Biochemistry
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Lecture -02
Amino Acids II

Ok, we start off with the discussion on amino acids.

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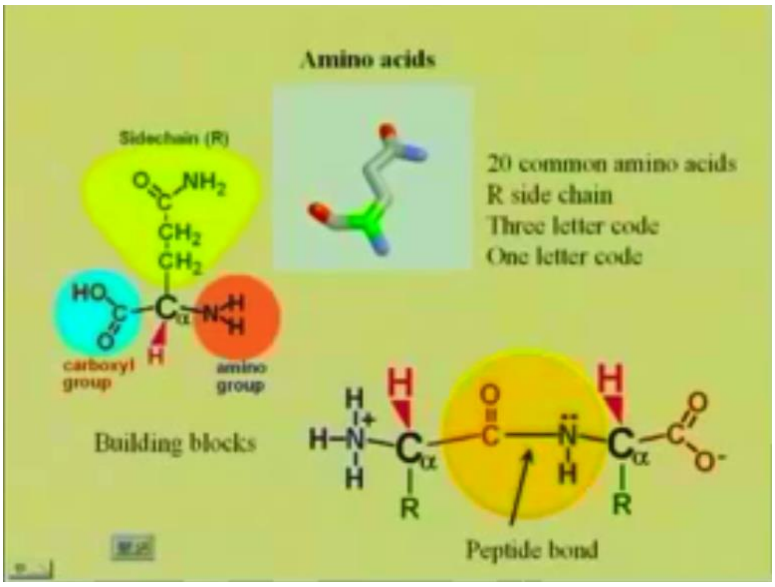

As we saw last time, the amino acids consider or have rather, an asymmetric carbon atom to them. To this carbon atom is attached an amino group and a carboxylic group. And we have the different side chains; we found out that we have twenty common amino acids, with twenty side chains, twenty odd side chains, which differ greatly in the property that they have and we have associated with them a three-letter code and one letter code.

If we go to this amino acid, this side chain corresponds to the amino acid, Glutamine. The three-letter code for Glutamine is GLN and the one letter code is Q. It is something that you have to remember regarding the amino acids. Now, if you notice in the structure that we have here, that is called as stick structure, in the stick structure, we do not see the hydrogens. The nitrogens are always marked in blue, the oxygen atoms are marked in red.

The carbon atoms are marked in green, that is for the asymmetric carbon and the others are marked in grey. Do you see that clearly? Now this is universal. Nitrogen atoms are always in blue, acidic or rather oxygen atoms are always in red and carbon atoms are in grey. Now, the

next thing that we looked at was the joining of the two amino acids. The joining of the two amino acids gave raise to what is called the peptide bond.

Now, when we look at the peptide bond, we see that, we have associated the carboxylic group of the previous amino acid, with the amino group of the next amino acid. So, this R (left side R in the peptide bond) group corresponds to the first amino acid of this particular dipeptide and this R (right side R in the peptide bond) group corresponds to the second amino acid of the dipeptide, which means that in a polypeptide chain.

You always have the first amino acid begin with an amino terminus. It is never written any other way. You always have the first amino acid in a protein, in a polypeptide chain, as the amino terminus. It always is an NH2 terminus. And the carboxylic terminus is always the end of the protein chain.

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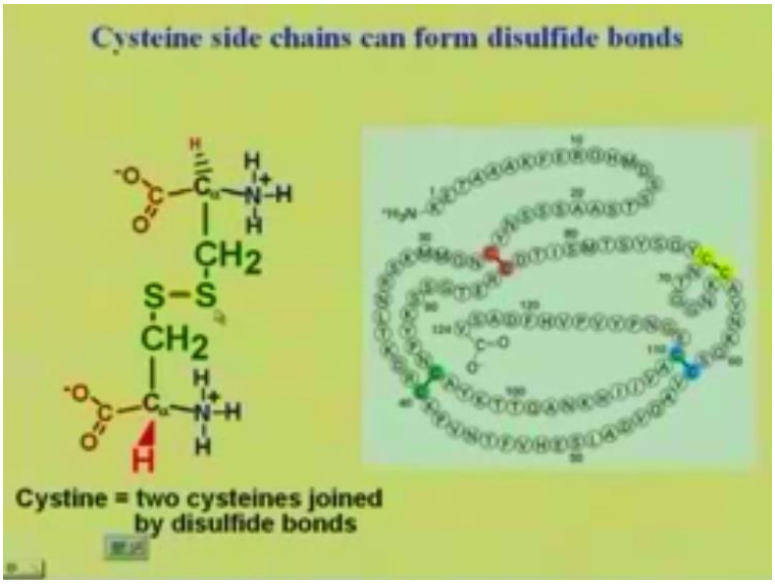

Now, if we go to the next Slide, we just have the one that I showed you earlier, where we have the elimination of water to form the peptide bond. And in this case, we have a tripeptide and from the R groups, we can identify the peptide. Now you understand that, as we have a protein chain or polypeptide chain, this is going to get longer and longer. Now, as it gets longer, we have here in the peptide bonds, Glycine because it has two hydrogens.

We have Alanine because it has just the methyl group attached to it and this is Cysteine because it has SH group attached to it.

