External Biochemistry
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Lecture – 07
Protein Folding and Denaturation- I

Welcome to the next lecture on Protein Folding and Denaturation. Before we start the understanding of protein folding and what we mean by denaturation our first understanding will be about amino acids that construct proteins and the forces involved to fold a protein.

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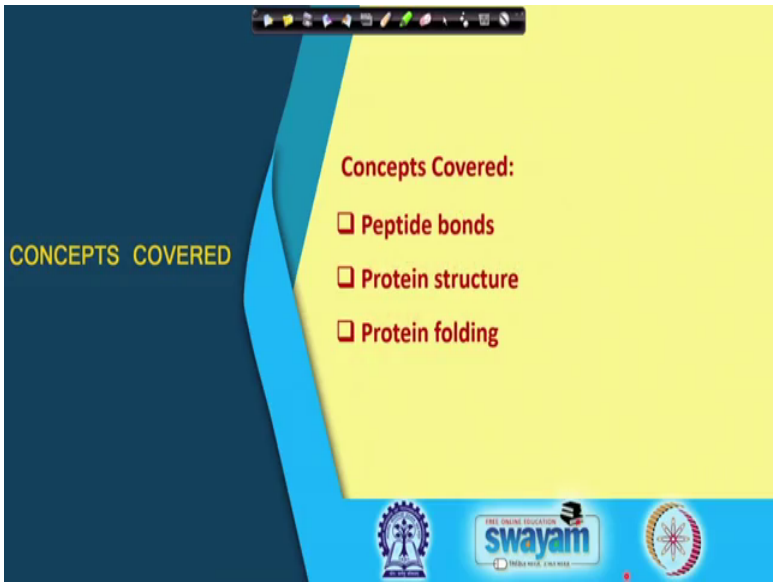

The concepts that are going to be covered in this course involve peptide bonds protein structure. And, a part of protein folding that is going to extend on to the next lecture, which we will understand in terms of protein folding and protein denaturation in terms of the interactions involved in the structure of proteins.

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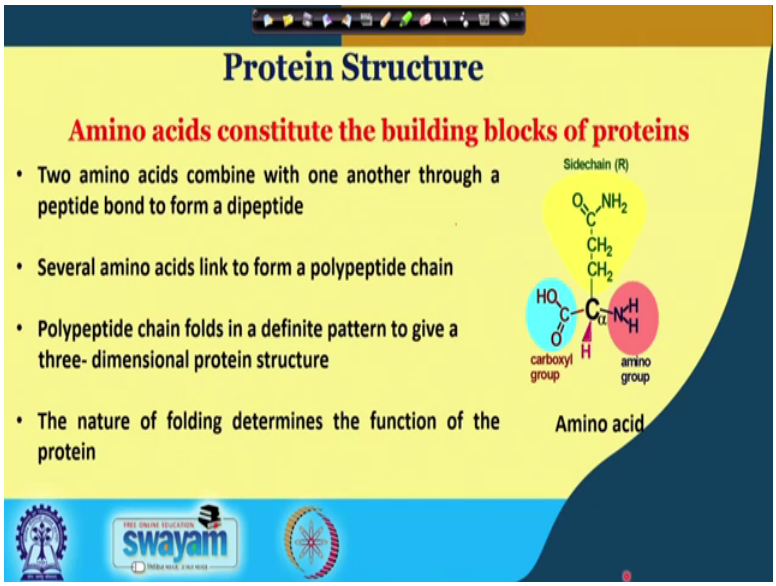

When we look at amino acids that constitute the building blocks of proteins; this is a structure that we looked at in our previous lecture where we looked at in one of the previous lectures, where we try to understand what we mean by an amino acid in terms of its structure. We have the amino group the carboxylic acid group and a specific side chain.

Now when we form the protein we say that these amino acids are the building blocks of proteins. This means that the amino acids will combine with one another through what is called a peptide bond to form a dipeptide. Now, several of these amino acids will then form a polypeptide chain. It is this polypeptide chain that will then fold into a definite pattern. And when it forms this folded pattern or folded structure it will have what is called a native structure for the protein.

Now the nature of the folding or the importance of the folding lies in the fact that a unique folded protein structure is going to render a specific function to the protein. And any disruption in the structure means a disruption in the function of the protein

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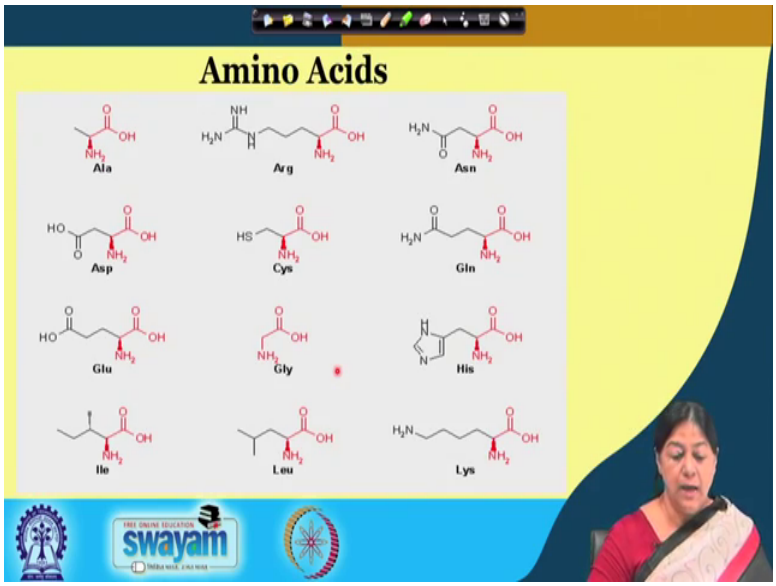

If we look at the different R groups we had seen this slide before where we looked at the different R groups. So, this is the amino group this is the carboxylic group and the rest of the structure that is in black here is the side chain; which is true for all the specific amino acids that we have drawn here. We know that we have different types of amino acids acidic amino acid basic some basic amino acids.

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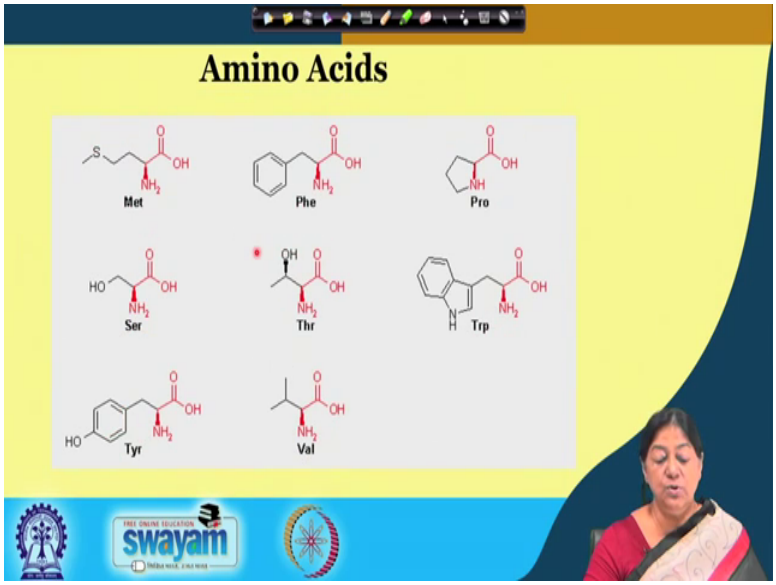

And those amino acids that are hydrophobic in nature. We will see how the structure or how the characteristics of the amino acids are extremely important in what we call protein folding.

