Fundamentals of Protein Chemistry
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Module – 01
Amino Acids and the Peptide Bond
Lecture – 04
The Peptide Bond

In the last lecture of Module 1, we will be talking about Peptide Bonds. What these peptide bonds do, their characteristics, their specific geometry, how they are extremely important in holding the amino acids together and the basic framework of what proteins are.

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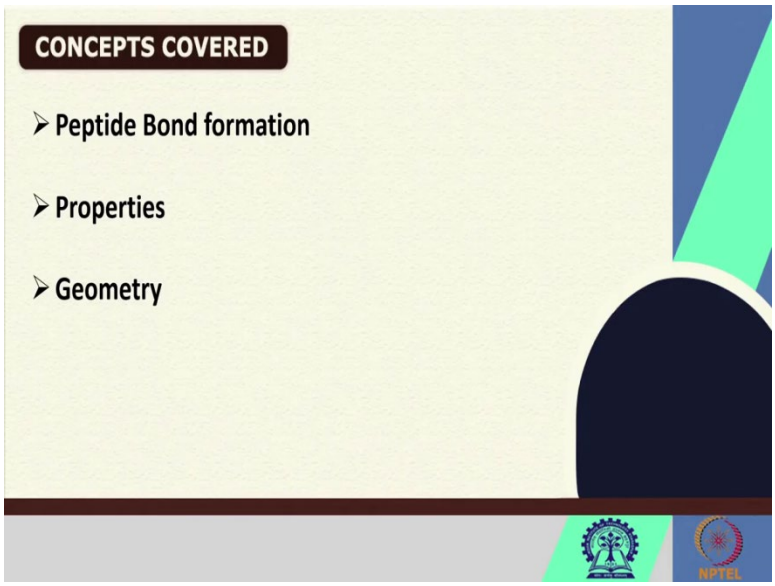

So, what we are going to look at, is we are going to look at peptide bond formation, their properties, their overall geometry.

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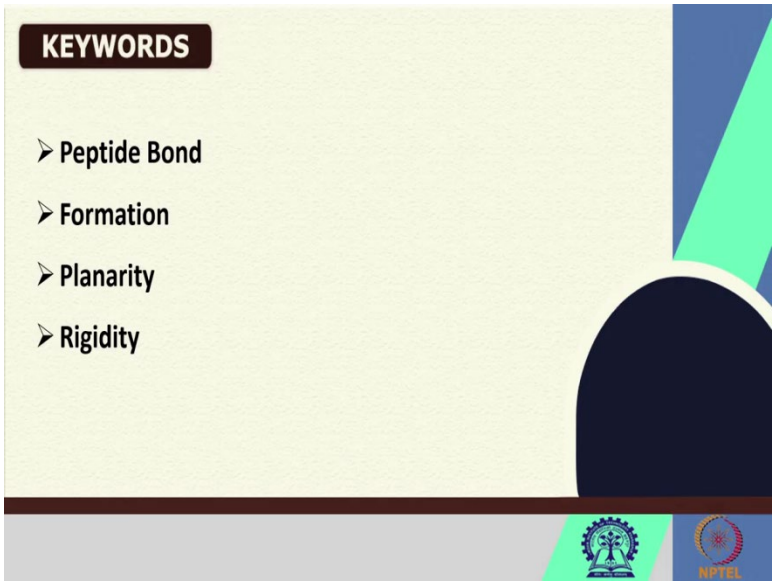

And the keywords for this lecture would be peptide bond, the formation, the planarity and the rigidity. So, by the end of this lecture, we should be absolutely clear as to how the peptide bond is formed, how it has a planar configuration and how it maintains a rigidity in its geometry. This is extremely important in an understanding.

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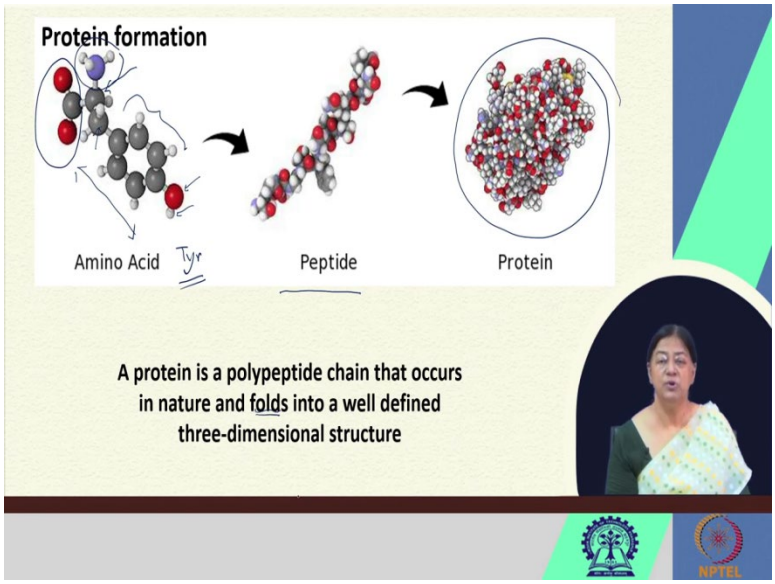

Let us try and identify the amino acid, considering that we already know the types of side chains that we have. The first thing that we have to identify is the α-carbon. We are going to try and understand, what are the specific characteristics of the α-carbon. To the α-carbon we have the NH_2 group attached. We also have the COOH attached. This therefore, is the side chain.

