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

Lecture - 06
Protein Structure IV

We complete our discussion on Protein Structures today. And just to recap on what we have done so far.

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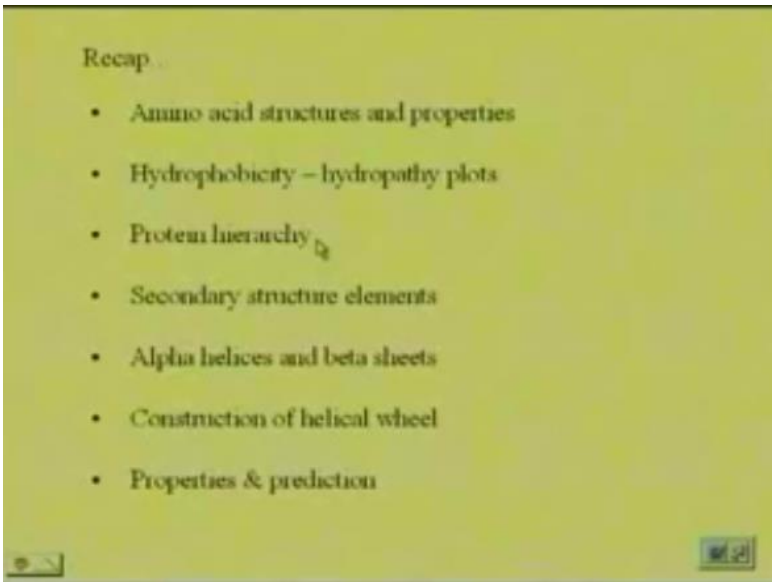

Initially, we studied about different amino acid structures and their properties. We consider the hydrophobicity as the most important property of amino acid and constructed hydropathy plots to determine, which regions of the protein amino acid sequence are actually hydrophobic in nature, which would occupy the core of the protein. We, then, went on to protein hierarchy meaning the different structural properties of proteins from the amino acid sequence to the final quaternary structure, which some proteins adopt.

Then, we consider the secondary structural elements mainly the alpha helices and the beta sheets and their different properties as to how they interact and what are their specific features be at the back bones or hydrogen bonding properties. Then, we constructed the helical wheel to figure out, which regions the alpha helices would be inside or facing the surface of the protein. And, then finally we went to certain properties of protein and predictions of secondary structure.

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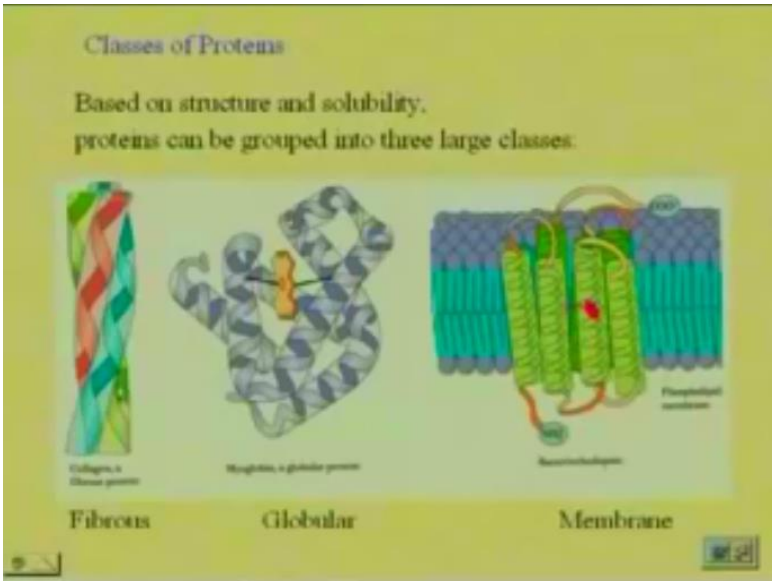

Now, that we know something about proteins, we have to consider the different classes of proteins that are formed based on their structure and solubility. Some of these we have gone over initially, for example, if we look at myoglobin, it is a globular protein, so, it is more or less shaped like a globule, which is the case with most proteins. We understand now on the surface we are going to have hydrophilic residues and within the core we are going to have hydrophobic residues, which tend to remain away from the solvent as far as possible.

The structure that you see here is the haem, which we will be studying later on when we consider myoglobin and haemoglobin specifically. Now, if you look on the right-hand side, we have the membrane proteins. The membrane proteins as we studied last time, are embedded in the phospholipid bilayer that forms the cell membrane and we have these helices that are transmembrane helices.

And, we also did hydropathy plots to figure out, how we can actually determine, which of these helices or where they are going to span the membrane and we also learnt on the surfaces of these helices, it is likely that we are going to have hydrophobic amino acid residues, so that they can interact with hydrophobic tails of these lipids. There is another class of proteins that are fibrous proteins, from the name you can figure out what this construction of it going to be. They are more or less fibres. And they are adopted to give strength to the structure of protein.

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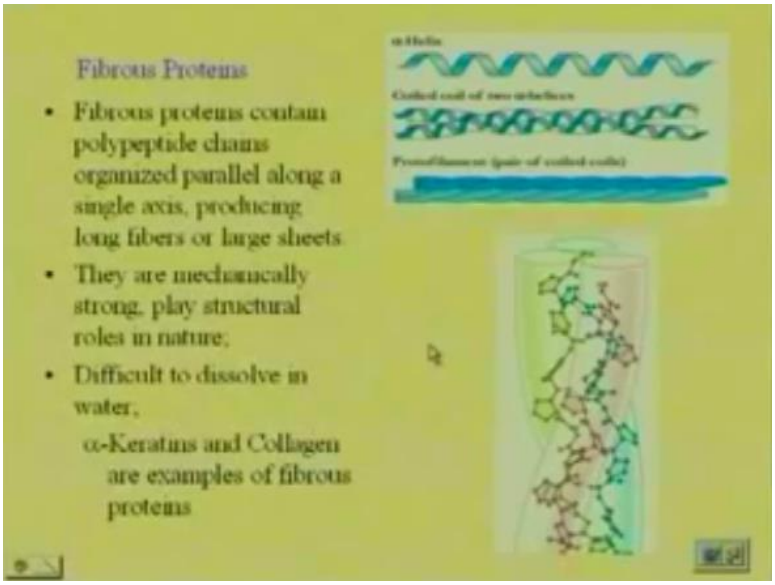

So, what we have in fibrous proteins, as you can see here, if you have a single alpha helix you can have a coiled, coil of 2 helix or you could have, what is called the triple helix as you have in this case. So, the interactions here would result in what is called a fibre and you recognise that these long fibres are large sheets that are actually formed are parts of structures that require mechanical strength.

For example – alpha keratin and collagen are two such examples of fibrous proteins. And because of their strength or because of their structure you find them, for example in hair or in muscles or because, they are fibrous in nature and they form these long parallel structure along a single axis. They give you this longitudinal structure and they give a mechanically strong nature to this.

