Biochemistry
Prof. S. Dasgupta
Department of Chemistry
Indian Institute of Technology – Kharagpur

Lecture - 12
Enzyme Mechanisms II

In the last class, we studied the enzyme mechanisms of ribonuclease A and lysozyme. Now what we are going to do now is we are going to study how protease is worked. Protease is other essentially enzymes that break peptide bonds, so there are extremely important in protein digestion, because they have to break the peptide bonds into different units, which are later used for protein turnover.

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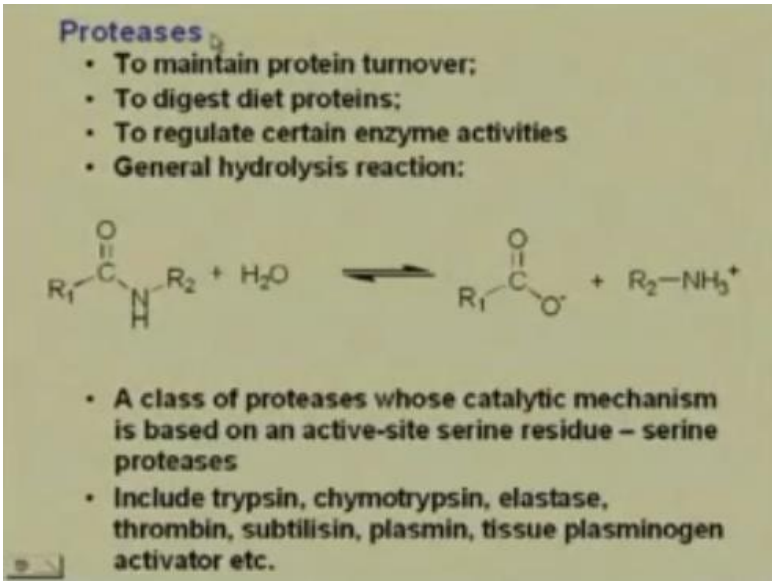

So basically what we have in proteases is to maintain the protein turnover, they are also essential because they have to digest the proteins that we take in the diet, because we consume proteins everyday and these proteins have to be broken down into smaller units okay. So these proteases that are specific types of enzymes, they break the peptide bond and they are also essential to regulate certain enzymatic activities.

So what do we have, we have a general hydrolysis reaction. In such a reaction, you recognise this CONH is our peptide bond. Now what is going to happen is this peptide bond is essentially going to be cleaved when it was formed if you remember, we had the NH2 and the COOH of two amino acids link up together to form the peptide bond with the release of H2O.

Now what is going to happen is we are going to have a general hydrolysis reaction in which we will have the cleaving of this bond and this is accomplished by these proteases.

Now of this, there are different types of proteases like a serine proteases, aspartic acid proteases and the one that we will be studying is serine proteases. Now basically the proteases, the names given to the different proteases are dependent on what residue the catalytic mechanism is dependent on okay. For example, if we have a class of proteases, the catalytic mechanism of which is based on an active-site serine residue, we call them serine proteases.

There are aspartic acid proteases where in that case, the active-site residue or the catalytic mechanism would be based on an aspartic acid. So the serine proteases basically include trypsin, chymotrypsin, elastase and so on, the other ones that I have listed here and if you remember about two three classes ago, I showed you a typical picture of how trypsin, chymotrypsin, elastase could be accommodated in the active-sites okay.

So these are all examples of serine proteases where the catalytic mechanism is based on an active-site serine residue and what are these proteases is going to do, they are going to cleave the peptide bond and we will see exactly how it cleaves the peptide bond and again what do we have to remember, the enzyme has to get back to where it started from, because it has to go on to cleave another peptide okay.

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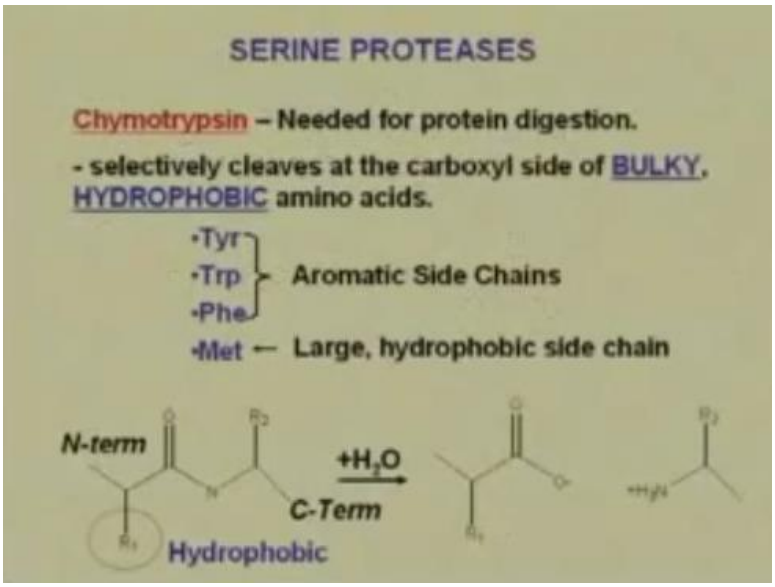

So what do we have, we have in general the serine proteases. This chymotrypsin is needed for protein digestion. It selectively cleaves, this you have to be careful of, at the carboxyl side, not at the amine side okay. You know that the, an amino acid has two sides to it, one is the amine side and one is the carboxyl side okay. This chymotrypsin will cleave at the carboxyl side of bulky hydrophobic amino acids okay. So usually it cleaves at the aromatic side chains and methionine okay. So this is where it cleaves.

So what do we have, we have an N-term, what is this, this is the N-terminal of the protein, these R1 and R2 are the residues, the side chain groups of the polypeptide chain and the CO and N would be the peptide bond and what are we going to have, we are going to have a cleavage, a cleavage where the cleavage is going to be at the carboxyl side. So this is where the cleavage is going to occur provided R1 is what a bulky hydrophobic residue, is that clear.

So if R1 is a hydrophobic residue, then the cleavage will occur here okay, not on the other side. It occurs at the carboxyl side of the hydrophobic group okay. So if this is R1, it is going to occur on towards the C-terminal end of the polypeptide chain. Now chymotrypsin is a serine proteases, what does it mean, it means that the active-site contains a specific serine residue that takes an active part in the catalytic mechanism of this protein okay.

So chymotrypsin is basically it is also a protein okay. These are all enzymes, so these are all biological catalysts that are proteins okay.

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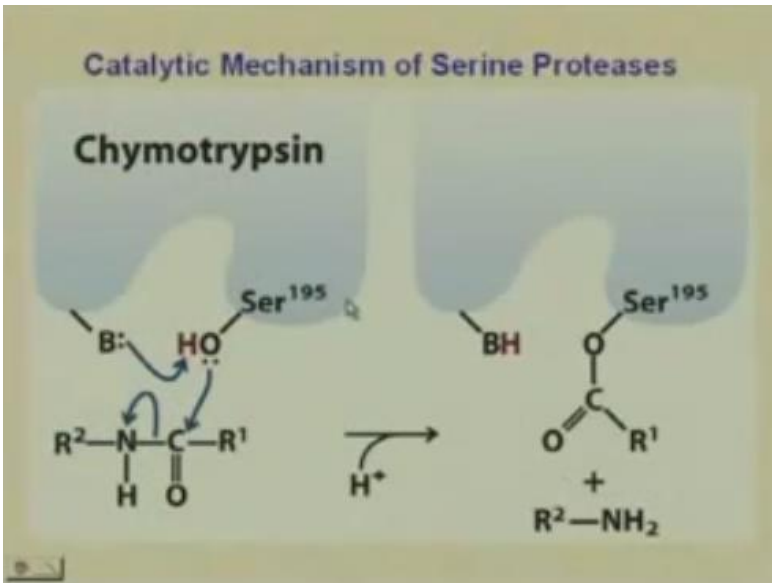

Now in this case, we will go in the mechanism in pretty much detail, but for now what we need to know is the serine that form mix chymotrypsin, a serine protease is serine 195 okay. So serine 195 is the serine that is part of the active-site of chymotrypsin that is involved in the catalytic mechanism and this we will go in detail because now we have this linked up together and obviously this cannot go back to form all cleave another peptide.

So essentially what is happening in here is we have a cleavage of the peptide group. Now which one is the hydrophobic group here; which amino acid is the hydrophobic group in this case. We have two amino acids here and you recognise, this is R1 that is R2, so which is our hydrophobic residue because chymotrypsin is going to cleave after the hydrophobic residue, so R1 is the hydrophobic residue.