Now, if we understand what the side chain is, we have a CH_2 and an aromatic ring, to which is added an oxygen and a hydrogen. So, this means the amino acid is tyrosine. Now, this in an

understanding of proteins, we have to know the side chains of the amino acids very well. So, after we know what the amino acids are we have to know their linkages. Their linkages form what is called the peptide. What is this peptide, is what we are going to understand.

The protein then forms a structure that is a measure of the folding of the polypeptide chain, which we will see in subsequent classes. So, the protein itself is a polypeptide chain that occurs in nature and folds into a well defined 3 dimensional structure. We will talk about folding and characterization and more about the structure in later classes.

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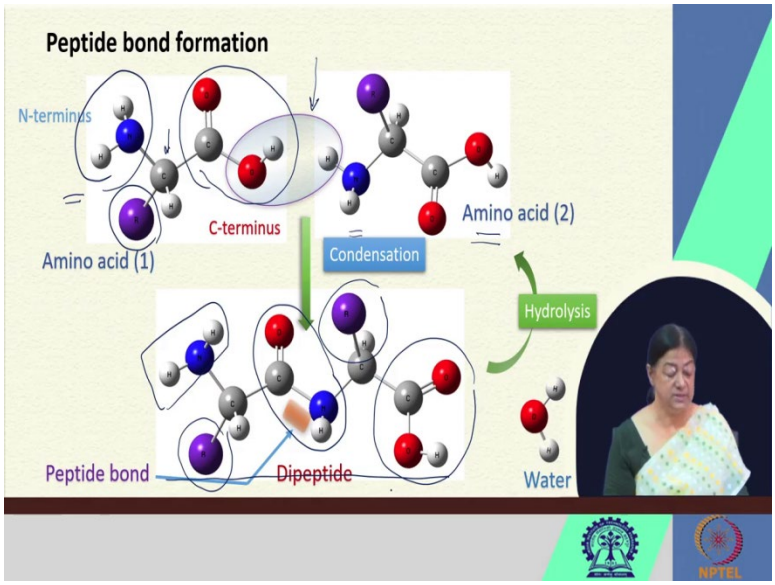

Now, when we look at peptide bond formation, what do we have? We have the amino acids that are going to be linked to one another. So, we have amino acid 1. How do we characterize the amino acid? We look at the amino acid by understanding that we have the α-carbon, we have the carboxylic acid group, we have the amine group, we have the R group. So, this we know is characteristic of all amino acids. We now have amino acid 2.

So, we have the N-terminus that is given by the NH_2 group, we have the C-terminus given by the COOH group and now we have amino acid 2. Now, they are going to link together. And what are they going to do? They are going to condense together in a process known as condensation and lose the water molecule. So, we have OH on one side and H from the amine N-terminus of the second amino acid and we lose the water.

So, in this process we have the formation of a dipeptide and this is the peptide bond. Now, this peptide bond has very unique characteristics, unique geometrical constraints, which we will understand in this lecture. This process is known as condensation, we can also have hydrolysis where we can break this peptide bond into the two individual amino acids that it was comprised of. But, in this formation we have to know that very importantly it is the N-terminus and the C-terminus of the peptide that is important here.

Now what do we mean by the sequence? We mean by the order in which the amino acids are linked to each other. So, when we have our first amino acid linked to the second amino acid, we know that the amino or the N-terminus is on amino acid number 1 and the C-terminus is on the end amino acid. In this case since we have a dipeptide, it is going to be on amino acid number 2.

Now, this is extremely important in understanding how peptide bonds form and how the sequence of the proteins is maintained.

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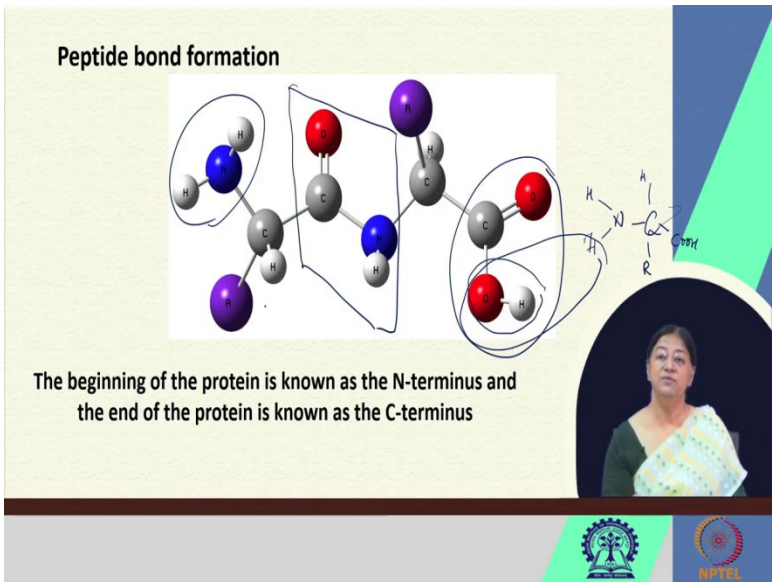

So, now that we have the peptide bond formation, we understand the beginning of the protein is known as the N-terminus and the end of the protein is known as the C-terminus. So what do we have now is the peptide bond, the amino terminus and the C-terminus.