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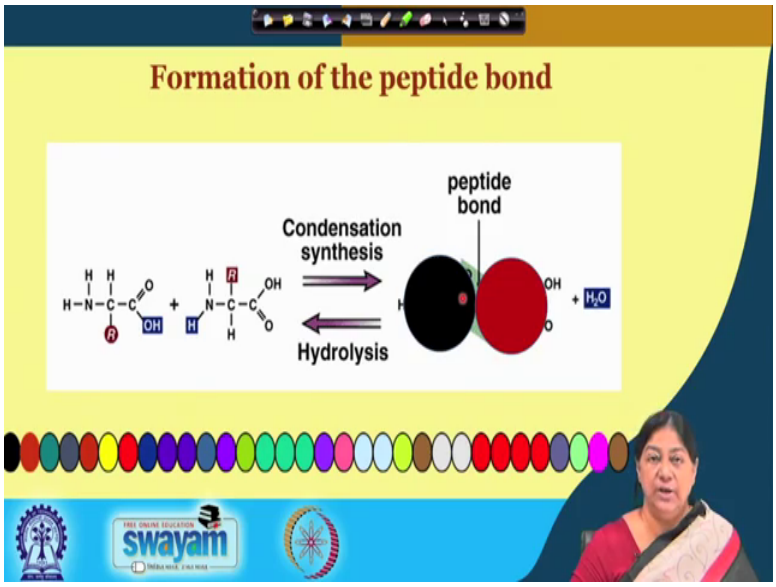

We are now going to look at the formation of the peptide bond. We mentioned that there are two amino acids that come together to form what is called a peptide bond. What do we mean by this peptide bond? When we have one amino acid which you see here; we have the amino group, we have the carboxylic group, and we have the R group. Similarly, we have a second amino acid where we have the designation or the square R group and the circular R group just to give a distinction between the structures.

In the process of what happens is called condensation we lose this molecule of water. In the loss of the molecule of water we have what is called a peptide bond created between the R group and this R group. And we will look at specific character characteristics not only of the peptide bond, but also of the specific R groups and how and why they can be located at specific points or specific parts in a three dimensional folded protein. So, when we represent the amino acids in a protein all we need to know is what this R group is; because we know that they are connected by this peptide bonds which is a constant in all such cases the C O NH that is the peptide bond.

So, the characteristics that we need to know since we know that the rest belongs to an amino group and a carboxylic acid group all we need to know is the characteristic of the

R group. So, we have these two that are linked together by a peptide bond. Similarly we can have a whole series linked together by peptide bonds that is going to form what we call R protein chain this is our polypeptide chain.

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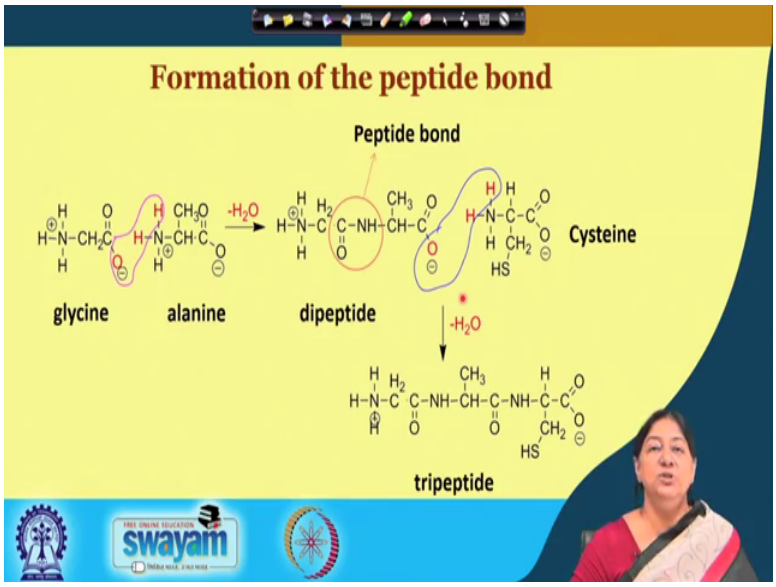

Now in the formation of the specific if we look at specific amino acids forming this peptide bond. Say, we have a glycine alanine and an alanine. We lose water to form a peptide bond and we have a glycine and an alanine side chain. If we add another amino acid residue the cysteine residue we will have form a tripeptide. Similarly, as I mentioned in the previous slide we can have a continuation of this and we will have a polypeptide. There are certain questions that we need to ask now.

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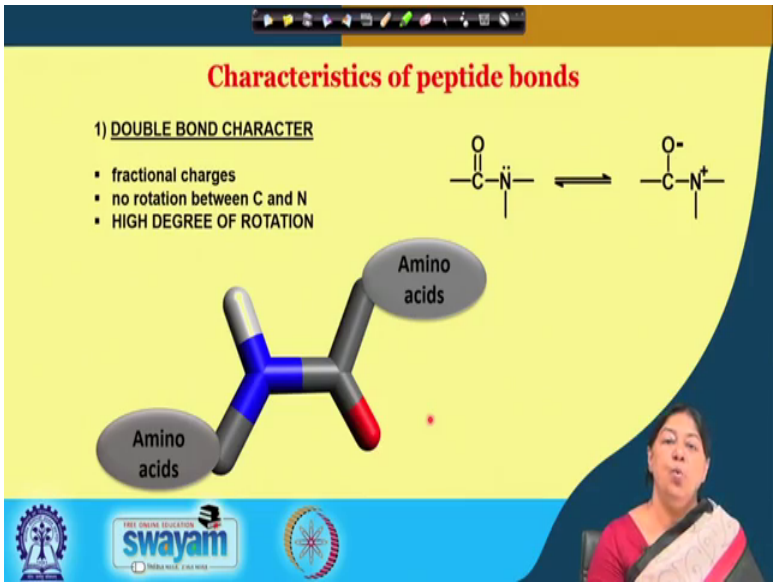

Certain questions related to what we mean by the characteristic of this peptide bond. So, here is the peptide bond this is the nitrogen this is the C, this is the double bond o, this is the amino acid residue, this is the other amino acid residue. We have fractional charges there is, if it were a single bond there would be a high degree of rotation but, because of the partial double bond character that is rendered through because of the lone pair of nitrogen this becomes rigid in nature.

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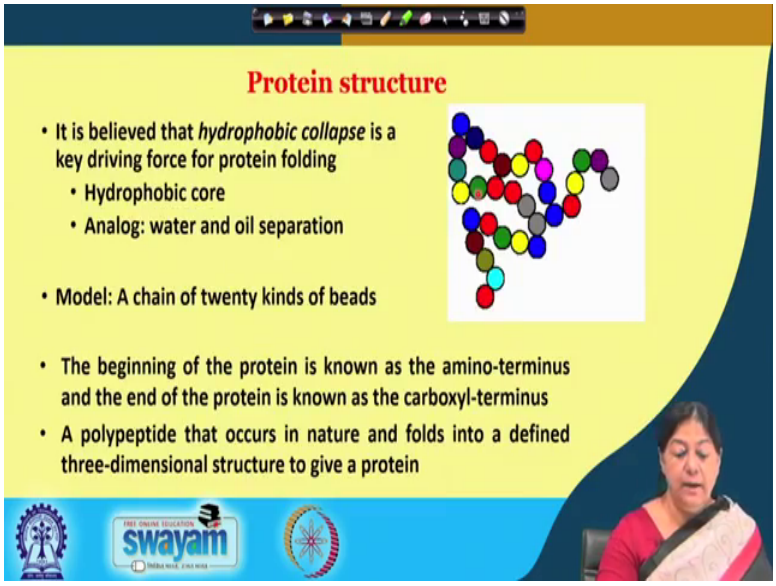

When we consider therefore, r polypeptide chain that can be formed by these 20 beads if we call them: so we can have the model as a chain of 20 kinds of beads where these 20 represent 20 different amino acid residues. Now what is going to make the protein fold? What we have here is we have different characteristics of the structures of the side chain residues. What do we have? We can have hydrophobic type what do we mean by a hydrophobic amino acid a hydrophobic amino acid is one that has only carbon and hydrogen and its side chain if it is so this would tend to be away from the water.

