Fundamentals of Protein Chemistry
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Module – 01
Amino Acids and the Peptide Bond
Lecture – 05
Discussion Class

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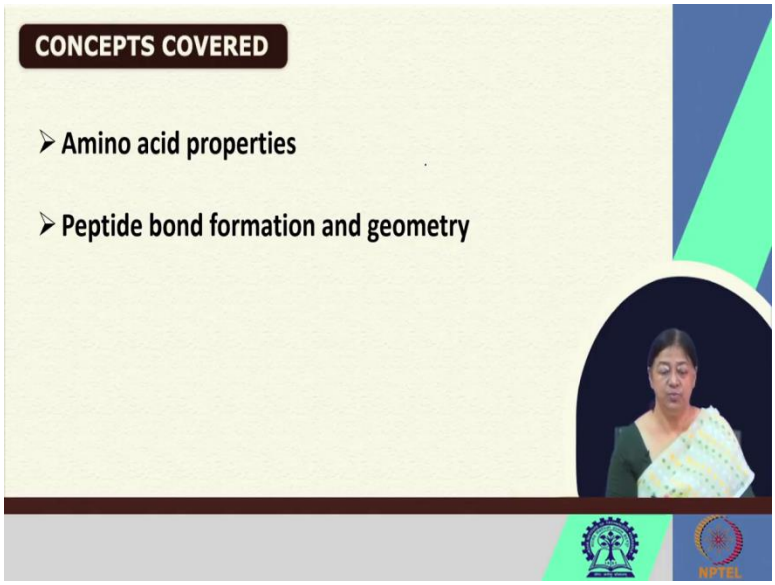

To complete module 1 that describe the amino acids and the peptide bond, we will have this discussion class. In this class we will revisit the amino acid properties, the peptide bond formation and geometry, and look at some more characteristics and their identification, how an understanding can be obtained from the previous lectures that we have had.

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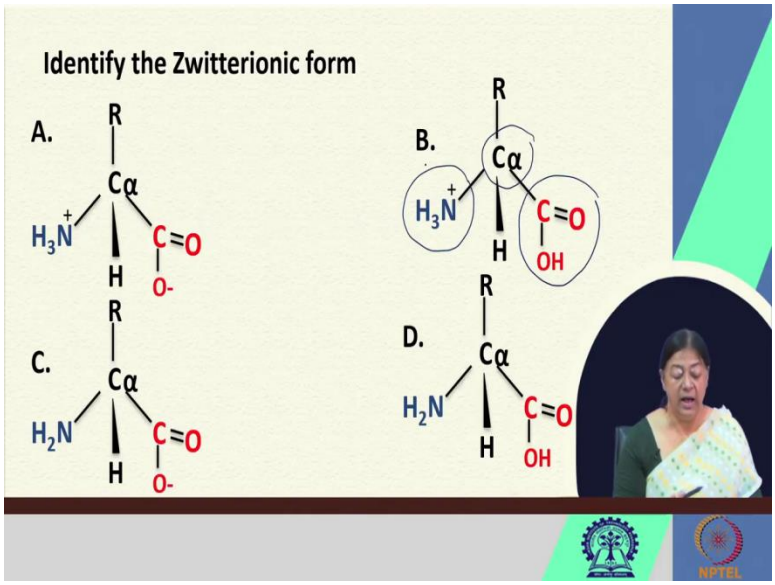

We first need to identify the zwitterionic form. Now, to understand the zwitterionic form we realize that [refer to slide] each of these structures drawn, is an amino acid structure. Each of them has a Cα carbon to the Cα carbon we have an NH_3 that is attached, we have a COOH that is attached and we have a hydrogen that is attached. Now, if we try and understand what a zwitterionic form is, we know that the zwitterionic form has a charge of 0.

So, if we study each of these structures carefully we will see that option B has a charge of +1, option D has a charge of 0, option C has a charge of -1 and option A has a charge also of 0. But a zwitterionic form usually means the option, where we have the charges in a manner that would be at a pH of 7. So, we have the form that would be the zwitterionic form.

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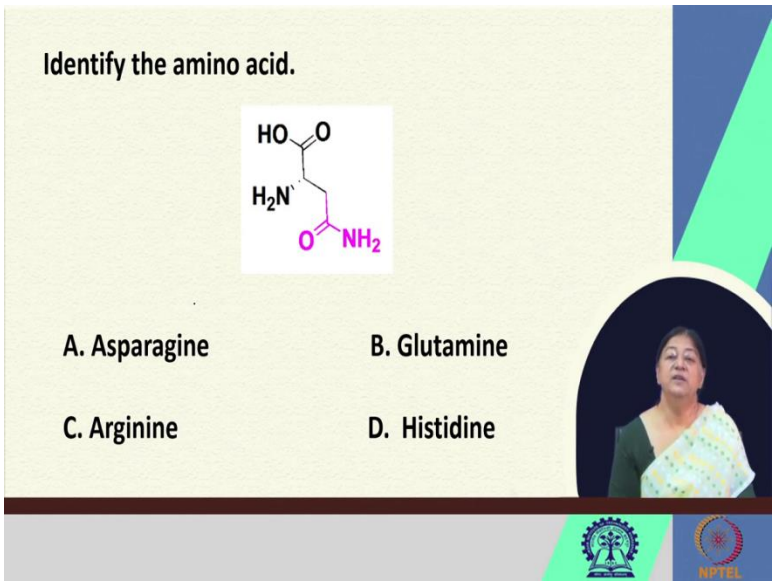

If we try to identify the amino acid, we have to know the structures of each of these amino acids for identification, because it is these amino acid properties that are going to identify or promote the property of the polypeptide chain as it is formed.

What happens if we have hydrophobic amino acids, what happens if we have hydrophilic amino acids, how do the interactions change because of the type of amino acid that we have, that is something that we will study in subsequent lectures, where we will look at the folding of a protein, where amino acids that are far apart in the polypeptide chain will come together to form specific structures, specific geometry with specific interactions.

So, we have to know what kind of amino acids we are dealing with. The first thing we see is we have the Cα carbon, we have the NH_2 and we have the COOH attached. We realize that the side chain is a C = NH_2, which rules out arginine, rules out histidine because we know the histidine has a imidazole group, the arginine has a guanidino group. This leaves us with glutamine and asparagine both of which have an amide group.

So, now when we want to look at this we have to realize what is the side chain. We see that there is a single CH_2 - C = O - NH_2, which means that it is asparagine.