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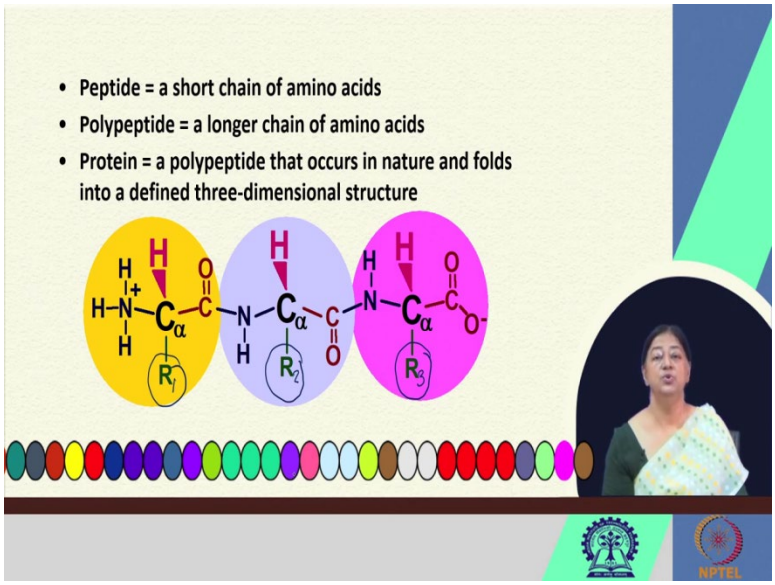

The peptide therefore, is a short chain of amino acids. What we saw was a dipeptide, we can have similarly a tripeptide. What will happen in the case of a tripeptide? In the case of a tripeptide the COOH of the dipeptide, [refer to slide] will lose the water and the hydrogen from the amino terminus of the subsequent amino acid, which will have the R group, the H group, followed again by the COOH.

So, when we have the loss of water in a condensation reaction, we will have another peptide bond formed. When we have three amino acids linked together, we are going to have two peptide bonds linking them. A peptide is going to be a short chain of amino acids; the polypeptide is going to be a polymer of amino acids and so we call it a polypeptide.

In the polypeptide we can have a very long polypeptide chain, that can fold into a unique three dimensional structure that defines what a protein is. So if we have these beads that are connected one after the other, symbolizing the amino acid type, we only need to know what R group we have.

Whether it is R_1 or R_2 or R_3, our identity of the side chain is what we need, because we know that the rest of the amino acids is common for all. If we know what R_1 or R_2 and R_3 are, we know how they are linked together in the polypeptide chain, because they are linked through peptide bonds. If we look at the linkage, we have the link through the peptide bonds - that tell us, that the different kinds of amino acids form a beaded structure one after the other, in a necklace formation. It will then fold into a unique three dimensional structure, giving the protein not only a unique structure, but also unique functionality.

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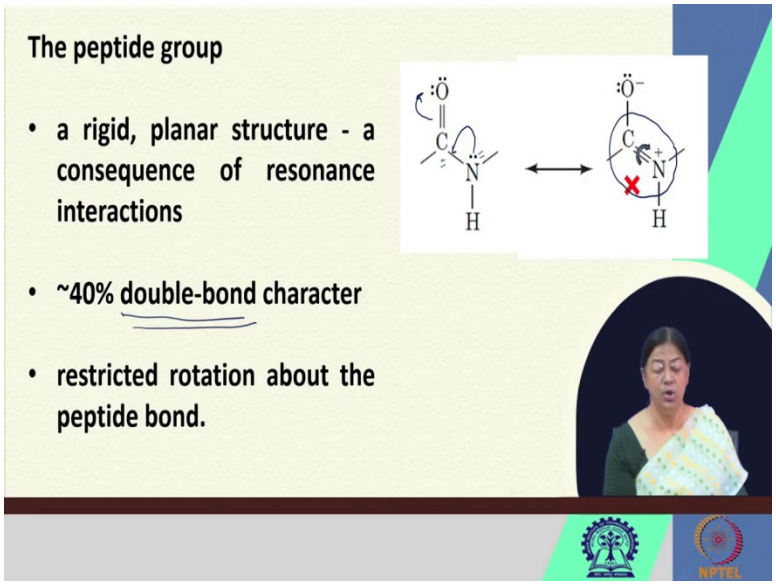

The peptide group now is what we need to study in terms of its chemistry. It is a rigid planar structure, which is a consequence of resonance interactions; it has 40 percent double bond character and it has restricted rotation above the peptide bond. So, this is what our peptide linkage was. How did we get this? We lost the OH from the COOH, we lost one of the hydrogen from the NH_2 and this resulted in the formation of a peptide bond.

Now, this lone pair of the nitrogen can actually go and form a partial double bond and it is going to result in restricted rotation about this double bond; the partial as we said of partial double bond character, renders this a relatively planar structure. Now, this gives a lot of conformational restraints to the rotation about this bond, which is unique to protein structures.