So, what would happen? It would tend to be what is called in the core of the protein. We will look at this in a bit more detail in the next lecture, but one thing we need to notice here is if we go back to a previous slide, and just look at the specific peptide bond formation you will notice that it starts off with the amino group and it ends with the carboxyl group. What happens again when we have the addition of another amino acid residue? Again this is the first amino acid residue and it continues in this fashion.

So, if we look at the specific chain the direction of the chain can be known because we know that the peptide bond is formed between the C O H and the NH 2 of the next amino acid. So, the beginning of the protein is known as the amino terminus and the end of the protein is known as the carboxyl terminus. And the polypeptide that occurs in nature and falls into a definite three dimensional structure is called a protein.

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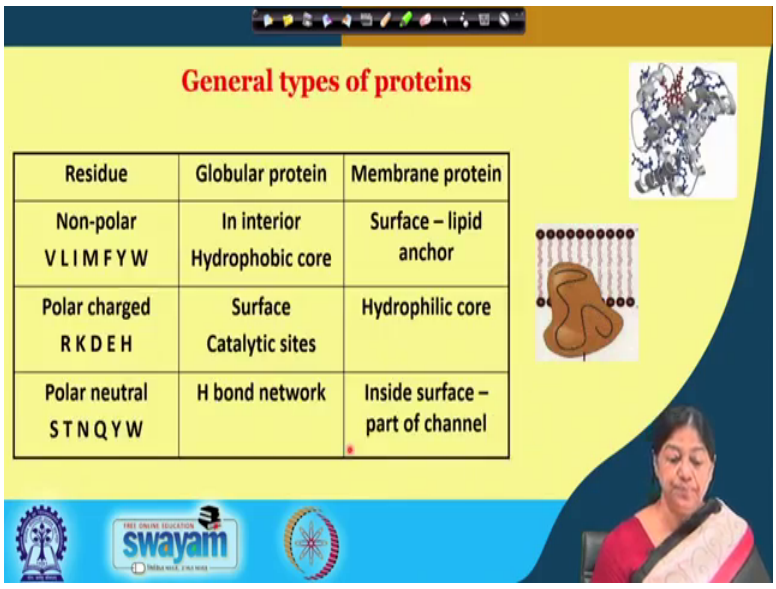

The general types of proteins that we know are globular proteins which are more or less shape like this which will have on the surface mostly hydrophilic type of residues. These are the one letter amino acid codes that we have the non polar in a globular protein is likely to be in the interior, why? Because they are hydrophobic in nature and they do not want to be in contact with the solvent. On the other hand if it is a membrane protein that interacts with the lipid bilayer the lipid bilayer you know where you have the fatty acid chains which are hydrophobic in nature.

So, any interaction between this protein and this would be hydrophobic in nature. So, in this case the surface the lipid anchoring ones would be the non polar type; for a globular protein again the polar charged amino acid type is going to be at the surface or at the catalytic sites of the protein. Whereas, in the membrane protein they are form the hydrophilic core. Now this is a very smart way because then we could have if we have a trans membrane protein as we know and we have a hydrophilic core, then that will allow the transport of ions from the inside to the outside of the cell or from the outside to the inside of a cell.

And the polar neutral amino acid residues are involved mostly in high bonding. Wherein, the membrane protein they form part of the channel and in the globular protein they are involved in the extensive hydrogen bonding network that is present in proteins that holds the protein structure together. So, the covalent bonds that we have in proteins are the peptide bonds. Apart from the peptide bonds the other covalent linkages that are present in proteins are the disulfide linkages between two cysteine residues.

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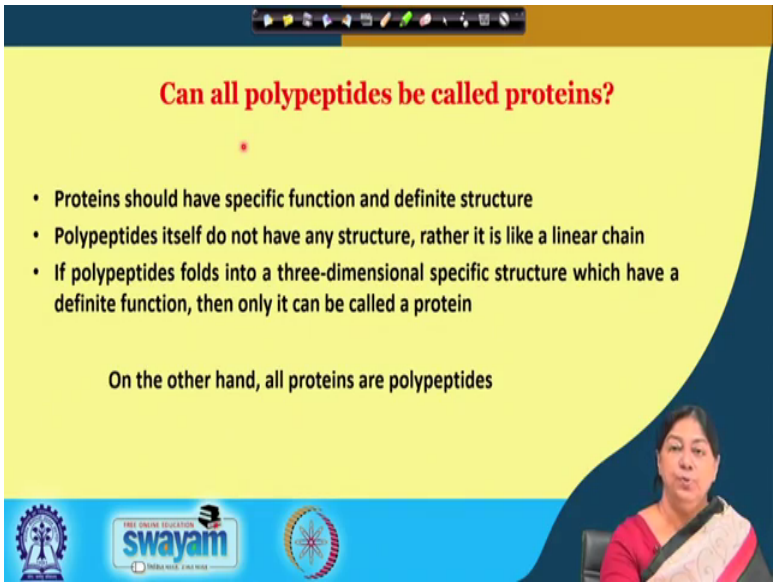

Can all polypeptides we call proteins? The answer here is, no. Proteins have a specific function and a definite structure all proteins are polypeptides because they are formed from the amino acid residues through the peptide bonds. But when the polypeptides itself they do not have any structure associated with them. And hence do not have any function, but increasingly there is research going on in intrinsically disordered proteins which is a separate topic and beyond the purview of this course.

But an understanding here would tell us that the polypeptides that we are concerned with in general fold into a three dimensional specific unique structure which has a definite function we then call it a protein of interest. This is the native three dimensional structure of the protein that has a function.

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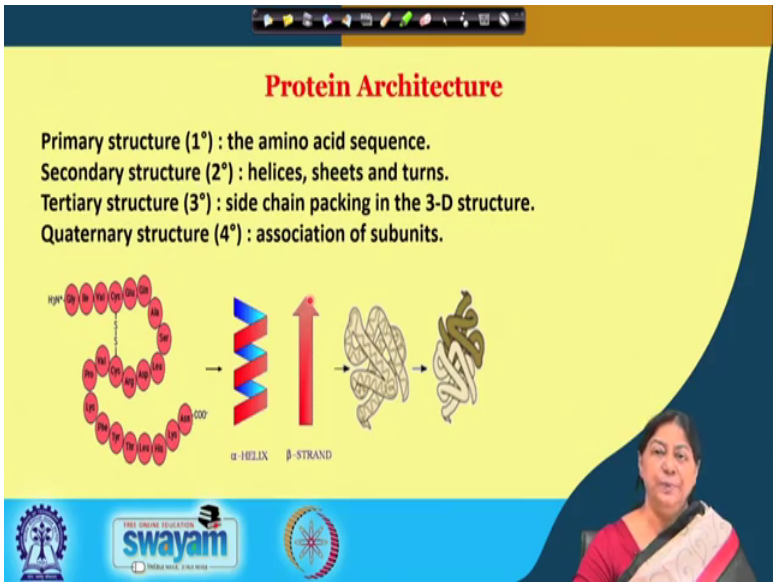

If we look at the protein architecture; what we mean by protein architecture? Protein architecture is the way the protein falls what the protein looks like and how we can assess it when we consider the unfolding or what is called the denaturation which will be the topic of our next lecture.