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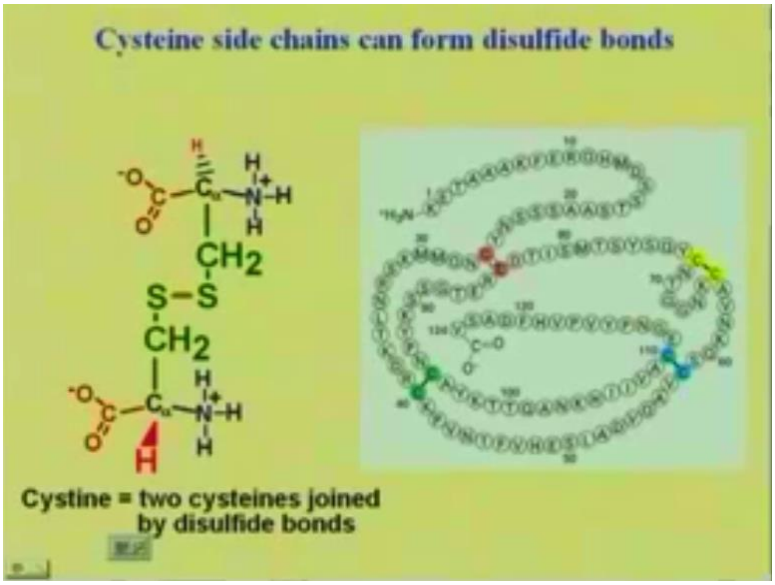

And we found out also, that this Cysteine amino acid can form what is called a disulfide linkage. So what we saw in the previous Slide was the formation of a peptide bond. How is the peptide bond formed again? It is formed by the elimination of water between two amino acids. So, we have the carboxylic group of one amino acid, lose its OH, with the H of the amino group of the next amino acid. And that is a strong covalent bond that is formed.

The only other covalent bond that is formed in proteins is this disulfide linkage that is formed by the linking of two Cysteine residues that may be following the protein chain, to form what is called the disulfide linkage that is the S-S linkage. This S-S linkage, that is shown in the above slide.

Now, I mentioned about this experiment, where the whole protein chain was unfolded and it was found that exactly the same disulfide linkages were formed. I will just go through that experiment once.

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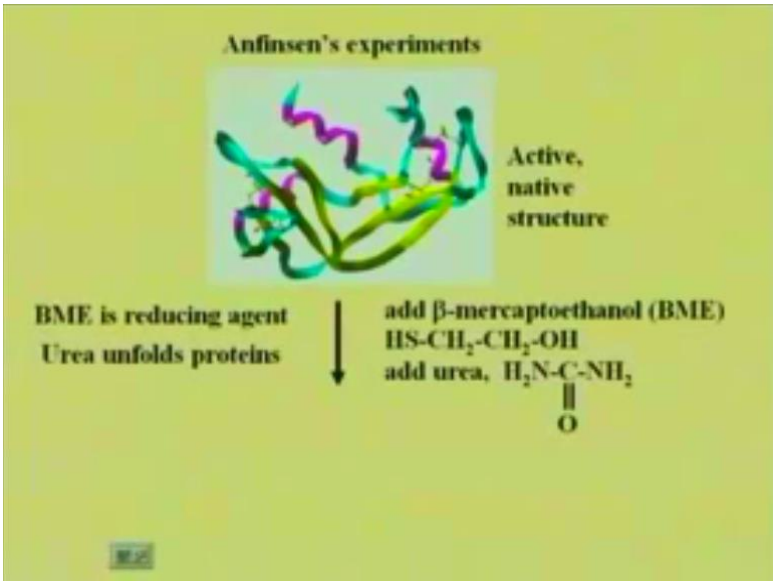

Now, when we look at a protein structure, which we will be doing in the other classes to come, this is what is called a native structure, an active native structure. What does an active structure mean? It means that, this protein or this enzyme has its function intact. So when we are looking at this active enzyme, we know that its enzymatic activity is to the fullest, because it is a folded protein, it is in its native structure.

Now, if you add beta mercaptoethanol to this, beta mercaptoethonal will reduce the S-S linkages, to form SH. So, your cysteine S-S disulfide bond is now, a cysteine residue over again. So now, we have added beta mercaptoethonal, the structure of which is given below, just the BME that is written here. Then we add urea. What urea does, is we learn again later, is it unfolds the protein.

Now, what do you mean by unfolding the protein? It means that all the interactions that occur in the folded protein, which we will be studying as we go along or understanding protein structures, the whole thing is unfolded to form a long polypeptide chain.

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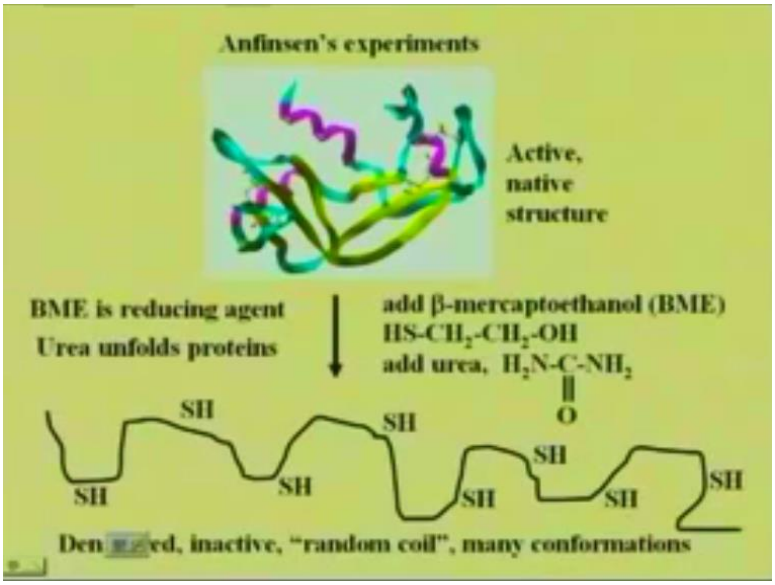

So, that is what it is going to look like now. Why, because we have the native structure, we had four S-S linkages. Now, what happened to those four S-S linkages? We reduced the S-S linkages to form SH. Then what we did is, after we form SH, we added urea. Once you add urea, what happens is it unfolds the protein completely. So, now we have this long chain, with eight SH bonds to it.