What is the order of the polypeptide chain here? Is it from left to right or is it from right to left? Why is it from right to left? Because we have remembered that this CO is actually the end of the amino acid, when it links you always start from the N-terminal. The N-terminal of the chain that we have drawn here is this side that is the carboxylic end, right because we have the NH, the C or the R2 linked and then will be the CO of this group.

So if the cleavage of this peptide bond is occurring it means that R1 is the hydrophobic amino acid bulky group after which the carboxylic group here and this is where the cleavage occurs okay and what is the important residue here, serine 195 okay. Now let us go in detail.

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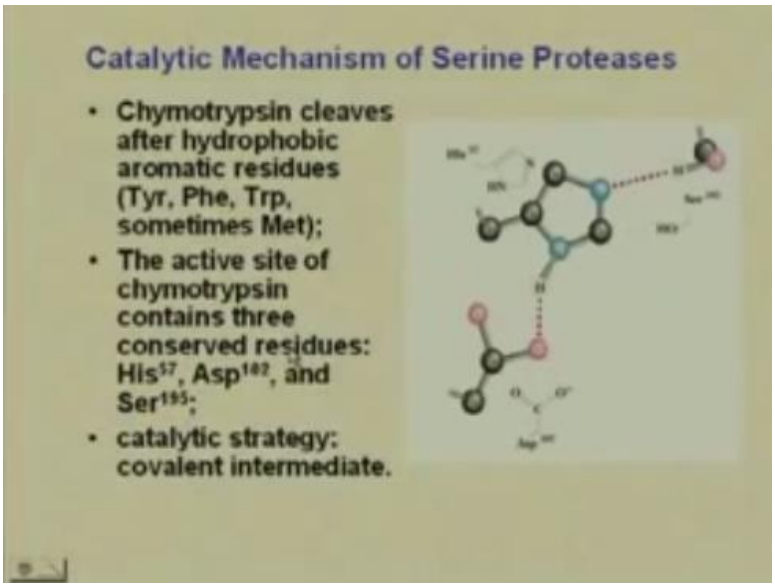

What happens here is we have three important residues. These three are histidine 57, serine

195 and aspartic acid 102 okay. You see the aspartic acid here, COO, this is histidine, what is histidine, you have two nitrogens, there is an H attached here and this is the serine, what is the site chain for serine OH okay. So this is just the schematic and this ball and stick models show you which residues are important.

So what do we have here, we have serine 195, histidine 57 and aspartic 102. Again chymotrypsin cleaves after hydrophobic aromatic residues usually tyrosine, phenylalanine and tryptophan and sometimes methionine. The active-site of chymotrypsin contains three conserved residues; it means it contains these residues conserved means it is the same for practically all species okay.

So we have histidine 57, aspartic acid 102 and serine 195 and the catalytic strategy in this case is to form a covalent intermediate, but we have remembered that this covalent intermediate has to be broken in two, because we have to get the serine back to where it is. So what are the steps involved.

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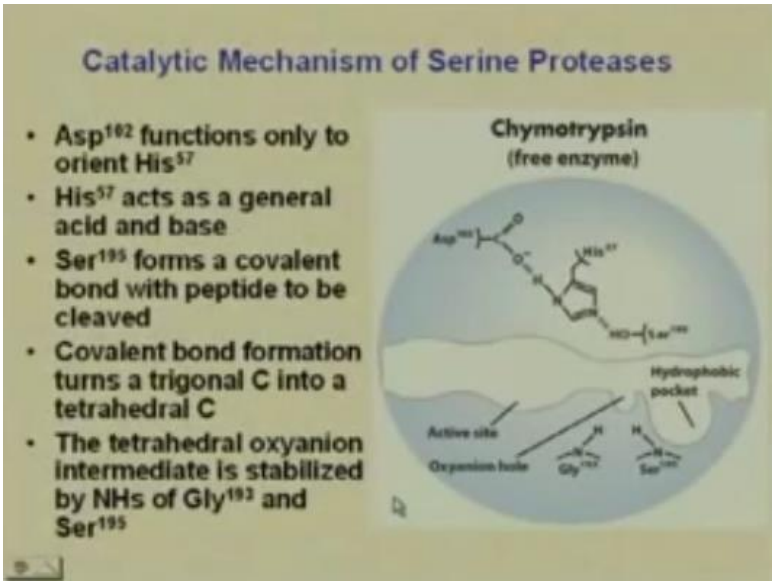

I am going to go through this quite slowly, but I want you to follow the mechanism because it is extremely interesting. Now the first thing that we have to know is what is important about the three particular residues that are part of this. Now these three residues are known as a catalytic triad okay. In the serine proteases, we have a catalytic triad that is composed of a histidine, a serine and an aspartic acid okay.

Now so each of these residues in the triad is going to have a specific role okay and this is

what we have to understand. The histidine again acts as a general acid and base. The aspartic 102 functions only to orient the histidine okay.

So what is the function of the aspartic acid 102, it just basically holds the histidine in place by an electrostatic introduction. It is holding or rather a hydrogen bonding type introduction, where it is holding the histidine in place then this histidine will act as a general acid and base and serine will form the covalent bond with the peptide that has to be cleaved okay. We will see how it does that but we have to know that we have the serine OH is its side chain.

The histidine N and N is this is its side chain and this is the side chain of the aspartic acid okay. So when we have a covalent bond formation, we have a trigonal carbon form into a tetrahedral carbon okay. We will go through the steps one by one. Now what is going to happen is this is the active-site pocket that you see here, the one in white. So this is our active-site. This is our hydrophobic pocket.

Now what is going to be there in the hydrophobic pocket, the bulky amino acid side chain; the carboxylic side peptide bond which is going to be cleaved okay. This small thing here is what is called an oxyanion hole. We will see why that is called an oxyanion hole and there are two other residues will rather the NH of the serine and NH of the glycine that helps in stabilising the oxyanion okay. We will go step by step into seeing how that occurs.

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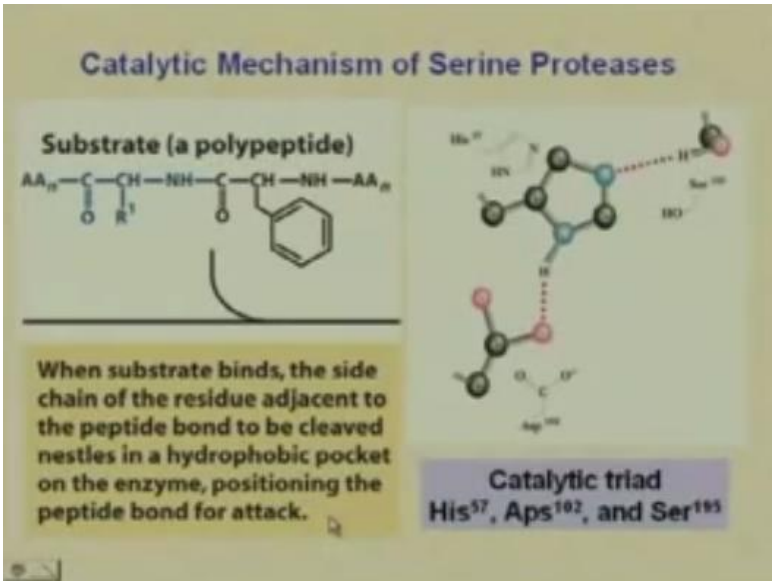

So what do we have, this is our substrate. What is a substrate, the substrate is a polypeptide chain, it is nothing but a polypeptide chain and the protease in this case is serine protease is

going to cleave a peptide bond? Which bond is it going to cleave? It is going to cleave this particular bond because this is at the carboxylase side of the hydrophobic residue. Is that clear okay.

So what do we have, this is the amino terminal and you now recognise this, this is the NH, the CH and this is the side chain attached to the CH, this is the carboxylic group, this is the NH of which one of R1 okay. So it is going to cleave right here. So what happens to this, this is going to fit into the hydrophobic pocket okay. Once it fits into the hydrophobic pocket, we will see how these groups will act in their different roles into cleaving this, chopping this up.

This as I mentioned is called a catalytic triad, the histidine, aspartic acid, that supposed to be aspartic acid and serine okay.

