Biochemistry - I

Prof. S. Dasgupta

Department of Chemistry

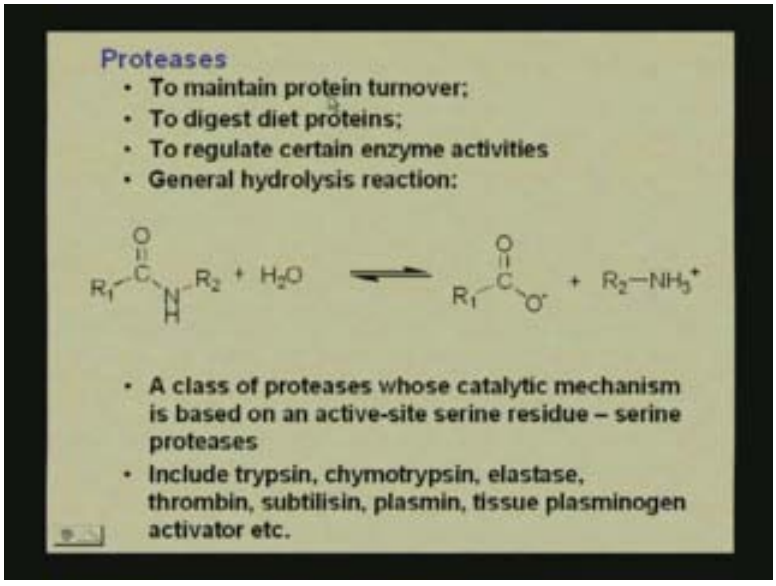
Indian Institute of Technology, Kharagpur

Lecture-11

Enzyme Mechanisms –II

In the last class we studied the enzyme mechanisms of ribonuclease A and lysozyme. What we are going to do now is we are going to study how proteases work. Proteases are essentially enzymes that break peptide bonds. So they are extremely important in protein digestion because they have to break the peptide bonds into different units which are later used for protein turnover. So basically what we have in proteases is to maintain the protein turnover.

(Refer Slide Time: 1:18)

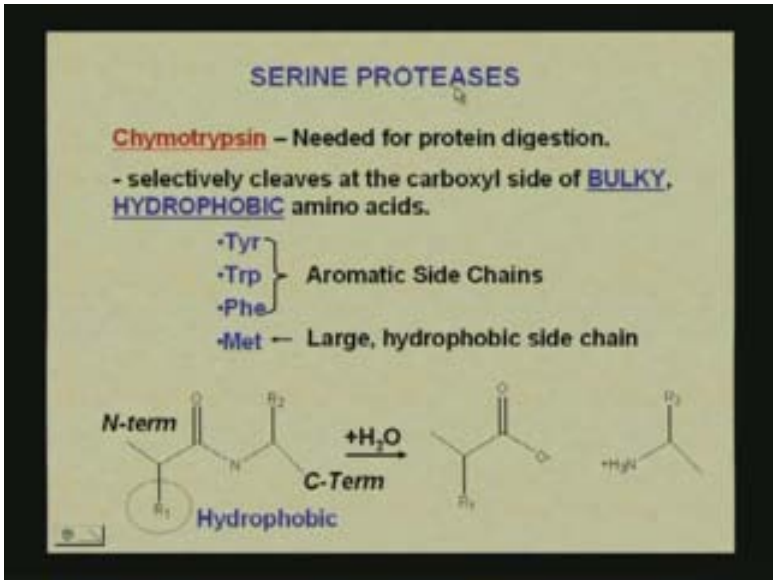


They are also essential because they have to digest the proteins that we take in the diet because we consume proteins everyday and these proteins have to be broken down into smaller units. So these proteases that are specific types of enzymes break the peptide bonds and they are also essential to regulate certain enzymatic activities. Here we have a general hydrolysis reaction. In such a reaction -C-O- N-H is our peptide bond. Now what is going to happen is that this peptide bond is going to be cleaved. When it was formed, if you remember, NH_2 and COOH of two amino acids linked together to form the peptide bond with the release of H_2O. Now 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. There are different types of proteases like serine proteases and aspartic acid proteases and

the one that will be studying is serine proteases. Basically the name given to the different proteases depends on what residue the catalytic mechanism is dependent on.
For example if we have a class of proteases where in the catalytic mechanism 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 of the catalytic mechanism would be based on an aspartic acid. So the serine proteases basically include trypsin, chymotrypsin, elastase and the other ones that are listed here. If you remember about two three class ago I showed you a typical picture of how trypsin, chymotrypsin, elastase could be accommodated in the active sites. These are all examples of serine proteases where the catalytic mechanism is based on an active site serine residue and what 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 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.

We have in general the serine proteases.

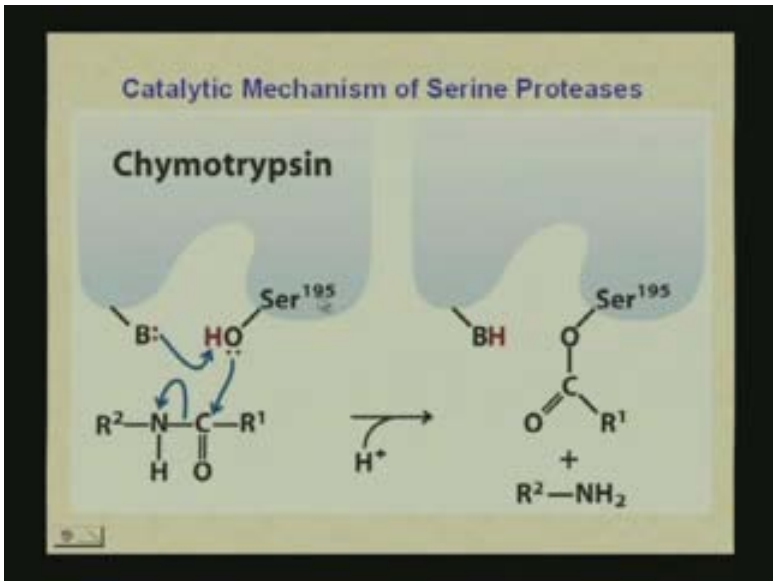
(Refer Slide Time: 4:12)



This chymotrypsin is needed for protein digestion. It selectively cleaves at the carboxyl side not at the amine side. You know that an amino acid has two sides to it one is the amine side and one is the carboxyl side. This chymotrypsin will cleave at the carboxyl side of bulky hydrophobic amino acids. Usually it cleaves at the aromatic side chains and also methyl. This is where it cleaves. We have a N-term. What is this? This is the N terminal of the protein, these R_1 and R_2 are the residues, the side chain groups of the polypeptide chain and -CO and N would be the peptide bond. We are going to have a cleavage. A cleavage is going to be at the carboxyl side. This is where the cleavage is going to occur provided R_1 is a bulky hydrophobic residue. If R_1 is a hydrophobic residue then the cleavage will occur here and not on the other side. It occurs at the carboxyl side of the hydrophobic group. If this is R_1 it is going to occur towards the C terminal end of the polypeptide chain. Chymotrypsin is a serine protease. It means that the active site

contains a specific serine residue that takes an active part in the catalytic mechanism of this protein. Chymotrypsin basically is also a protein. These are all enzymes and biological catalysts that are proteins. Now in this case we will go in mechanism in pretty much detail but for now what we need to know is the serine form that makes chymotrypsin in serine proteases is serine^{195}.