So, what is this? It is the denatured, inactive, random coil. What is the random coil mean? It means, it is not folded any more. It is just like any necklace or to speak with beads on it. It is not folded. So, now what happens is, the SH is now a free to link with any other SH. You have eight SHs. So, number one could link with number five or it could link with number six or with seven. So now, when we look at this structure, we believe that the SHs could practically or possibly link with any others.

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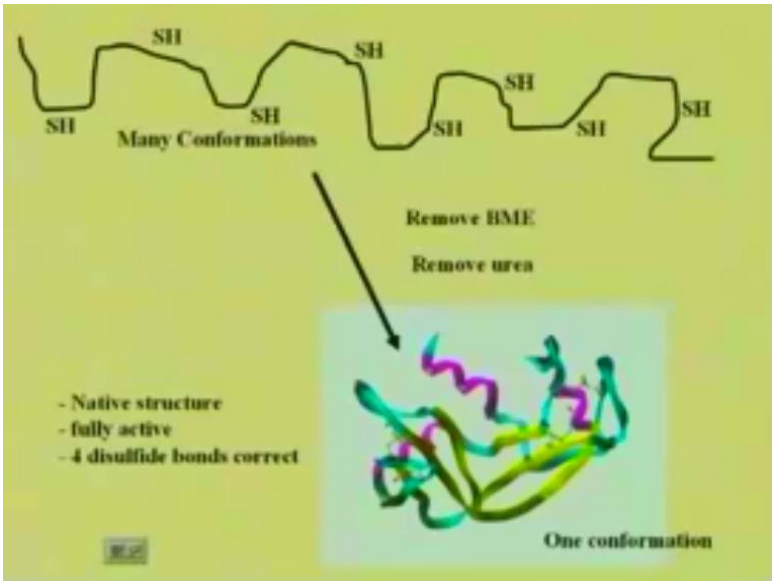

Because, there are many such conformations possible. But now, what actually happens is, we remove the beta mercaptoethonal. In the removal of BMA, what we are ensuring is that, these SH linkages or rather the S-S linkages can now reform. Because, we are now, instead of reducing it, we are oxidizing it. So, if we look at these SH linkages that can form, we understand that since there are many confirmations possible, it might form any one.

Then what we do is, we remove urea. So, what I am doing? I am allowing the formation of S-S linkages; I am allowing the protein to refold. In doing so, it is found that it forms the exact same structure that was there before. What is this structure? It is exactly just one conformation. And what is this there? It is the native structure of the enzyme that is fully active, with the four disulfide bonds, correct to what was there in the original protein.

If you just think of the whole thing, it is extremely fascinating, considering that this protein has hundred and twenty-four amino acids linked together. It has a hundred and twenty-four amino acids linked together and it had eight SH linkages, which could have bonded with any other one. I allow the protein to unfold. So, when I have this unfolded structure, it could have essentially formed any or folded into any conformation, but that is not happened.

It always forms this one conformation that is the native structure of the fully active protein, with the exact four disulfide bonds, correctly linked with one another. And that is what extremely intriguing about protein folding. And it is still not understood to date. Nobody understands how a protein folds into the same structure, each and every time.

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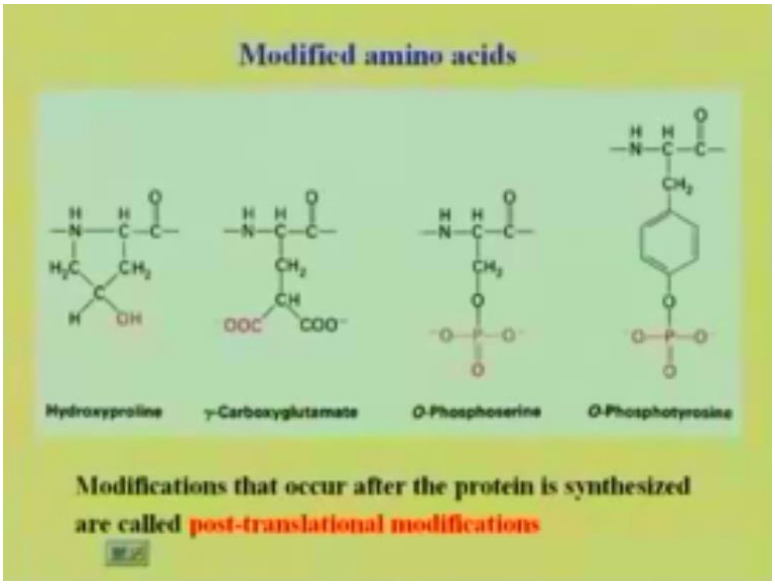

Now, these are some modified amino acids, which we just need to know the names of, considering that these amino acids are present in proteins, to some extent. The names are Hydroxyproline, What is different here is, there is specific OH attached to this. Can you see the OH attached to the Hydroxyproline? That is one other amino acid, not among the common amino acids, that you observe in proteins.

Another one, the second one is gamma carboxyglutamate. What is glutamate? Glutamate is, when you have the single carboxylic acid group here. If you have another carboxylic attached to it, it becomes gamma carboxyglutamate. And this is the structure, which you see in some proteins. Another one is phosphoserine, linked to the O. What is serine? Serine is, now you recognize this as part of the polypeptide chain.

How is this part of the polypeptide chain? That is just kept here and I will show you how this is part of the polypeptide chain. If you look at this region (showing the phosphoserine), what do we see here? This is the NH of which amino acid? The serine, the phosphoserine in this case, and what is this (showing C=O), the part of? It is carboxylic part of this phosphoserine (()) (13:15). So what is happening next?

