is when we have the facilitation of binding of the other ligand molecules that is assisted by the binding of the first one so that is called cooperativity.

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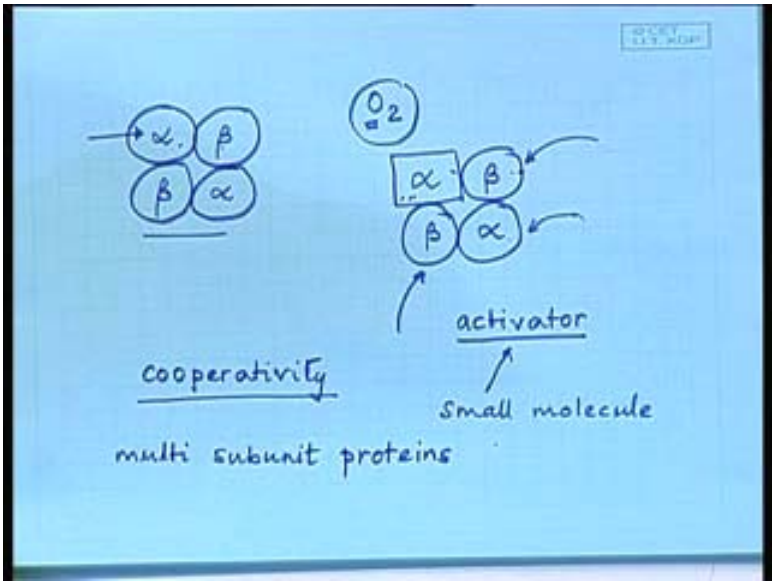

There are many enzymes or many proteins actually show cooperativity and once that show cooperativity are usually multi sub unit proteins because you have these sub units together. If you have one of them activated or one of them bound to the small molecule then it activates the binding of the other sub units to the ligand as well. So these are usually for multi sub unit proteins. Now therefore we have a small molecule that can act as an effector or regulator to activate or inactivate an action of the protein. So what is the action of hemoglobin? It is to bind oxygen. What is it doing is when this small molecule is bound it activates the binding of the other oxygen molecules to the other sub units. Then we say the protein is under allosteric control. Basically this is allosteric regulation (Refer Slide Time 42:09 min).

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. There are many types of proteins will show allosteric control for example hemoglobin but not myoglobin. The myoglobin is a single chain protein that also binds oxygen but it does not show any allosteric control primarily because it is not multi sub unit it just has a single poly peptide chain.

There are other various enzymes that show allosteric control and there are the gene regulating proteins which can also show allosteric control. So let us have this as an example. We have an allosteric regulation a less active enzyme. So these are two sub units of the enzyme we have a positive modulator and the substrate

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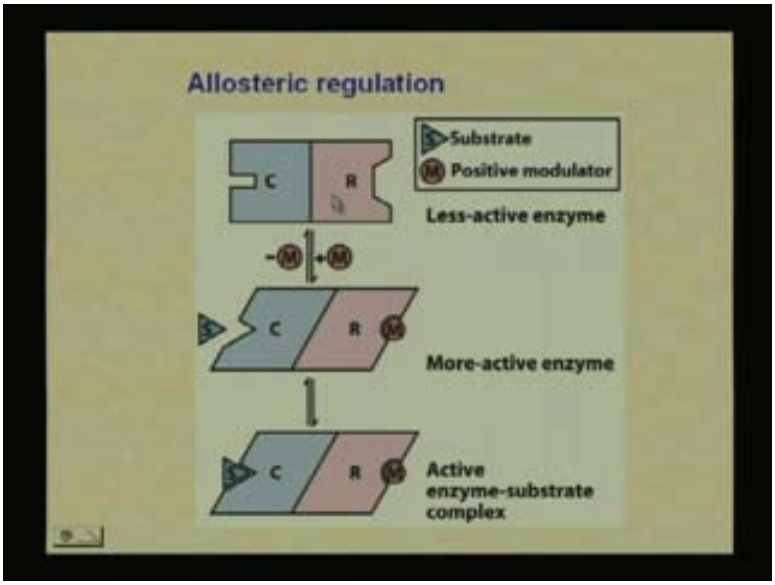

Now if you look at that say we can consider a lock and key mechanism for this. Then basically what happens is if we just look at lock and key mechanism this substrate cannot fit anywhere it does not fit in the active site and this is basically a less active enzyme.
As soon as we have a modulator fit into this so there is an induced fir of this particular sub unit that will link to the modulator. Once this linkage occurs we will have a conformational change here. Then what happens to the conformational change? This can accommodate the substrate easier so initially we had a less active enzyme, we have a modulator that makes the more active enzyme and then we have the enzyme substrate complex (Refer Slide Time 44:08 min).