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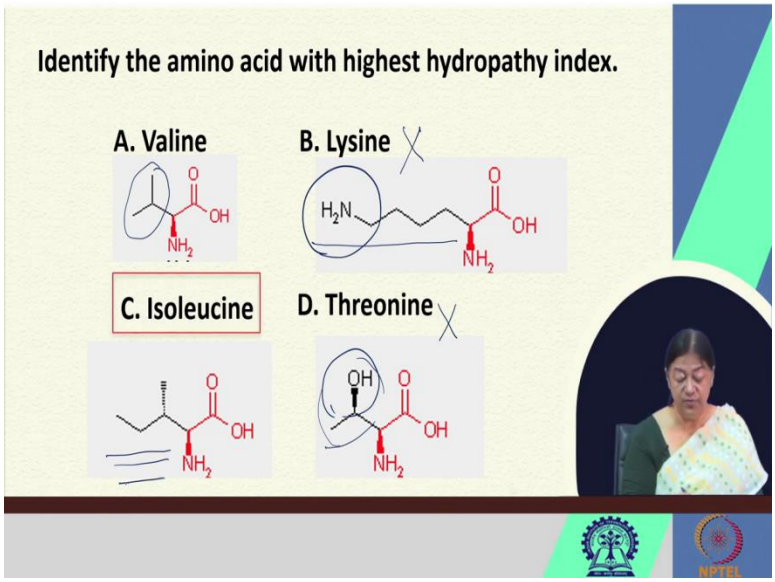

If we want to identify an amino acid with the highest hydropathy index. What is this hydropathy index? It is a measure of the hydrophobicity of our amino acid. So, let us look at the structures of each of these. This is valine [refer to slide] and the side chain is this one in black. Lysine has as we know, an additional NH_2 in its side chain rendering more polar. Isoleucine has only carbon and hydrogen atoms in its side chain, threonine has an OH.

Now, when we are looking at hydrophobicity the first thing we will have to realize is that it has to be a side chain that has only carbon and hydrogen atoms in it. So, we can rule out lysine, we can rule out threonine, because we have a nitrogen group in lysine, we have an OH group in threonine.

So now, among valine and isoleucine, which one has a more hydrophobic character, would depend upon the number of carbons it has and the number of hydrogens it has in association. So, we realize that isoleucine has a greater hydrophobic character, rendering it the amino acid with the highest hydropathy index.

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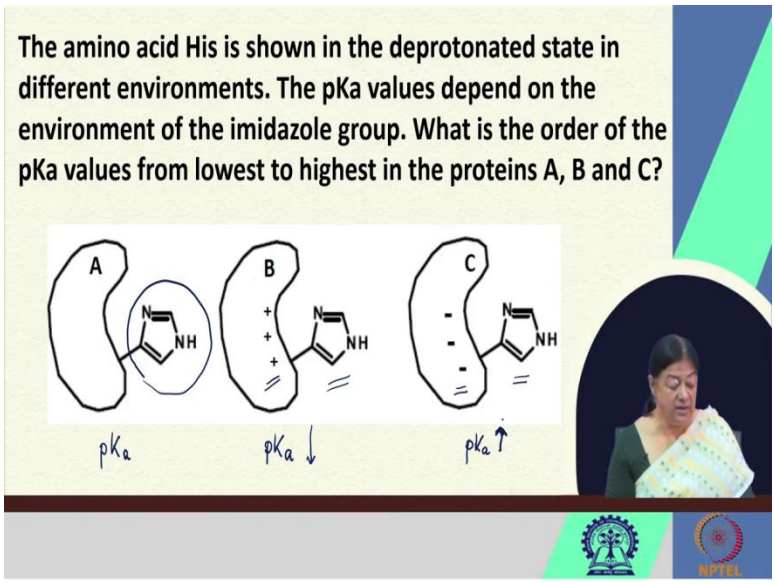

The amino acid histidine now is shown, in the deprotonated state in different environments. We had looked at an example like this in a previous lecture. The pKa value of the imidazole group depends on the environment. Now, this is as I mentioned earlier an extremely important factor, because it tells us or it gives us information about when the imidazole can behave as an acid in donation of a proton and when the imidazole can behave as a base, where it will accept a proton.

Now, this is going to be important as I have mentioned because it covers the range of physiological pH. But for now we want to order the pKa values from lowest to highest in the proteins that are depicted in A, B and C. Now, we have the imidazole group that is in a deprotonated state.

Now, the point is that if we have a positive environment then it is unlikely that the imidazole group is going to hold on to its proton, because we already have there a positive environment. So, an additional proton is not required. If we have a normal pKa for this case, we want to know whether pKa is going to be higher or lower. How do we know that?

We know at what point the proton is going to be lost. So, if we look at the environment, we realize that because of the positive environment it is going to lose the proton at an earlier pH value. So it lowers the pKa value. When we have a negative environment, a positive charge would help because we want to go for a neutrality. Then what will happen? It will lose its proton at a higher value of pKa.

So now these can be put in order, depending upon the environment that the imidazole group is in. These are important factors in understanding the amino acids and we will see a bit more of it.

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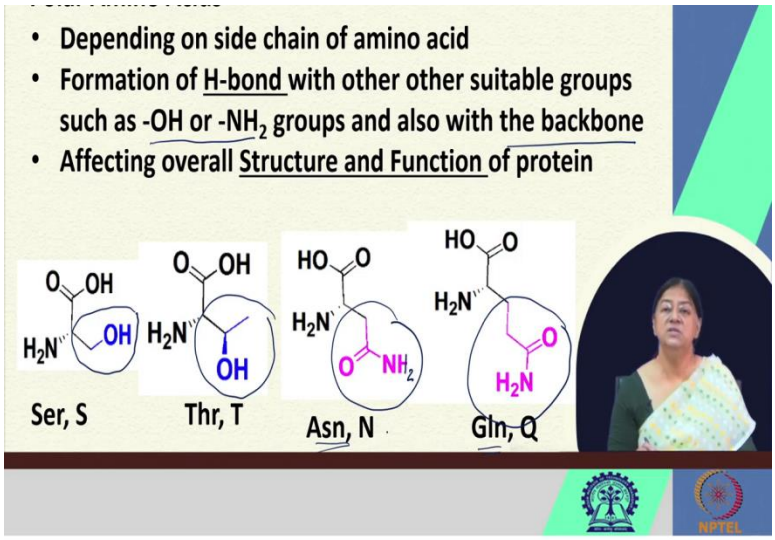

Depending upon the side chain of the amino acid now, there are characteristics related to the hydrophobicity, there are characteristics related to the size, there are possibilities of mutation. What are mutations? Mutations are where a single amino acid or even multiple amino acids are altered to another amino acid.