Here, if I link these two together, the covalent bond of the peptide one bond would be this, linked to the NH of the next amino acid. (Interacting with the students) So, what group is this (showing the lower part of phosphoserine)? Your R group. Now, you can see again. If you look at the carboxyglutamate, what do I have here? I have part of the polypeptide chain. And

you always notice, what is the one that you have on the left, the nitrogen, the amino part of the carboxylic acid always. Because, that is the way you read the protein.

Because now, you are not always going to get structures, you are going to get letters, one after the other. So, what do you have to do? You have to read the sequence of the protein. When you read the sequence of the protein, then you have to recognize the symbols and these are redundant. What is the information that you need, you only need to know, what R is there? That's all you need to know. Because, if I tell you that I have glycine, alanine, valine.

If you know the structures, you can draw the polypeptide chain. Because, you know how they are linked to one another. You know that the NH2 has to be on the glycine side. The carboxylic part has to be on the valiant side. And the Alanine would be intermediate like one of these. So that is the way, we consider all the amino acids. All the information you actually need, is the series of letters.

The alphabet, the amino acid alphabet that comprises proteins or polypeptide chains. So what are the ones, the modified ones that we could have, there are all these, but these are the common ones. We can have hydroxyproline, in which are, hydroxyl is attached here, we can have carboxyglutamate, where the carboxy is attached here, we can have phosphoserine, where the phospho is attached here, the phosphate grouped to the inside of OH.

we can have phosphor tyrosine, tyrosine remember is one of the aromatic amino acid that we studied in the last class. What are the other two? They are phenylalanine and tryptophan. So what do we have here, instead of the OH that we would have for tyrosine, we have the phosphate attached to it. So, it is now phosphor tyrosine. Now usually, when the polypeptide chain is formed, this is not a common occurrence. All these happen, all the modified amino acids that you see, occurred after the protein is found.

So these are usually as I have mentioned, written here, these are modifications that actually occur after the protein is synthesized and what we call them are post-translational modifications. Because, the process of translation is how you get from RNA to protein. What is the central dogma of biology? It is DNA to RNA to protein. The process of DNA going to RNA is known as transcription.

The process of RNA going to protein is known as translation. So after the translation, it means you have RNA, translating to the protein. So, you have the synthesized protein. Now, if you have what is called the post-translational modification, you would have these specific modifications, that I have shown on the Slide that occur in the already synthesized protein.

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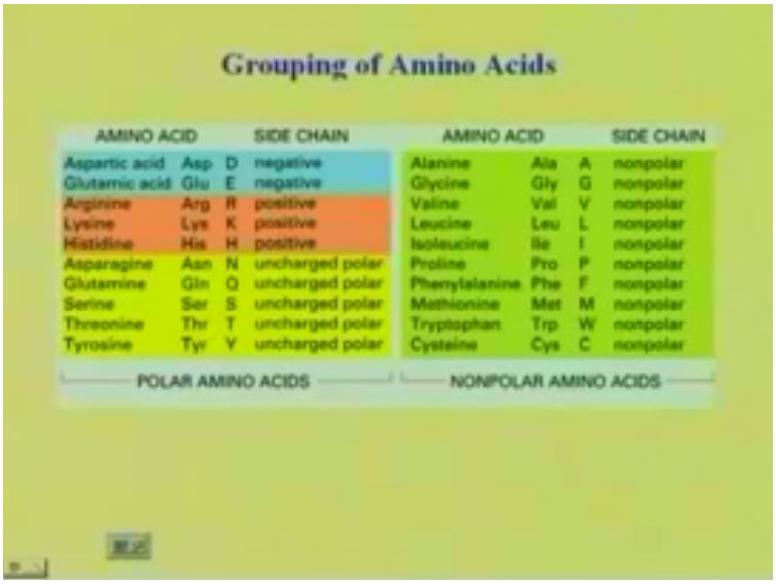

Now, what we see on this Slide are grouping of amino acids. We mentioned some groupings last time, when I showed you the individual structures of the amino acids. But these are extremely important in the properties of the proteins, in the catalyst of the enzymes and the catalytic properties of the proteins, in their functional properties, in their surface properties, of the proteins. The grouping of these amino acids is very important.

Now, if you go to different sites or even different books, there are certain differences in the groupings. But that does not really matter because what we will be looking at is, we will be looking at the closed groupings, meaning that we will be looking at Aspartic acid and Glutamic acid. These are the acidic amino acids. We look at Arginine, Lysine and Histidine. Now this is where, there is one change at times, where the Histidine at times occurs in the positive set and at times occurs in the uncharged polar set.

We will see why that occurs, in a few Slides from now. So the ones that definitely negative are Aspartic acid and Glutamic acid. The ones that are definitely positive are Arginine and Lysine. We also see Histidine in this group, in this particular grouping of amino acids. Now, what does this mean, if we have a negative amino acid or we have a positive amino acid,

these amino acids, as I mentioned earlier are likely to be on the surface of the protein. Why? Because, they are going to interact with the solvent.

You know that when we are speaking about the solvent, the protein is where? It is in a solution of physiological PH that is, say you have protein in your body, it is in the blood, the blood is primarily a lot of water in there, so what kind of solution is it, it is a polar solvent. So, all of the ones that we are looking in here, would favorably interact with the polar solvent. If you look at the second set that we have here, these are uncharged polar.

So, where do these charges come from, they come from the specific side chains that they have. And if you have a knowledge of the side chains you know exactly, what they are. Then, we have the Arginine and Lysine. In the uncharged polar set, you see how we have the changes, these are the three letter codes and these are the one letter codes. Then we have the nonpolar amino acids.