Why is this essential? When you have a biological activity you do not want the enzyme-substrate complex always to be formed because you probably do not need the product in the first place.

So you should have regulate it so that when the product is needed they will be a small molecule a positive modulator they may be usually called cofactors or you have metals even, metal ions that act like this. Where only in the presence of the metal ion or in the presence of a particular small group you will have the enzyme active because you do not have the enzyme to be active all the time. That might lead to like if you have say hemoglobin active all the time it is going to keep on adding oxygen but we also wanted to deoxyfy. So it has to be deoxygenated at times.

So we have to have regulation. 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 a conformational change. Now the conformational change is going to amount to a fit of the substrate to the enzyme which was not possible previously. (Refer Slide Time 45:42 min). So it was not possible earlier which is now possible and you form an active substrate complex that can go on to form the product.

So let us just summarizes to what we did today. We looked at a 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 cyclization step. In the cyclization step Histidine 12 acts as the base and Histidine 119 acts as the acid. We have a cleavage of a phosphodiester bond. In the second step we have hydrolysis. In hydrolysis we have a reversal of rules so Histidine 119 acts as base and Histidine 12 acts as acid. (Refer Slide Time 47:22 min)

now when we have this phosphodiester bond and we have the cyclization we still have not studied DNA and RNA structure but we are looking at Ribonuclease A. now if we 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. Here what did we have is we had an O and we had a P and here we have the O and the H that is what we had initially. Now what happens to this H is this H was taken up by the Histidine in the first step and we have a formation of a cyclic phosphate ring so then this attached here and basically we had a cyclization it is called the cyclic phosphate ring. That is formed here and then we have the hydrolysis (Refer Slide Time 48:33 min).

Now if this particular carbon is called the 2' so we have the 2' -OH of the ribose ring where the H is abstracted. If I have DNA and if I want to know whether Ribonuclease A is going to cleave DNA or not, where the DNA is deoxyribose.

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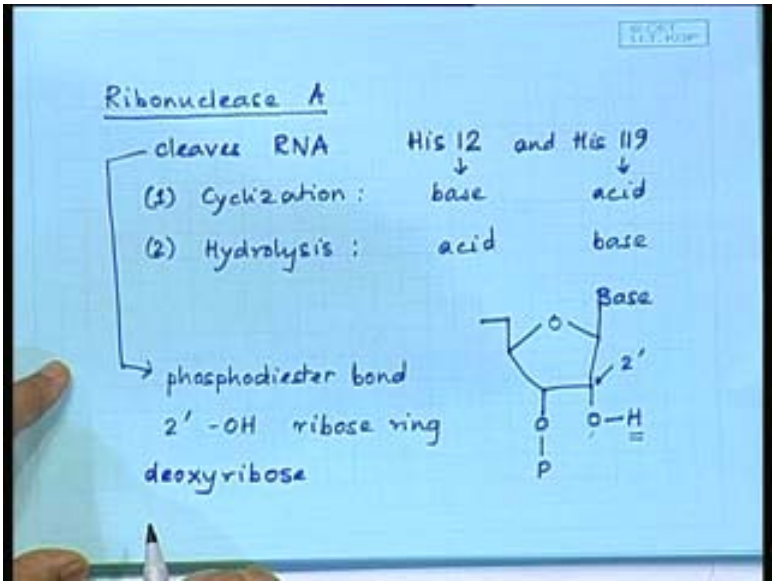

So what is deoxyribose? We have DNA, we have deoxyribose it is essentially the same this is P and so on, and this is missing. It is deoxy at the 2' position. What did I have for RNA? I had this O-P and rest of the chain and the base. Therefore if I have Ribonuclease A which is written in short form like this RNase A act on these two it will not be able cleave DNA because there is nothing here. It cannot abstract the proton here, it cannot form a cyclization step or anything here. So this can be act as a sort of an inhibitor to Ribonuclease A because it looks very similar but Ribonuclease A will not be able cleave DNA but they will be other parts are similar.