Now, we have the side chain of the amino acid that has a specific characteristic. It can be hydrophobic in nature, it can have heteroatoms that will result in hydrogen bonding with other suitable groups, that may be on the backbone or also may be as another side chain. So, we have the OH or the NH_2 groups, with the backbone. This affects the overall structure and function of the protein.

Now, if we look at serine and threonine we see that for serine we have CH_2OH and for threonine we have an additional CH_3 and an OH. If we look at these structures, we see that the difference between their structures is not large.

So, this means that their characteristics of hydrogen bond formation or the way they would behave in a protein in terms of their size, in terms of their function, would be pretty much similar. Why? Because their side chains are not that much different. Similarly, if we look at asparagine and glutamine [refer to slide], we see that this was our side chain that we saw initially in one of the problems and here we have the $CH_2CH_2CONH_2$ that is the glutamine.

So what we see here is, we see that again the side chains are similar. The glutamine is slightly longer, but the characteristic interactions that these are going to take part in are not going to be very much different. Why? Because the side chains have similar characteristics.

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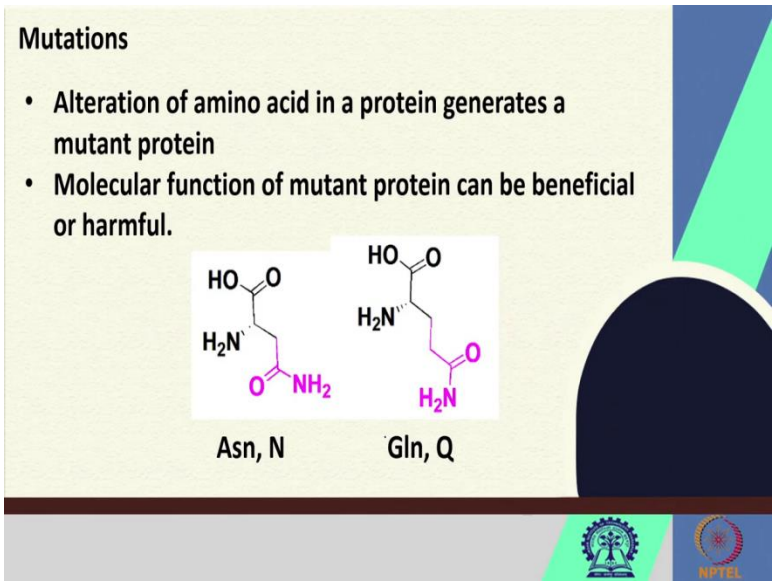

The mutations as I just mentioned, is the alteration of an amino acid that would generate what is called a mutant protein and the function of this mutant protein can be beneficial or harmful. Now, if we look at a mutation say from asparagine to glutamine, we wouldn't expect much of a difference, because the characteristics are pretty much similar. The accommodation that would be required would be for the extra CH_2 group that we have.

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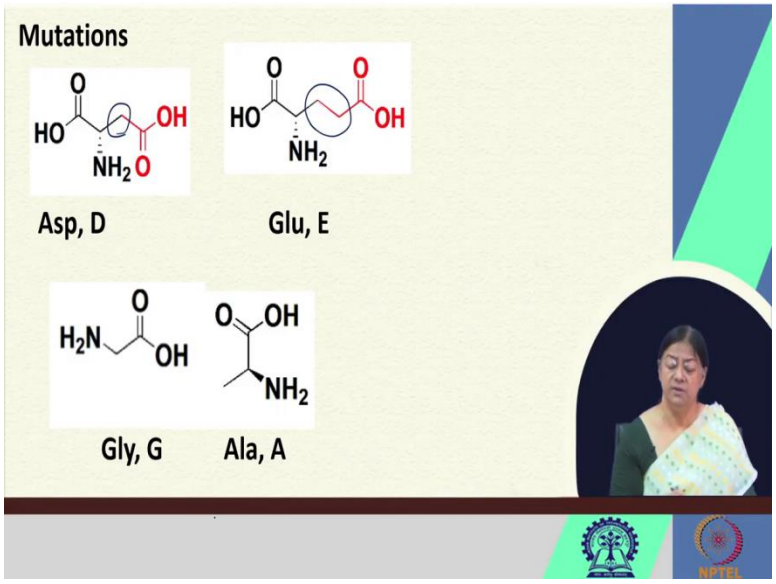

If we now look at aspartic acid and glutamic acid the same argument follows. We have an additional CH_2 that we would have to take care of in terms of glutamic acid, but again the side chain characteristics are the same. Glycine and alanine both of these are very small amino acids, a similar result.

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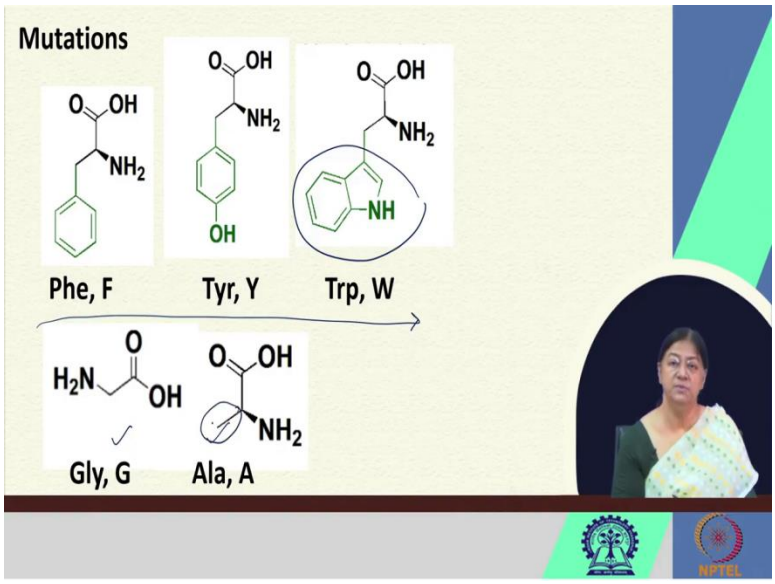

But, if we have mutations that are going to result in changes between the amino acids or the aromatic amino acids that should not be much of a difference, except for the bulky tryptophan group. Glycine and alanine are pretty much the same in terms of a slight additional CH_3 group in terms of alanine.