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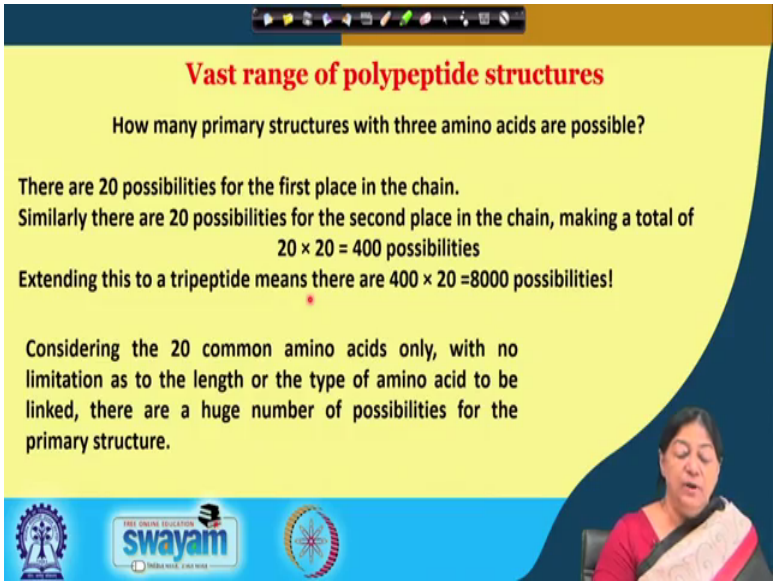

If we look at the specific different types of: how many primary structures with three amino acid residues. Let us consider the first amino terminus residue; this could be 20 of the different amino acid residues the common essential amino acid residues. There are 20

possibilities for the second there are 20 possibilities for the third, which means there are many many possibilities of proteins that can form with the 20 common amino acids that we know of. And there is no limit to the length or the type or how the amino acids linked to one another.

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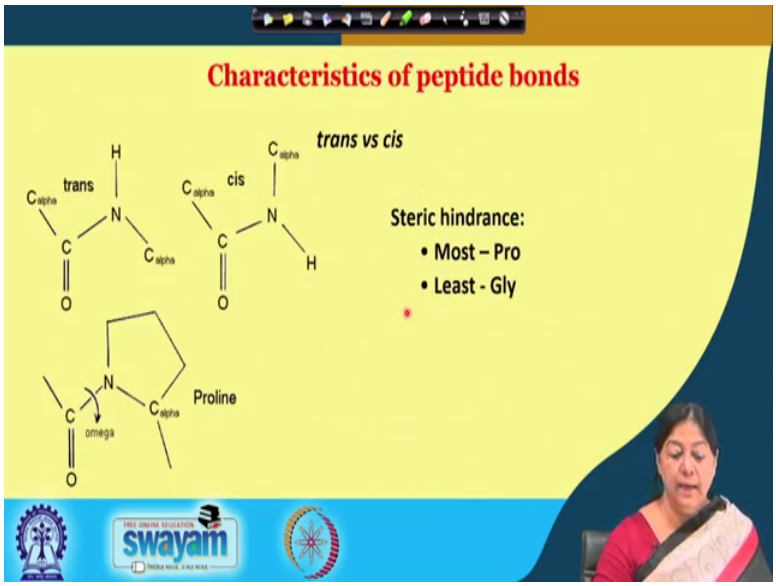

The peptide bond in general has a Tran's structure. So, if we look at the C O N H; this means that this C alpha atom that we are talking about or this C alpha atom that we are talking about. This we call the i this is the i plus 1, because we know that the C O H, C O O H of the previous amino acid linked with the NH 2 of the next amino acid. This is something that is trans in nature because the C alpha are facing in opposite directions.

However, there is a possibility of a C alpha C alpha, cis conformation which is less likely because of the bulk of the side chains because this C alpha now is going to have a side chain linked to it. So, if we have a side chain linked to it there may be steric interaction which means that the transfer conformation is observed to a much larger extent than the cis conformation. Another unique amino acid or other amino acid is the proline, where it falls upon itself informing the peptide bond. And as such what happens is it has a steric hindrance inherent to it.

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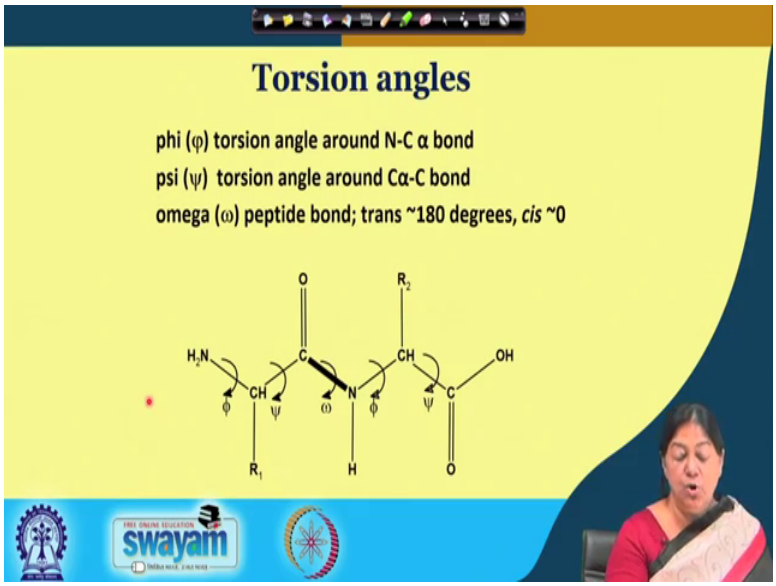

The torsion angles are another very important part; the one marked in dark here we as I mentioned, because of the part the lone pair on the nitrogen this has a partial double bond character; which means there is restricted rotation about this because of the partial bond character associated with it. We have an addition here specific torsion angles the psi angle and the phi angle between specific atoms in the residue.

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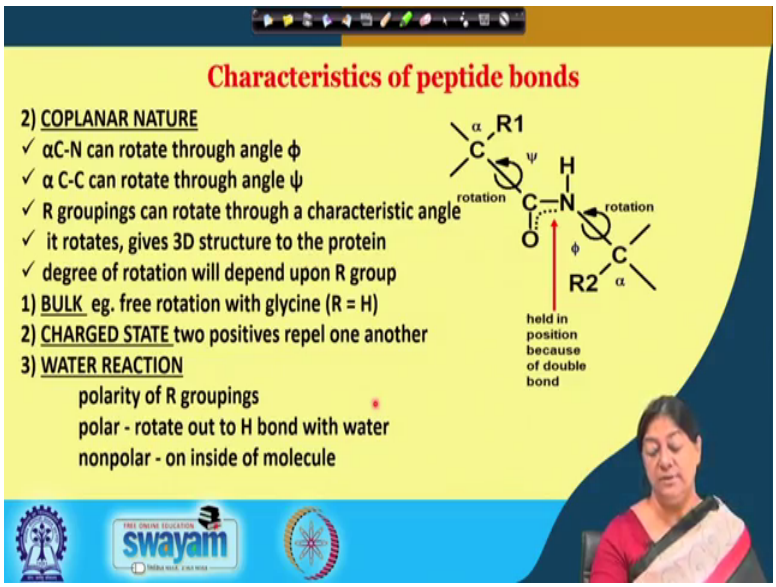

We will look at this a bit more when we are talking about rotation about the C C bond here; that is the psi angle rotation about this which is restricted is called the omega angle.