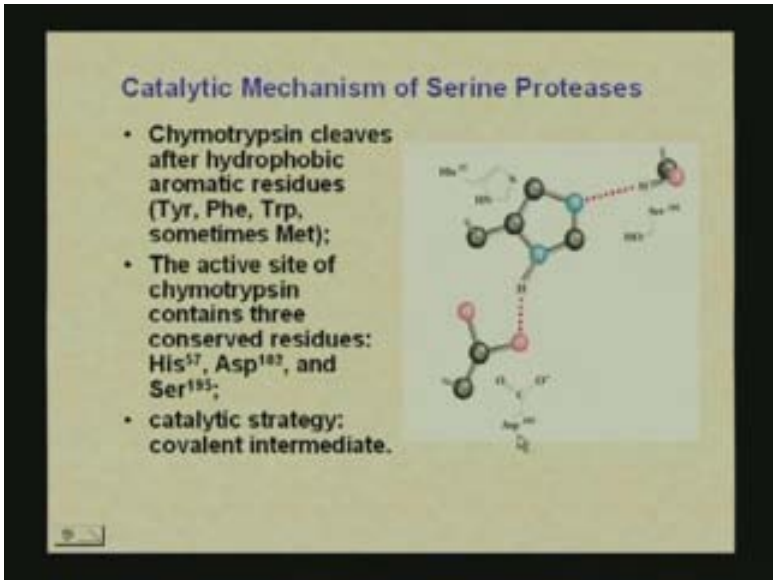
(Refer Slide Time: 6:42)



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 together and this cannot go back to form or cleave another peptide. What is happening here is we have a cleavage of the peptide group. 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 recognize this is $R^1$and that is R^2. So which is our hydrophobic residue? Because chymotrypsin is going to cleave after the hydrophobic residue R^1 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 to remember that this C-O 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 because we have the -N-H, the C of the R^2 link and then will be the C-O of this group. If the cleavage of this peptide bond is occurring it means that R^1 is the hydrobhopic amino acid bulky group connected to the carboxylic group here and this is where the cleavage occurs. What is the important residue here? Serine^{195}. Now let's go in detail. We have three important residues. These three are histidine^{57}, serine^{195} and aspartic acid^{102}.

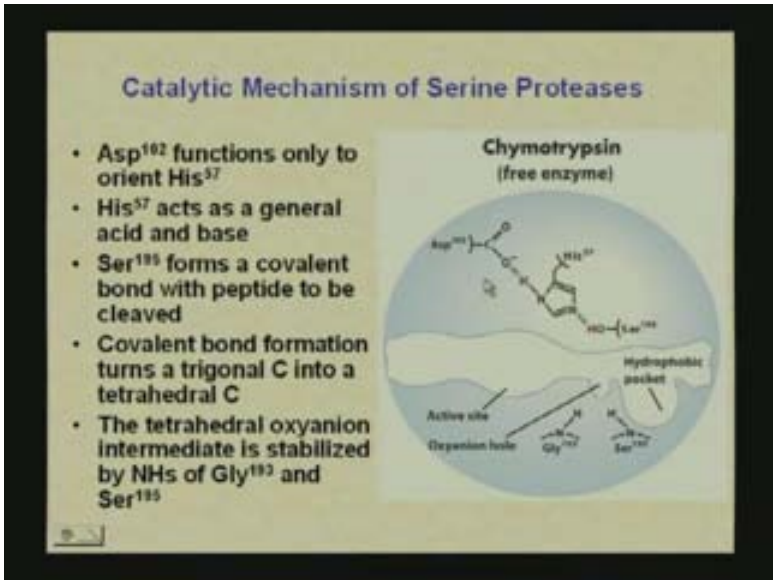
(Refer Slide Time: 9:25)



You see the aspartic acid here -C-O-O. This is histidine. What is histidine? You have two nitrogens and there is an H attached here and this is serine. What is the side chain for serine? -OH. This is just the schematic and these ball and stick models show you which residues are important. We have serine[195], histidine[57] and aspartic acid[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 three residues. Conserved means it is the same for practically all species. 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 to remember that this covalent intermediate has to be broken into two because we have to get the serine back to where it is.

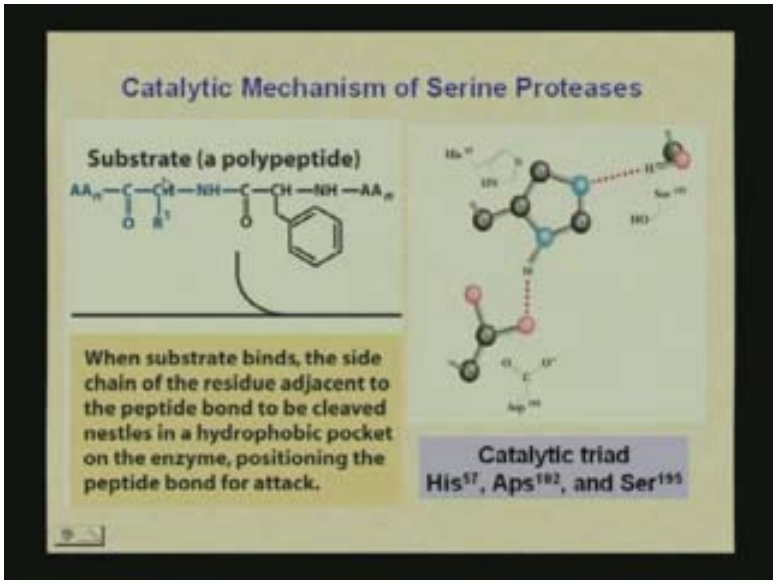
What are the steps involved? I am going to go through this quite slowly and I want you to follow the mechanism because it is extremely interesting. The first thing that we have to know is what is important about the three particular residues that are part of these? Now these three residues are known as a catalytic triad. In the serine proteases we have a catalytic triad that is composed of a histidine, a serine and an aspartic acid. So each of these residues in the triad is going to have a specific role 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. What is the function of the aspartic acid[102]? It just basically holds the histidine in place by an electrostatic interaction. It is holding out a rather hydrogen bond type interaction where it is holding the histidine in place and 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. 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 it's side chain and this is the side chain of the aspartic acid.

(Refer Slide Time: 12:46)



When we have a covalent bond formation, we have a trigonal carbon form into a tetrahedral carbon. We will go through the steps one by one. This is the active site pocket that you see here, the one in white. So this is our active site. This is hydrophobic pocket. 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. 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; rather the N-H of the serine and N- H of the glycine that helps in stabilizing the oxyanion. We will go step by step to see how that occurs. This is our substrate.

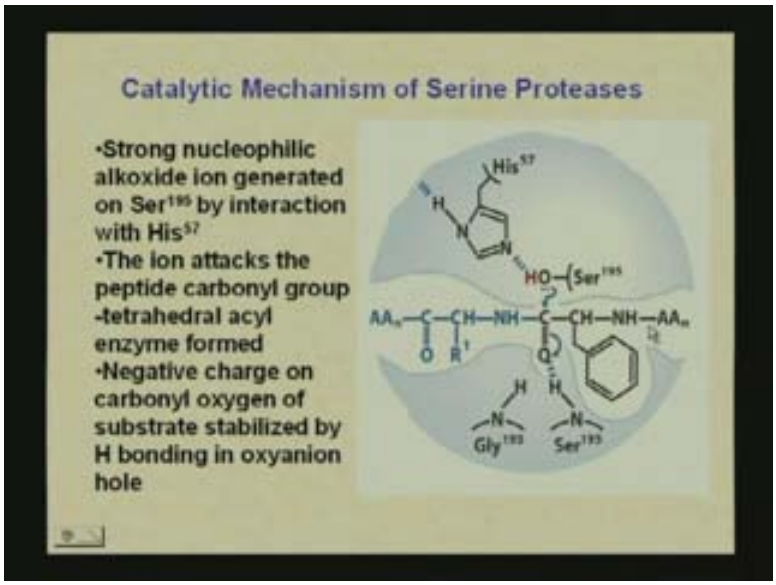
(Refer Slide Time: 14:00)



What is the substrate? The substrate is nothing but a poly peptide chain and the protease in this case the 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 carboxylic side of the hydrophobic residue. This is the amino terminal and you recognize 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 R^1. It is going cleave right here. What happens to this is it's going to fit into the hydrophobic pocket. 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 and this is called a catalytic triad - the histidine, aspartic acid and serine.

Here is our poly peptide chain inside the active site.