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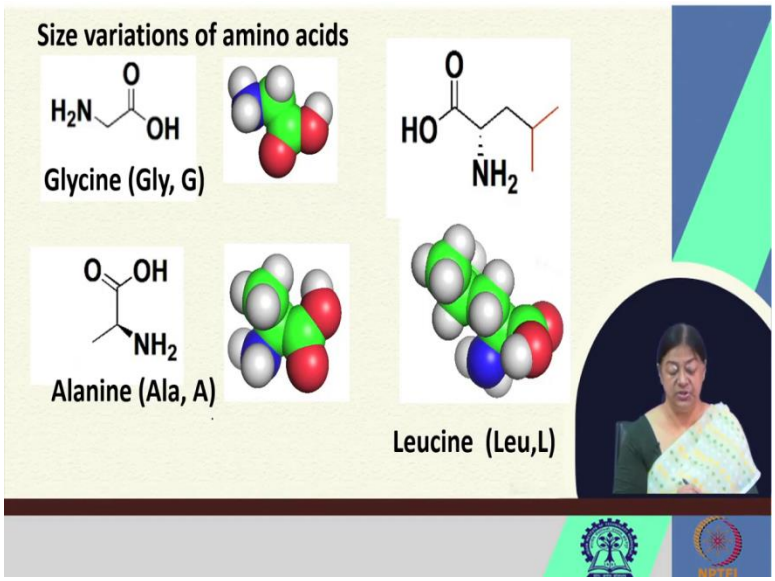

But if we look at the size variations now, we have glycine that is the smallest amino acid we have alanine that gets a bit larger then we have leucine that is quite large. So, if we have a mutation that is taking us from glycine to leucine there may be accommodation or steric factors that are involved.

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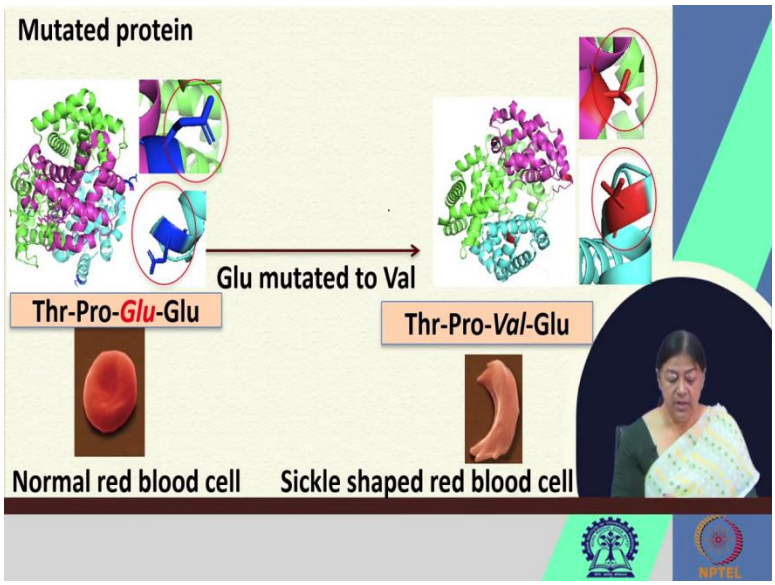

Characteristics: this is an extremely important mutation that was the first description or the first identification of what was called a molecular disease in the formation of sickled shaped red blood cell leading to sickle cell anemia. Now, what happens in this case is we have a mutated protein. In this mutated protein if you look at the sequence, we know now that this is a sequence of the protein and we know that these are connected by peptide bonds. The change or the mutation in this case is from a glutamic acid to a valine. Now, a glutamic acid as we looked at the structure has an acidic group to it. Valine on the other hand is a hydrophobic amino acid.

So, the glutamate that is on the surface of the protein and the valine which is formed on mutation would not prefer to be on the surface of the protein, rendering it a different character altogether and this characteristics leads to the red blood cell going from a normal type to a sickle shaped red blood cell giving you this deadly disease.

So, we see that the glutamic acid with its characteristics and valine with its characteristics can create such a variation in the terms of interactions that are going to lead to mutated proteins that are going to be harmful.

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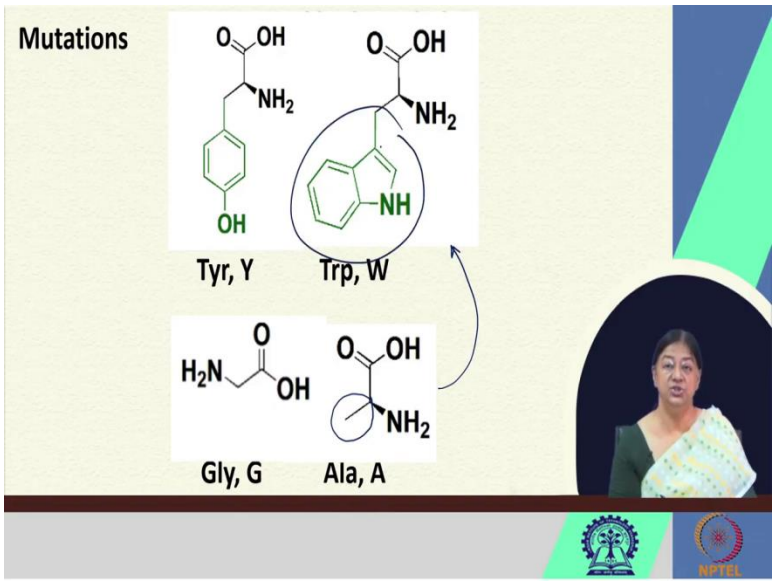

So, the mutations that are going to take us say from alanine to tryptophan. They are going to have steric issues as well because the accommodation that we had with alanine now has to accommodate this large side chain. So, these are also important issues that have to be taken care of in mutations.

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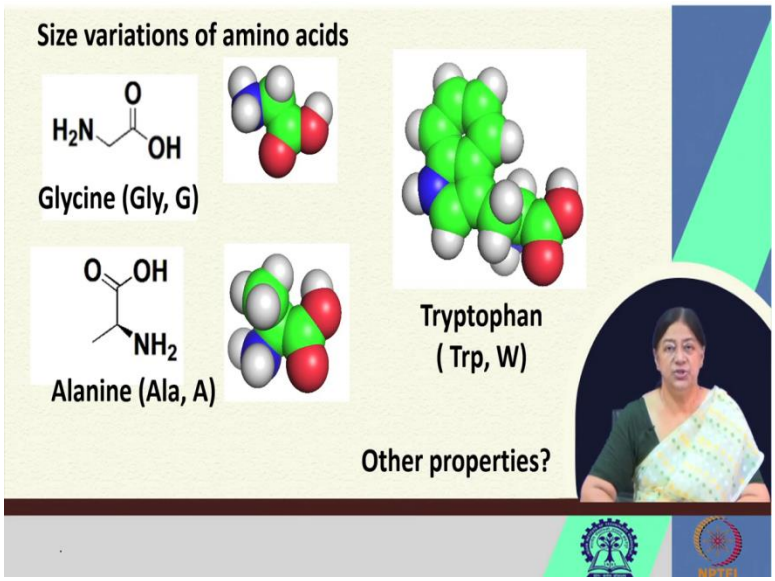

If we look at the size variations of the amino acids say glycine and alanine that we have drawn here, along with tryptophan, we see that there are size variations, which means there are going to be steric factors that are going to play a role. But, what are the other properties that are going to be important here?