Here we have an active site and when it fits into the active site it may so happen that it probably will fit but it will not cleave because it cannot form the cyclize phosphate intermediate just because of the absence of this OH. So you see how extremely is specific this particular protein is in cleaving just RNA. so this is the way we have the enzymatic mechanisms extremely specific for what ever particular enzymatic reaction that they are suppose to catalyzed. So what we did next was we consider the mechanism of Lysozyme where we saw Glutamic acid 35 act as a proton donor in the first step and then a proton acceptor. So it is basically only the Glutamic acid that is taking part and the Aspartic acid 52 was there for just for stabilization that is the role of the Asp 52.

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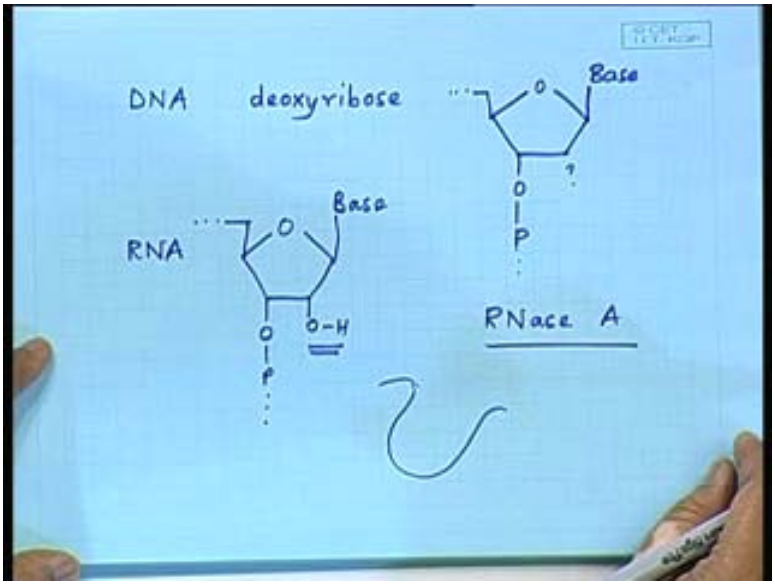

So can you me tell me at what pH this would be active? The pH at which Lysozyme would be active would be lower then the RNase A definitely because you have to have a proton donation and a proton acceptor activity of Glutamic acid. That is possible because you know the pKa of Glutamic acid is low so the pH has to be low for particular protonation deprotonation to occur.

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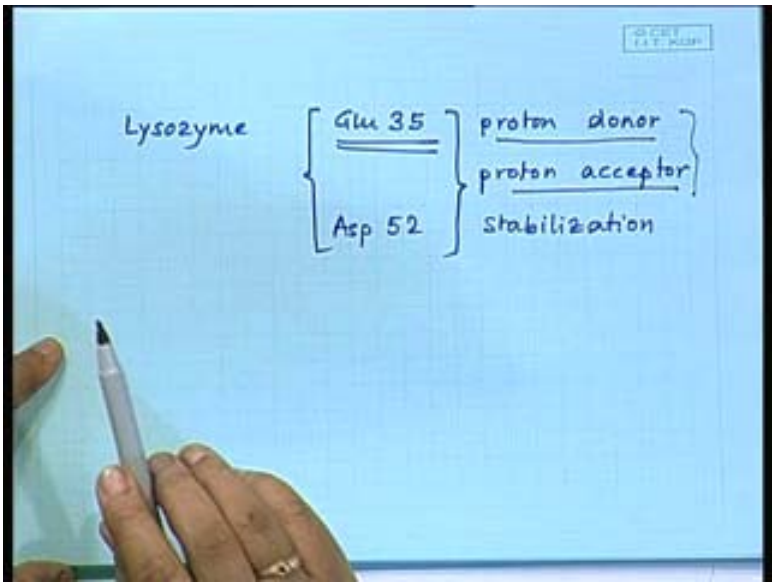

But for Ribonuclease A it is Histidine so it is going to occur around six seven or in that range. So we will stop this lesson and we will go on to the next one later on.
Thank you.