(Refer Slide Time: 15:29)

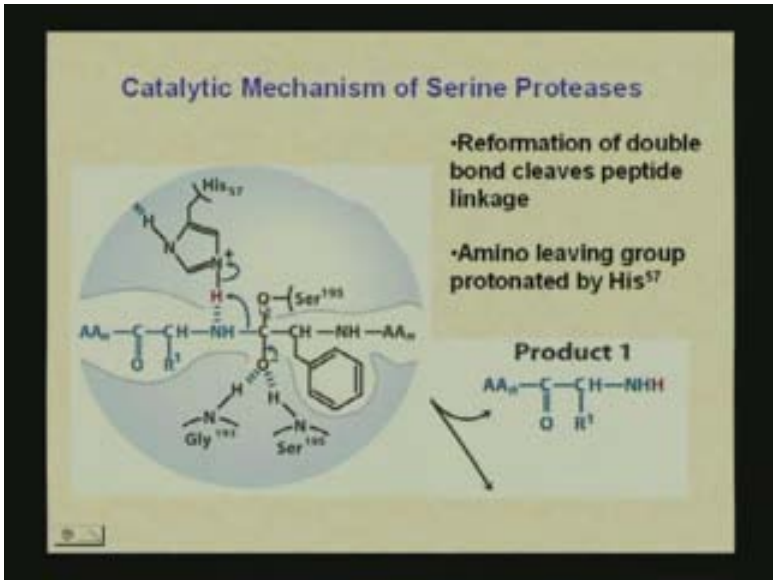


Now what is going to happen? The first thing that is going to happen is that this histidine[57] is going to abstract the proton from serine. After this abstraction there is an ion at the serine side chain. This is going to attack the peptide carbonyl group and we have an acyl intermediate form. What is an acyl intermediate? An O-CO group. 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 it is acting as here is 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^- formed here. So what is happening is this O after the abstraction of the H to the histidine forms an O^- that sits in this oxyanion hole and is stabilized by the -NH of glycine[193] and serine[195]. This is our first step.

What has been formed now? In the first step we have linked the enzyme with the polypeptide chain. 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.

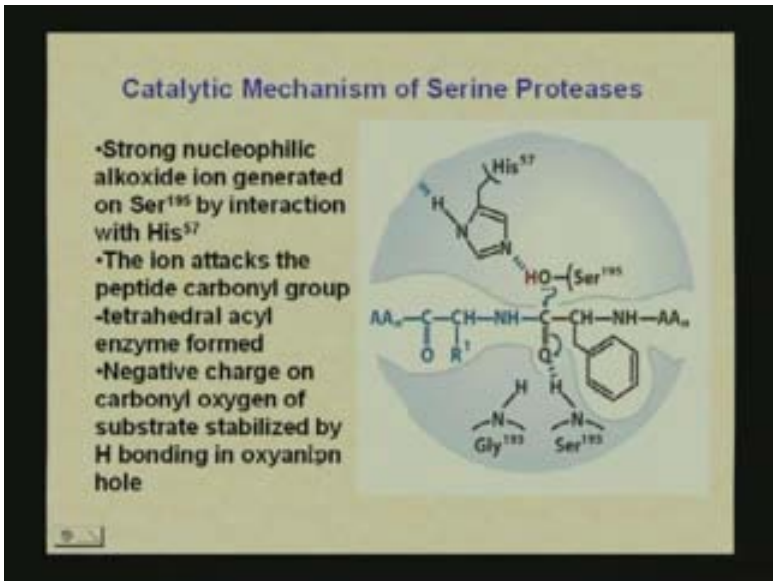
this is a direct linkage. This is our linkage. What did serine[195] do? It linked with the carbonyl group. How? After the H was abstracted from serine.

(Refer Slide Time: 18:31)



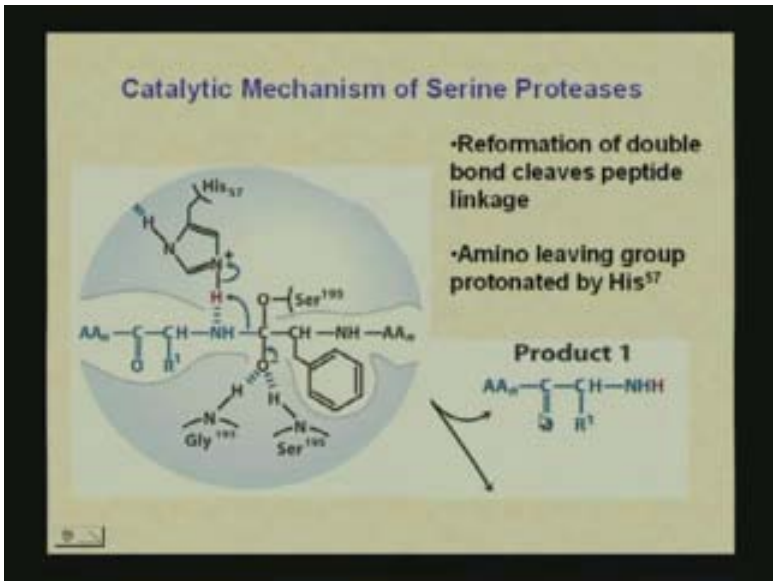
The H that is marked in red here originally belonged to serine. If we go back to the previous step, this is the -OH that originally belonged to serine. What has happened is histidine has taken up this H. In this event what has happened is this alkoxide ion that has been generated attacks the carbonyl which becomes O^-. The O^- is stabilized by the -NH groups of glycine[193] and serine[195].

(Refer Slide Time: 19:14)



We have to cleave the peptide bond. What happens is this O^- that was sitting in the oxyanion hole goes back to form the C=O. This now is cleaved. What does this do? This takes up the hydrogen that was originally on serine and what does it form? NH_2. 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 that is leaving.

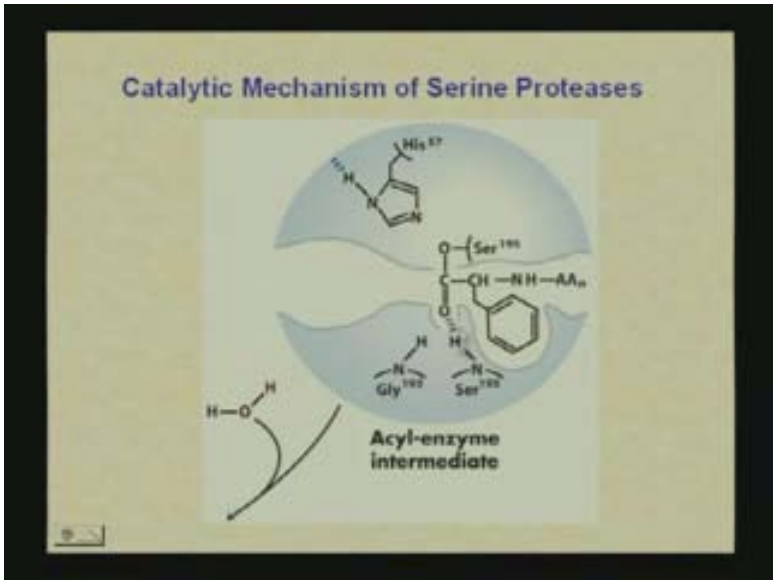
(Refer Slide Time: 20:32)



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. Serine of 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 the serine. 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 $-NH_2$. This is the first product. But what are we still left with? Still we have to get serine back to –OH. If we want chymotrypsin to act on again we have to get an acyl intermediate now. We have to get this back to –OH.

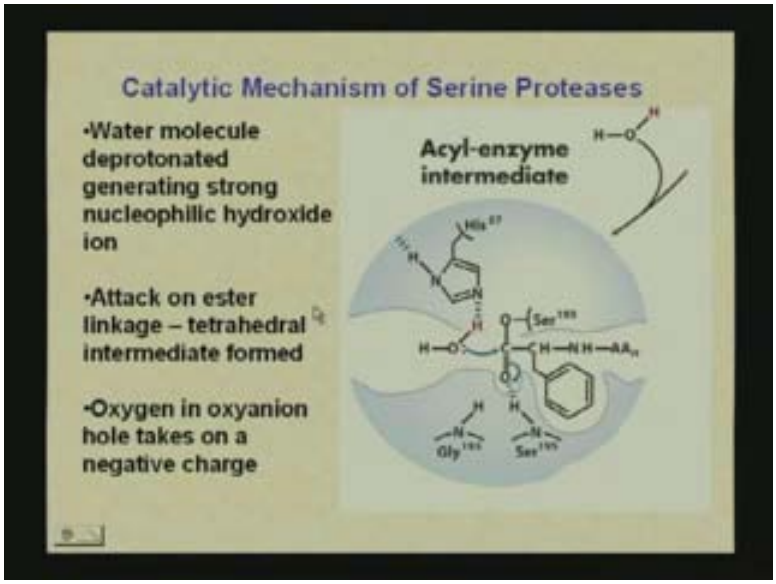
Our first product has gone. This part is empty now. We have our C=O formed back again but we still have this linkage and this is the carboxylic part. This is our hydrophobic pocket. So we have what is called an acyl-enzyme intermediate. Why it is called acyl-intermediate?