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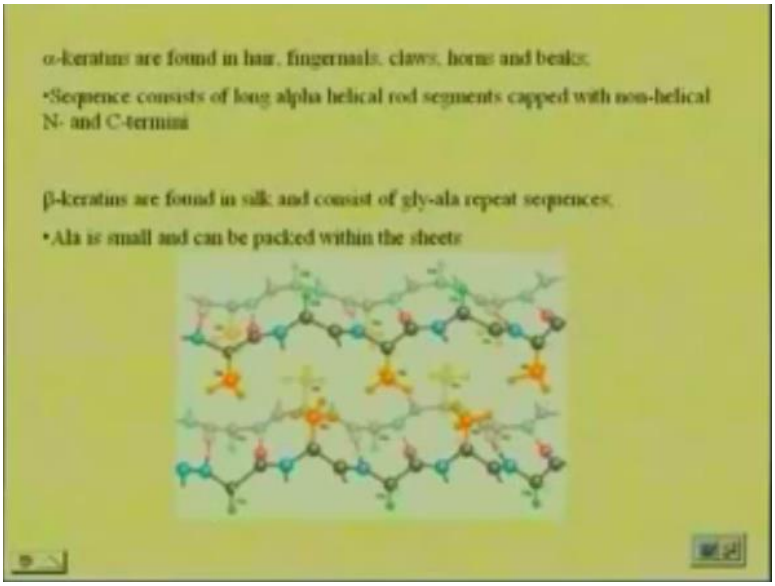

For example – Alpha keratins, they are found in hair. And their sequence consists of long alpha helical rod segments as I showed you in the previous slide. Beta keratins, the ones that we see here, they form as beta sheets. They are found in silk, which is also a fibre but over here, if you look carefully, you can see alanine and glycine repeat sequences as they are called. So, you have alternating, glycine and alanine, the reason being that you know the structures of these amino acids.

They are small, in size and they can also be accommodated in beta sheet structure of beta keratins. So, the alpha keratins are the ones that form the alpha helices. Beta keratins are the one that forms the beta sheets that consists of gly-ala repeat sequences. And, in alpha keratin that are found in hair, what do we see? We see longitudinal parallel alpha helices that give mechanical strength to the fibres that are formed.

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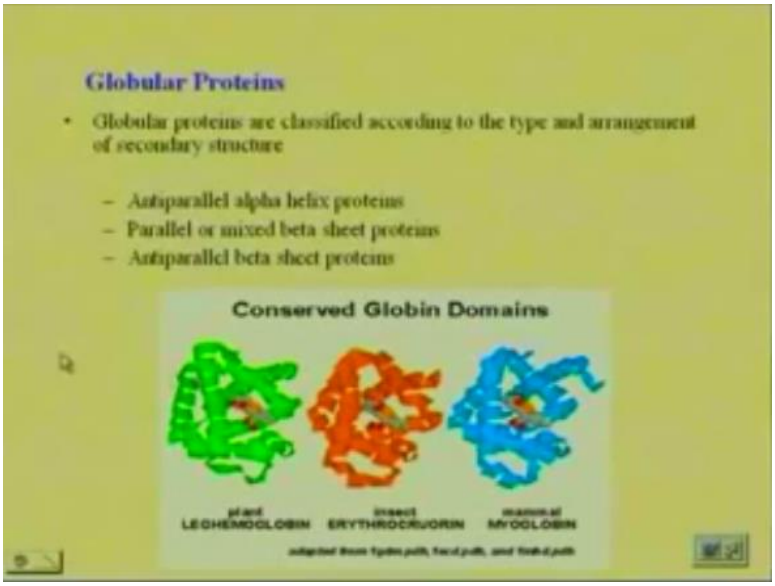

In consideration of globular protein, usually they are classified according to their type and the arrangement of secondary structure. So, what you find is you will probably see anti-parallel alpha helix protein that consists only of alpha helices. You might find beta sheets protein. You might find alpha plus beta or alpha by beta. There are different types of nomenclature, depending on how the protein actually looks.

So in this case, you would have a largely alpha helical protein. You could have, for example, when you are looking at a globular protein what you are looking at this essentially is the globular structure of the protein. Now, if you look at this case, these are all globins. They are globular proteins. This one is from plant. The middle one is from the insect. And the last one

is from a mammal. What do you notice here? You notice that each of them have very similar structure.

The reason being that they are going to perform the same function. So, whether it is from a plant or an insect or a mammal, because these globins perform similar functions, they have similar structures. Now, what we are going to study today is what keeps these structures together? Because, now we know that the only types of covalent bonds that you have in proteins are the linkages between the amino acids that give the peptide bonds and what are the other ones.

The disulphide linkages that you see that are covalently linked together which can do what? They can bring different parts of the chain close by. And, we also now know of the alpha helices and the beta sheets that also bring parts of the structure together and what are they connected by, not covalent bonds but hydrogen bonds.

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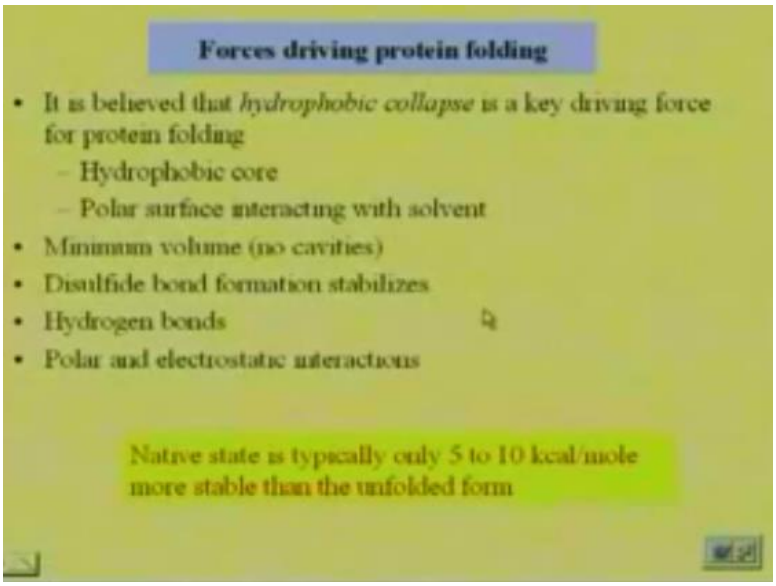

So, what are the forces that are going to drive protein folding, because now we do not have our polypeptide chain anymore, we have a folded protein structure. And, what we need to know is how this protein folded structure is formed? Now, there are certain terminologies here that I am going to go through. One is called hydrophobic collapse. Now, what we mean by this is that you know, the core of the protein is hydrophobic in nature.

So, it may so happen that initially when the polypeptide chain is formed, there is a hydrophobic collapse meaning that there is a hydrophobic core formed first and the rest of the

protein chain, then will gradually form alpha helices or beta sheets or inter connectors such as loops or turns to bring about the three-dimensional structures. So, initially you are going to have a collapse. The polar surface is going to interact with the solvent.

And we are going to have gradual formation of the alpha helices. There may be another case, where the alpha helices are formed first, so there would be local interactions first followed by gradual collapsing of the overall structure. What you are looking for is minimum volume, where you do not have very large cavities because that would open up the proteins. The disulphide bond formation you recognise will stabilise the protein, why? because it will bring different parts of the polypeptide chain close to one another.

Then, you have hydrogen bonds which we studied in detail in case of alpha helices and beta sheets, which will bring different parts of the protein structure together. And again, we have polar electro statistic interactions, which we are also going to study and consider how we actually form these ionic interactions or other polar type hydrogen bonding between amino acid residues.

Now, what we have now is we have what is called an unfolded form that was polypeptide chain and we have native folded form of the polypeptide chain.