Rotation about the N C bond here is called the phi angle. Now this is important in the geometry of the molecule because it gives us interactions possible favorably with for example, if this amino acid residue preferably has an interaction along this side of the protein chain, then what is going to happen on the protein structure. There is going to be a specific rotation that allows the side chain to form a specific interaction. So, we have the polarity of the groups the characteristics of the amino acid residues that are going to result in a specific structure of the protein which we will see in more detail later on.

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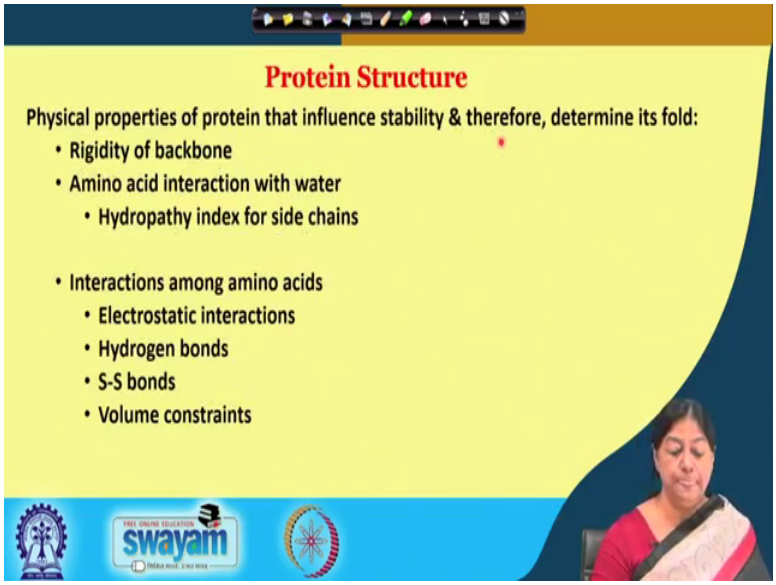

So, the physical properties of the protein influence stability and where does this come from: it comes from the inherent property of the amino acid residues. Because the amino acid residues themselves are involved in electrostatic introductions in hydrogen bonds in disulfide linkages and in volume constraints that give a specific characteristic to the geometry of the protein that can be involved.

Another very important interaction is or rather another very important property of the amino acid is the hydrophobicity; where there is a specific hydropathy index for the side chains that actually mention how hydrophobic a specific amino acid residue is. Now, that is going to have a very important impact on the location of this specific amino acid or the range of amino acid residues if it is hydrophilic or hydrophobic in nature.

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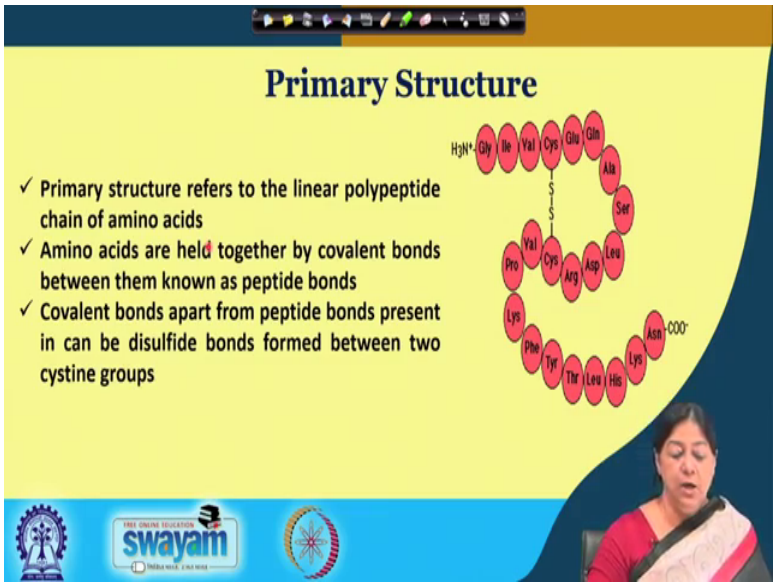

So, the primary structure of the protein refers to the linear polypeptide chain of the amino acids. What are these linked by? These are linked by peptide bonds this is the amino terminus and this is the carboxyl terminus of this short chain. And the only other covalent linkage is the disulfide linkage between two cysteine residues. So, apart from the covalent bonds present in proteins through the peptide linkages we have those of the disulfide linkage.

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For example if we look at the sequence of amino acids in lysozyme this is the primary sequence. All we need to know is the type of amino acid side chain, what amino acid residue because we know how these are linked to one another based on the peptide bond.

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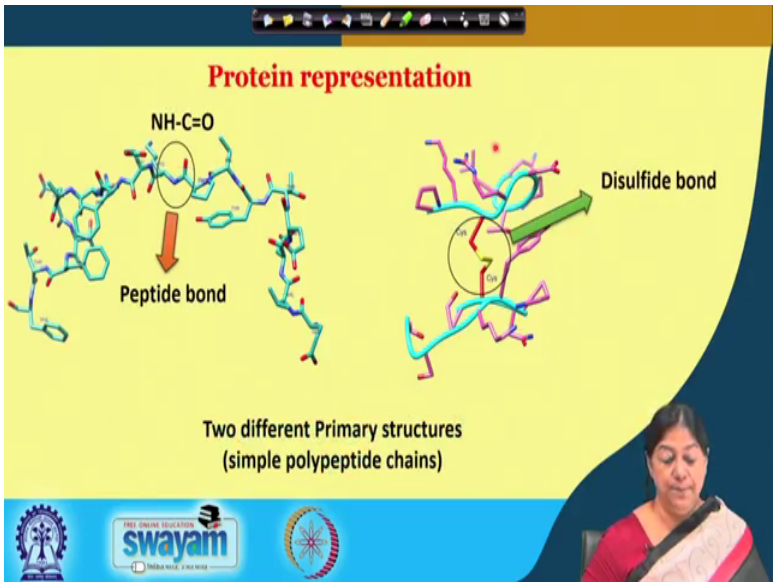

So, if we look at the specific protein representation we look at a peptide bond here. So, we see the NH and the C double bond O which means based on this we can actually look at the directionality or which. So, this is the CO, this is the NH, this is the NH of the next amino acid, and this is the C double bond O of the previous amino acid. And just by looking at the specific structures we can say that this is a phenylalanine then this is a tyrosine and so on and so forth, because we know that each of these amino acid residues has a unique structure associated with it.

This is an example of two cysteines linked together to form what is called a disulfide bond. So, these this is how proteins are represented in their three dimensional structure; that gives us an idea about where the amino acid residues are in three dimensional space. And the when we study enzymes we will see which amino acid residues are presented what is called the active site of enzymes or what functionalities are there.

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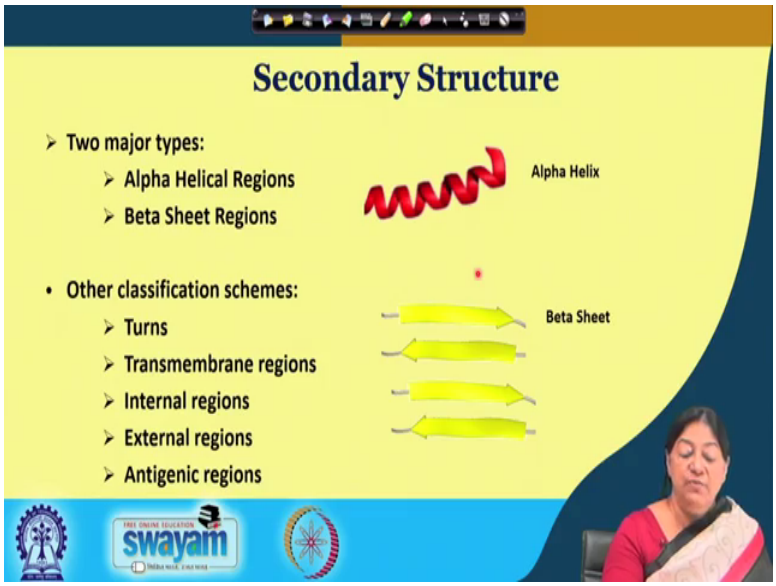

If we look at the secondary structure of proteins in the secondary structure rather there are two major types alpha helical regions and beta sheet regions. We see this is just a typical alpha helix representation a typical beta sheet representation, where these are called strands beta strands they can be parallel to each other or they could be anti parallel to one another.