(Refer Slide Time: 22:15)



Because we have an –O-C-O group here and this is the aycl group and we have the enzyme still covalently linked to part of the polypeptide chain that is supposed to cleave. Now we have a water molecule come in. Water molecule is in a lot of abundance in our body. The water molecule now is deprotonated. Who is the one that is going to take up the hydrogen? Histidine.

(Refer Slide Time: 22:50)

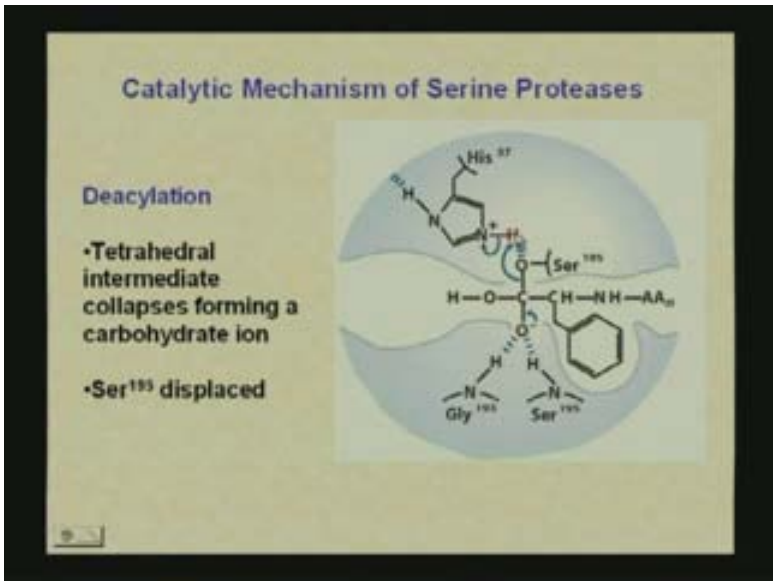


Histidine then will take up the hydrogen from water. It abstracts hydrogen from water and what is going to happen to the O^-? It is going to attack the carbonyl which again forms an O^-. We have again the oxyanion hole that has been occupied by the O^- and is

stabilized by the -NH groups. 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 contains the bulky 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. We have an intermediate basically. We have a water molecule. The water molecule proton is abstracted by histidine. Then we have the $–OH^-$ hydroxide ion attack the carbonyl to form a $–CO^-$.

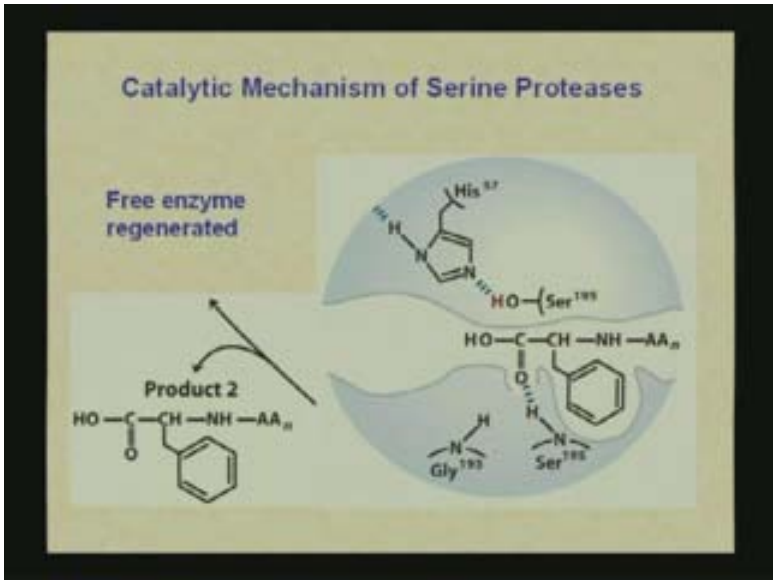
This $–CO^-$ and the -OH is linked here. What happens is this O^- is going to come back to form what? A C=O and what is going to get cleaved? The CO, the acyl linkage to serine and where is it going to get H from? From histidine.

(Refer Slide Time: 24:48)



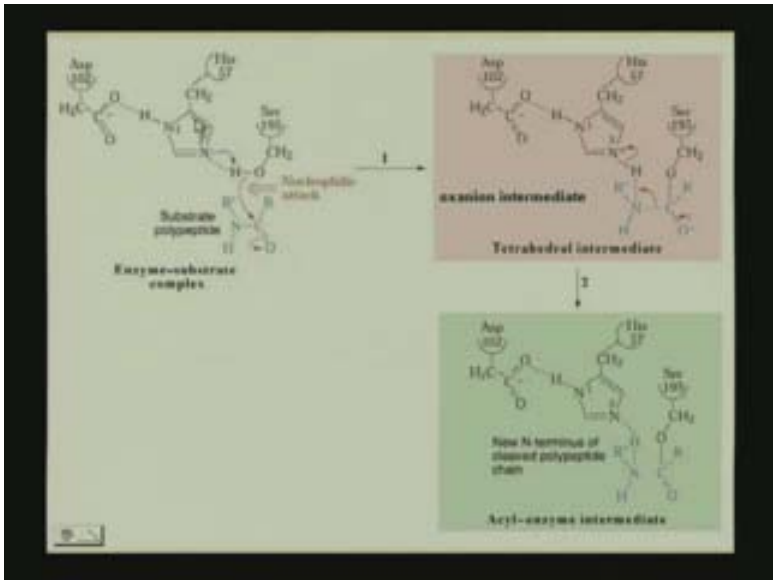
What does histidine in the first series of steps do? It takes the hydrogen from serine and gives it to the leaving amino part of the polypeptide chain. In the next series of steps it takes the proton from water and gives it to serine. We have basically deacylation and we have what was O^- here form a 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. Chymotrypsin can then go and further cleave another peptide. What is the second product then? This is now generated C=O -OH with this. This is our second product. What happens 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. What can happen again? Histidine can again take this H; this O can again link with the peptide in another cleavage of another peptide. All we have to remember is the roles of the catalytic triad.

(Refer Slide Time: 26:25)



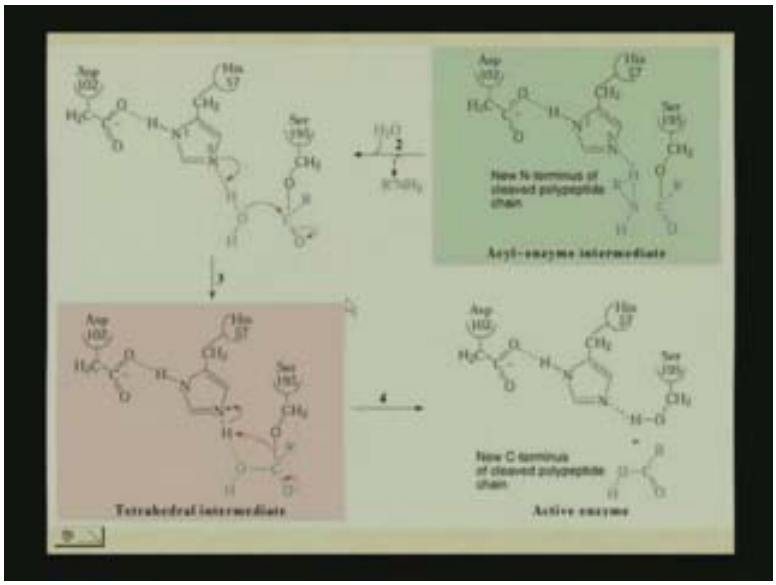
What is the role of the aspartic acid? Just to hold the histidine in place because you see aspartic acid[102] has no role to play here but it does act in holding this histidine in place by formation of this particular hydrogen bond. We have a particular hydrogen bond and this is possible only if the aspartic acid is in place. The aspartic acid holds the histidine so that the histidine can take and donate protons to either water or serine so that chymotrypsin is regenerated. Basically this is the summary of what has been done here. What is aspartic acid[102] doing? It is just holding the histidine in place.