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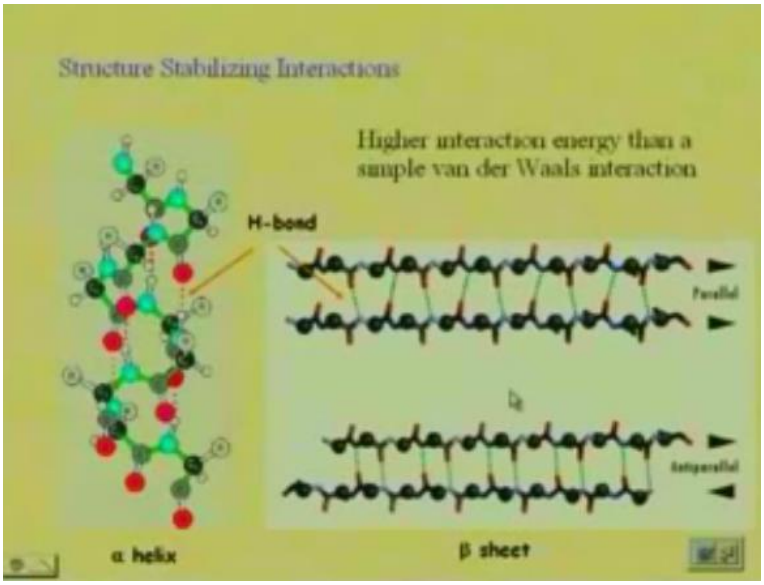

Now, if we look at the hydrogen bond once more and see how it stabilises the interactions, for example - in Alpha helices and Beta Sheets, you recognise that we do have a lot of hydrogen bonds here and what happens is the accumulation of these hydrogen bonds renders

a large amount of stability to the protein. Because, it is not just one hydrogen bond that you have series of hydrogen bonds both in alpha helices as well as in beta sheets.

And you also recognise here that in an anti-parallel case, the hydrogen bonding is between similar amino acid residues and what happens in the parallel case, they are between different amino acid residues. That is very clear here and we also did this in the last class.

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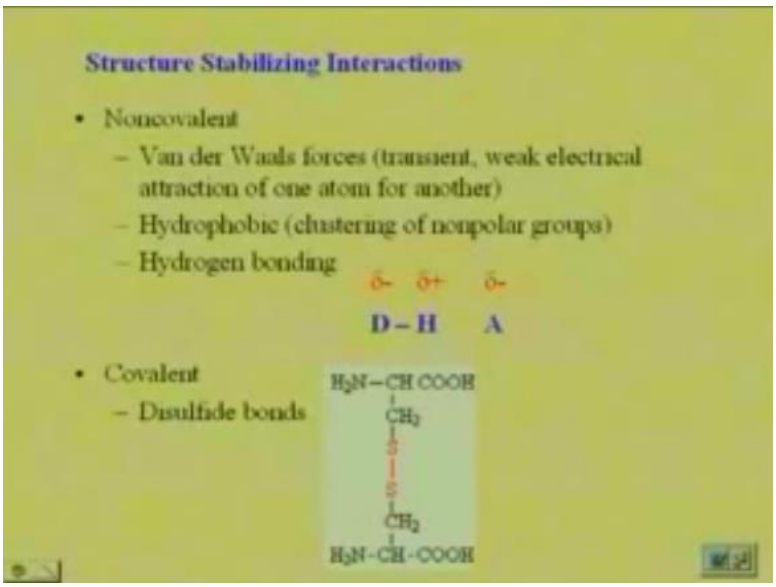

So, what are these structure stabilising interactions? The covalent…we are not talking about the peptide bonds anymore because we already have amino acid sequence. So these amino acid sequence means that the amino acids are linked together to form the polypeptide chain already. But, now we also know that we have these disulphide bonds that actually form from the link of two cysteine amino acid residues in forming a cystine bond, where we have this SS linkage.

Now, these can be very far part in the structure and bring the polypeptide chain close to one another. So, we would have a structure stabilising interaction in the form of a covalent bond in the form of disulphide bonds of the cysteine residues. What are the other cases? The Van der Waals forces that are weak electrical interactions. They are transient in nature. We have hydrophobic. What are these? These are clustering of non-polar groups together, like we form the hydrophobic core of the protein.

Then, we have hydrogen bonding. What do we have in that case? We have a donor atom that has the hydrogen attached to it and we have an acceptor atom. And what happens in this

case? We have the hydrogen atom that is sort of partially shared because of the acceptor atom and the donor atom having partially Delta negative charges and the hydrogen being Delta positive. So, what are all these interactions? All these interactions are non-covalent in nature.

There is no covalent linkage. But, what happens is when you add these all up, you have a substantial amount of energetic favourability for the folded confirmation. So, we are going to study that in a bit more in detail as we go along.

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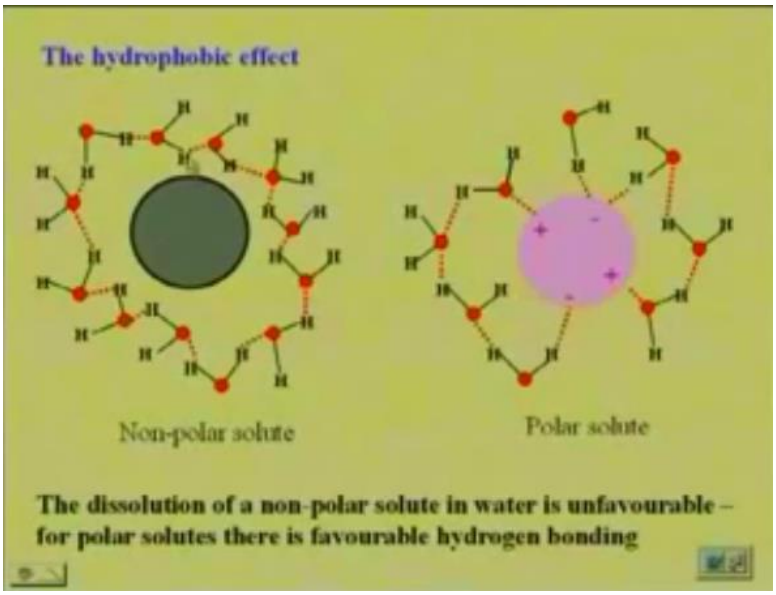

Now, what do we have here? This is called what we mentioned about hydrophobic collapse or hydrophobic effects. When we have a non-polar solute, there is no possibility of hydrogen bonding. Why? Because we do not have any polar group or any group that can act as a donor. What do we have to have? We must have a donor and an acceptor. But, in the case of polar solute, what do we notice? In case of polar solute, we see that there is the possibility of hydrogen bonding.

So, when we have, what do we expect on the surface of the protein? All these polar residues that can interact with the solvent will preferentially be on the surface of the protein. However, the non-polar solutes that have no such interactions with the solvent around it, would tend to be in the central part of the protein or rather what we call the core of the protein, the hydrophobic core.