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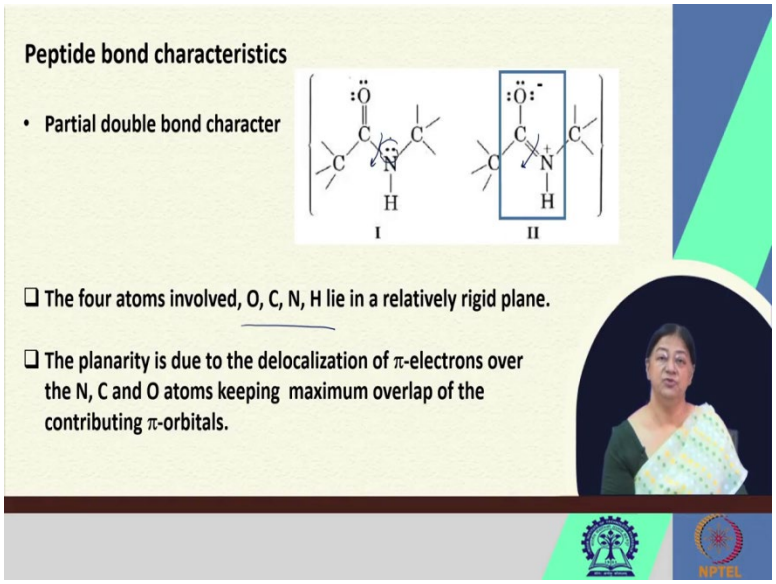

So, the peptide bond characteristics include a partial double bond character and the planarity that we see is due to the delocalization of the π electrons over the N, C and O atoms, keeping a maximum overlap between the contributing π-orbitals. This is what results in the partial double bond character, which is important in its geometry, in its constraints in rotational motion.

The four atoms therefore, lie in a relatively rigid plane. That is important or rather restricts the rotation about this bond, that has lost its single bond characteristics to a certain extent, due to the double bond character formation. Why is that, because of the lone pair on the nitrogen.

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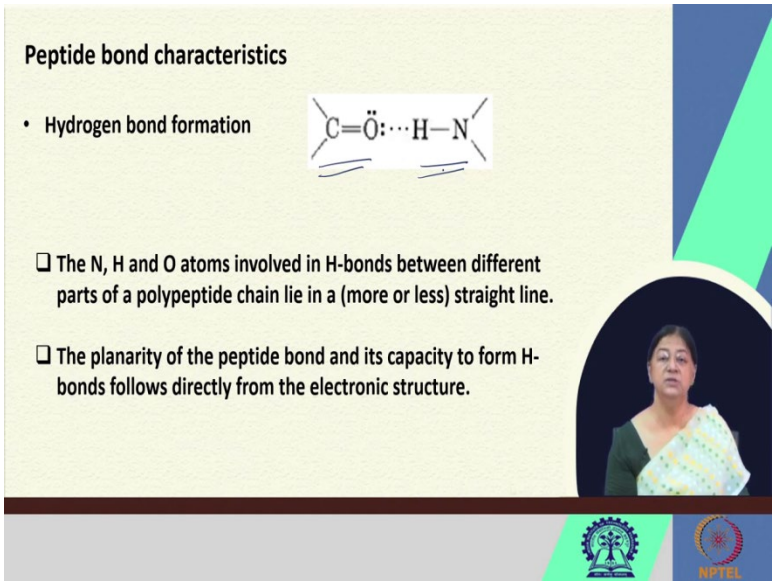

Another important factor involved in peptide bonds, is hydrogen bonding possibilities between the C = O of the backbone and the NH of the backbone as well. When we look at these hydrogen bond formations, they actually are formed between the different parts of the polypeptide chain.

So, if we look at a peptide bond on one part of the polypeptide chain, there can be hydrogen bond interactions between different parts of this chain that assist actually in the folding characteristics, which we will see in a subsequent lecture, and the planarity of the peptide bond and its capacity to form hydrogen bonds follows directly from the electronic structure that we just discussed.

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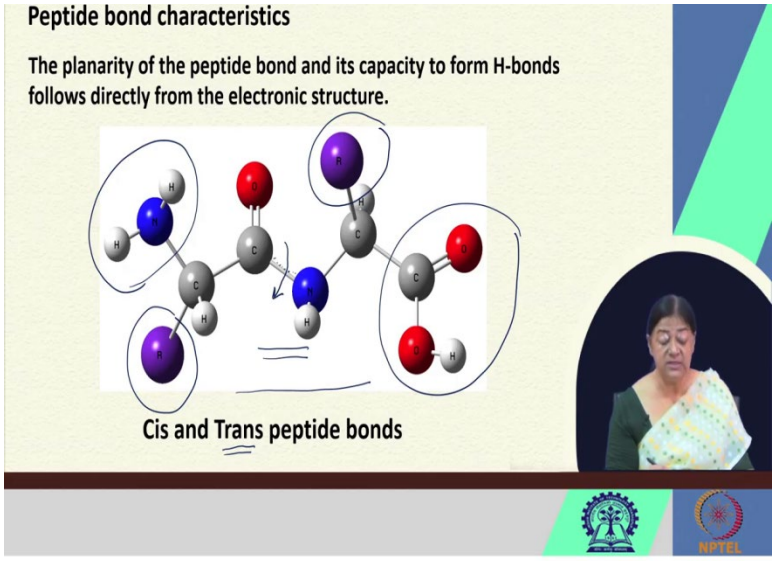

Another very important characteristic about the peptide bond is its geometry. What is this geometry in terms of the rotation about the single bond? The planarity of the peptide bond has the capacity to form hydrogen bonds. Now, [refer to slide] this is where we have restricted rotation, this being our peptide bond. So this is the way we have our dipeptide return. How do we know this is a dipeptide and how do we know the direction? We know that it begins from the N-terminus ends at the C-terminus. And what we have here is the formation of a peptide bond, because this is amino acid number 1, this is amino acid number 2.