But these from the beta sheet and they have characteristic patterns in what is called hydrogen bonding. The secondary structural elements or the secondary structure components of proteins are held together by hydrogen bonds. Other classifications are turns and loops and specific regions.

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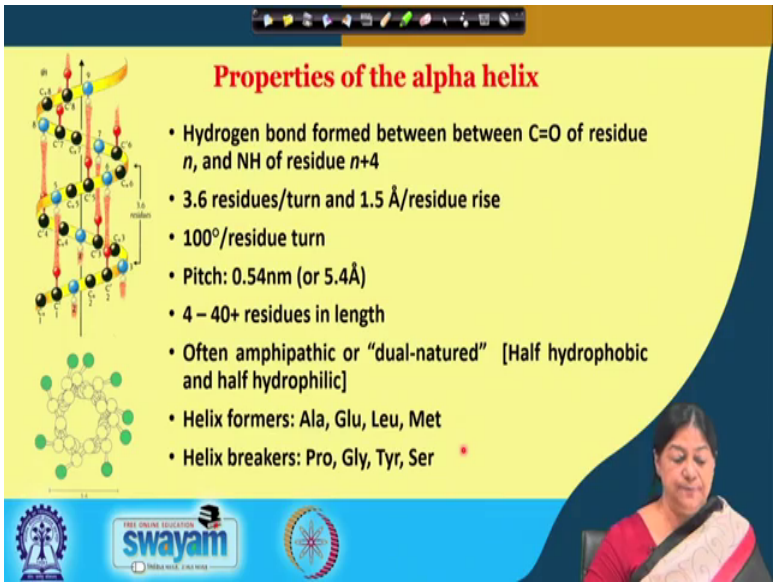

The hydrogen bonds between the C O of residue N and the NH of residue N plus 4 this forms a characteristic that is seen in the alpha helix is present in protein. These are all characteristics of alpha helixes there are 3 point 6 residues per turn which means this is a typical alpha helix what is called when we look at the shape or how it is represented we look at a right handed helix in this fashion.

If we know this is the rotation of the helix and this is the direction of the helix that is what is called a right handed helix. Similarly if we look at a left handed helix we have a rotation in this fashion and the directionality here. Now sometimes these mostly this alpha helix can have an amphipathic characteristic which means that part of it is hydrophilic in nature and part of it is hydrophobic in nature.

If we look down the axis, if we look down this axis for the alpha helix we will see something that looks like a wheel; where, the amino acid residues are at the surface and some of these may be hydrophobic in nature some of these may be hydrophilic in nature. In that case if it is a specific globular protein it would be expected that this hydrophilic part is facing the exterior of the protein, whereas this hydrophobic part is facing the interior of the protein.

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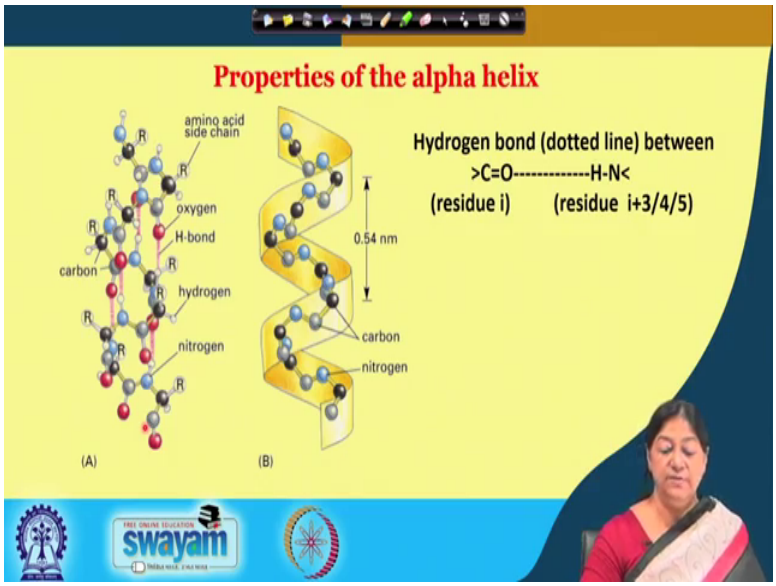

Other properties of the amino the amino acid residues present in the alpha helix as I mentioned is the hydrogen bond characteristic which is a specific type i 2 i plus 4.

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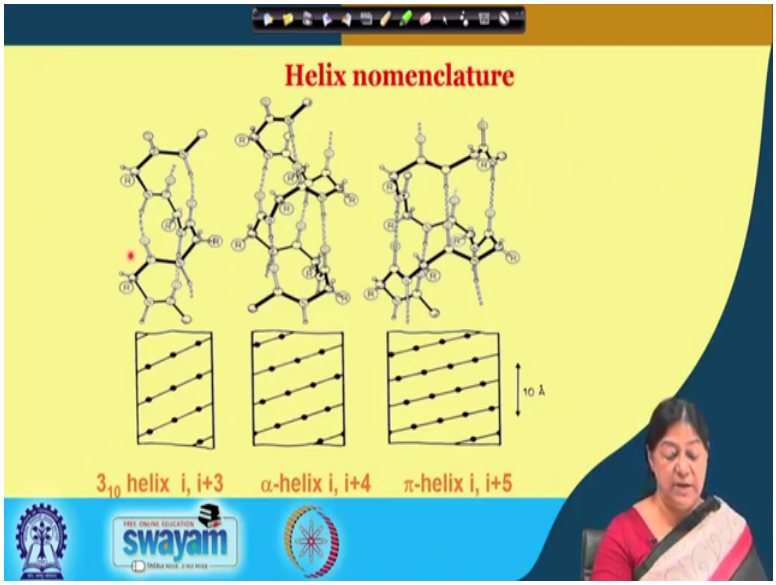

But they can also be of different types where we have specific helix called a 3 10 helix where the hydrogen bonding is i to i plus 3 and alpha helix the regular right hand in helix that is i to i plus 4 or what is called a phi helix from an i to i plus 5.

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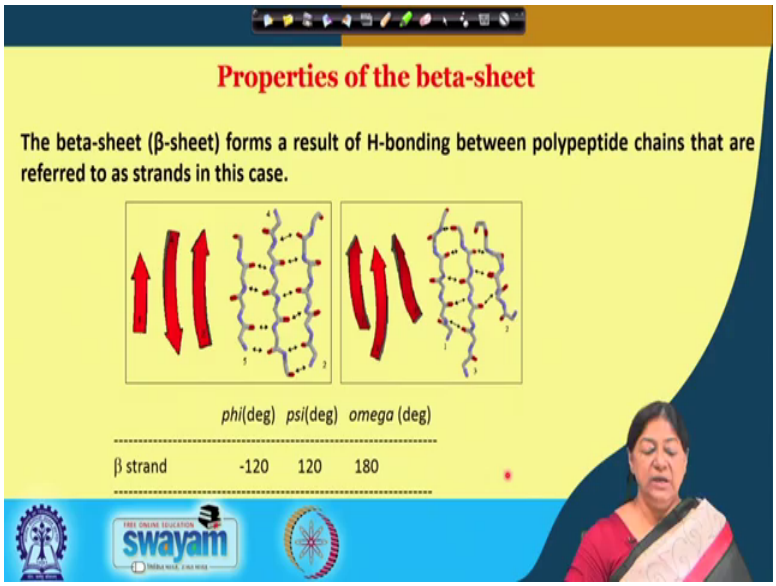

Now, if we look at the properties of the beta sheet in this case also the strands as I mentioned they could be parallel to one another, they could be anti parallel they have specific phi psi angles to them and again they have hydrogen bonding that is between the different strands. Now the differences between the alpha helix and the beta sheet is that the beta sheet can be from if the strands that form the beta sheet can come from different parts of the protein. Whereas, when we look at an alpha helix this is a consecutive or continuous stretch of amino acids that form the alpha helix.