These nonpolar amino acids would also have called hydrophobic amino acids, would tend to be away from the solvent. Why? Because, they have no favorable atoms, no favorable interactions with the solvent. Why is that, because all the side chains mostly contain carbon and hydrogen. So, what do we have here? We have Alanine, we have Glycine, we have Valine, we have Leucine, Isoleucine, Proline, Phenylalanine, Methionine, Tryptophan and Cysteine.

Last three of these have hetero atoms in them. Methionine has a Sulphur. Tryptophan has a nitrogen and Cysteine also has a Sulphur. But, since they have a predominant amount of carbon and hydrogen to them, they are put in the nonpolar group. So, where would these favorably be? Unless they have specific reasons to be on the surface, they would rather be breading in the protein. What we mean by breading in the protein is we study later when we look at protein structures.

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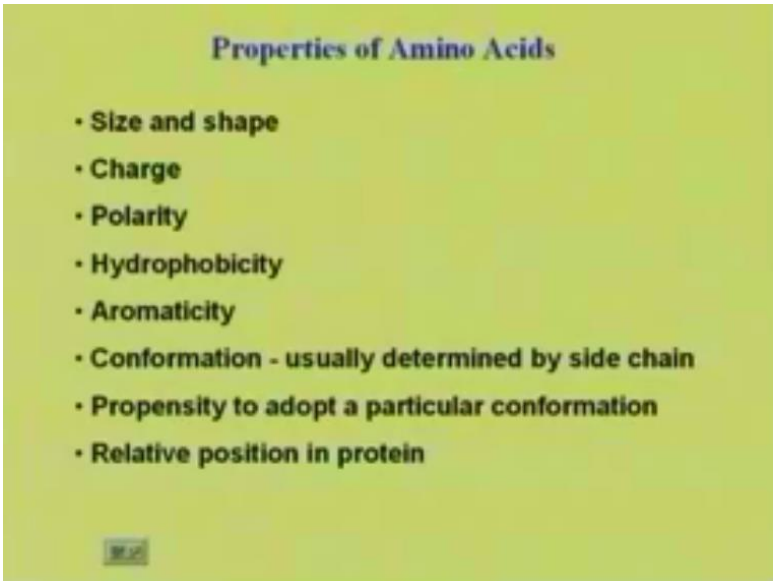

Now, we have to consider specific properties of amino acids. What are these properties? One thing that we looked at is, we have looked at the size and the shape. Shape means, what do I mean by shape or size, size means molecular mass. The smallest one is Glycine. It has the hydrogen atom in that. What is the next smallest one? Alanine, why? Because it has the methyl group.

Then gradually we can go there, what is the largest and bulkiest one we could have, Tryptophan is the bulkiest one and the longest is Arginine, Lysine. Arginine is the longest in and we could have Lysine. So, these are the long chain amino acids that you could have. So the size and the shape of the amino acid is dependent on what? On the R group. Because the rest of it, it is the same. What is the rest of it?

We have the isometric carbon atom that is referred to as the alpha carbon atom, then we have the amino group, the carboxylic group and a hydrogen that is common to all amino acids. Then, it is only the size and the shape of the R group that differs. What else? The charge. We just saw in the previous Slide, how we have the groupings. So, we can have positive amino acids. What are the positive amino acids? Lysine and Arginine. What are the negative amino acids?

Aspartic acid and Glutamic acid. The next could be the polar amino acids. The ones, now what is the difference between the charged ones and the polar ones? The polar ones, preferably some of them being small like Serine, can occur in the center part of the proteins. They will form hydrogen bonds. Because, they have an OH to them, like Serine has an OH,

so it can preferably form a hydrogen bond. Threonine has an OH, that can also form a hydrogen bond.

What about the amide ones? What do we have? We have two amines. What are they? Asparagine and Glutamine. So, we could have sets of hydrogen bonds formed with them as well. So, we could have them being polar, forming hydrogen bonds, but uncharged. That is what the differences. Then we have, probably the most important property of amino acids, the hydrophobicity. That is extremely important because it determines the protein folding nature.

That is what is, we will study a lot of this hydrophobicity as we go along. Another one is aromaticity. We find three amino acids that are aromatic in nature, Phenylalanine, Tryptophan and Tyrosine. And as I mentioned before, in the last class that it is these three aromatic amino acids that contribute to the absorbents of proteins. The optical density or the UV absorbents that you see of proteins is solely due to the aromatic amino acid residues.

And this we monitor at two hundred and eighty nanometers. That the UV absorbents of proteins is usually monitored at two eighty nanometers and the absorbents of the optical density that is absorbed is solely due to the presence at to eighty nanometers of the aromatic amino acids, Phenylalanine, Tyrosine and Tryptophan.

Then, we have the confirmation that is usually determined by the side chain. What you mean by the confirmation of the amino acid? The side chain, when I have a long side chain, it has single bonds in it. It is free to rotate in three-dimensional space. So, I am going to have confirmation, associated with the property of the amino acid. If I have an Alanine, I have just the methyl group.

So, that will not as such have any confirmation because all of it will look identical. But, if we have a long side chain like Glycine or Arginine, we have a lot of single bonds in there. What can happen to those single bonds? We can rotate. As they rotate, see I have the Lysine rotate. Then what is going to happen? The NH2 group that forms the end of the Lysine group, will what? that will also rotate? That would also change and as it changes, it is going to change the confirmation of its side chain.

This we will study, the propensity to adopt a particular conformation. This comes, when we will between the secondary structure of proteins, which we will be doing in detail later on. And the relative position in proteins we can say from the properties that we already know. What are these? One is the hydrophobicity.

From the hydrophobicity, I can straight away say that, say for example of Phenylalanine or Leucine or Isoleucine, is expected to be in the central part of the protein. Lysine and Arginine are an acidic amino acid, like Aspartic acid or Glutamic acid, is expected to be where? On the surface of the protein.