Now, if we look at the orientation with respect to the peptide bond, we see that they are trans to each other, meaning they are on opposite sides of this partially double bonded peptide bond. How does that help us or what more can we understand from this?

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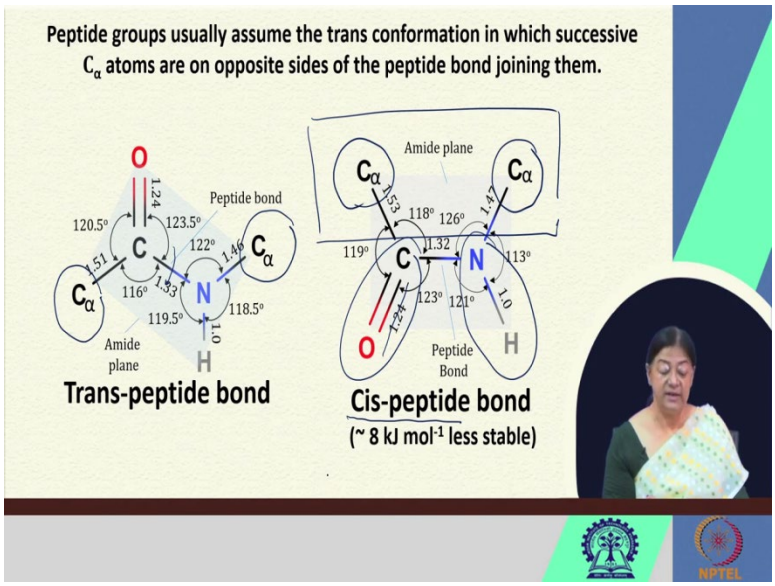

So, when we have a trans-peptide bond, we usually see that peptide bonds assume this trans conformation, in which successive α-carbon atoms are on opposite sides of the peptide bond. We have the α-carbon of the previous amino acid and the α-carbon of the subsequent amino acid and they are on the opposite sides of the peptide bond.

But since this still has some rotational characteristic possible or some rotational property possible, because of its single and the double bond formation; the partial double bond formation restricts its rotation. But, it also remains partially in a single bond form, which will allow rotation. And if that rotation does occur, we will form what is called a cis peptide bond. In the cis peptide bond what do we see, [refer to slide] this is our peptide and this is the C=O and this is the NH. So, there has been rotation about the single bond between the C and the N. What are the Cα atoms here? These Cα atoms correspond to 2 consecutive amino acid residues. And what do we observe here? We observe that they are on the same side of the peptide bond, making this a cis peptide.

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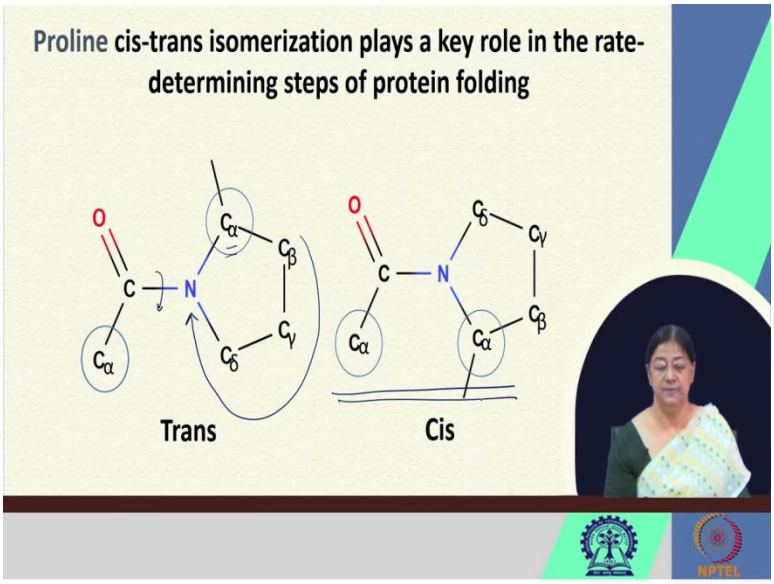

The important or interesting characteristic is what we see in proline. In proline cis-trans isomerization is an extremely important factor. This is the side chain of proline [refer to slide]. As we have discussed earlier, it is an imino acid that folds back upon itself, where you can see the Cα, then the Cβ, the Cγ, the Cδ and the amino terminus of the proline amino acid. Now if we look at this, this is the Cα of the previous one, what do we see about the Cα of the next. We see a possible trans formation. But, what happens in this case is there can be rotation again about this and then what do we observe. We observe that the 2 Cα are on the same side. Giving us a cis conformation, which is more prevalent in proline, because of the side chain property being an imino acid.