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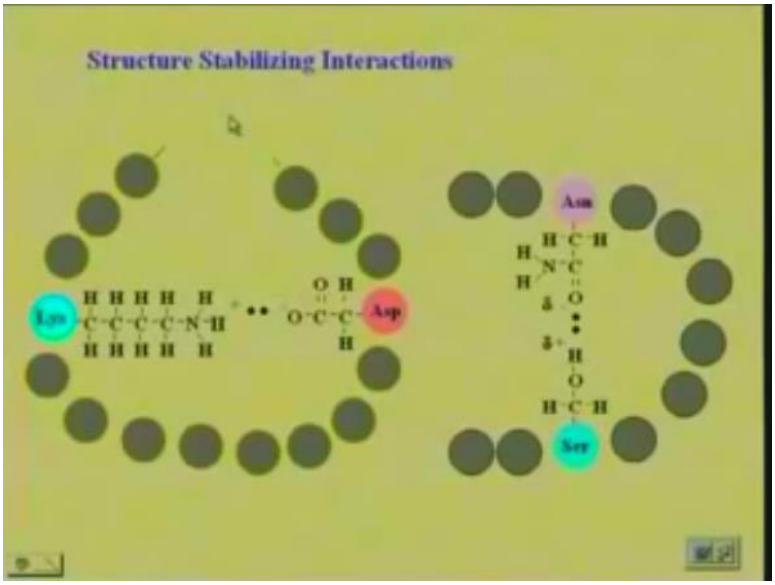

Now, this is an example of a structure stabilising interactions. What do we have here? We have an ion pair. What is an ion pair? An iron pair links lysine and aspartic acid together because you have to remember that it is not always possible for all the lysines or all the aspartic acids or all the basic acidic charged amino acid residues to be on the surface. The reason being that it may so happen that it follows the large hydrophobic region.

So, that would tend to be in the core. Then, the covalent bond is not going to break. But, the lysine is there, so what is going to have, is what is called an ionic interaction or what is called an ion pair formation with a charge of an opposite kind in the protein chain itself, so that is going to effect, the folding because the lysine is going to be looking for an acidic amino acid residue close by, so that it can form this iron pair.

So, what it is that going to do? It is going to change the folding in sense. It is going to effect, the folding. If you have all these hydrophobic in nature, then the nature of the folding would definitely have been slightly different from what you see here. What do we observe here? We observe the lysine that is a basic amino acid residue have an ionic interaction and ion pair formation with an aspartic acid residue. What do we see here?

This is the case, where you can have hydrogen bonding. We do not have ion pair formation here, but what do we have. We have a donor. Who is donating? Serine is donating. And we have an acceptor that has the carbonyl oxygen of the amide group of the asparagine. So, this would be the formation of a hydrogen bond and this would be the formation of ion pair. Both of these are extremely important in forming a final protein folded structure.

So, what do we have? Now, when we consider energy terms, what are we talking about an energy terms? Just look at what energy terms, we can have.

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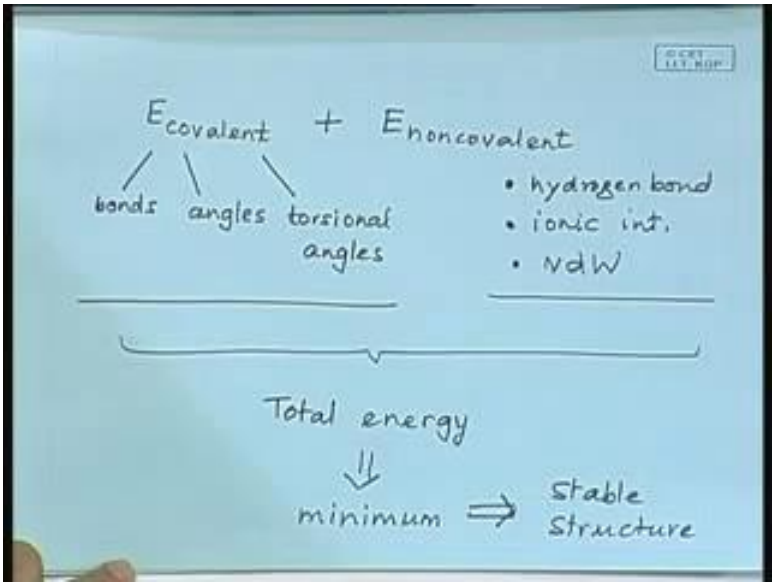

If we are looking at energy, then we can have total energy covalent or we can also have a non-covalent component to this. When we are looking at the covalent energy components because this is contributing to our total energy. Now, under covalent ones what do we have? We have bond formation. Normal bond formation that is going to contribute. So, we have bonds. What else do we have? There is a contribution from angles.

There is a contribution from… what other do we have, Torsional angles. So, we have energy contributions from these three components under my covalent set because, these are formed from direct linkages. What about the non-covalent? What are the ones that we listed? We listed a hydrogen bond, we listed ionic interaction and what else do we have? Van der Waals interaction.

So, these are the three we could have in case of a non-covalent formation. And, these are the three we are going to have for covalent bond formation. And summing these together, we are going to get the total energy. Now, what do you want this total energy for the protein to be a minimum? We want this to be minimum, so that we have a stable structure. So, total energy we have to get a minimum, which is going to result in a stable structure.

What is the structure? It is the final native folded form of the protein. Now, what are these in terms of energetics? If we look at the bonds, how do you represent bonds just normally for a compound? You represent them as springs.

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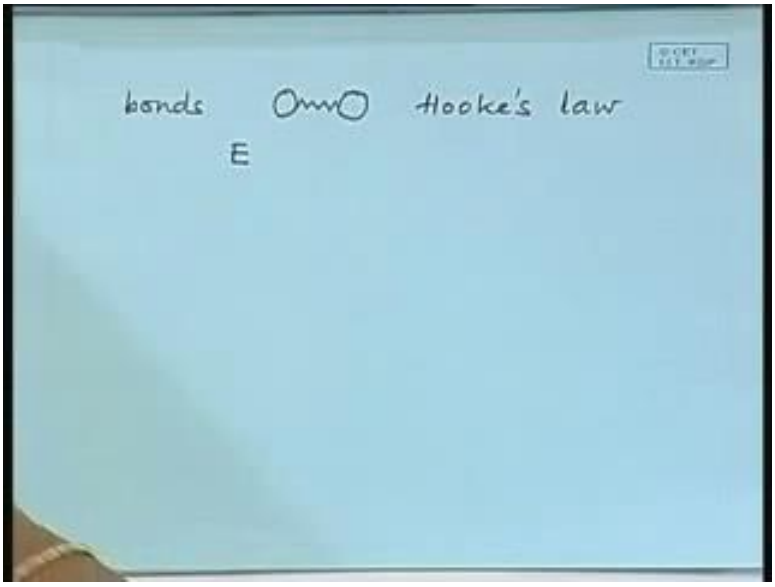

What do we have in case of bonds? We represent these as springs and they follow Hooke's law. All of you know what Hooke's law is? When you have 2 masses connected by a spring they are going to give some energy following Hooke's law. What is that? That is this. What is this energy due to? It is due to stretching energy.