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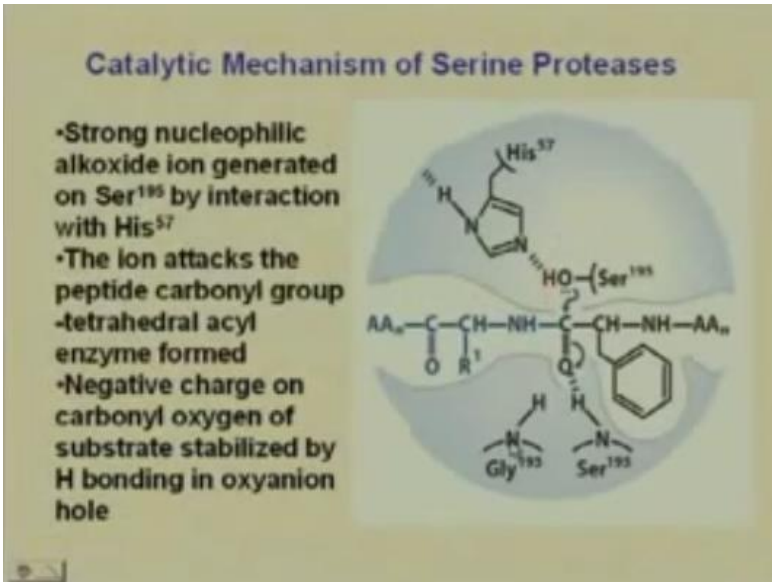

So here is our polypeptide chain inside the active-site okay, now what is going to happen. The first thing that is going to happen is this histidine 57 is going to abstract the proton from serine okay. So after this abstraction, there is an ion at the serine side chain. What is this going to do, this is going to attack the peptide carbonyl group and we have an acyl-intermediate form, what is an acyl-intermediate, an OCO group okay.

So what do we have, let us go through this once more. We have the histidine that we know, what can the histidine act as it can act as a general acid and the general base. What is it acting as here, it is abstracting the H from serine and the serine now forms an alkoxide ion that is generated by the interaction with histidine 57, the ion attacks the peptide carbonyl group

forming what is called an acyl-enzyme and we have an O minus formed here.

So what is happening is this O after the abstraction of the H to the histidine forms an O minus that sits in this oxyanion hole and is stabilised by the NH of glycine 193 and serine 195. So this is our first step. What has been formed now, in the first step, we have linked the enzyme with the polypeptide chain okay.

So now there is a direct covalent link between the polypeptide chain through serine 195 to the carboxylic group of the peptide bond that is going to be cleaved. So this is a direct linkage okay.

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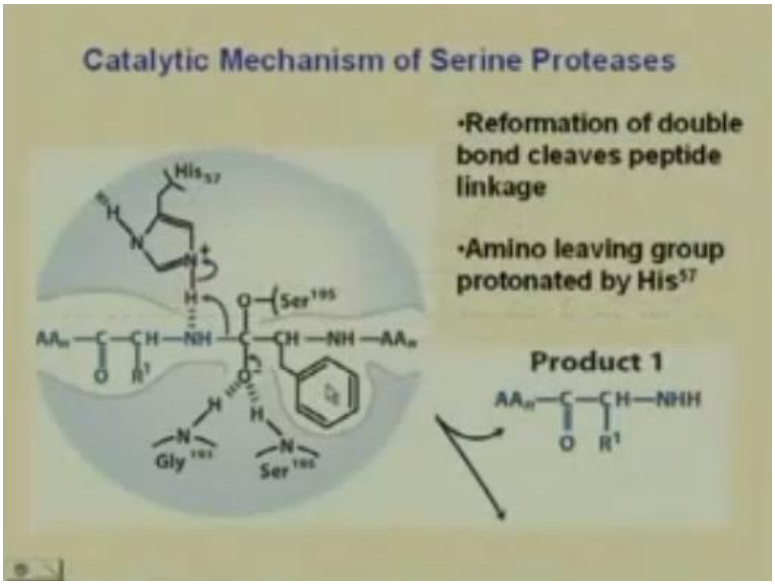

So what do we have this is our linkage. What did serine 195 do, it linked with the carbonyl group how after the H was abstracted from serine. The OH that is marked in red here originally belongs to serine right. If we go back to the previous step, this is the OH, that had originally was belonging to serine.

What does happen, histidine has taken up this H in the event, what has happened, is this alkoxide ion that has been generated attacks the carbonyl which becomes O minus, the O minus is stabilised by the NH groups of glycine 193 and serine 195. Now what do we have to do, we have to cleave the peptide bond. Now what happens is this O minus that was sitting in the oxyanion hole goes back to form the C double bond O, this now is cleaved.

What does this do, this takes up the hydrogen that was originally on serine and what does it

form NH2. What is this, this is the amino terminal of the product that has been formed, because we have a cleavage of the peptide bond here, you are going to have a carboxyl terminal and you are going to have an amine terminal.

So this H that had been abstracted by histidine 57 from serine 195 into forming this intermediate is now part of the first product which is the amino part of the amine terminal part of the part that is leaving right. So what do we have, we have the amine part of the polypeptide chain forming the product that is cleaved off, but we still have a covalent linkage with serine right.

Serine or the enzyme is still linked to the other part of the polypeptide chain. Now we have to remove that. So, basically the amino leaving group is protonated by histidine. So what is histidine acting now as, it is acting as an acid, because it is protonating, this initially it acted as a base in taking up the H from, where did it get the H from, from the serine. So it got the H from the serine and now it is giving the H to the NH part of the polypeptide chain to form the NH2. So this is the first product form right.

But what are we still left with; we still have to get serine back to OH right. If we want chymotrypsin to act on again, we have to get, we have an acyl-intermediate now. We have to get this back to OH, so what do we have to do.

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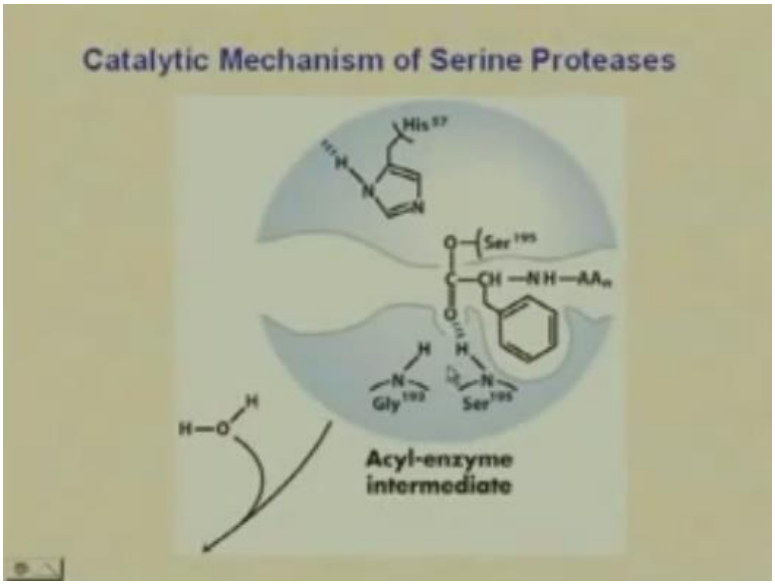

So our first product has gone. So this part is empty now. We have our C double bond O formed back again, but we still have this linkage and this is the carboxylic part of, so this is a

hydrophobic pocket. So we have what is called an acyl-enzyme intermediate. Why is it called an acyl-intermediate, because we have an OCO group here okay and this is the acyl group and we have the enzyme still covalently linked to part of the polypeptide chain that will be supposed to cleave.

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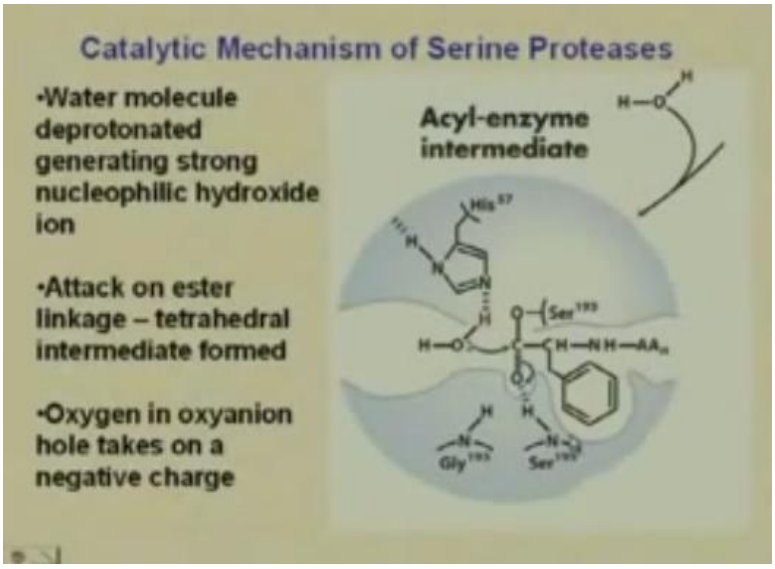

Now we have a water molecule come in okay. Water molecule is a lot of abundance in our bodies. So what happens, the water molecule now is deprotonated. So who is one that is going to take up the hydrogen, histidine. So histidine then will take up the hydrogen from water so it abstracts hydrogen from water and what is going to happen to the O minus, it is going to attack the carbonyl, which again forms an O minus, is that clear.