(Refer Slide Time: 27:15)



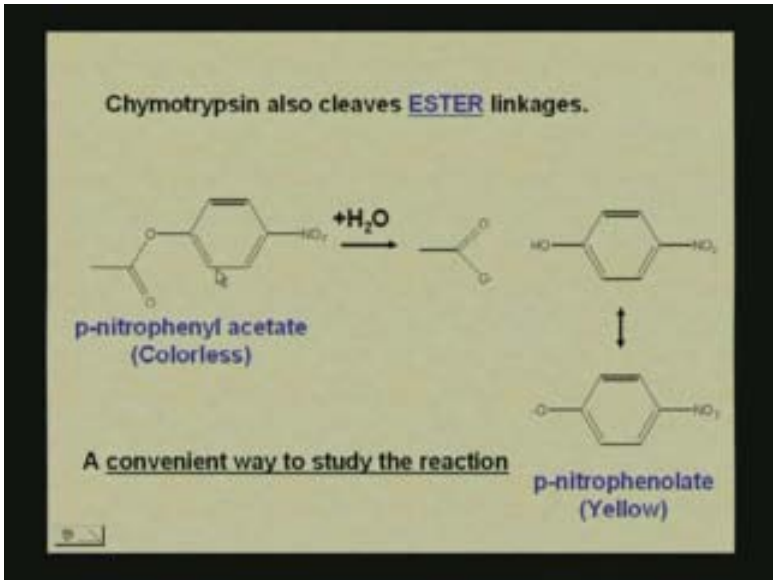
The N base abstracts the H from serine. This O is linked to the carboxylic one that is going to be cleaved of the peptide. Then what happens is we have what is called an oxyanion intermediate, a tetrahydral intermediate and we have a cleavage of the peptide bond. That is essentially what the whole step 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. But in this case it is doing its role twice.

(Refer Slide Time: 28:05)



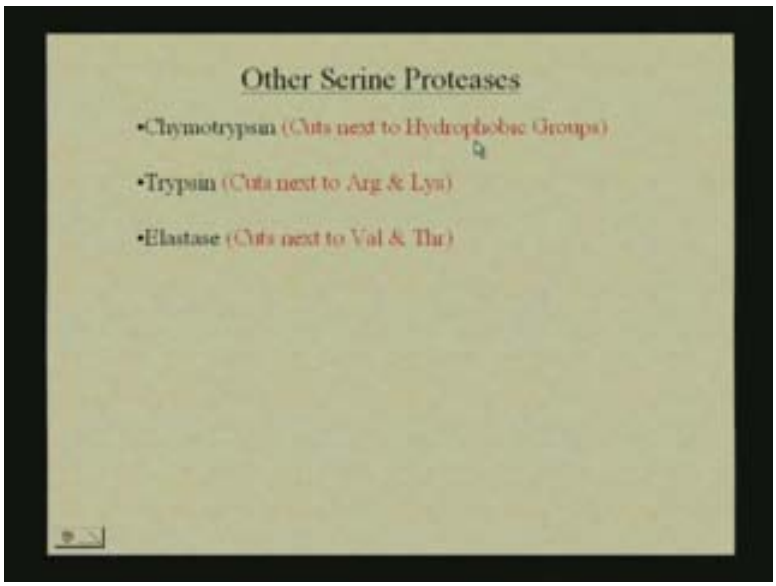
It is taking up H from serine giving it to the leaving part that had the amino group to it. It is donating the H from the serine to that. Then in the next step it takes H from the water and gives it to serine. We have now our catalytic triad back in place to form the active enzyme. A chymotrypsin can also cleave ester linkages. This is usually done as an experiment. It does not have any biologic relevance as such but we can have a colorless entity like para nitrophenyl acetate form para nitrophenolate that will tell you how active your enzyme actually is.

(Refer Slide Time: 28:59)



This would be an enzymatic general reaction that could be conducted where we have a convenient way of studying the reaction as to how it can cleave an ester linkage. We have other serine proteases. Chymotrypsin. Where does it cut? It cuts next to hydrophobic groups.

(Refer Slide Time: 29:23)

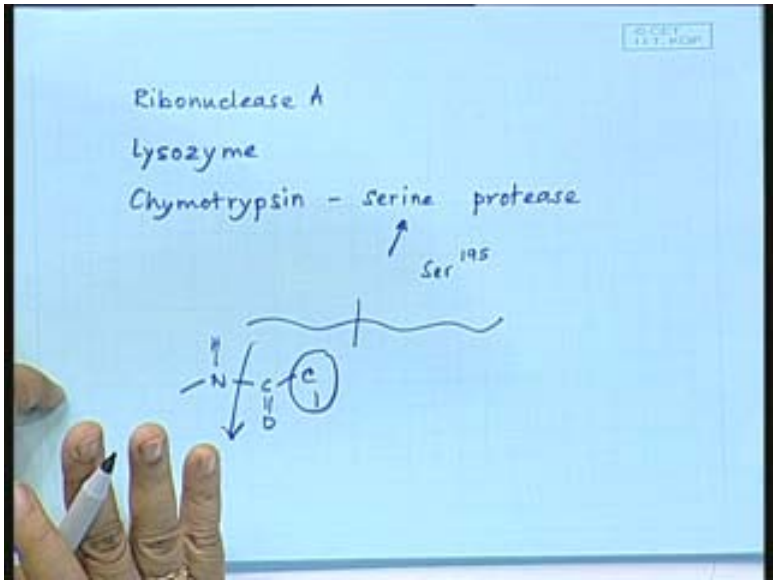


What do we mean by cut? Means it chops of the polypeptide chain. That's essentially what it is doing. It is chopping up the polypeptide chain. Trypsin will cut next to arginine and lysine. So it is cutting or it is cleaving after basic amino acid residues. Why is that? Because its active site is such that it will fit basic amino acid residues and it has specially

very carefully positioned aspartic acid that is going to form a covalent link, a formal electrostatic interaction with the arginine and lysine so that it sits in the active site. Elastase cuts next to valine and threonine. 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. If we have arginine and proline it is going to distort the polypeptide chain. It will not fit into active site. If it cannot fit into the active site it cannot be cleaved.

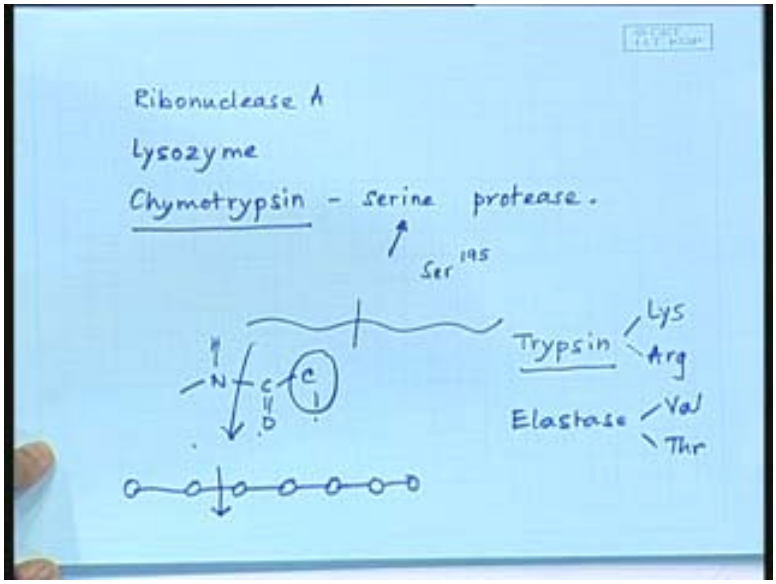
We studied about the different enzymatic mechanisms. We studied Ribonuclease A, we studied lysozyme and we studied chymotrypsin. Chymotrypsin is a serine protease. What does that mean? It means that its active site has the serine. That is why it's called a serine protease. Which is the serine? Serine^{195}. What does the serine^{195} do? It forms a covalent link with the specific polypeptide chain that has to cleaved. What are we doing is we are cleaving a peptide bond. We are cleaving a peptide bond only if this group attached is what? What type of group has to be attached here? A bulky hydrophobic group.