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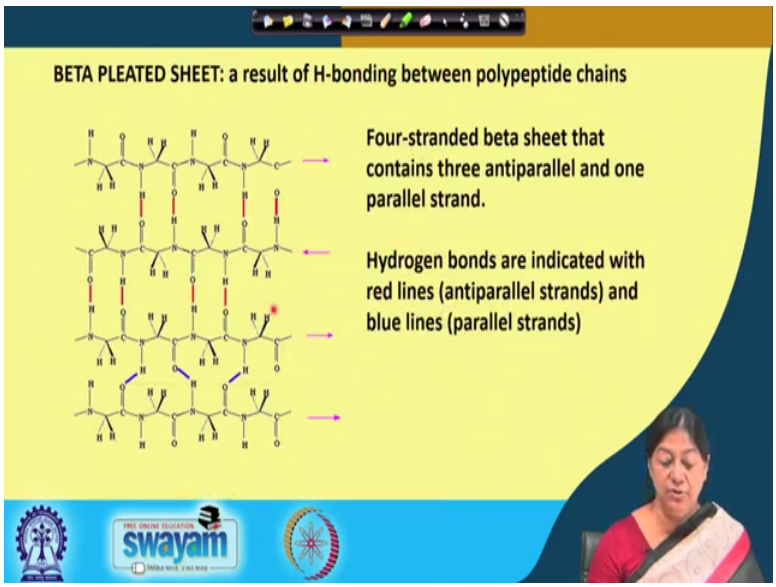

The characteristic hydrogen bonding pattern for these if we look at this anti parallel type we will look these are marked by the hydrogen bonding characteristics; where again we see that the hydrogen bonding observed we have to remember is between the backbone atoms. The backbone CO and the backbone NH this is characteristic of the secondary structure elements of proteins, the secondary structure of proteins. In addition they may be side chain hydrogen bonds that are in addition to what we see say for example, in an alpha helix there could be hydrogen bonds between the side chains.

But that is not what defines an alpha helix the i to i plus 4 is the characteristic definition of a hydrogen power bond for the regular alpha helix and the NH the CO NH again hydrogen bonding pattern which you will see for specific type for an anti parallel a specific type for a parallel. Where the hydrogen bonds are indicated by the red lines here for the anti parallel strands and by the blue lines here for the parallel strands.

And what is important here is when we look at the specific characteristics when we look at what actually forms a secondary structure, what forms a folded protein. When we consider what we mean by protein denaturation we are actually going to disrupt or we are going to destroy these weak bonds. And how we are going to do that is what we will see in a next lecture.

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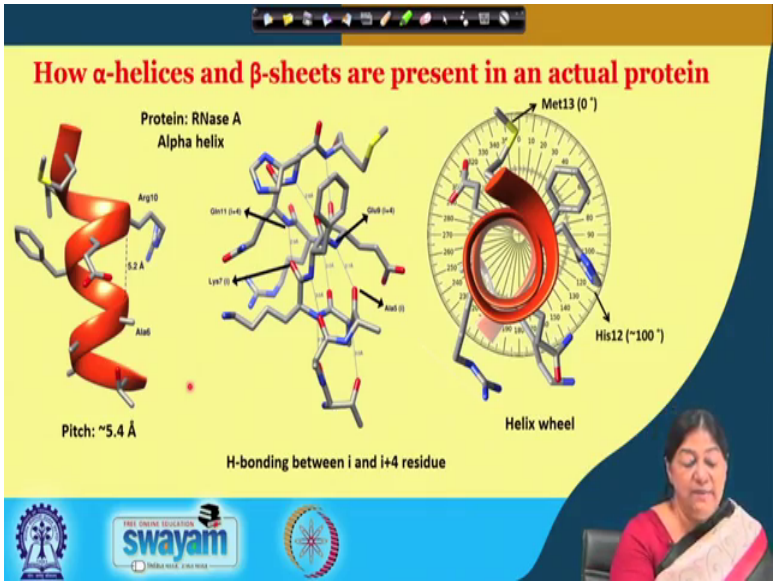

So, we have the alpha helix in a protein we have the hydrogen bonding between an i and i plus 4 res residue represented in an alpha helix and what is called a helical wheel of an

alpha helix. So, this is how we can actually look at the hydrogen bonding pattern in an alpha helix. And we can also see the side chains of the alpha helix that are protruding out from the axis of the alpha helix.

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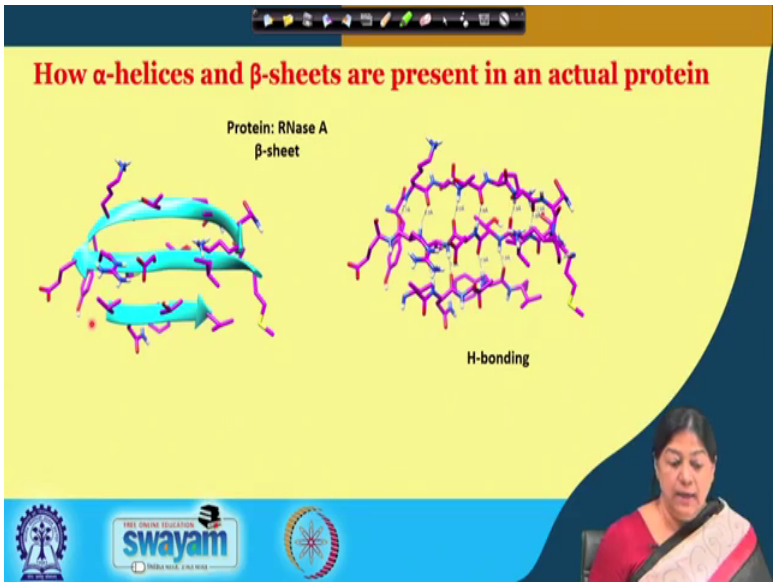

If we look at different representations now for example, this is how the alpha helix and helices and beta sheets are present in an actual protein. So, look this is where we have the hydrogen bonds again between the different strands of the protein. This is where we have the strands represented it in the blue arrows here that tell us that this is a specific distance away. So, we look at the specific hydrogen bonding characteristics between the amino acid residues that comprise the strands that make up the beta sheet.

Similarly when we saw the alpha helix for the alpha helix we have to remember that the nitrogen is represented by the blue and the CO is represent the O is represented by the red atom here. And the hydrogen bonding pattern in the backbone is between the C O and the NH for the alpha helix as well as for the beta sheet which comprise the secondary structure of proteins. Because when we look at a disruption in the structure we will have to see by any method possible how the secondary structure is getting destroyed.