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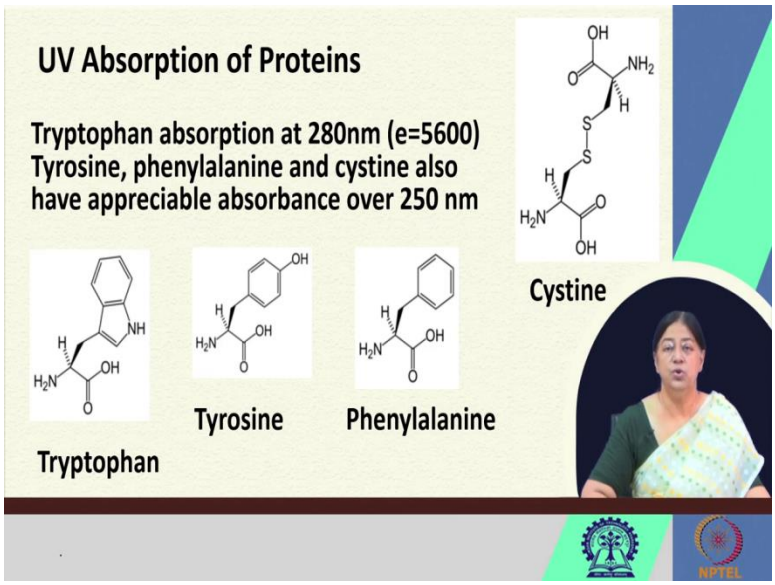

The first thing that comes to mind is the UV absorption of proteins. When we looked at UV absorptions of amino acids and we try to understand what important factors were involved we looked at tryptophan absorption and found out that this had the highest extinction. In addition to this there was tyrosine, phenylalanine and cystine that also had appreciable absorbance above 250 nm.

Now, the question is when we have changes in the amino acids say due to mutations or for whatever reason when we want to determine the presence of the protein, these are factors that come into mind. An aromatic amino acid residue that would result in the UV absorption of proteins and what we were trying to understand was apart from size variations and steric factors, whether a change in a particular amino acid is going to affect the UV absorption and this it does.

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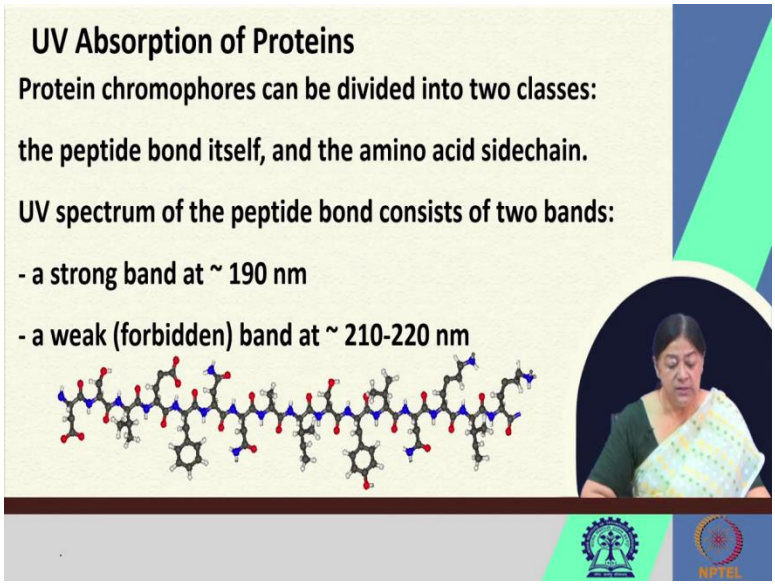

So, the protein chromophores that we looked at previously have divisions in terms of the peptide bond itself and the UV spectrum of the peptide bond consists of these two bands that we looked at. The strong band and a weak forbidden band that corresponded to specific transitions, but in addition to these we have the UV presence of the aromatic amino acids, that also give us additional absorbance values that are of use in protein chemistry.

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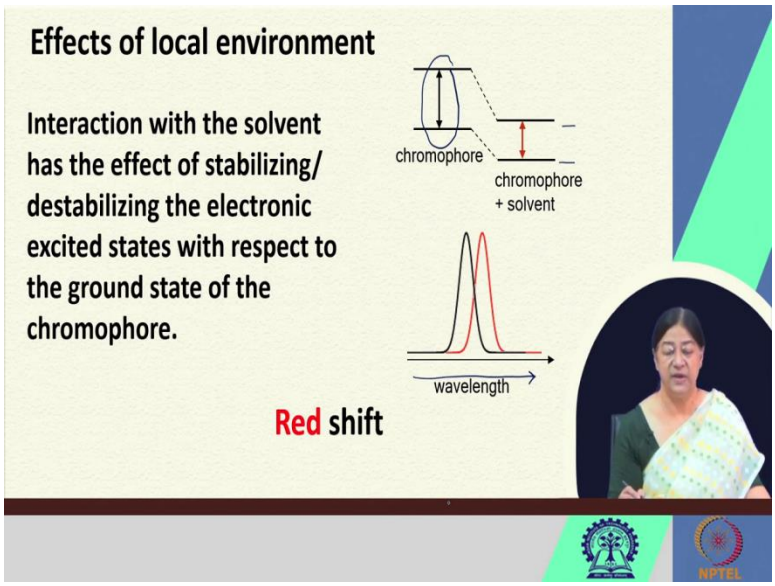

Now, an interesting feature here that we could look at, is the effects of a local environment. What happens when there is an interaction? Say the peptide is interacting with the solvent. Can we look at the wavelength difference and have any idea of whether there is a stabilization or a destabilization?