So what is happening, we have again the oxyanion hole that has been occupied by the O minus and is stabilised by the NH groups, so we are now going back. Initially, what happened was the serine 195 gave up its hydrogen to histidine. Histidine then donated this hydrogen to the leaving amino part of the polypeptide chain, but we still have the other part of the polypeptide chain, which contain the (()) (23:53) hydrophobic group that was in the hydrophobic pocket.

And now what do we have to do is we have to get serine back to where it was. So we have an intermediate basically. So we have a water molecule, the water molecule proton is abstracted by histidine okay. Then we have the OH minus hydroxide ion attack the carbonyl to form a CO minus.

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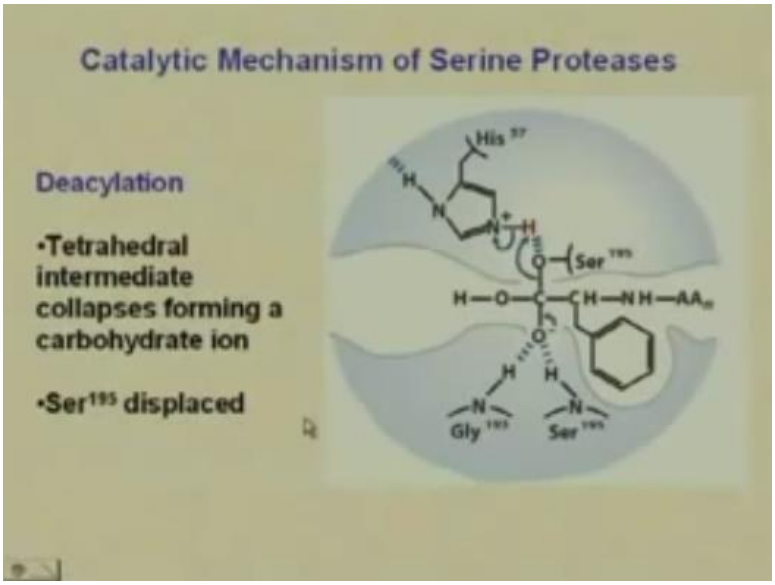

Now what is going to happen is the COO minus and the OH is linked here, what happen is this O minus is going to come back to form what C double bond O and what is going to get cleaved, the CO the acyl linkage to serine and where is it going to get an H from, histidine. So what does histidine in the first series of steps do, it takes the hydrogen from serine and gives it to the leaving amino part of the polypeptide chain.

In the next series of steps, it takes the proton from water and gives it to serine okay. So we have basically deacylation and we have what was O minus here formed double bond O, this is cleaved and this takes up the H and histidine is back to where it started from and so is serine. So chymotrypsin can then go and go further cleave another peptide.

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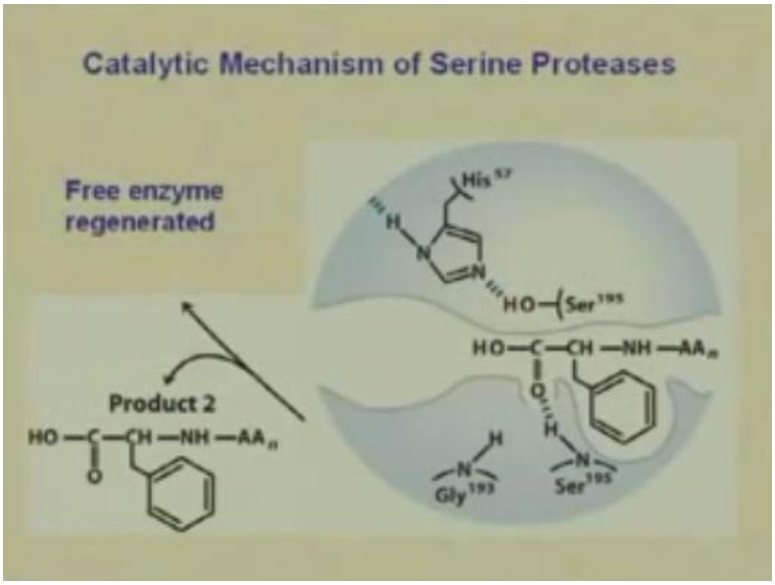

So we have, what is the second product then, this is now generated C double bond OOH with

this, so this is our second product. What happen then, we have the cleavage of a peptide bond. What do we have, we have histidine back to where it was, serine back to where it was. So what can happen again, histidine can again take this H, this O can again link with the peptide in another cleavage of another peptide okay.

So all we have to remember is the roles of the catalytic triad okay. What is the role of the aspartic acid, just to hold the histidine in place because you see aspartic acid 102 had no role to play in here okay, but it does act in holding this histidine in place by formation of this particular hydrogen bond okay.

So we have a particular hydrogen bond and this is possible only if the aspartic acid is in place okay. So the aspartic acid holds the histidine, so that the histidine can take and donate protons to either water or serine, so that it can rather chymotrypsin is regenerated.

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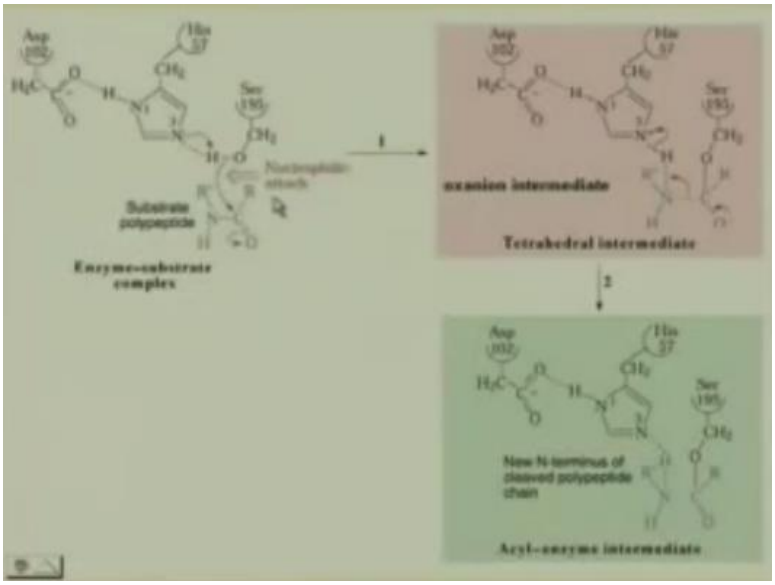

So basically, this is the summary of what has been done here. So, what is aspartic acid 102 doing, it is just holding the histidine in place. The N base here, what does it do, it abstracts the H from where serine and what happens, this O is linked to the carboxylic 1 that is going to be cleaved of the peptide. Then what happens is we have what is called an oxyanion intermediate, a tetrahedral intermediate and we have a cleavage of the peptide bond okay.

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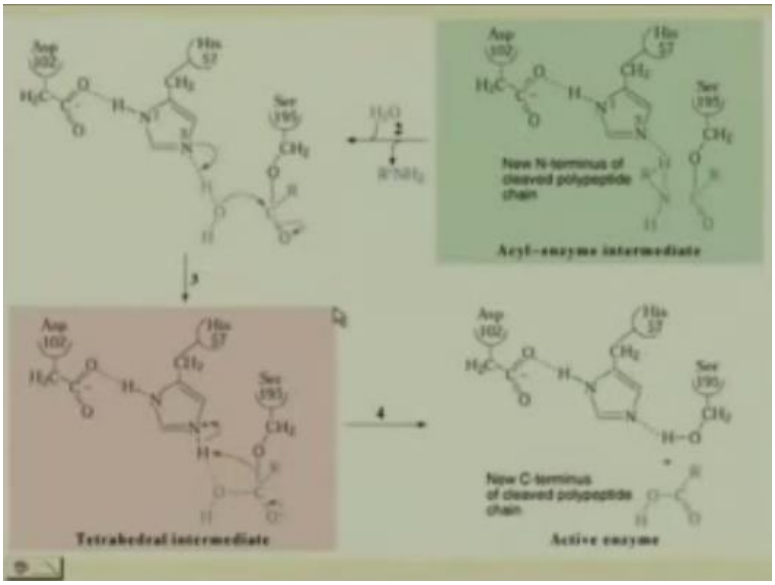

That is essentially what the whole step is all about and all we need to know is we have to regenerate the enzyme. Regenerating the enzyme means the histidine usually reverses its role into, but in this case it is doing its role twice. It is taking up H from serine, giving it to the leaving part that had the amino group to it; it is donating the H from the serine to that. Then in the next step what does it do, it takes H from water and gives it to serine. So we have now our catalytic triad back in place to form the active enzyme.