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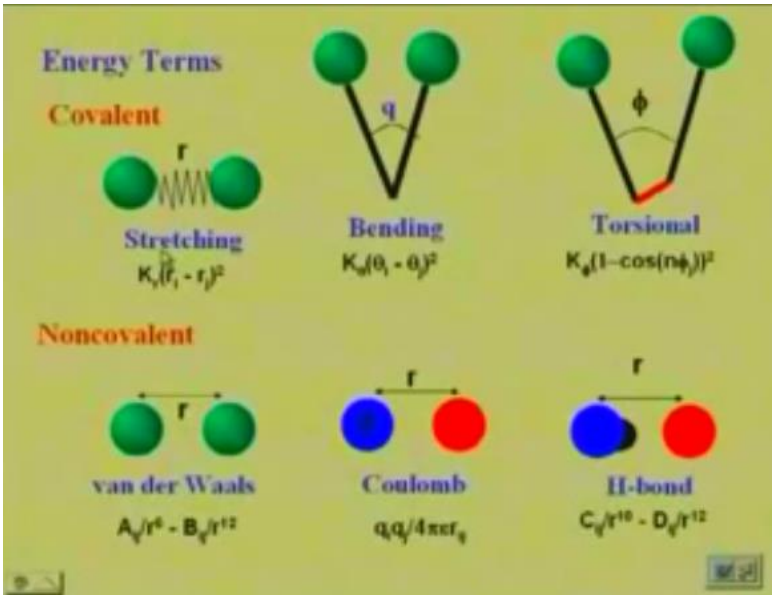

This follows Hooke's Law. What is this R? The R is the variation that you will get because the bond is not going to stick to one specific distance, it acts like a spring. Why? Because it is going to form certain interactions. So, it has to act like a spring, where it is going to have

some favourable interactions that it is going to try to adopt or it is going to have some unfavourable interactions, which is trying to going to get away from.

So, what we have here is we have our covalent. What do we have in our covalent? We have our bond. Our bond is going to give us an energetic contribution to the total energy in the form of Hooke's law. What do we have for the angles? We have a bending that is going to contribute to the energy also. These are called energy terms that are added to the total energy that the protein is going to have.

Now you understand, this is going to get extremely complicated if you want to do a competition. Because the number of atoms are huge. But, never the less these are the components that form the total energy. We also have the torsional energy. What you need to know is what are the contributions. You need to know that the covalent terms come from the stretching, the bending and the torsional.

What about the noncovalent? What are they going to come from, we are going to have an ionic interaction, we are going to have Van der Waals interaction and we are going to have a hydrogen bonding. So, Van der Waals interaction, all of you know about the Lennard Jones potential curve, where we have an attraction due to the negative part of this, A 6 12 potential is, this is called, where we have a contribution for attraction and a contribution for repletion, because you know these atoms have a repulsive force acting on them, when they get too close to each other.

Then, we have Coulombic attraction. What is this? This is due to the ionic introduction between the two ion pair formers. What else are we left with? The hydrogen bonding. So, this is another feature, where we have the hydrogen bonding, again we have 10 12 in this potential. Now, let us look at this one once more, what are we talking about here? We are talking about the total energy that a peptide would have.

A poly peptide would have based on its energy terms. Now, what are these energy terms? We have energy terms contributions from the covalent component and contributions from a noncovalent component. Now, the covalent component is comprised of three parts. These three parts you have to remember from the name itself, why are these covalent? Because, these are directly linked.

You have to remember that if you have a spring, it means that the two atoms we are talking about here are directly linked to one another. If we are talking about an angle bending energy, these are linked to one another. If we are talking about torsional energy, the atoms are linked to one another. But, none of them are linked in a noncovalent formation. I do not have any bonds linking the two atoms that have been drawn in the noncovalent set.

So, when we were talking about the covalent contribution, we are talking about energy contributions from parts of the protein structure that are linked to one another. One is the bond, second is the angle and third is the torsional part. For the noncovalent set, we have the Van der Waals, the coulombic attractions and the hydrogen bonding.

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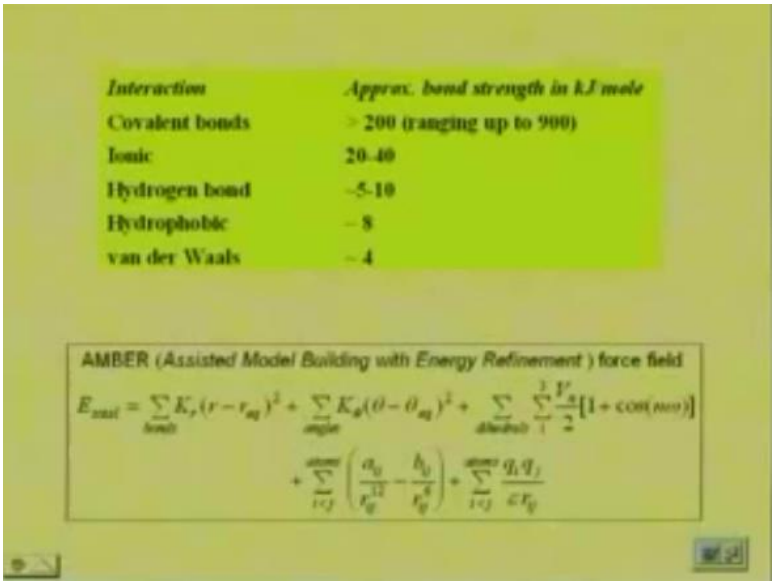

Now, if we look at the interactions and their approximate bond strength in kilojoules per mole, you see that covalent bonds are extremely strong. Ionic bonds are 20 to 40 kilojoules per mole. Hydrogen bonds are approximately 5 to 10 kilojoules per mole. Hydrophobic are 8. Van Der Waals are approximately 4. But, when we consider a combination of the large number of hydrogen bonds, Van Der Waals, hydrophobic interactions that are possible.

They add up together, making it a strong folded structure, because of the multitude of interactions that are possible. Now. what have I listed here, is what is called an energetic contribution due to, suppose for example you were to do a calculation to figure out, what the total energy of protein was? You would use something called a force field. I am not going into the details because it is beyond this course.

But, since we consider the different energetic terms, I just want to emphasis here that when you are calculating the total energy, what you want to do is, you want find the summation of all the possible variations from equilibrium bonding for bonds. So, what are we looking at now? We are looking at all the covalent interactions the bonds, the angles, the dihedrals. In this case, here this is a crude one because I have not considered the hydrogen bonds here.

What do I have here? This is for Van Der Waals, this is for coulombic attractions. But, I am missing out hydrogen bonds because you understand that when you are doing a computational calculation here, it is going to be extremely difficult to try and calculate all the bonds. Because, as soon as you form a dipeptide, you recognize, how it is going to change or increase the number of bonds, increase the number of angles and increase the number of dihedrals.

So, we have an extremely large number as you go from a single amino acid to a dipeptide to a tripeptide. So, if you consider even the smallest protein that would have approximately 30 amino acid residues that would be extremely difficult to compute. Because you can understand the multitude of introductions that are going to form, based on the number of atoms that you are going to have in it.

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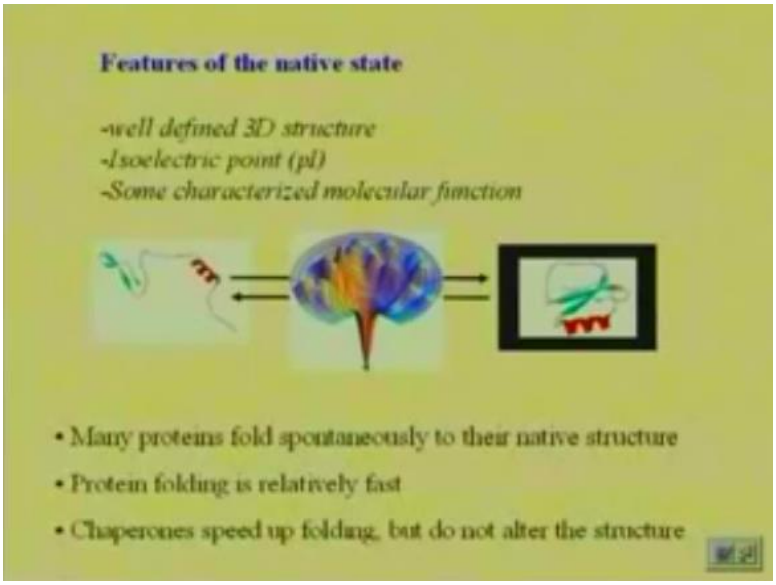

So, what do we have? We have a final native structure. What is this native structure? We have a well-defined 3D structure, it has a specific function and we remember we spoke about the specific isoelectric point due to the type and number of amino acid residues, it has. So

what are we going from? We are going from a relatively unfolded structure to a folded structure. So, basically, I spoke about (()) (27:18) last time.