Interestingly, when we look at a specific chromophore that has a specific transition and the chromophore and the solvent are stabilized, it means they have lower energy difference. Now what is going to happen is they are going to shift to a longer wavelength. This is referred to as a red shift. These are observations that we make in our protein solutions to monitor effects.

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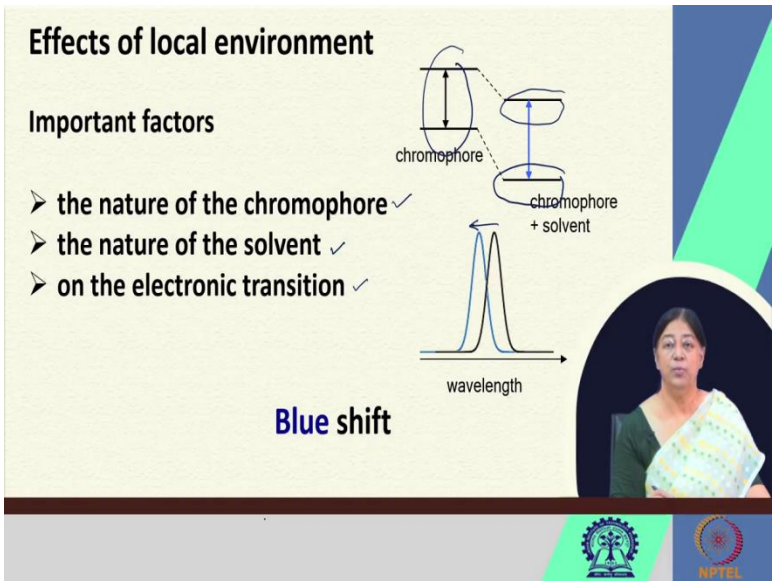

Similarly, we will see the effects of a local environment in terms of the important factors that are going to play a role. For example, it may so happen that the ground state is stabilized more than is the excited state. So, what happens is the energy difference is larger indicating that the wavelength or the shift is to a lower wavelength. So, this is called a blue shift.

So, the important factors that play a role are the nature of the chromophore which would be involved in the specific transitions, the nature of the solvent because that is going to describe or give the interaction specifics and of course on the electronic transition. So, these are the factors that are important in our spectroscopic aspects of the peptide bond or for that matter for the aromatic amino acid residues.

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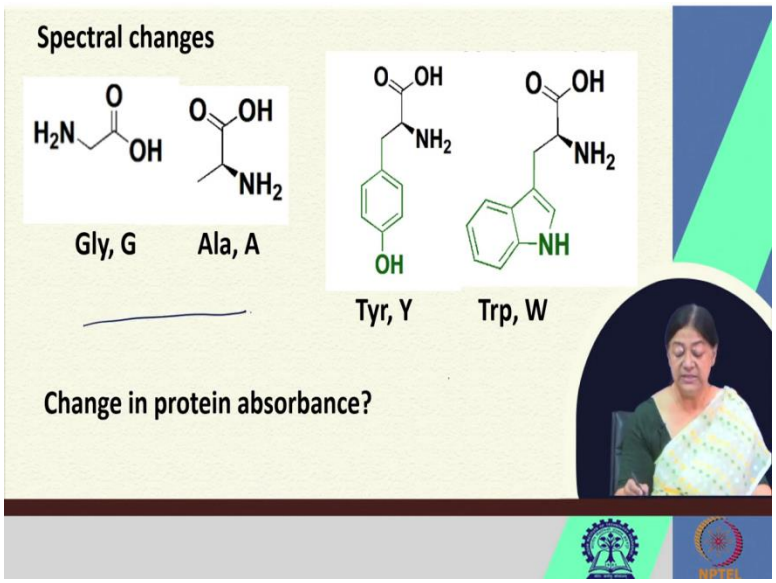

So, when we look at the spectral changes we will see whether there are going to be any spectral changes associated with glycine alanine, tyrosine tryptophan. So, these are important in understanding what is occurring in a protein.

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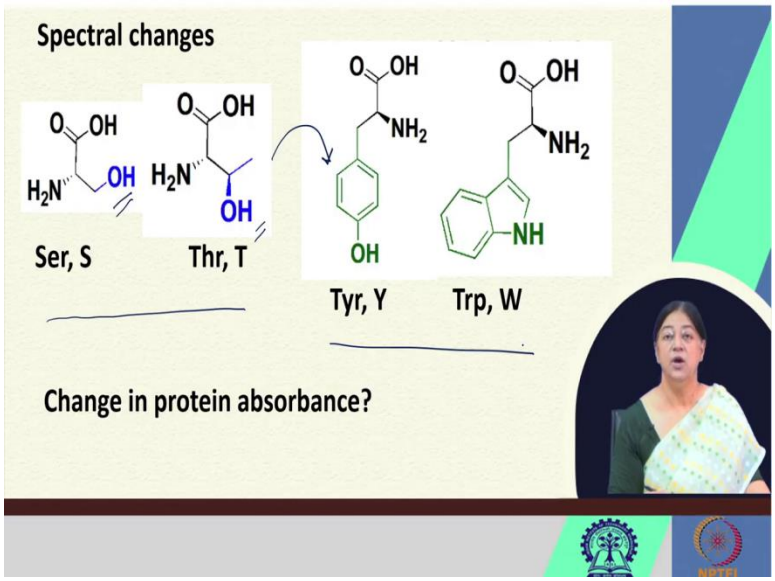

So, when we have changes related to serine and threonine we know that the interactions are going to be pretty much similar, because of the polar possibility of hydrogen bonding. But, when we try and change any of these amino acids to one of the aromatic ones apart from the steric factors that are going to be involved, we are going to have a definite change in the overall UV absorbance or the spectral characteristics of the protein.