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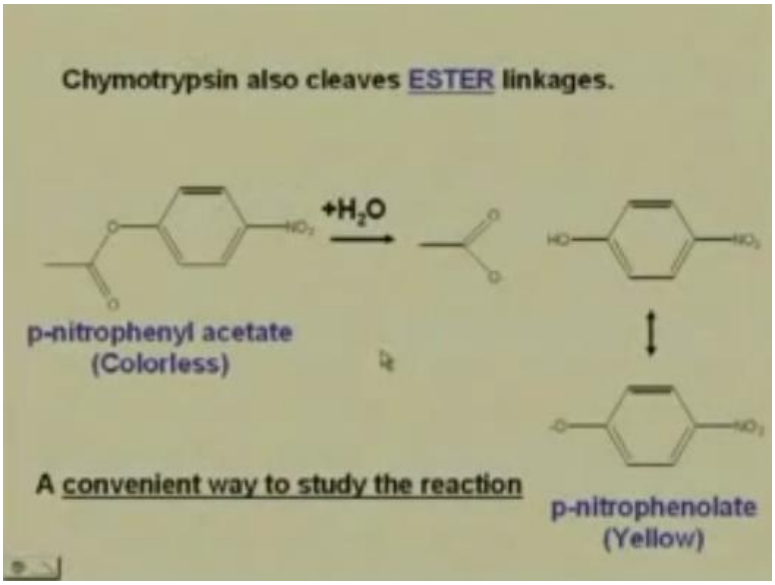

A chymotrypsin can also cleave Ester linkages, now this is usually done as an experiment. It does not have any biological relevant as such, but we can have like a colourless entity like para nitrophenyl acetate formed para nitrophenolate that will tell you how active your enzyme actually is okay. So this would be an enzymatic general reaction that could be conducted okay where we have a convenient way of studying the reaction as to how it can

cleave an Ester linkage okay.

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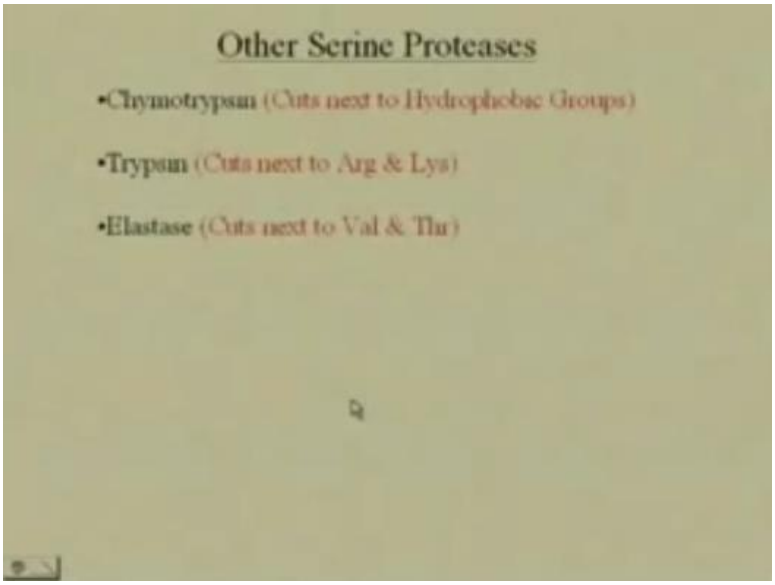

So what do we have, we have other serine proteases. Chymotrypsin where does it cut, it cuts next to hydrophobic groups. What do you mean by cut, means it chops up the polypeptide chain that is essentially what is doing it? chopping up the polypeptide chain.

Trypsin will cut next to arginine and lysine, so it is cutting or it is cleaving after basic amino acid residues. Why is that because its active-site is such that it will fit basic amino acid residues and it has especially very carefully position, aspartic acid that is going to form an electrostatic introduction with the arginine and the lysine, so that it sits in the active-site.

Elastase cuts next to valine and threonine okay. So another thing that we have to remember for trypsin is that it will cleave arginine and lysine provided it is not followed by a proline okay. If you have arginine and proline, why do you think so because it is going to distort the polypeptide chain, so it will not fit into the active-site as simple as that. If it cannot fit into the active-site, it cannot be cleaved okay.

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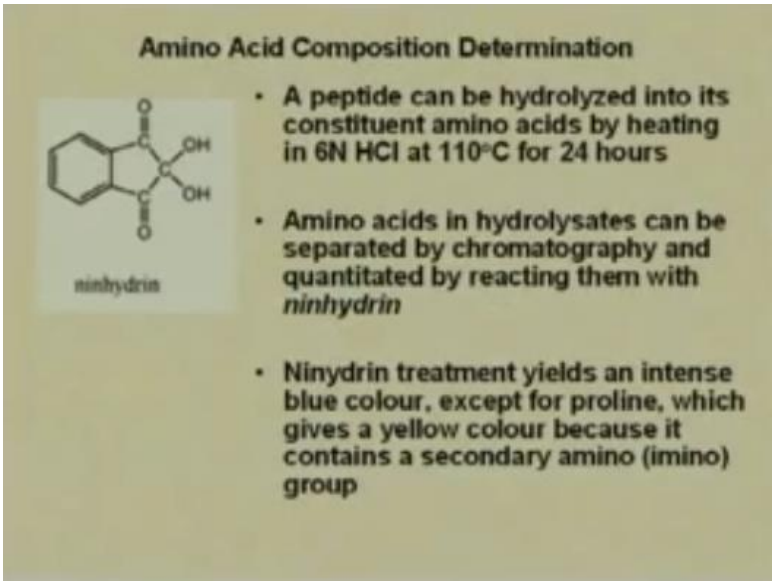

What else can we do now okay. So we studied about the different enzymatic mechanisms okay.

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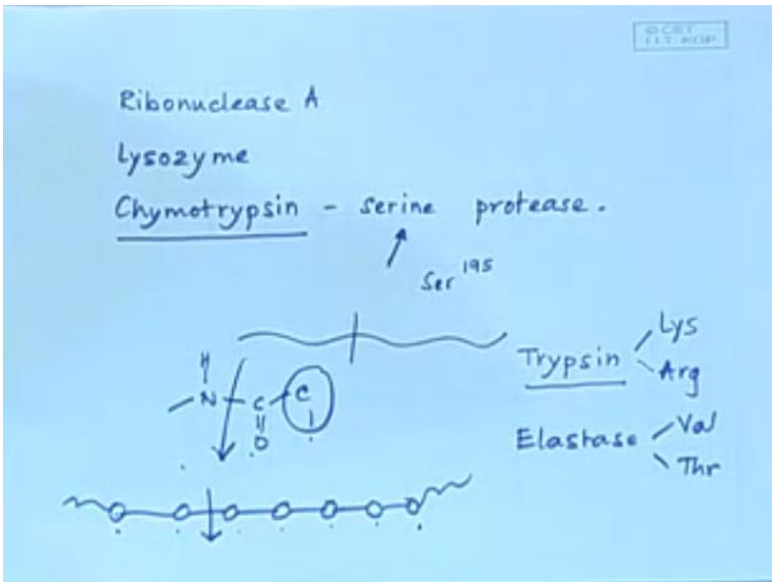

What we studied was? we studied our ribonuclease A. We study lysozyme and we studied chymotrypsin. Chymotrypsin is a serine protease. What is that mean? it means that its active-site as a serine, that is why it is called a serine protease, which is this serine, serine 195 okay. What is the serine 195 do? it forms a covalent link with the specific polypeptide chain that has to be cleaved.

So what are we doing, is we are cleaving a peptide bond right. We are cleaving a peptide bond only if this group attached this what, what type of group has to be attached here, a bulky hydrophobic group okay. So when we have chymotrypsin, we have a particular type of

breakage when we have trypsin, we will also, trypsin is also a protease, it is also a serine protease, but it will cleave only if this is lysine or arginine.