(Refer Slide Time: 32:09)



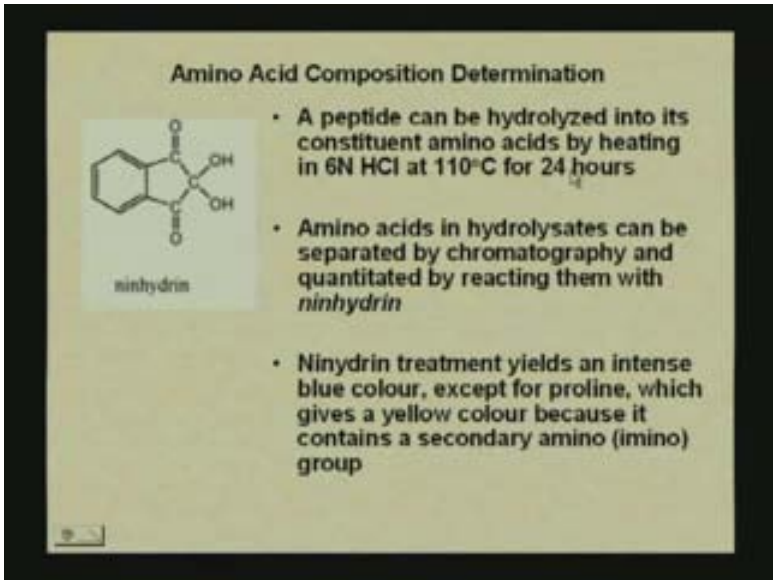
When we have chymotrypsin we have a particular type of breakage. When we have trypsin, which is also a serine protease, it will cleave only if this is lysine or arginine. We have elastase, which is also a serine protease it will cleave after valine and threonine. These are used a lot in amino acid analysis. You will see amino acid or peptide sequence determination. We will see how we can do that. Now we have a protein sequence. We know that these amino acids linked together. We know that if we are going to use trypsin it is going to cleave at a specific point.

(Refer Slide Time: 33:11)



We know that if we have elastase it is going to cleave at some other point. We know that if we have chymotrypsin we are going to cleave at some other point. So based on that we can use this very cleverly to figure out what the sequence of our amino acid in a poly peptide chain is. We are going to see how we can actually do that. It is like a puzzle. If we want to look at the amino acid composition, we can have a peptide. A peptide can be hydrolyzed into amino acids by heating in 6N HCl at 110^{o} centigrade for 24 hours.

(Refer Slide Time: 34:01)

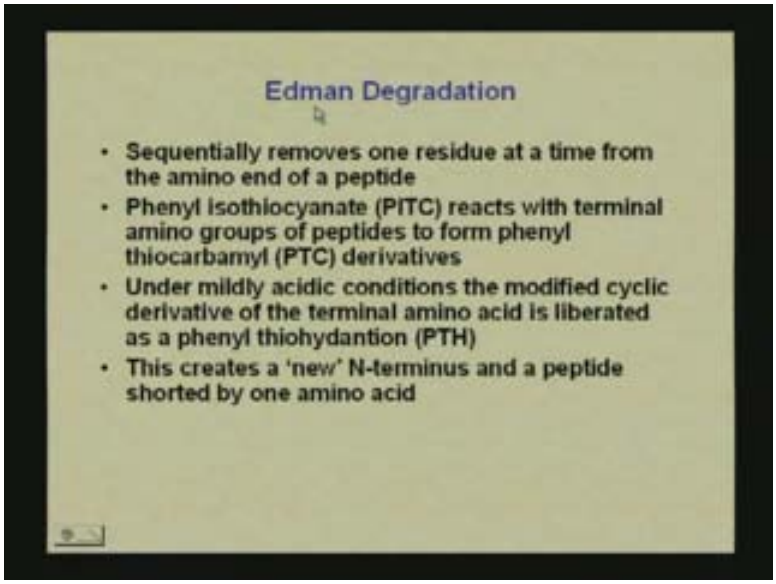


What is going to happen? We are going to chop down everything; break it up into fragments completely. But this will not tell you in what order they are. It will just give

you an over all 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 forth. But it gives no information about which order they are in. This is usually done using a compound called ninhydrin which gives a purple color for all amino acids except proline. 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 color except for proline, because proline is an imino acid. It is a secondary amine. You must have done dye formation in your organic chemistry laborites. This basically forms an intense blue dye and you can quantitate this dye into how many of each amino acid you have and how many of each amino acid types you have.

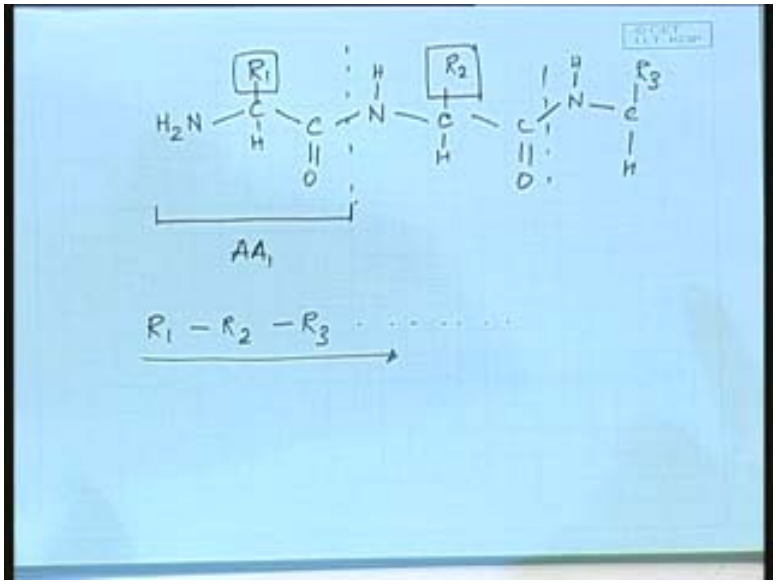
How does that help us? That does not help with the sequence. It just tells you how many of each you have. But you want to know the sequence. So we do what is called degradation. Degradation is the chemical method that you can use to chop it up.

(Refer Slide Time: 35:40)



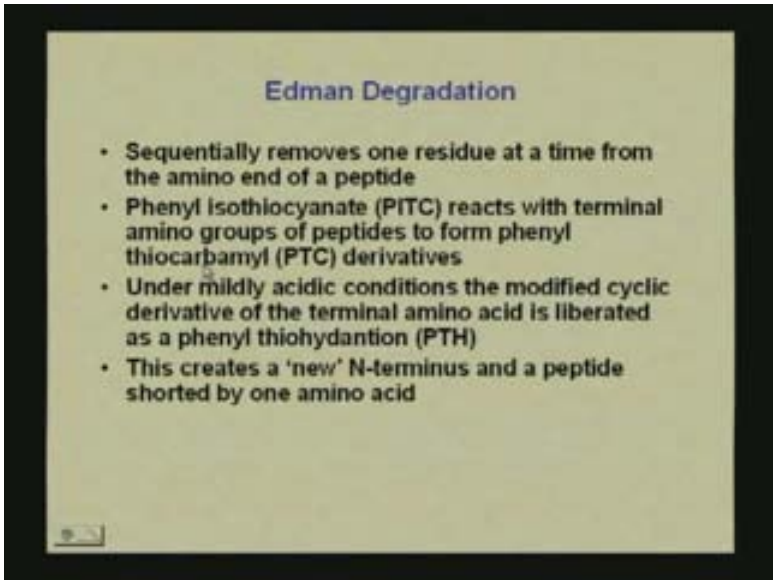
What we studied for chymotrypsin was an enzymatic mechanism where we have a break up. In Edman degradation what it does it sequentially keeps on removing one residue at a time from the amino end of the peptide. So what does that tell us? If we have our polypeptide chain we have a R_1 group here. We are going to have a second R group here that is going to be linked to the third R group here. Which is our first amino acid? The amino acid number one (AA_1). What Edman degradation does is it chops off here. Then you can figure out what this amino acid is. What does this become? This becomes now the n terminal. So then you chop off again. Then you determine what R_2 is. First you know what R_1 is. Then you know what R_2 is. Then you know what R_3 is and then keep on going till you come to the end of your chain. What is this giving you? It is giving you the amino acid sequence of the protein. It is not just telling you how many of each. Once you know everything you know how many of each of them are there anyway.

(Refer Slide Time: 37:47)



But it is telling you in which order they are linked and for this we have certain reagents because we have to have a reagent that is going to link up with amino acid and cleave it off the rest of the polypeptide chain. What are these reagents? We have one that is called PITC. PITC is phenyl isothiocyanate that reacts with the terminal amino groups of peptides to form what are called PTC derivatives.