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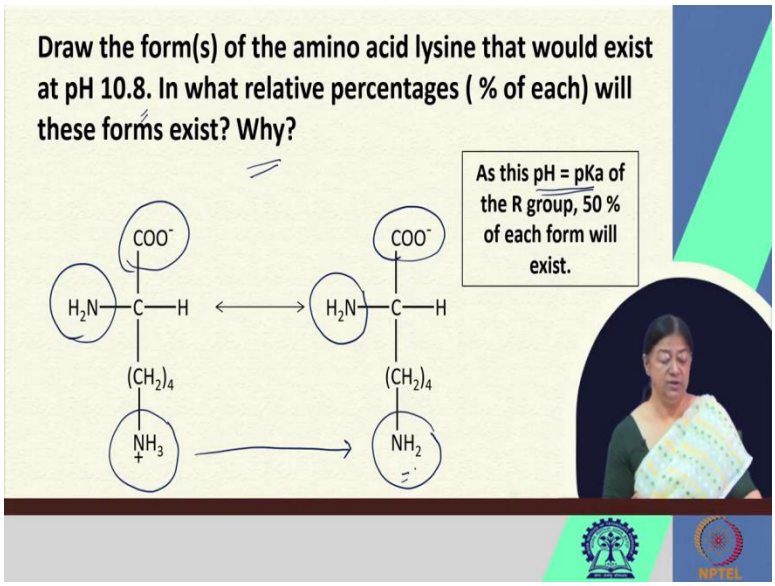

We come back to some questions about amino acids. To draw the form of the amino acid lysine that would exist at pH 10.8 and in what relative percentages of these forms would they exist and why. This [refer to slide] is what we call the Fischer projection of the lysine amino acid residue where we have the COO^-, we have the NH_3^+ here and we have the NH_2 here. Here we have the COO^-, the NH_2, where we have lost this proton. So, at pH 10.8 if you remember from a previous class, pH 10.8 was the point at which the proton was lost. The proton that would have originally been present at a pH less than 10.8 is no longer present, because we want to draw the forms of amino acid at this particular pH. So, we have lost this proton very early, we have also lost this proton at 10.8 we are losing this proton that is associated with the side chain of the amino acid lysine.

Given the Henderson-Hasselbalch equation we have a pH equal to pKa of the R group. If this pH is equal to the pKa of the R group, we have an equal amount of the protonated and the deprotonated forms.

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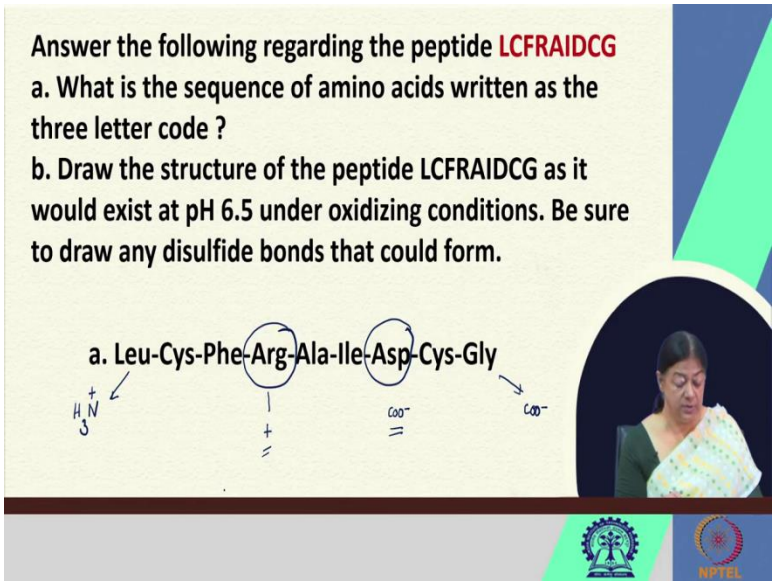

Now, we have to answer the following regarding this peptide LCFRAIDCG. These are one letter codes. So we want to understand what is the sequence of amino acids written as the three letter code, and then draw the structure of the peptide as it would exist at pH 6.5 under oxidizing conditions and we have to draw any possible disulfide bonds. We know that the disulfide bonds are formed between two cysteine residues.

So, we have this in three letter code [refer to slide]. We know that leucine is the amino terminus, glycine is the C-terminus. We can also actually find out the pI of this peptide given. Now if we want to look at the structure of the peptide at 6.5 we realize that the NH_3^+ at leucine is going to remain protonated and glycine is going to have lost its proton.

What are the other non ionizable side chains? We have arginine, we have aspartic acid. Aspartic acid being an acidic amino acid will also lose the C, the proton associated with the COOH of its side chain, but arginine will hold on to its + charge because of the guanidino group. Let us see what the structure looks like.

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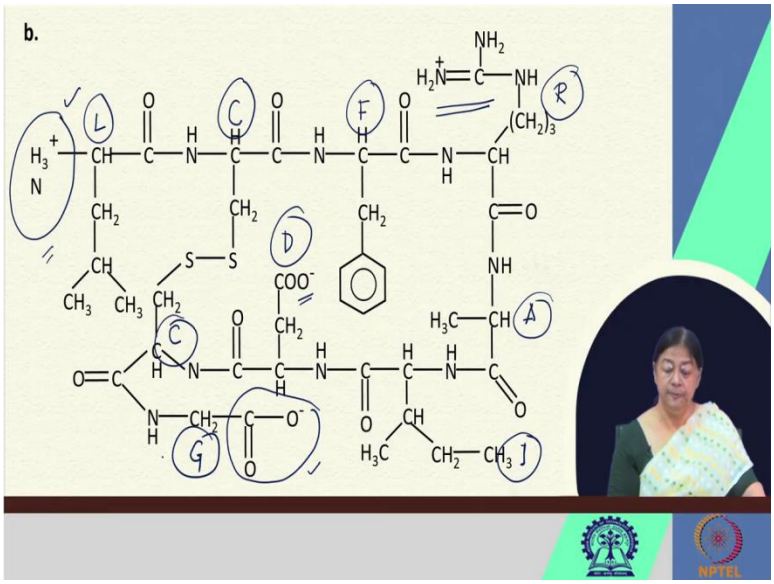

[Refer to slide] so we have the NH_3^+, we have lost the proton for this one. So, let us see. This is our leucine, this is our cysteine, this is our phenylalanine, this is our arginine, which has its positive charge. Now, we come here. This is alanine, this is isoleucine. Then there is CONH, this is CH_2COO^-, this is our aspartic acid, where we have lost the proton here. We come back here. This is where we have another C and coming back here this is where we have our glycine, this is the CH_2.

So, this is the way we have to know how to identify the amino acids present, identify their specific characteristics not only in terms of their charge, but also in terms of their geometry. This is just a straight Fischer projection type that has been drawn, where the connectivity of the amino acids have been shown. The NH_3^+, that is the N-terminus is the first amino acid and the last amino acid is the glycine.

So we have L, C, F, R, A, I, D, C, G, the representation that we need at this specific pH. If we are asked the question what is this at a very low pH. We realize that the terminal COOH and the aspartic acid side chain would remain as such at a low pH value.