We have elastase, which is also a serine protease; it will cleave after valine and threonine okay. So these are used a lot in amino acid analysis. We will see amino acid, a peptide sequence determination. We will see how we can do that, valine, threonine okay. So now we have our protein sequence. We know that we have these amino acids linked together. We know that if we are going to use trypsin, it is going to cleave at a specific point.

We know that if we have elastases, this is going to cleave at some other point. We know that if we have chymotrypsin, we are going to cleave at some other point okay. So based on that we can figure out, we can use this very cleverly to figure out what the sequence of our amino acid polypeptide chain is okay. So we are going to see how we can actually do that, it is like a puzzle okay.

What do we have here, so if we want to look at the amino acid composition okay, we can have a peptide, now a peptide can be hydrolysed into amino acids by heating in six normal HCL at a 110 degree centigrade for 24 hours. What is going to happen is you are going to chop down everything. Break it up into fragments completely, but this will not tell you in what order they are right.

It will just give you an overall identification where you can then separate them by what is called chromatography and figure out how many of each you have. How many alanines, how many glycines, how many aspartic acids, how many glutamic acids and so on and so for, but it gives you no information about which order they are in okay. This is usually done by what is called a compound called ninhydrin, which gives a purple colour for all amino acids except proline okay.

It is just like a dye that links up with the amino acids, they are quantitated by reacting them with ninhydrin. It gives an intense blue colour except for proline because proline is an imino acid okay, it is a secondary amine. So basically, you form, you have done dye formation in your organic chemistry laboratories. So, this basically forms an intense blue dye and you can quantitate this dye into how many of each amino acid you have.

How many of each amino acid types you have okay. But how does that help us. That does not help us with the sequence okay. It just tells you how many of each you have, but we want to know the sequence.

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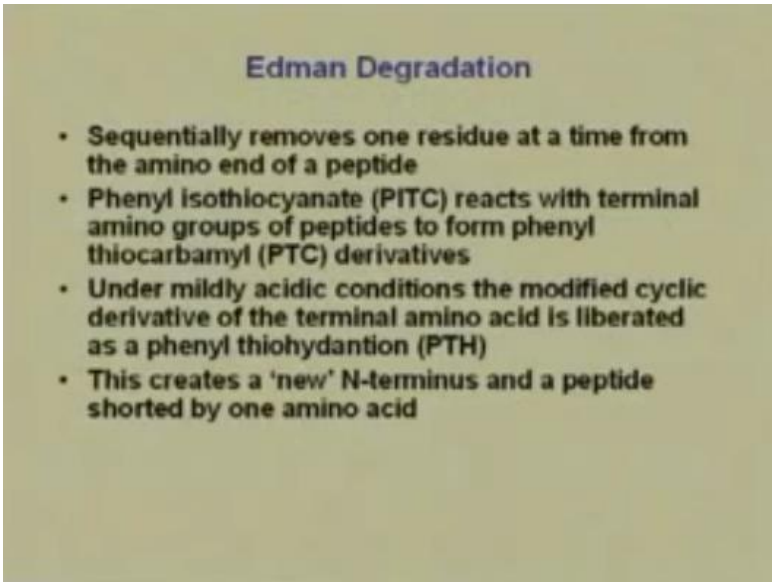

So we do what is called degradation okay. This degradation is this is the chemical method that you can use to chop it up. What we studied for chymotrypsin was an enzymatic mechanism right, an enzymatic mechanism where we have a breakup. In Edman Degradation, what it does it sequentially keeps on removing one residue at a time from the amino end of a peptide.

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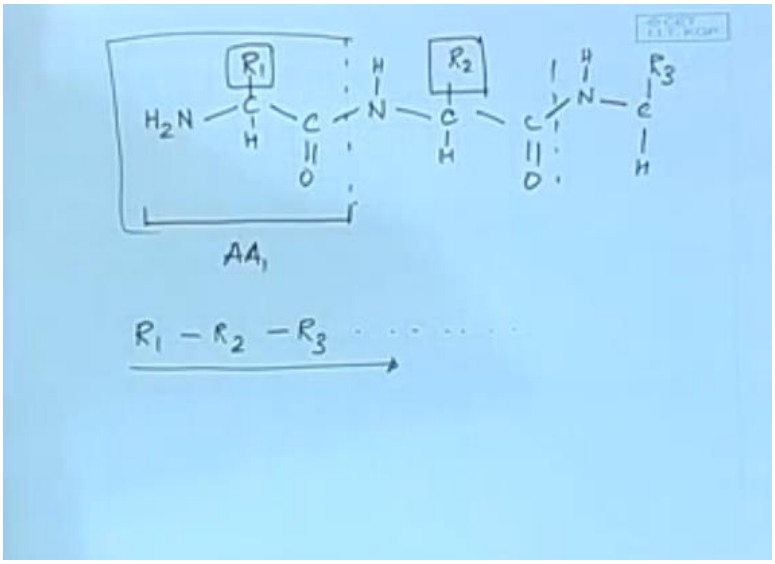

So what is that telling us, so if we have our polypeptide chain, what do we have, we have our, what is this going to be linked, this is going to be linked, so we have an R1 group here right.

Then what are we going to have here, we are going to have our second R group here that is going to be linked to the third R group here right.

So, which is our first amino acid, this much is amino acid number 1 right. What Edman Degradation does is chops off here, then you can figure out what this amino acid is okay. So now what is this become, this becomes now the N-terminal right. So then you chop off again. So, you then determine what R2 is, so what can you do. So first you know what R1 is, then you what R2 is, then you know what R3 is, then you can keep on going till you come to the end of your chain right.

So what is this giving you, it is giving you the amino acids sequence of the protein. It is not just telling you how many of each, obviously once you have everything you know how many of each of them and there anyway. But it is telling you in which order they are linked right and for this we have certain reagents, because we have to have a reagent that is going to link up with this amino acid and cleave it off the rest of the protein polypeptide chain.

So what are these reagents, we have one that is called PITC, PITC is Phenyl isothiocyanate okay. That reacts with the terminal amino groups of peptides to form what are called PTC derivatives okay, but all you need to know is what we are doing with Edman Degradation is we are just chopping off the N-terminal end every time, not the C-terminal.

We go through the C-terminal; it' is a different thing altogether okay. So what happens is the PITC reacts with the terminal amino groups of the peptides to form a PTC or a Phenyl thiocarbamoyl derivative. Then we have a slightly acidic condition that is going to liberate this by what is called a Phenyl thiohydantoin derivative okay.

What you need to know again is that in Edman Degradation, we are removing one residue at a time from the amino terminal of the protein. We have a certain what is called a thiohydantoin formed that has with it the amino acid that belonged to the N-terminus. Then happens is it this is chopped off, it creates a new N-terminus.

Once a new N-terminus is formed then the peptide is already shortened by one amino acid, you keep on doing that till what, till you identified all the amino acids and what do you know by this, you know the exact sequence of the polypeptide chain okay that is what is important

here. So we have a PITC that acts with the terminal amino groups and then we have certain derivatives form okay.

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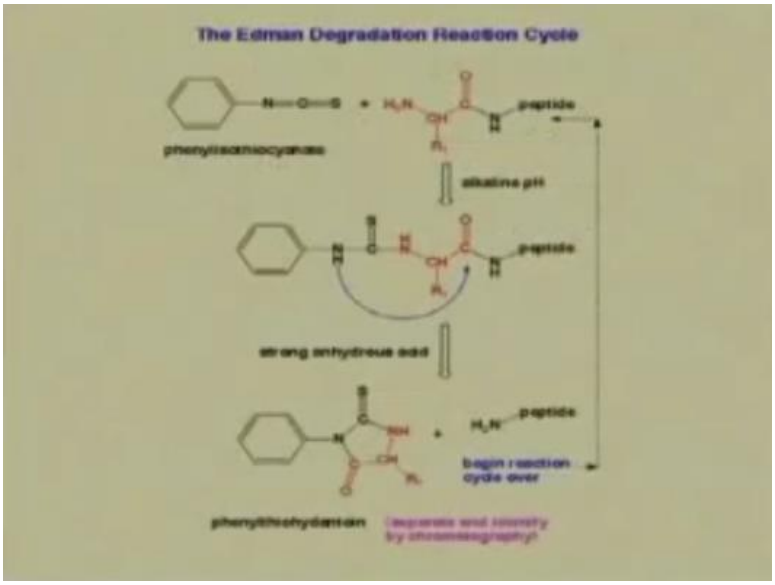

This is basically what happens. We have a phenyl isothiocyanate, this is our peptide, what is this; this is the terminal amino group. We have our peptide here. If we have an alkaline PH then this basically forms a link here. So, we have a Phenyl thiohydantoin derivative. So we have you see the CS, this H we have a linkage here a CN bond formation okay.