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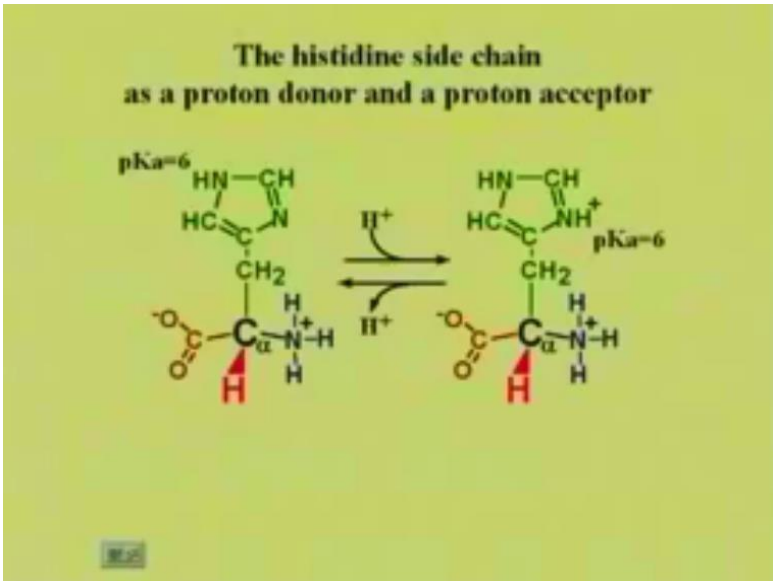

Now, this is very important, Histidine. Remember I mentioned two Slides ago, why Histidine is sometimes considered to be a positively charged amino acid and sometimes it is not. The reason is, all of you know what pKa means. We will be studying this in great detail, because this is extremely important for protein structure. Now the side chain that you have here, is the side chain for the Histidine.

Now if you notice, what Histidine can do is, it can accept a proton and as it accepts the proton, the side chain gets protonated. So what has happened here? What is this? This is my asymmetric alpha carbon atom. The nomenclature of this is such, that if this is the alpha carbon atom. Since the carboxylic acid part and the amino part is common to all amino acids, this carboxylic carbon is always referred to as just C.

This C alpha means that the side chain is connected to this carbon atom. Then we have this CH2. What comes after alpha? Beta. So, this is the C beta. There is a specific nomenclature, which we will study as we do protein structure later on. But for now, it will (()) (29:57) say that two C alpha is attached C beta of the side chain. So, Alanine will just have the C beta. The next one in this case would be, what would this be? This would be a C gamma. Then we would have two deltas, a C delta and an N delta and so on and so forth.

So, just by the nomenclature alone, you can actually identify which amino acid it belongs to. Why? Because the odd chains are different for each case. So, an Alanine is just going to have a single C beta. What about Valine? It is going to have the C alpha. Every amino acid has a C alpha. What about Valine? It is going to have a C beta and two C gammas, a C gamma one and a C gamma two.

So, let us get back to the understanding of the protein donor and protein acceptor capabilities of the Histidine side chain. The pKa of six means, that when there is an equilibrium at a certain PH, where it can either accept the proton or it can donate the proton. Now, apart from what is here, which we will be studying in a moment, the ionization of amino acids. At this particular point, I can protonate this nitrogen to form NH plus.

It can also deprotonate to form this pack. So this is an equilibrium. Now this equilibrium occurs at a PH close or rather between 6 and 7. Now if you look at what the physiological PH is, that is the PH of blood, the PH of which all the enzymatic reactions in our body are taking place, that is at a PH of 7.4. The physiological PH is 7.4. Now, what happens to Histidine is that, because of this protonation and de-protonation that can occur.

Histidine forms a large a number of the catalytic sites of proteins. Just because, it can accept and donate a proton, close to the physiological PH, which is not the case with the other amino acids. If you are too basic, your pKa is going to be too high. The PH of the body does not reach that amount of protonation or de-protonation. Because the physiological PH of our blood, where all the reactions that are taking place in our body, is close to the pKa value of the Histidine side chain.

This forms a component in many enzymatic reactions, in many catalytic sites of proteins. So, what are we talking about? We are talking about basically, the ionization of amino acids. So,

what do I mean by ionization of amino acid. Initially, if I am at a very low PH, what is going to happen to the carboxylic group? It is going to remain as COOH. What is going to happen to the amine group? It is going to remain as NH3 plus. Because I have larger amount of protons in solution. That is what we are going to exactly see here.

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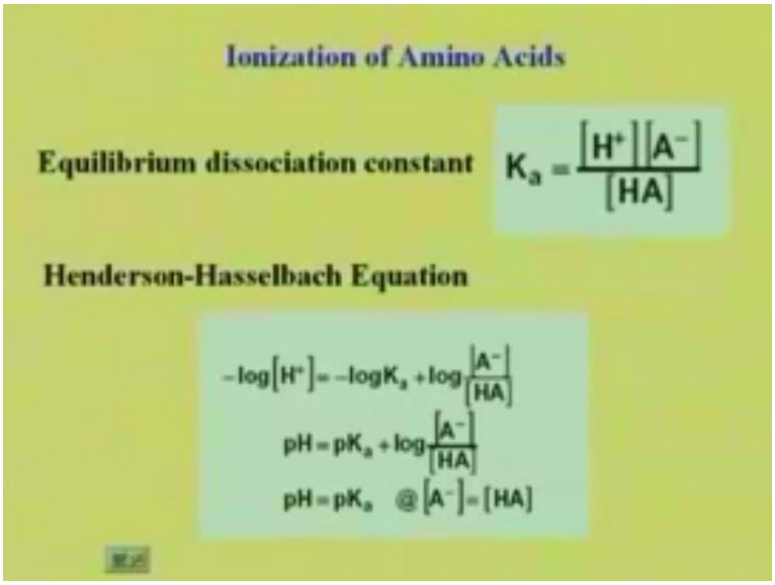

So, at the PH of one, when the PH is very low, I have COOH. I also have NH3 plus. We all understand that. Why? Because I have an acidic solution, which has sufficient amount of protons, to protonate all the side chains or all the amino groups. We are not talking about the side chain right now. But, since the amino acid itself has a carboxylic group and amine group, we can consider the protonation of those two sides alone. So, what do we have here?