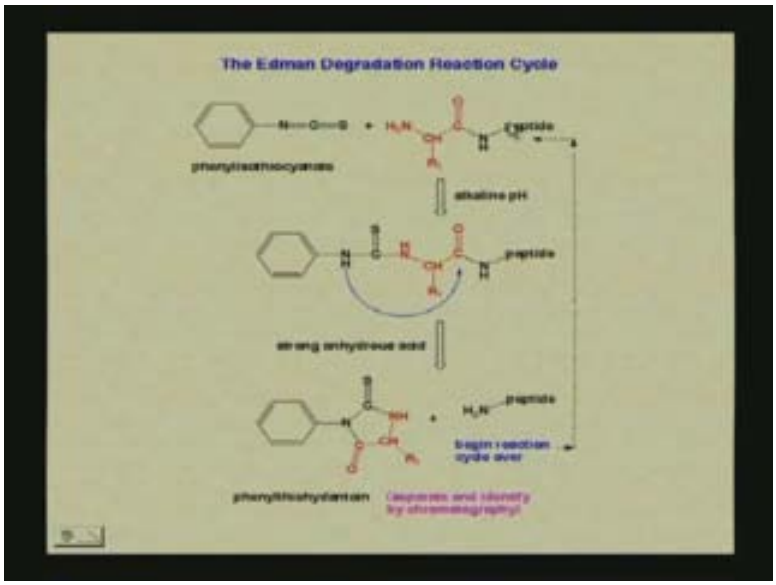
(Refer Slide Time: 38:24)



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 and not the C terminal. We go to this C terminal it is a different thing all together. The PITC reacts with the terminal amino

groups of the peptides to form a PTC or a phenyl thiocarbamayl derivative. Then we have a slightly acidic condition that is going to liberate this by what is called a phenyl thiohydantion derivative. 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 thiohydantion formed that has with it the amino acid that belongs to the N terminus. What then happens is that 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 can keep on doing that till you identify all the amino acids and what do you know by this? You know the exact sequence of the polypeptide chain. That is what is important here. We have a PITC that acts with the terminal amino groups and then we have certain derivatives formed. This is basically what happens. We have a phenyl isothiocyanate. This is our peptide. What is this? This is the terminal amino groups. We have our peptide here.

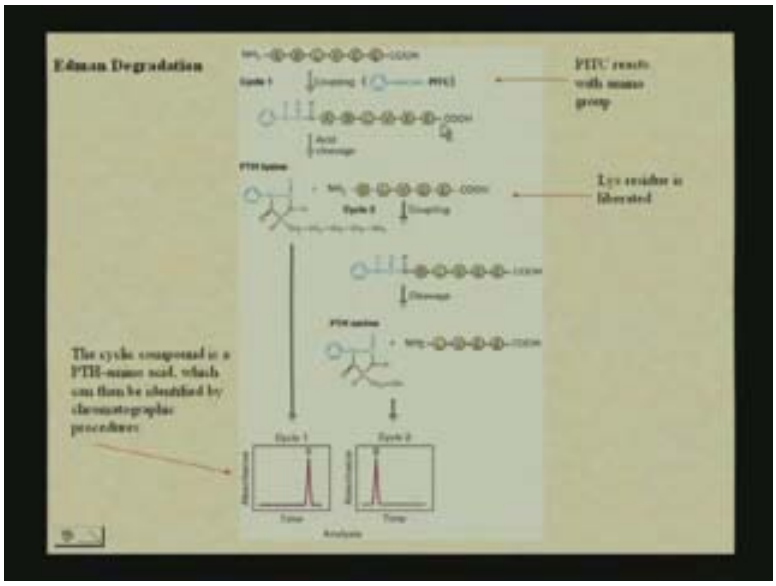
(Refer Slide Time: 40:31)



If we have an alkaline pH this basically forms a link here. We have a phenyl thiohydantoin derivative. We have the C=S, this H and we have a linkage here C-N bond formation. This C-N bond formation then with acid forms this phenyl thiohydantoin. What is linked here? What is this? This is R_1. 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 the new amino terminal. What does this do? This can then go through another cycle. When we have another cycle what we are going to get then? We are going to get another hydantion derivative with R_2. So 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 happened to be part of original polypeptide chain. This R_1 was part of the original polypeptide chain. It has now been chopped off. Now I have the rest of the peptide with the new N-terminus. If I go back to do it, what am I going to get? I am going to get another derivative with R_2

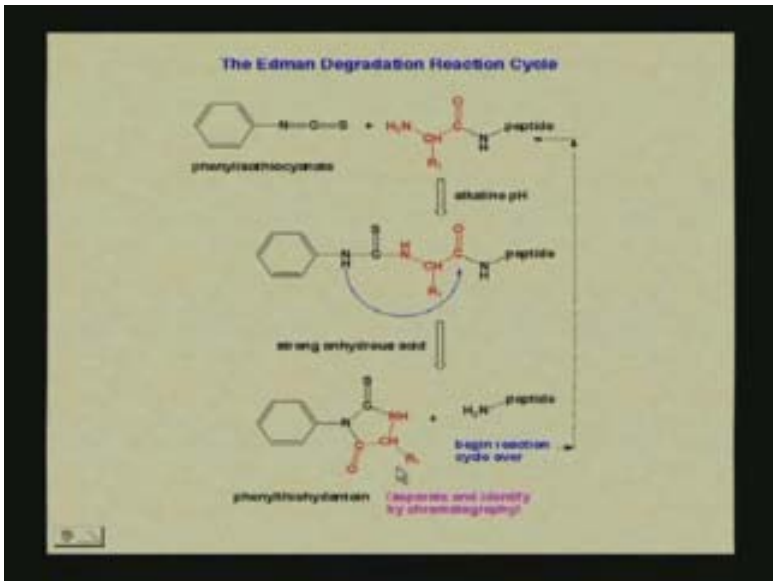
here. I can keep on doing the cycle till I have completed the whole sequence of the protein. So this is basically what is done. It's just you have the PITC coupling. So this is my sequence.

(Refer Slide Time: 43:02)



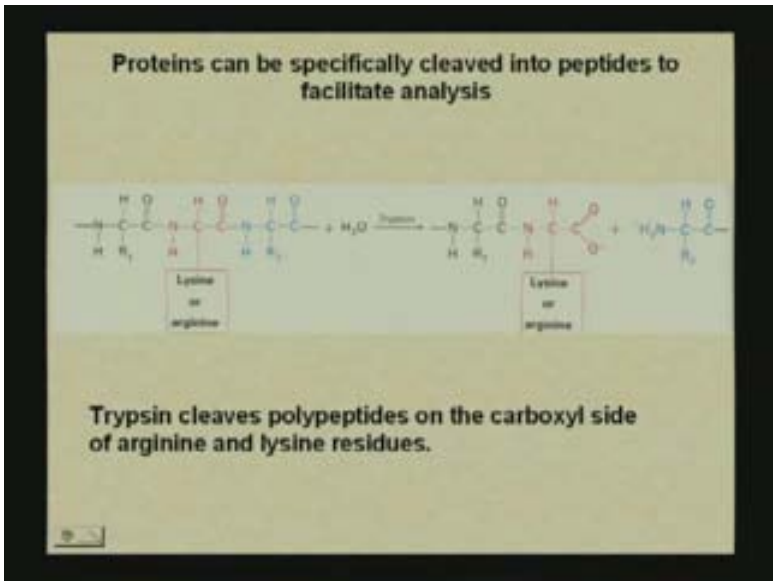
I have K, S, L and V. In my first step I am going to release the first amino acid, in the second step, the second amino acid. I keep on doing the cycle till I go through it. Cycle one will release one amino acid cycle two will release another amino acid. What do I know? I know how this behaves in HPLC.

(Refer Slide Time: 43:30)



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 $R_1$is if I match it with the standard. That is all you have to do. Even for lysine this is going to look the same. But you will now know the order in which it has come up so we can have cleavage. What did we have for Edman degradation? We had cleavage at the N-terminal.

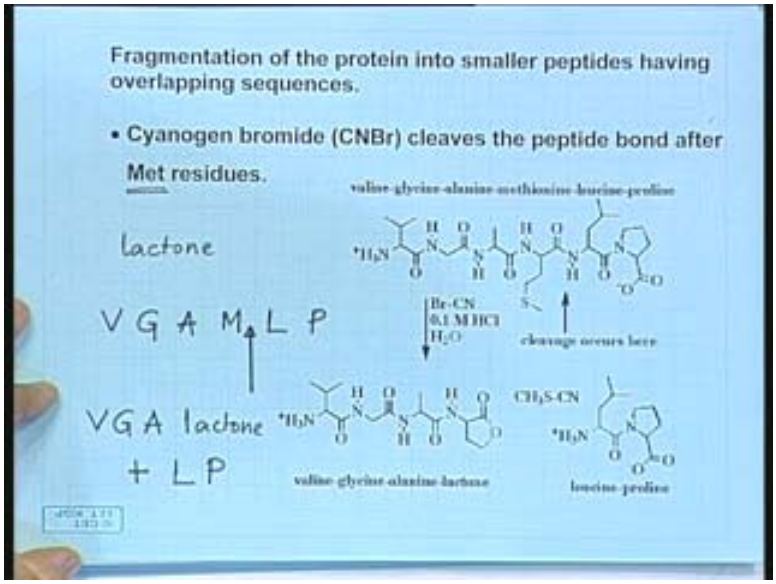
(Refer Slide Time: 44:14)



For trypsin we can have cleavage after lysine or arginine provided it is not followed by a proline. 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 at the carboxylic bulky hydrophobic it would be chymotrypsin because you have to understand each of these enzymes are extremely specific in the way they work.