Where there were a multitude of open conformational was possible for the polypeptide chain. But, it formed a single native structure. So what is happening now, is when it forms the native structure, we have all these interactions that are taking place now. Hydrogen bonds between the alpha helices, hydrogen bonds in the alpha helices, hydrogen bonds between the strands of the beta sheets.

Then, what are the other interactions that we can have? Disulphide interactions that are going to form the covalent interactions, hydrophobic interactions, Van der Waals interactions and other electrostatic and coulombic hydrogen bonds and all others between the other amino acids in the protein.

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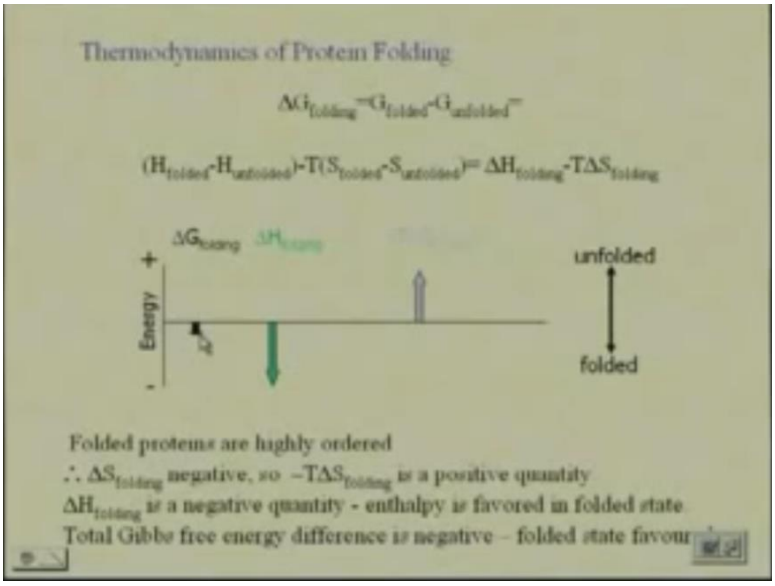

Now, we are going to consider the thermodynamics of protein folding. If we consider an initial and final state, what do we have? We have a Delta G. what is this Delta G? We want to find the free energy change due to folding of the protein. So, initially we had an unfolded state that is G initial. We have now a folded state, so that is G final. So, how do we find the Delta G folding? We know that it is the G final which is G folded minus, G initial which G unfolded.

So, what do we have to consider? We have to consider; we all know that Delta G is what? Delta H - T Delta S, so I have Delta H folding minus T Delta S folding. So, what is my Delta

H folding? My Delta H folding is again going to be my H final minus my H initial. So, H folded minus H unfolded. Now, I am doing this in a particular temperature so the temperature is constant. What is my Delta S? S folded minus S unfolded.

Why? Because my unfolded is again my initial state and my final is my folded state. Now, we are going to look at the specific terms of these. Now, what happens to my Delta, my S unfolded and my S folded? When, I consider my entropy, what is entropy? The entropy is the disorder of the system. Now, if I have an unfolded protein and I am now having the protein fold, I am bringing order into the system. Right?

Now if I bring order into the system, what happens to my S? My Delta S is negative. It is not positive anymore. Why? That's because I have ordered the disordered polypeptide chain into an ordered folded native structure. So, my Delta S folding is negative. So, what happens to my T Delta S? My T Delta S is therefore a positive quantity, minus T Delta S because my Delta S folding is negative, minus T Delta S is a positive quantity, which is what I have drawn here.

So, if you look at the energy on the y axis and on the energy axis here, we have an unfolded form here and a folded form here. Now, obviously the entropy of the unfolded form is going to be much more than the entropy of the folded form. So, my overall minus T Delta S is going to be positive, but the enthalpy or the folding interactions that are formed are all favourable, why? because the hydrophobic interaction is going to be favourable.

Hydrogen bond is going to be favourable and ionic interaction is going to be favourable. What is not going to be favourable is, if I have a specific hydrophobic interaction trying to make with the solvent. That is going to give an unfavourable situation, but for most of the cases in the folded protein, I am going to have a large number of interactions contribute to the enthalpy and this multitude of interactions which makes is a high negative number.

So, what happens is these more or less compensate one another and I eventually have a favourable Delta G of folding. Let's go with this once more. We have a Delta G of folding that I am considering. What is Delta G of folding? I am going from an unfolded state to a folded state. So, I have to consider in the thermodynamic quantities at Delta H minus a T Delta S of folding.

I know that the entropy of a polypeptide chain in the open form is going to be much more than it is going to be in its closed folded form. So my Delta S folding is negative, which makes my minus T Delta S, a positive quantity. Then, I have Delta H folding, which compensates for this positive ordering of this system because of the large number of favourable interactions that are formed on protein folding, which are all the energy terms that we spoke about in the non-covalent states.

We have the Delta G of folding that is a small negative quantity. What does this mean? It means that this is a favourable spontaneous process. So, I am going to go from my unfolded form to the folded form in a spontaneous process. Why? The Delta S is not going to make it spontaneous. It is the enthalpy that is going to make it spontaneous. We have thermodynamics of protein folding.

Now, what we have here is, if we consider the Delta G of folding, we have the initial part, which is the unfolded part and the final part, which is the folded part. So, when we are considering the Delta G, we go from the initial unfolded form to a final folded native structure. So, the Delta G is G final that is G folded minus G initial that is G unfolded. If, we now consider the form of Delta H folding minus T Delta S folding, we recognize that we can also open these up into the initial and final components.

So, the Delta H folding is going to be H final that is H folded minus H initial that is H unfolded. Similarly, for Delta S we can have S folded minus S unfolded. Now, if we consider the entropic consideration, if we go from an unfolded form that is disordered to a folded native conformation the entropy contribution Delta S is going to be a negative quantity, why? because we are ordering the system.

A positive quantity is when we have more disorder. A chaotic situation would give you favourable entropy, but we are bringing order into the system because we are folding the polypeptide chain. So, our Delta S is negative. So, minus T Delta S is a positive quantity which is what we have here. It is positive in its energy contribution, but the Delta H folding is a negative quantity due to the different interactions that give favourable energy to the folded conformation.

Due to that, we have this positive energy that is due to the entropical considerations compensated by the enthalpy due to the large number of interactions possible. So, the overall Delta G folding is a small but negative quantity meaning that you have favourable spontaneous folding from the unfolded to the folded native protein.