So, we are going to look at specific we studied a bit of spectroscopy. We are going to look at other spectroscopic methods in general that will tell us that we have a disruption in the alpha helix structure or we have a disruption in the beta sheet. This means that the hi bonding between the strands in the beta sheet or the hydrogen bonding between the i

and the i plus 3 i plus 4 or i plus 5 residue is being destroyed being disrupted by certain chemical certain characteristics that we are going to look at.

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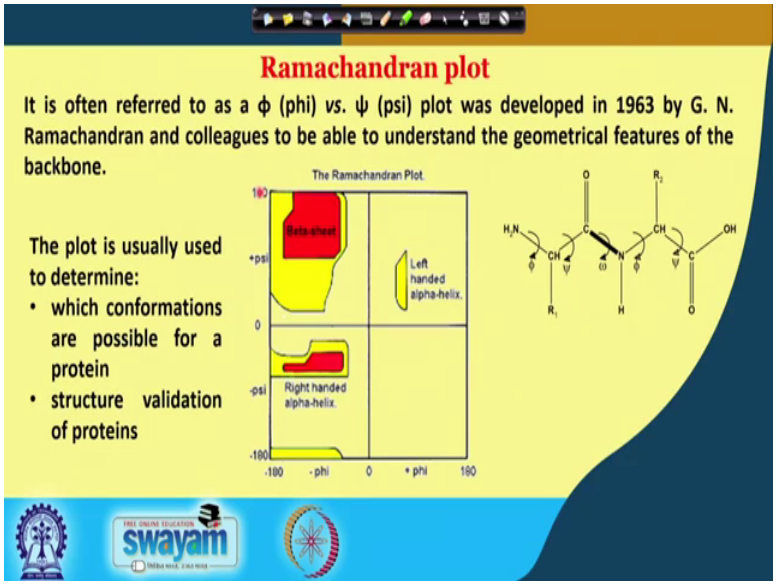

The torsion angles that we consider where we have the alpha or the phi angle and the psi angle and the omega angle. This is a very important plot that tells us something very unique about the protein geometry the secondary structure geometry; where the phi angle is on the x axis this psi angle is on the y axis. It is actually a circular plot where were looking we are going from minus 180 to 180 here minus 180 to plus 180 here it determines which confirmations are possible for a protein and structure validation of the protein. It was developed by Professor G. N. Ramachandran and his colleagues at a that gives us a possibility to understand the geometrical features of the protein and is an extremely important plot.

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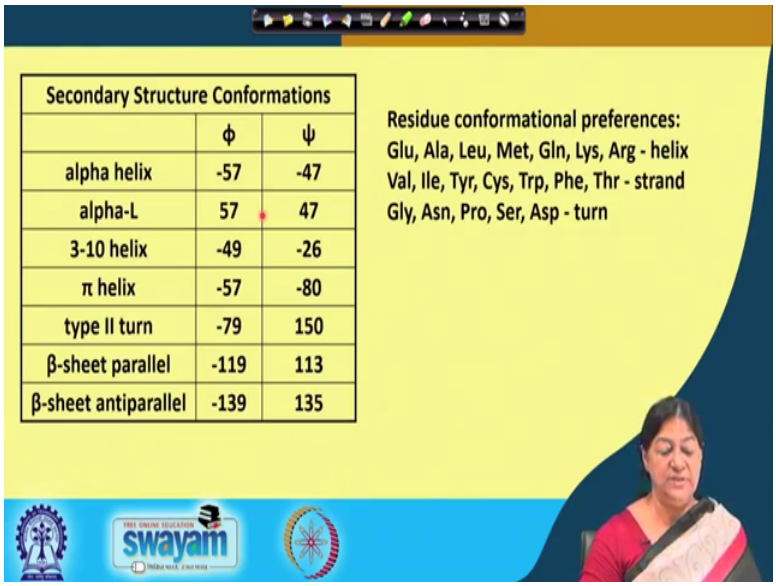

The specific confirmations are given for the alpha helix the different types; a left handed helix, the 3-10 helix, pai helix, the beta sheet parallel, and anti parallel. And there are residual conformational preferences where say that a specific type of amino acid would prefer to remain and specific conformation.

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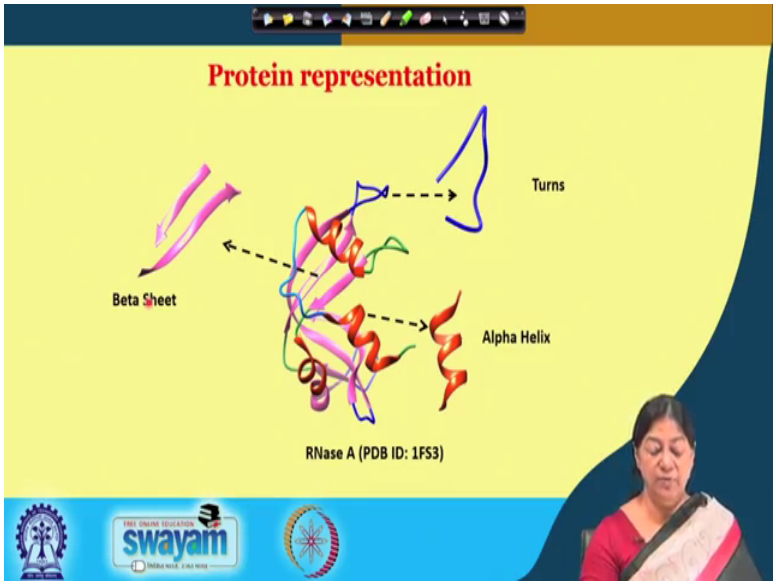

So, if you look finally, at a protein representation the beta sheet represented like for this part the turns that actually form the linkers to the beta sheets and the alpha helix and the

alpha helix represented like this. This gives us a complete protein structure which is ribonuclease a which has a specific protein data bank identifier 1FS3.

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The references that we have considered here are specific books that will have the basics of protein structure. And how they are the stability and their folding is something that we will continue in our next lecture.

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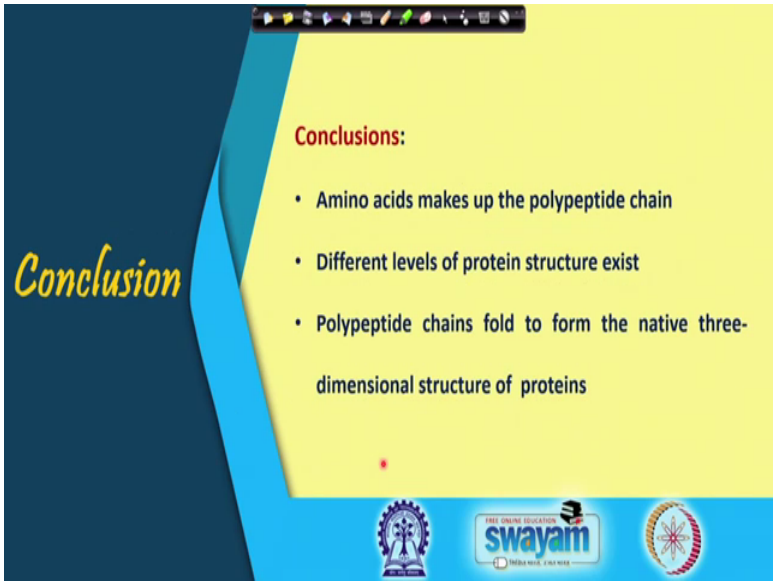

So, what we do understand is that amino acids make up the polypeptide chain the building blocks of proteins. There are different levels of protein structure that exist. We

will be looking at the tertiary structure and the specific folding characteristics and the fold forces that actually hold the protein together in our next lecture. And if the native form that folds to form the three dimensional structure that is functional in nature.

Thank you.