We have act, a PH of one. We have our carboxylic acid protonated. We have our amine group protonated. There is no other place, where a proton can be accepted here. As you increase the PH or rather, if you consider a titration, say you are adding base, so what is going to happen, the base is going to abstract the protons from the amino acid, in the order or in the ease of its abstraction. So, what is the first one that is going to get lost? It is the carboxylic acid one.

So, at a PH of seven, I have lost this. So, I am no longer C double bond OOH. I am now COO-. But, the amine group does not lose its proton at this PH. I keep on increasing the PH or rather, I keep on adding more base. At a point, I will lose the proton that is attached to the amine group. So, if I do an ordinary titration of the amino acid, I will get how many inflection points? Two. Why? One corresponding to the loss of the carboxylic acid H, the other corresponding to the amine H plus.

So this is where, I get one set, what is this pKa? This is pKa two. What does that mean? It means, as I close on and reach the PH close to two, I am going to lose this proton. This pKa is ten. So, after I reach a PH of ten, will I lose this proton? So at a PH of twelve, I am now at this position, where I have lost all the protons possible (())(37:32). Is that clear? So initially what did I have? I had everything protonated at a low PH. As I go on, what do I have?

I have a form. What is the charge on this form? It is a Zwitterionic form. It has a positive charge to it and a negative charge to it. So it is effectively charges zero. That is Zwitterionic form of the amino acid. Every amino acid is going to have this form. Now, what becomes fascinating is, suppose my R group also has a charge to it. So, where is it going to become interesting?

When I have acidic group or an amine group attached to it, so this will come into the picture, when I have Aspartic acid or Glutamic acid, Lysine and Arginine and the Histidine for the pKa six.

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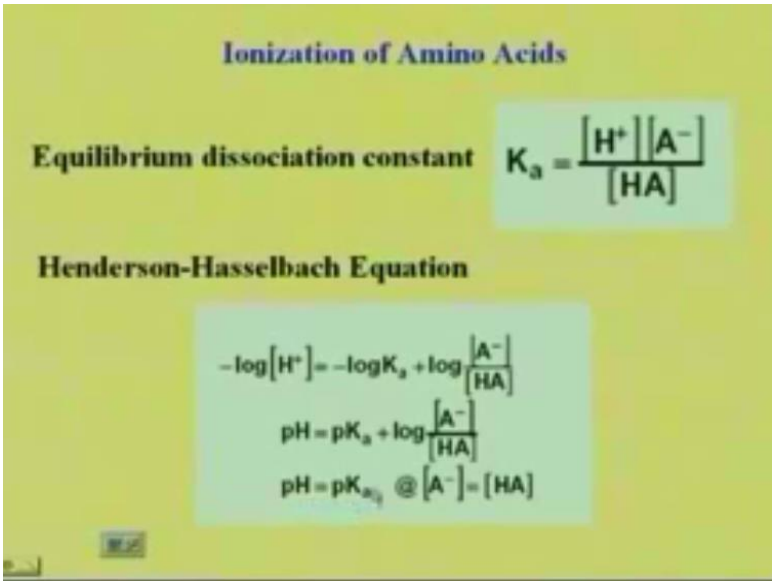

If I consider, the ionization of the amino acid, I have an equilibrium dissociation constant associated with it. This is true for all acids. What is the equation that we have here? We have [HA], dissociating into [H+] and [A-] and what is this Ka associated with it? It is this equilibrium here, where I have [H+], [A-] and [HA]. Now, you all studied about buffers, where we are looking at what is called as Henderson-Hasselbalch equation.

What is that equation? You basically rearrange this equation, rearrange the expression rather and we write it in such a manner that we get the [H+] on the left hand side. And, I know that the negative logarithm of hydrogen ion concentration corresponds to the PH. So what do I have here? I have the pH of the solution equal to the pKa, right, what is the pKa? It corresponds to this equilibrium + log [A-]/[HA]. What is this? This is the base form. This is the acid form.

Rather the salt form or the acid form. That is the way, we write the Henderson- Hasselbalch equation and if the concentration of these two are equal, it means, I have equal amount of un-dissociated and dissociated forms. Then, this is going to have a ratio of one, making the logarithm 0 and my pH double bond pKa. So, what are we considering here, we are considering our amino acids as 1HA form.

And we consider the dissociation from [HA] to [H+] and [A-]. And what am I getting from there? I am getting a pH double bond pKa, when my [A-] double bond [HA].