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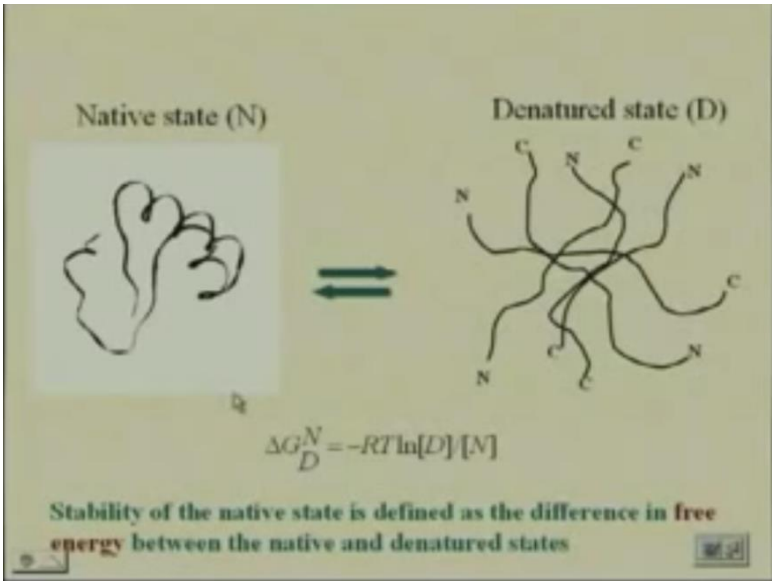

Now, if we consider the native state and the denatured state, this is in an equilibrium situation. The beginning of the protein begins at N and it ends at C. So, we have a disordered orientation here that is also called an unfolded state or a denatured state and we have a native state in this case. So, if we have Delta G associated with it, we are going to have equilibrium associated with it. So, we have K that is minus RTLn, K being the equilibrium between the denatured state and the native state.

So, the stability of the native state if defined as the difference in the free energy between the native and the denatured state.

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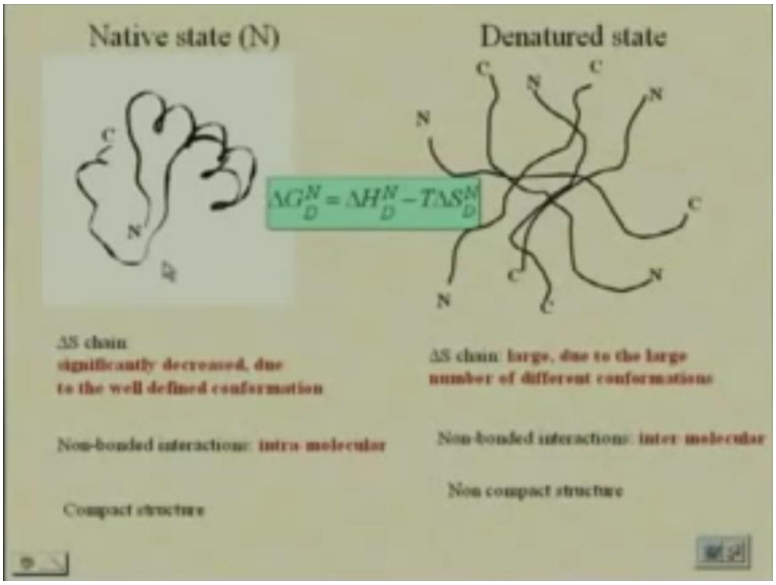

So, what do we have in the native state? We have a compact structure, intra molecular non bonded interactions and the entropy significantly decrease, because of the well-ordered conformation. What are the features that we have for the denatured state? We have a non-compact structure. We have inter molecular non bonded interactions. We have a large Delta S due to the large number of different conformations.

All of these contribute to the Delta G of the system, the Delta G of folding, which is going to be a small but negative quantity because we are going to have spontaneous folding from the denatured state to the native state.

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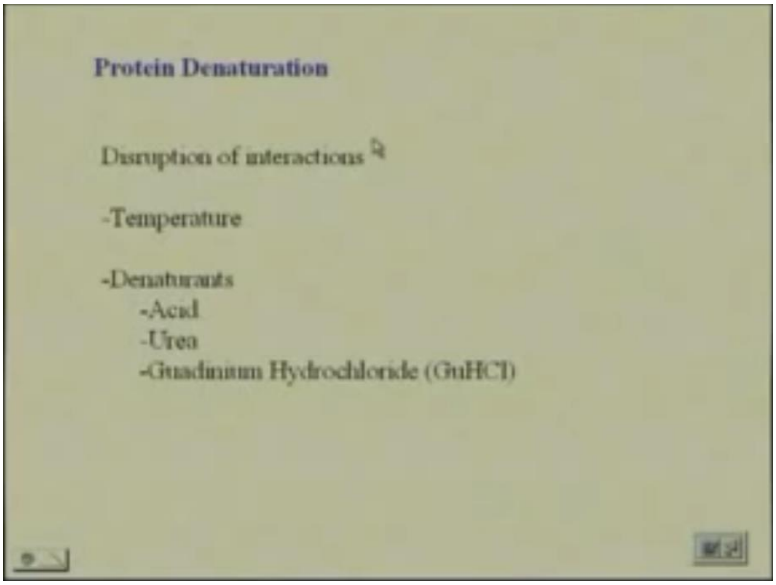

Now, if we consider the different interactions that we had, what were the different interactions that we had? We had hydrogen bonding, electro static interaction and Vander

wall's interaction, hydrophobic type interactions. Now, I can denature the protein if I add certain denaturants to it or I increase the temperature. Now, we have to consider that in the process of denaturing, I am disrupting the actual interactions that were holding the protein folded together. So, if I look at all the conformations that I had.

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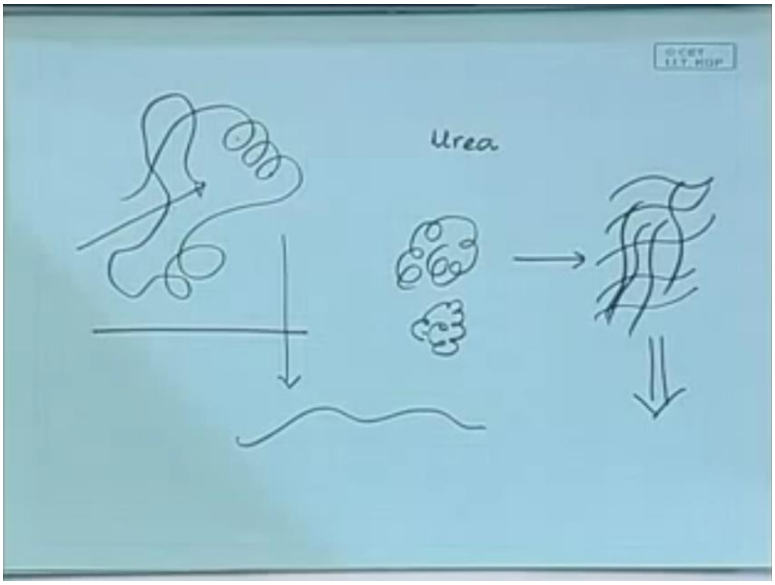

So, if I had a protein helix structure and I had some sheets, what do I have? In this folded structure as I add denaturant, say I add urea. Urea disrupts the hydrogen bonding. Now, if I disrupt the hydrogen bonding of the alpha helix, what is it eventually going to do? It is going to open up the helix, because the hydrogen bonds that were present here would preferably form with urea. So, urea sort of gets into the protein structure and disrupts the interactions that were holding the folded or the protein polypeptide chain together.