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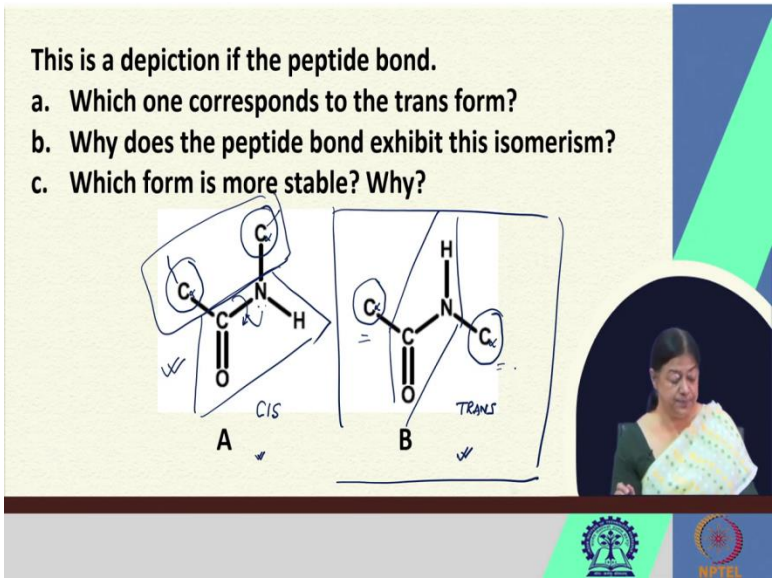

Now, this is a depiction of the peptide bond and we try to understand what differences we see. So we want to know which one corresponds to the transform. We now know that this [refer to slide] is the peptide. This is the peptide, the CO-C=ONH. We have to look at the positions of the α carbon. So, these are the positions of the α carbon. We realize that this is the previous amino acid linking up with the next amino acid. So, this is our Cα atom, this is our Cα atom. Again this is our Cα atom, this is our Cα atom; both connected through peptide bonds. For this particular one we see that they are on opposite sides of the peptide bond. Here they are on the same side of the peptide bond. So, we know that this is the cis and this is the trans.

Why does the peptide bond exhibit this isomerism? We know that not only this largely has a single bond character, but also a partial double bond character, for the restricted rotation that occurs because of the lone pair on the nitrogen that is delocalized over the bond here, rendering it a partial double bond character; thus restricting its rotation. But nevertheless rotation is possible. So, the peptide bond can exhibit this cis to trans isomerism, though we know that this form is more stable. And why is that; it is because of the side chain characteristics. We know that to the Cα we are going to now have the Cβ or the rest of the side chains present. Because of that, if it is in the trans form, there is a less or a lower possibility of any steric interactions or any possibility of their coming together.

So, the clash of the side chains is more possible when we have a cis conformation less so when we have a trans conformation.

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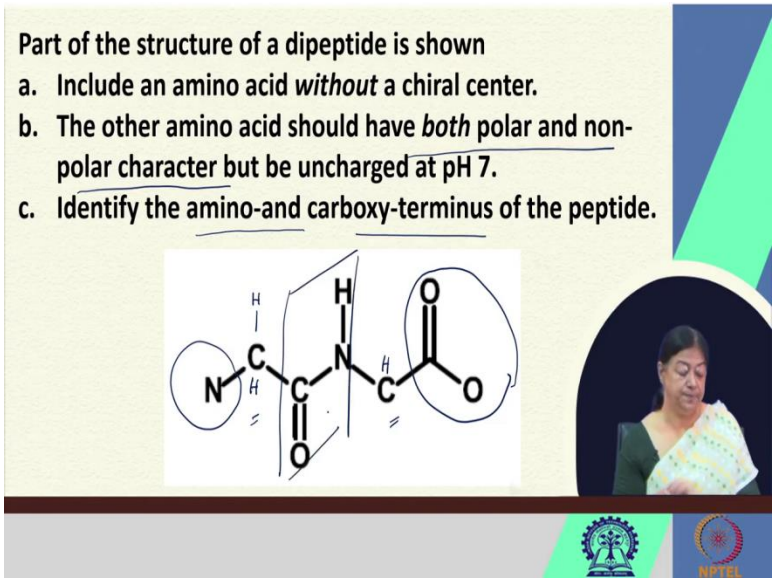

We have now part of the structure of a dipeptide shown. We have to include an amino acid without a chiral center and the only amino acid without a chiral center is glycine. So, we just have to put a hydrogen atom here [refer to slide] and another hydrogen atom here, which we know is present anyway.

So now when we do this, the other amino acid should have both polar and non polar character, but be uncharged at pH 7. This would preferably be an amide group. So, we can put an asparagine or we can put a glutamine and then we have to identify the amino and the carboxy-terminus.

We know that this [refer to slide] is the amino-terminus, this is the carboxy-terminus of this specific dipeptide. And depending upon what we have present here, we can determine even the pI. And where is our peptide bond? This is our peptide bond.

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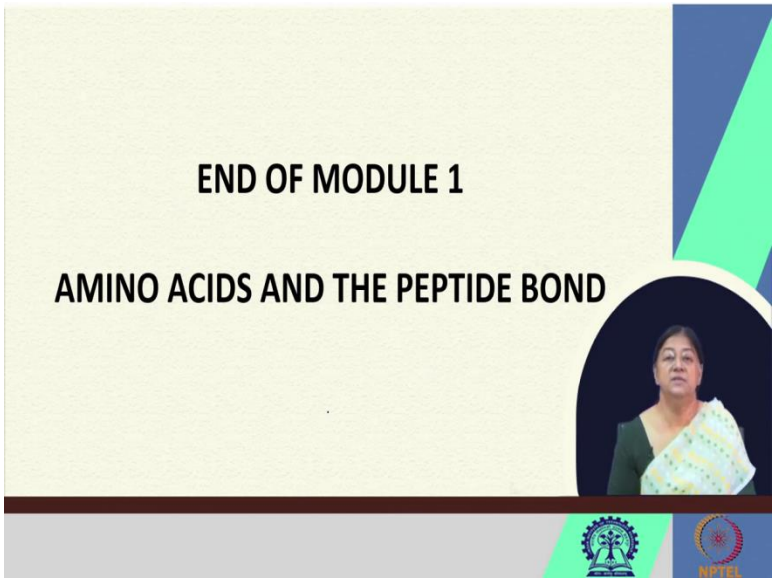

So this completes module 1, where we looked at amino acids and the peptide bond. We looked at specific characteristics of the amino acids, what the side chains are, what types of side chains we can have and how they can have different properties. We then went on to look at the peptide bond formation, its geometry and its specific characteristics. We will begin module 2 with an understanding of the primary structure and look at protein architecture and protein structure.

Thank you.