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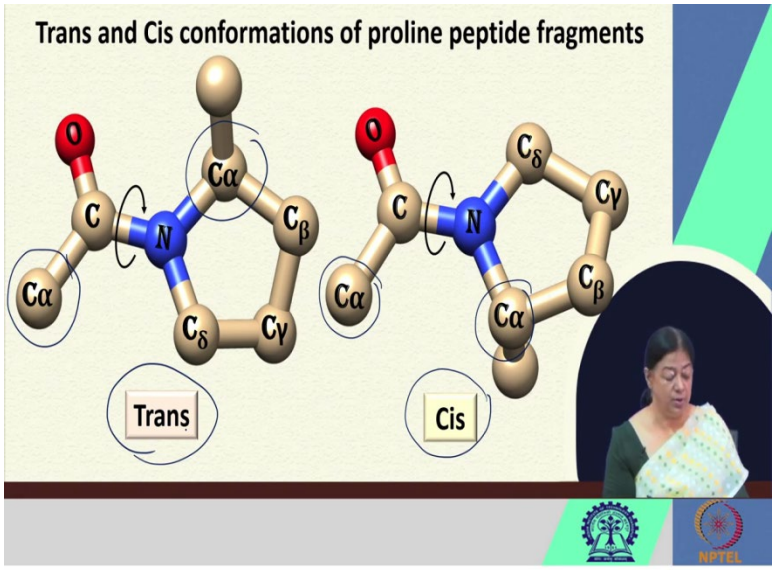

So, this gives us some idea of the trans and the cis conformations. When we see the Cα on the same side, we know it is a cis, when we see the Cα on opposite sides we know it is a trans and this gives us a very important geometric characteristic of our peptide bond.

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So, what did we see now, we saw the peptide bond formation, the relative polarity, the planarity, the rigidity and its isomerism. But now we have amino acids linked together to form a peptide bond and in the formation of the peptide bond the characteristics of the individual amino acids also come into play.

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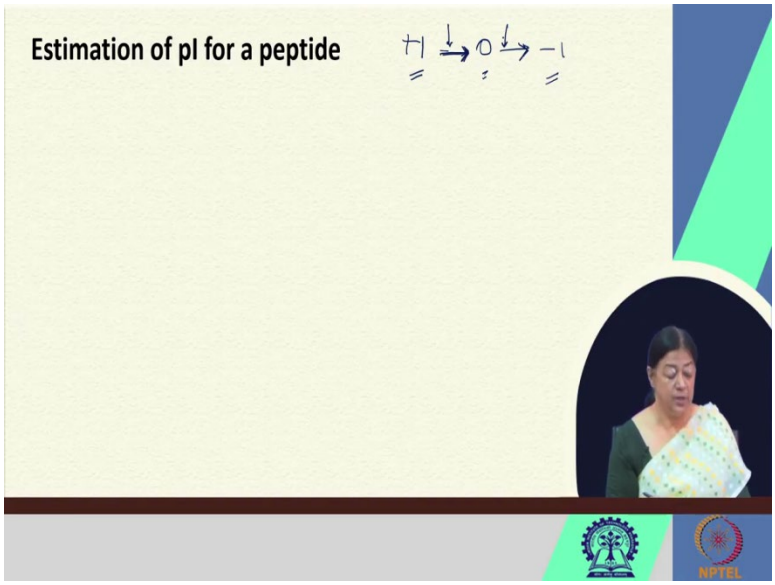

So, how can we estimate the pI for a peptide? The first thing that we have to know is what are the ionizable groups present, what are the protons that can be lost during the titration of this peptide. And based on that we know the pKa values (which are responsible for taking the species, be it an amino acid or a peptide, from +1 to 0 and from 0 to -1), is the average of these 2 pKa values.

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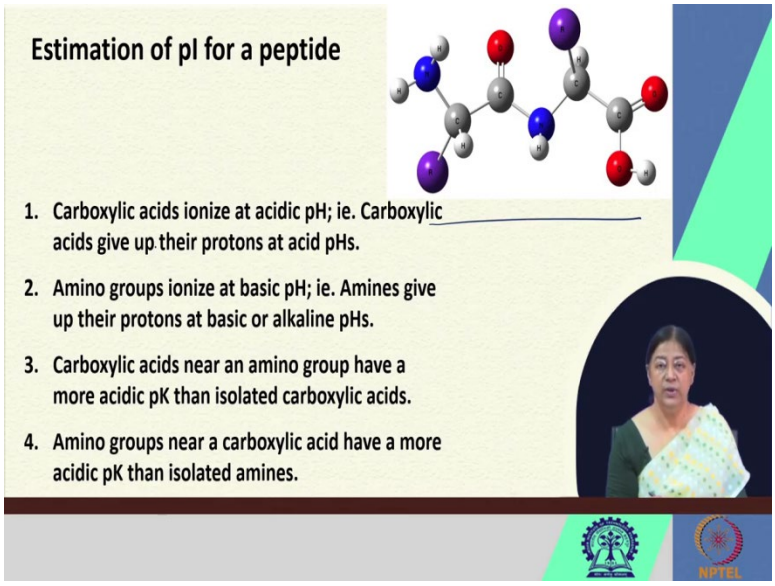

In our understanding of the peptide, we are going to look at what are the important characteristics here? If, we have the carboxylic acids, we know that they ionize at acidic pH; that means, they give up their protons. The amino acids that ionize at basic pH, means they would give up their protons at a relatively alkaline pH range. In addition to that, the characteristic of the environment is also important.