This CN bond formation then with acid forms this phenyl thiohydantoin. What is linked here, what is this, R1, what is this R1 that is the first amino acid of your peptide chain, then what do we have here, we have the peptide with a new amino terminal, what does this do, this can then go through another series of another cycle basically. When we have another cycle what are we going to get then, we are going to get another hydantoin derivative with R2 and so on and so for.

So essentially in Edman Degradation, we have a Phenyl isothiocyanate interact with the amino terminal of a peptide chain and what happens is, we have the cleavage of the peptide into forming a new amino terminus and we have a cyclic phenyl thiohydantoin derivative, which has linked to it the first amino acid that happen to be part of the origin polypeptide chain okay.

So, this R1 was part of the original polypeptide chain. It has now been chopped off. So now I have the rest of the peptide with a new end terminus. If I go back to do it what am I going to

get, I am going to get another derivative with R2 here right. So I can keep on doing the cycle till I have completed the whole sequence of the protein.

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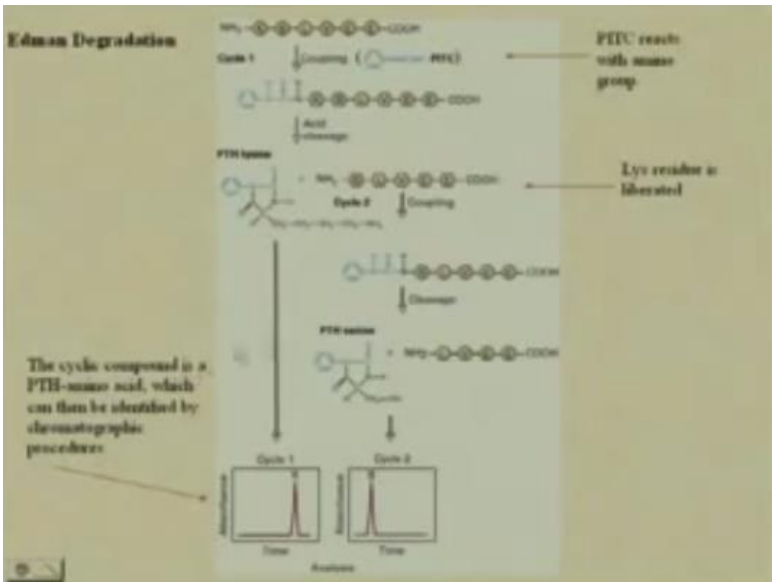

So this is basically what is done, it is just you have the PITC coupling. So this is my sequence. I have KSLV and so on and so for. So what is going to happen, in my first step I am going to release the first amino acid, in the second step, the second amino acid. So I keep on doing the cycle till I go through the cycle will release one amino acid, cycle two will release another amino acid okay.

What do I know; I know how this behaves in CHPLC. If I know how this particular derivative with lysine here behaves in a chromatographic column then I know, I have a standard for every amino acid. So I can figure out what R1 is if I match it with the standard that is all you have to do okay. Once you have this because R1 is going to (()) (43:53) even for another lysine, this is going to look the same right. But you will now know the order in which it has come off.

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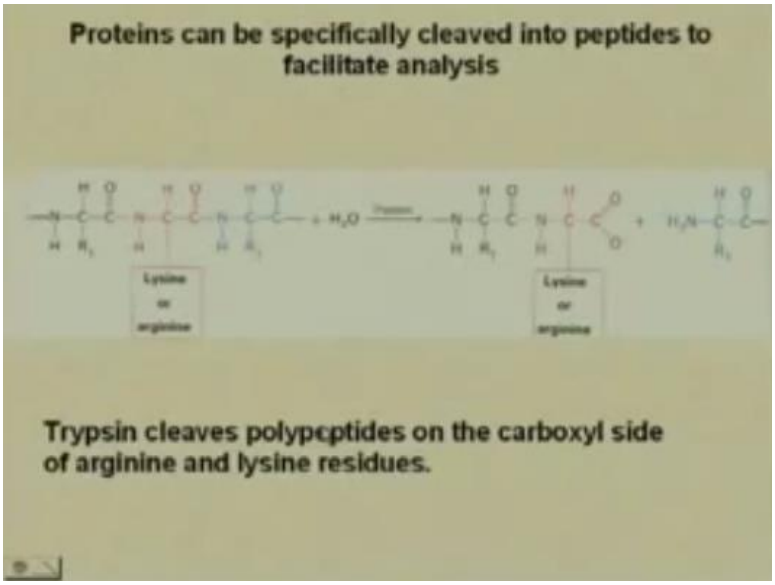

So, we can have what, we can have cleavage. What did we have for Edman Degradation, we had cleavage at the N-terminal. For trypsin, we can have cleavage where after lysine or arginine provided; it is not followed by a proline okay that is where we have a cleavage. So we have to be careful when we are looking at lysine or arginine. We have to be careful that there is no proline here.

If there is no proline, then this is our side chain that is lysine or arginine, again it will cleave at the carboxyl end of a basic amino acid residue. If an enzyme, a protease cleaves at the carboxyl side of lysine or arginine that is a trypsin. If this were a bulky residue and it cleaved that the carboxylic bulky hydrophobic, it would be chymotrypsin okay, because you have to understand each of these enzymes are extremely specific in the way they work okay.

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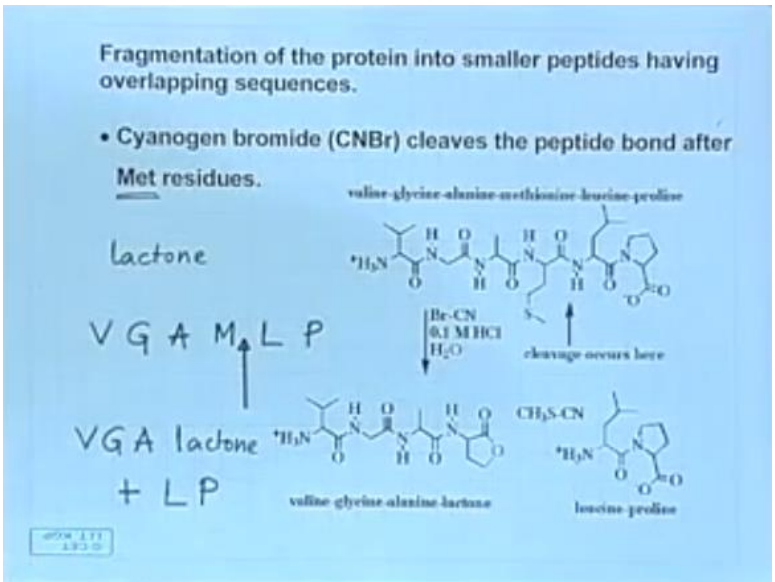

So what we can have is we can have another type which I have printed out here were we can figure out how we can actually form smaller peptides by overlapping sequences. What can we do is this, for example, is Cyanogen bromide? Now in Cyanogen bromide, Cyanogen bromide cleaves after methionine residues okay.

There are certain things that we have to remember. So, we have Cyanogen bromide that cleaves after a methionine residue. So, what happens in this case what you need to know, I have just given the mechanism here but that is not important. What we need to know is that a certain lactone is formed, so we have the formation of a lactone, the lactone is formed where the methionine residue was in the polypeptide chain.

What is that mean, it means that if you had a valine and a glycine followed by an alanine, a methionine, a leucine and a proline, then if you treated this with Cyanogen bromide you would have a cleavage at this point here okay. What would happen is the side chain of the methionine group would go and link up with the Cyanogen bromide okay. So what you are actually left with is a VGA lactone plus a leucine and a proline, is that clear okay.