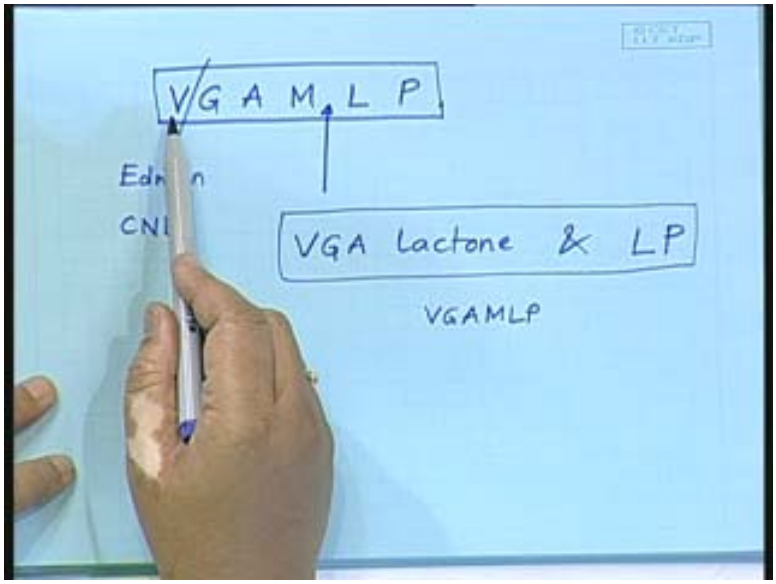
We can have another type where we can figure out how we can actually form smaller peptides by overlapping sequences. This for example is cyanogen bromide. In cyanogen bromide, cyanogen bromide cleaves after methionine residue. These are certain things that you have to remember. We have cyanogen bromide that cleaves after methionine residue. I have just given the mechanism here but that is not important. What we need to know is that a certain lactone is formed. We have the formation of a lactone. The lactone is formed where the methionine residue was in the polypeptide chain. What does that mean? It means that if you have a valine and a glycine followed by an alanine, a methionine, a lucine and proline then if you treated this with cyanogen bromide you would have a cleavage at this point here. What would happen is the side chain of the methionine group would go and link up with the cyanogen bromide. What you are actually left with is a V G A lactone plus lucine and proline.

(Refer Slide Time: 47:36)



Let us just go through it once more. When we have a lactone formation we have a polypeptide chain which is V G A M L P. 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 am I going to get? Since the methionine is going to interact with the cyanogen bromide I am going to be left with VGA lactone and L, P. After treatment of this particular polypeptide with CNBr, if I am left with VGA lactone and LP then I know that my original sequence was VGAMLP. Now if I tell you that I have a particular sequence that is VGAMLP if I treat it with CNBr I am going to get 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.

(Refer Slide Time: 49:50)



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. 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 iodoso benzoate that is going to cleave on the carboxyl side of tryptophan residue 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.

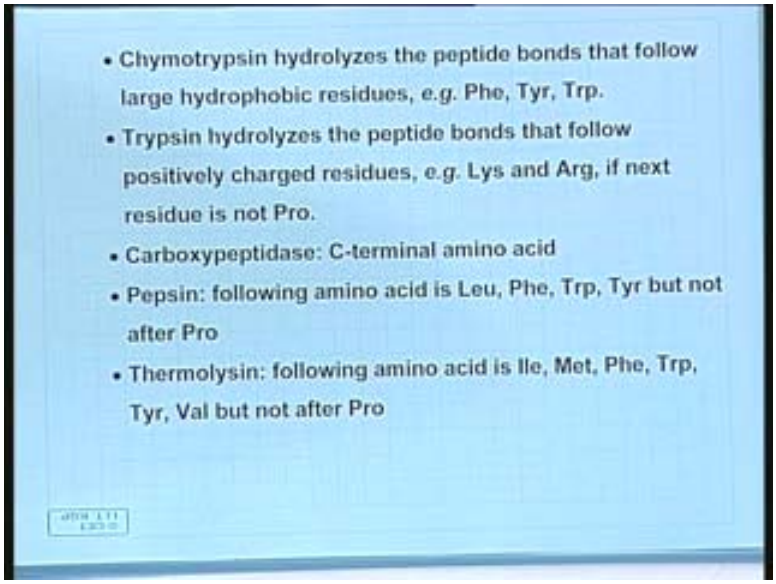
(Refer Slide Time: 50:51)

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

You have a protein. For example let us look at ribonucleus A which has eight cysteine residues. We know that it has four disulfide linkages so I can reduce the disulfide linkages and what I can do is I can get the long chain. But then if I use this particular reagent to chop off at the cysteine residue where on the amino side of the cysteine residues then I will get fragments say of the length 20, 50 or something like that. But if I do Edman degradation I will get this one amino acid and 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 we need to know is that trypsin will cleave on the carboxyl side of lysine and arginine. Chymotrypsin will cleave on the carboxyl side of aromatic residues.

We can use this information to figure out what we can do with the amino acid sequences of proteins into determining how the utilization of the chemical cleavage and enzymatic cleavage will lead us to the polypeptide chain. This is the information we need.

(Refer Slide Time: 52:26)

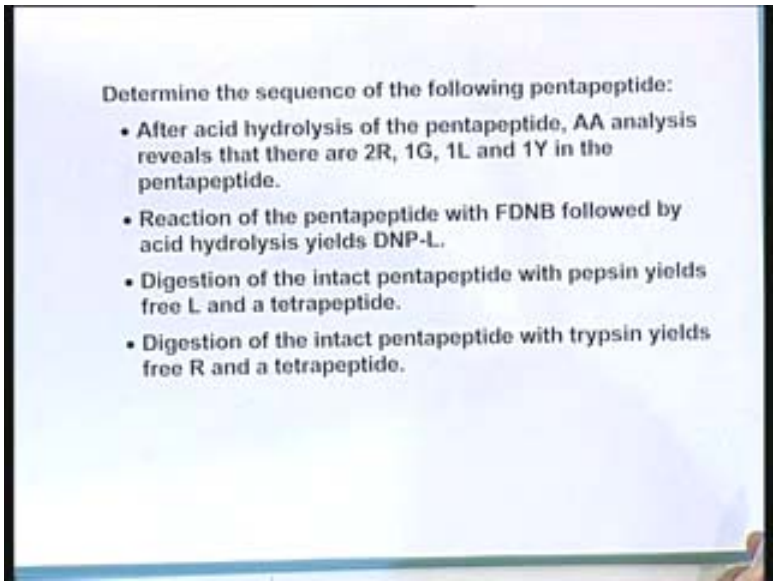


We need to know that chymotrypsin hydrolyzes the peptide bonds that follow large hydrophobic amino acids. What are they? Phenylalanine, tyrosine and tryptophan. We need to know that trypsin will hydrolyze peptide bonds that follow positively charged residues. That's lysine and arginine if the next residue 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. These are all enzymatic cleavages. Thermolysin will cleave if the following amino acid is isoleucine, methionine, phenylalanine, tryptophan, tyrosine, valine. 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.

I have small problem for you where you can determine the sequence of a pentapeptide. What is pentapeptide? It just has five amino acids.

(Refer Slide Time: 55:04)



What are these five amino acids? There are two arginines, one glycine, one leucine and one tyrosine Reaction of the pentapeptide with FDNB which is another reagent will tell you what the N-terminal is just like Edman degradation. So the Edman degradation methods 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 1, 2, 3, 4 and 5. Digestion 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 2 R's, 1 G. There are 2 R's, 2 G, 1 L and 1 Y. You just fit these in correct boxes. We will discuss this in the next class. I will give you more peptide sequences to solve. Thank you.