Now, what happens if I heat it? For example, if I heat albumin like egg, what happens? You form a solid mass. Basically, what are you doing? You are denaturing the protein. So, if you have solid globular albumin structure when you heat it up, what are you doing? You are decreasing the solubility or aggregating it because you are opening the polypeptide chain up. As you open the polypeptide chain, what happens?

You have this mass formed and each of these structures are supposed to look identical are opening up into a denatured form, forming a solid mass of the aggregate and you have now, a denatured protein now. This is the denaturation due to temperature. So, what we are doing is, if we go back here, essentially what we are doing in protein denaturation is we are disrupting all the interactions that are possible.

What were these interactions? They are hydrogen bonding, Vander wall's interaction and ionic interaction. What can we denature proteins by?

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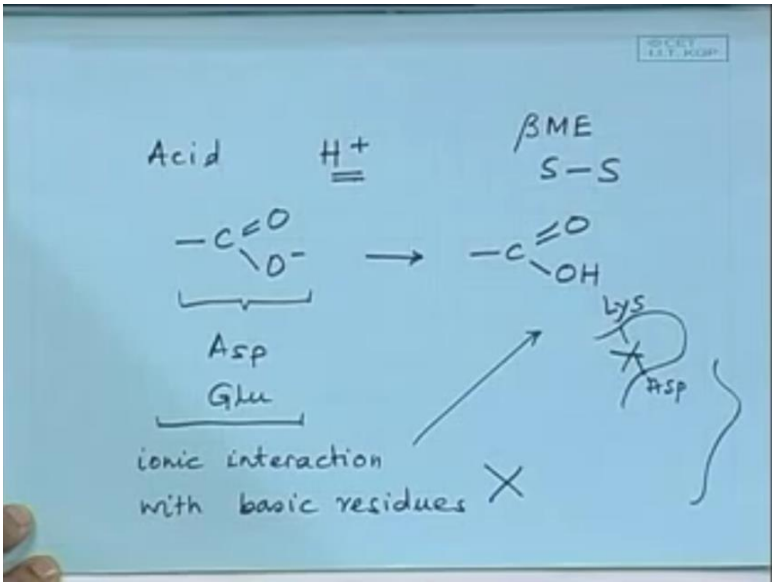

By increasing the temperature and we can also add different denaturants for example acid. What happens if I add acid? It means I am adding H+. What happens when I add H+? For example, all the acidic groups, what comprises these groups? It is aspartic acid or glutamic acid. What is going to happen to them? They are going to get protonated. So, if they get protonated, what do I get? This is what is going to happen on addition of acid.

Now, if this aspartic acid or glutamic acid happened to be involved in ionic interaction with silicine, what would then happen? It would disrupt the ionic interaction. Right What are we saying? We are adding acid to our protein. Now, when we add acid to our protein any acidic group that had the free carboxylic group in its ionized form would get protonated and if this aspartic and glutamic acid that could form an ionic interaction with basic amino acid residues.

What would happen to those? They would no longer form. Once I get into this form, it is not possible for the ionic interactions to form. So any folded structure that would have this ionic interaction with Asp and Lys, what would happen? This would no longer form. What would that do? That would open my chain up. So eventually, the idea here is that when you have a denatured structure you are disrupting the energetics.

Denaturation usually does not mean that you are disrupting covalent bond formation. So, if you had a disulphide linkage you would not actually be disrupting that unless you would put in say beta mod cap to ethanol or you would have some other reagents that would break the S-S linkages, because that you have to remember is a covalent linkage. You are also not breaking the peptide bond.

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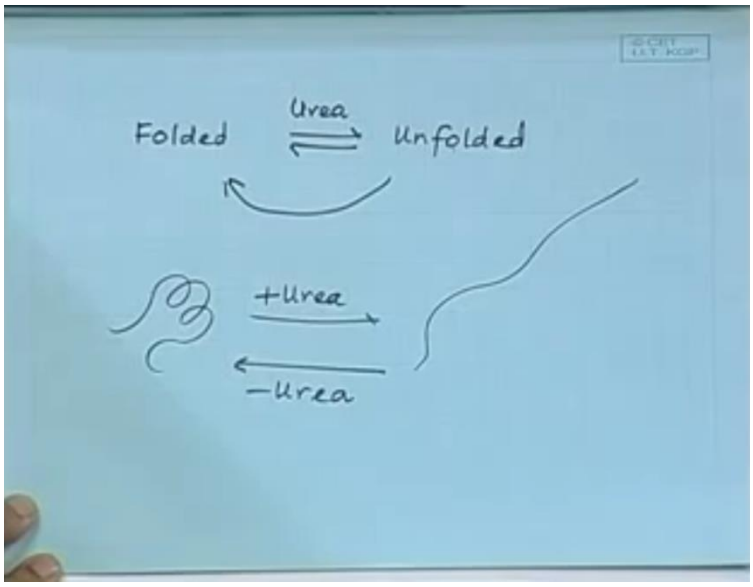

The polypeptide chain is intact. So, what we can do is we can go from a folded form to an unfolded form by denaturing the protein. You can also what is called renature the protein in some cases. So, what you would have to do? Say you added urea. What you would have to do? You have to remove the urea if you want to get back here and in some cases, you can reform the folded protein.

So, you are going from your native folded structure to an unfolded structure and you can reform this back if you, so this would be plus urea, minus urea. So, you can form your folded structure back again.

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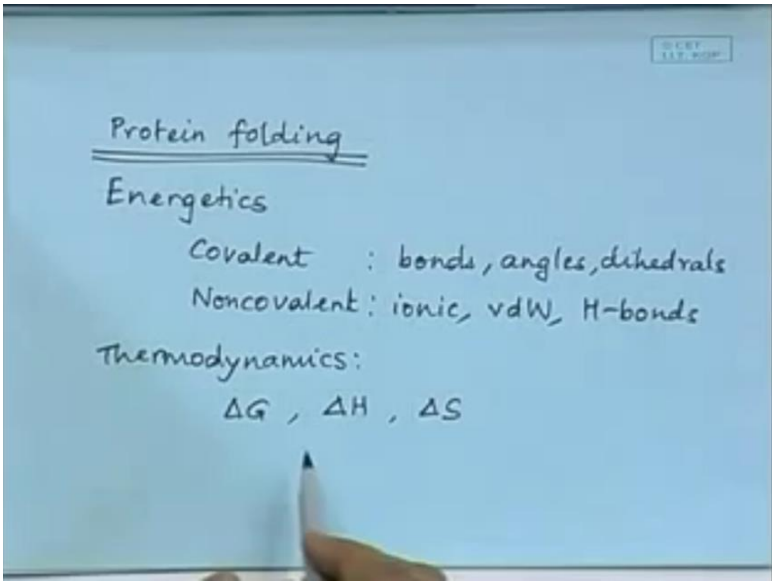

So, what we learnt today was we learnt about the protein folding, the energetics of protein folding, where we have covalent and non-covalent contributions. The covalent contributions come from bonds, anything that's connected, angles and dihedrals. Non covalent has ionic, Van der Walls and hydrogen bonds. Those are going to contribute to the energetics of protein folding. Then, when we consider the thermodynamics of protein folding, what do we have?

We have Delta G associated with the folding. We have Delta H associated with it and we have Delta S associated with it. All of these fall under the preview of protein folding, where we are considering the thermodynamics, the Delta G, Delta H and Delta S. And we have an equilibrium consideration here as well. We will stop here. Thank you.