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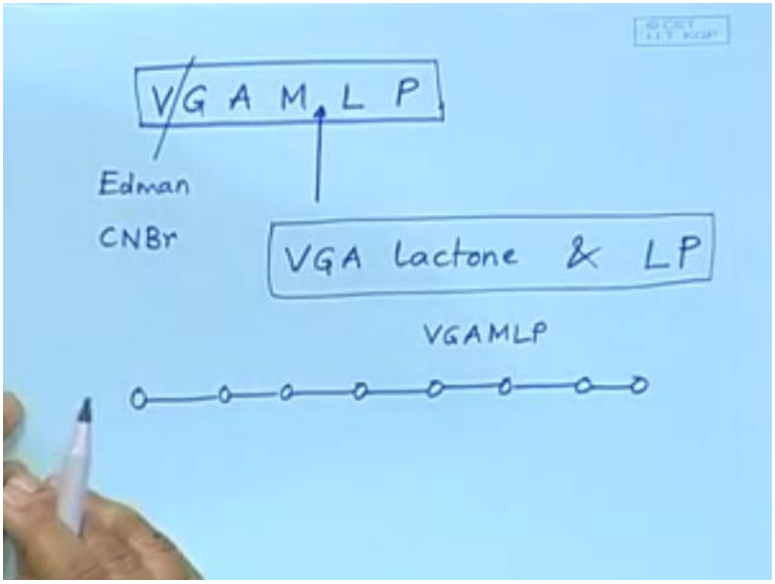

Let us just go through it once more. What I am saying is that when we have a lactone formation, so what we have is we have our polypeptide chain, which is VGAMLP. If I use Edman Degradation, what will I get from Edman Degradation here, the first amino acid I will get is V, if I do Edman's method. If I treat this with Cyanogen bromide, I am going to have Cyanogen bromide act here and what I am going to get is since the methionine is going to interact with the Cyanogen bromide I am going to be left with a VGA lactone and LP.

So after treatment of this particular polypeptide or this particular peptide with CNBr, if I am left with a VGA lactone and LP, then I know that my original sequence was VGAMLP okay, because I know. So now if I tell you that I have a particular sequence that is VGAMLP, I know that I treated with CNBr, I am going to get a VGA lactone and LP. If I do Edman's Degradation, I am going to figure out which one is the amino terminus of the peptide chain okay.

So this gives us a way to actually fragment out the polypeptide chain. What do I mean by that, I mean that if I have a long chain now, I can either use an enzymatic cleavage or I can use a chemical cleavage, what is that if you get back to the slide here?

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Reagent	*Cleavage site*
Chemical cleavage	
Cyanogen bromide	Carboxyl side of Met residues
O-Iodosobenzoate	Carboxyl side of Trp residues
2-Nitro-5-thiocyanobenzoate	Amino side of Cys residues
Hydroxylamine	Asn-Gly bonds
Enzymatic cleavage	
Trypsin	Carboxyl side of Lys and Arg residues
Chymotrypsin	Carboxyl side of aromatic residues
Staphylococcus protease	Carboxyl side of Asp and Glu residues

I can have a chemical cleavage where I can have Cyanogen bromide, which will cleave on the carboxyl side of methionine residues. I can have Iodosobenzoate, that is going to cleave on the carboxyl side of tryptophan residues and the useful thing here is that since proteins do not have many methionine tryptophan or cysteine residues, it is easier for you to get fragments.

So say you have a protein, for example let us look at ribonuclease A, it has 816 residues okay. We know that it has four disulfide linkages, so I can reduce the disulfide linkages and what can I do is, I can get the long chain, but then if I use this particular reagent to chop off at the 16 residues where on the amino side of the 16 residues, then I will get fragments say of the length 20, 50 or something like that.

You understand that but if I do Edman Degradation, I will get this one amino acid and a 123 on the other side. But it would be smarter if I cleaved off with some other reagent and then try to do my sequencing of the shorter fragments that I now get. It is going to be an easier job. I can also use enzymatic cleavage. For the enzymatic cleavage, what do we need to know, we know the trypsin will cleave on the carboxyl side of lysine and arginine.

Chymotrypsin will cleave on the carboxyl side of aromatic residues okay. So how can we use this information to figure out what we can do with the amino acid sequences of proteins into determining how this utilisation of the chemical cleavage and the enzymatic cleavage will lead us to the polypeptide chain okay.

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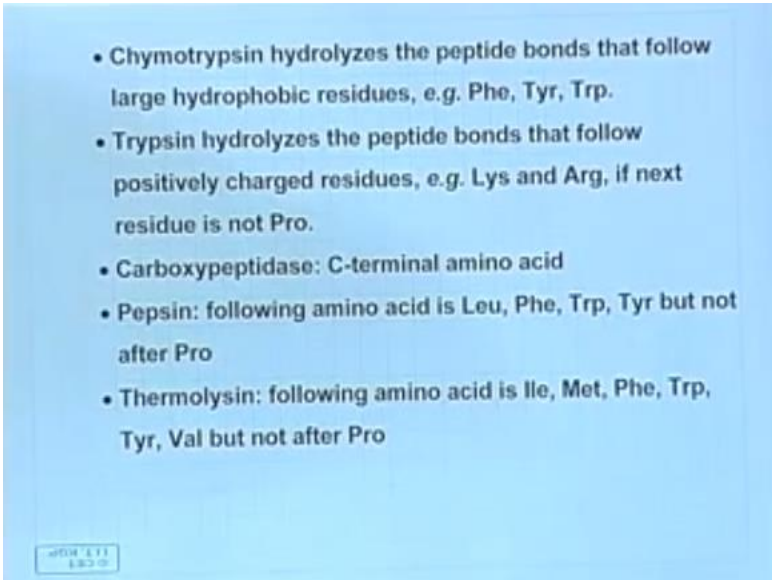

So this is the information we need. What do we need, we need to know that chymotrypsin, hydrolysis, the peptide bonds that follow large hydrophobic amino acids, what are they? phenylalanine, tyrosine, tryptophan. We need to know that trypsin will hydrolyse peptide bonds that follow positively charged residues, that is lysine and arginine, if the next residue is not proline. Another enzyme carboxypeptidase will tell us what the C-terminal amino acid is.

Pepsin will cleave if the following amino acid is leucine, phenylalanine, tryptophan and tyrosine. So where is pepsin cleaving, only if the following amino acid is either leucine, phenylalanine, tryptophan or tyrosine okay. You see these are all enzymatic cleavages. Thermolysin will cleave if the following amino acid is isoleucine, methionine, phenylalanine, tryptophan, tyrosine, valine okay.

So we have isoleucine, methionine, phenylalanine, tryptophan, tyrosine, valine for Thermolysin. For pepsin, we have leucine, phenylalanine, tryptophan, tyrosine. For carboxypeptidase, it will tell you the C-terminal. How will I know the N-terminal, Edman Degradation. After I cleave my sequence with trypsin, I will know there will be a lysine or an arginine, that was present. Then I have chymotrypsin okay.

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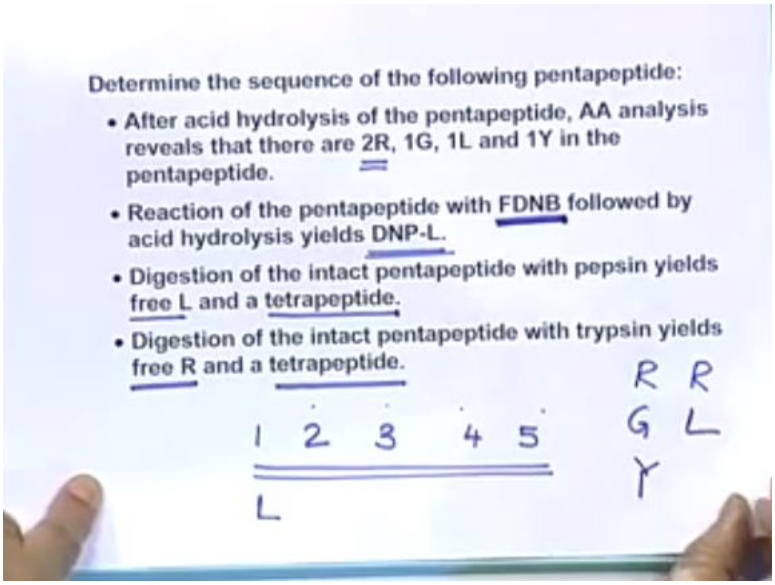

So, I have a small problem for you where you can determine the sequence of a pentapeptide, what is a pentapeptide, it just has five amino acids. So what are these five amino acids, there are two arginines, one glycine, one leucine and one tyrosine reaction of the pentapeptide. FDNB is another reagent that will tell you what the N-terminal is just like Edman Degradation okay.

So the Edman Degradation method tells you that the first amino acid is L, if you digest the peptide with pepsin, you will get L and a tetrapeptide. You know it is a pentapeptide, so you have one, two, three, four, five digestions of the pentapeptide with trypsin gives you free R and a tetrapeptide. What you have to figure out is the sequence of the pentapeptide. You know that this is L and you know that there are two R's, one G, one L and one Y. You just got to fit these in the correct boxes okay.

We will discuss this in the next class, I will give you more peptide sequences to solve. Thank you.