So, the carboxylic acids near an amino group have more acidic PA. What does it mean? It means that they would lose their proton easily, because they have more protons in the environment due to the amino group present. Similarly, amino groups near a carboxylic acid would have a more acidic pK than isolated amines. So, if we look at a specific dipeptide now and try to determine the pI of this dipeptide, based on the information that we already know, let us see how it can be achieved.

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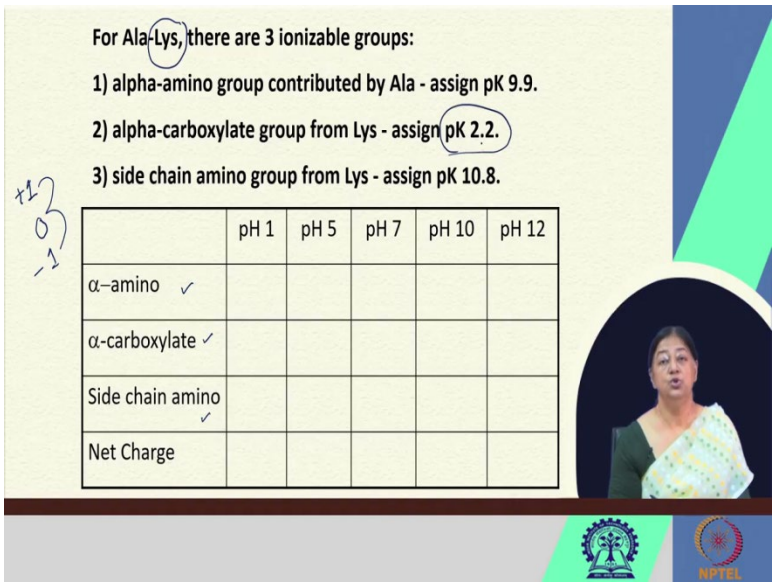

So, say we have the ala-lysine dipeptide. That means, the ala has the N-terminus, the lysine has the C-terminus. We have 3 ionizable groups; one is going to be for the 2 termini; the N-terminus, the C-terminus. And the third one is because lysine itself being a basic amino acid has an ionizable group as well, an ionizable side chain.

So, we have an alpha-amino group that is contributed by the alanine (with a pK of 9.9), the alpha-carboxylate group from lysine, because we know the way we have written the dipeptide, means that the lysine forms a C-terminus. In addition to that, we have a side chain amino group from lysine and we have a pK associated with that.

To do an exercise like this, the best thing is to construct a table with all the possible ionizable groups that are present. For this case we have the alpha-amino group contributed by the alanine, we have the carboxylate group contributed by the lysine, and we have the side chain amino group contributed by the lysine - and we want the net charge.

Why do we want to look at the net charge? Because, when we do our calculations, we have to know at what point we are at a charge of +1, what pK value is taking us to a charge of 0, and subsequently what pK value is taking us to -1? So, we have to go from a +1 to 0 to -1 to determine the pI of the peptide.

So, at pH 1 what are we going to expect? We are going to expect that the amino terminus is going to be completely protonated. The lysine side chain is going to be protonated and the carboxylic acid group will not have lost its proton, because it is going to lose it at 2.2.

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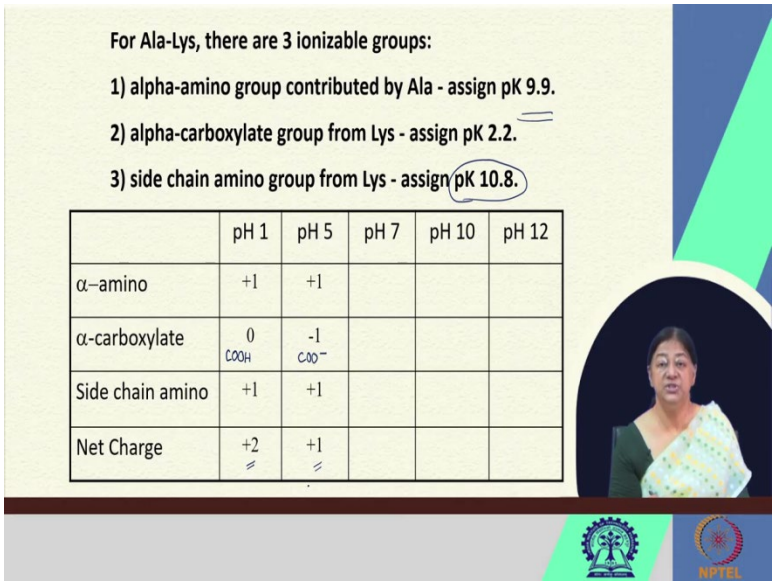

So, at a pH of 1, we have a +1 charge for the NH_3^+ at the amino terminal - the N terminals, we have the side chain +1 because of the NH_3^+ on the lysine side chain, and the α-carboxylate has a charge of 0, because it is still COOH. Now, as the titration continues, as soon as we cross 2.2, we will lose the carboxylate proton; the COOH is going to become COO^-. This means, that at pH 5, what is going to happen?

We are going to have a +1 charge for the amino, because we still have not crossed the pK value of 2.2, the carboxylate has now gone from COOH to COO^-. So, we have lost the proton and the charge is now -1. The side chain amino group is going to be lost at 10.8, so we still retain the +1. The net charge at pH 1 was +2, the net charge at pH 5 is now +1. Now let us proceed with the titration and see what happens at pH 7.

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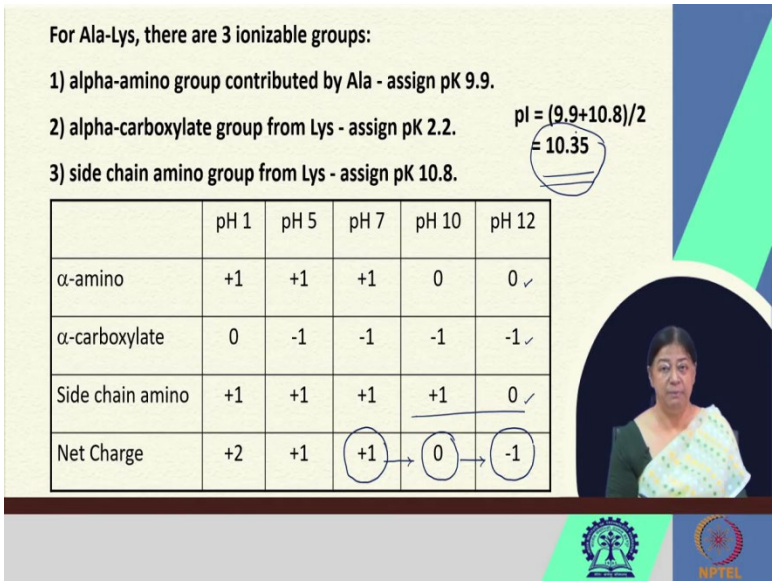

If we look at the values at pH 7, we see that the α-amino group still does not lose its proton. The α-carboxylate group has already lost its proton. So, it retains its charge. The side chain amino group has not lost its proton and is +1. The overall charge is still +1. At pH 10, now α-amino group has now lost the proton. So, the charge is now 0. The α-carboxylate group is -1 and the side chain amino is still +1, because we have not exceeded the pK value of lysine.

Now the overall charge is 0, rendering this dipeptide neutral at this pH. At pH 12, we are going to lose the charge on the side chain amino group. So, the α-amino has already lost its proton, the α-carboxylate has lost its proton a long time ago, because of it's a carboxylate group. Then we have the side chain amino group that has now lost its proton.

So, the charge now is -1, the charge at pH 7 was +1 and the charge at pH 10 was 0. What are we interested in? We are interested to know which pK has caused the transition from +1 to 0 and which pK has caused the transition for 0 to -1. And based on that, we will find out what the pI of the dipeptide is. Now we realize that because of the presence of the lysine group, this is definitely going to have a pI that is going to be in the alkaline range.

So, this exercise can be done for a very varied number of peptides, longer peptides, there are now sites available that will actually calculate the pI for you, given the sequence of amino acids. But, it is good to know how to do this calculation in determining what the pI of a peptide is going to be.

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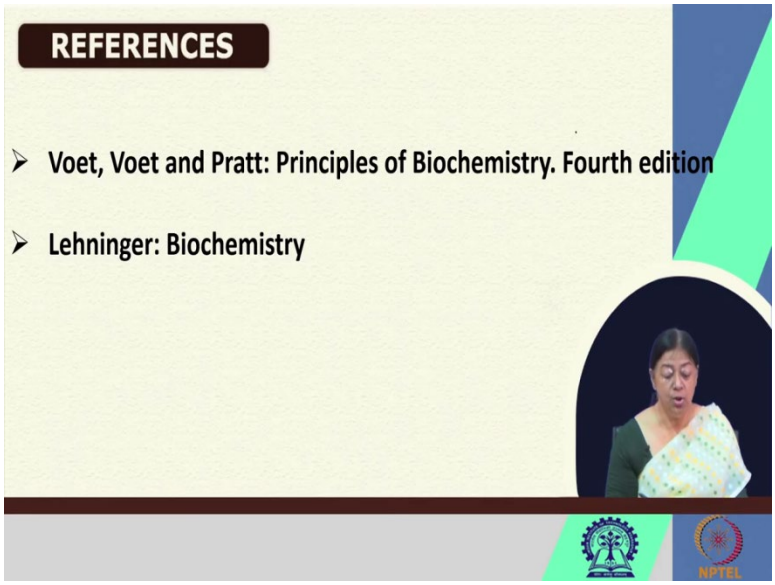

We have these specific books for reference [refer to slide]. At the end of this lecture we now have an overall understanding of the peptide bond, the characteristics of the peptide bond, in terms of its geometry, in terms of its chemical characterization, why it has a partial double bond character - and so restricted rotation.

We have looked at conformations such as the trans and the cis conformation, and we also learned how we can find out or estimate the pI value of a peptide bond, or a polypeptide and understand how we can estimate the charges on the side chains, in an example that we did for ala-lysine dipeptide.

Thank you.