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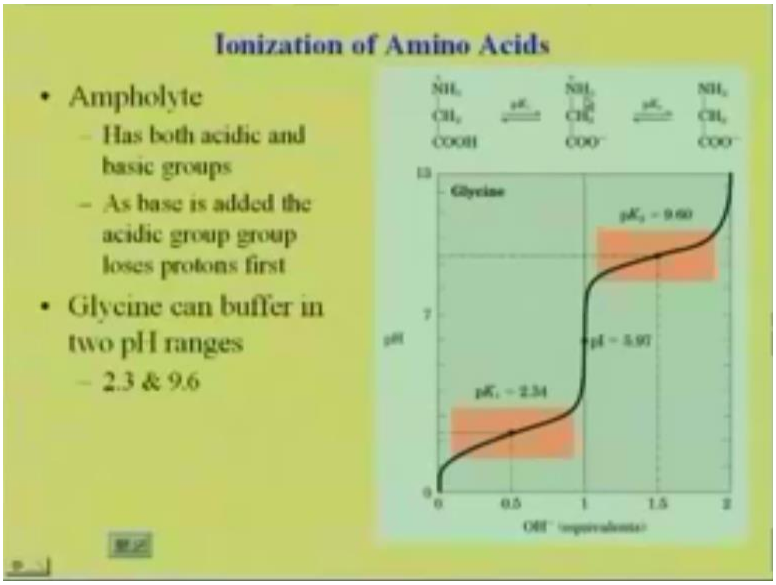

Now, this is what happens when we titrate an amino acid. The titration of an amino acid is going to show a diagram like this, which you will understand very clearly. First of all, we consider these as Ampholytes, why? Because they have both an acidic and basic group attached to it. That is true for all amino acids. You have an acidic group, which is the carboxylic acid group. You have a basic group, which is the amino group. So, these are called Ampholytes.

Now, as the base is added, what is here along the X axis, we have number of OH- equivalence added. So, basically what you are doing? You are adding NaOH to your amino acid solution. Initially, if we make the pH very low, what does my amino acid look like? All of its protonated. So, I have COOH and NH3+. Now, what am I doing? I am abstracting H+. How? By adding OH-. So, if I add OH-, I abstract protons so there is a dissociation immediately.

As soon as, I hit upon this pH, what happens? What do I have at this pH? From the previous Slide, I have equal amount of this species, which is my A- and this species. Now, I want you to understand this very clearly, because you have to remember that as soon as you start OH- addition, this H+ starts getting abstracted. So, as soon as I start the first drop of OH-, does not bring you to the pKa, but it starts abstracting H+.

So, I come to a point, as I keep adding my OH-, I come to a point, where this is equal to this. What is that point? That is when my pH is equal to my pKa. So, the pK1 value as it is called, is 2.34. What does this mean? It means at this point, I have equal amount of the carboxylic acid anion here and the COO- and COOH. Now, what I am doing? Now, in its deterionic form (()) (44:50), I keep on adding OH-.

As I add OH- further now, what is going to happen? At some point, I am going to lose the amine proton. The NH3+ has to lose its proton, when I cross its pK2, then after I met this level, say. So, I am at pK1, where I have equal amount as I keep on adding OH- now, I have more amount of A- than HA. Now, I keep on going with the titration, then, when I come to this point, what do I have?

I have equal amounts of this form, which is now my A- and my HA, which is this. So, this is my H as this is my acid, which has this additional H+ added to it. As I add OH-, I abstract this proton at a particular pH, the pK2 will correspond to a pH, where I have equal amounts of this species and this species. So, now, if I keep on adding OH-, I will just be increasing the pH, because, I now have just OH- in solution and this. This would be the titration of Glycine.

You would have two inflexion points, the first corresponding to the carboxylic acid proton, the second to the amine proton. But, now the fun comes, when we do not have H2 here.

Suppose, I have come thing else, suppose I have Glutamate, what does Glutamate have now? It has an additional COOH to it.

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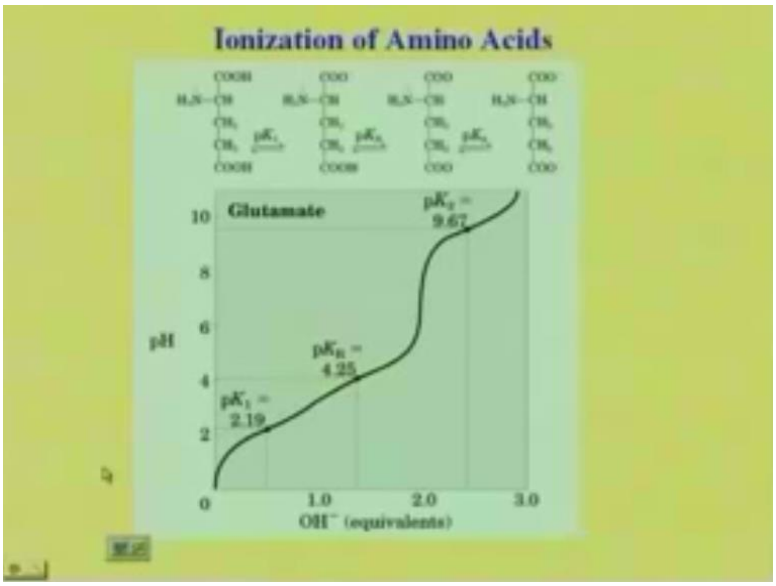

So, what do I have at low pH now? At low pH, look at this species, this is our side chain. This is the carboxylic acid part of the amino acid; this is my NH3+. At this point, all of them are protonated. Why? Because my pH is very low. So, at my low pH, that is at this region here, all of these are protonated. I am now adding OH-. What is going to happen? I will start abstracting the proton or rather the group that will most easily lose its proton will start the equilibration.

That will be this proton. The proton that belong to the carboxylic acid of the amino acid not of the side chain will be lost first. So, what is the species that is being formed? It is this. This is still protonated; this is still protonated. Now, I am continuing with the titration. I still have how many more protons that I could lose? Two. One belonging to the COOH of the side chain and one the NH3 protons.

As I go on further with my OH- addition, my titration, the next easiest one to be removed is the side chain one. Now, I keep on going. Now, what is going to happen? As I am now going to lose the last proton that is left, that is here, the amine group. So, the three pK values that I now have correspond to the, what does it correspond to? We have 3 protons that could be lost.

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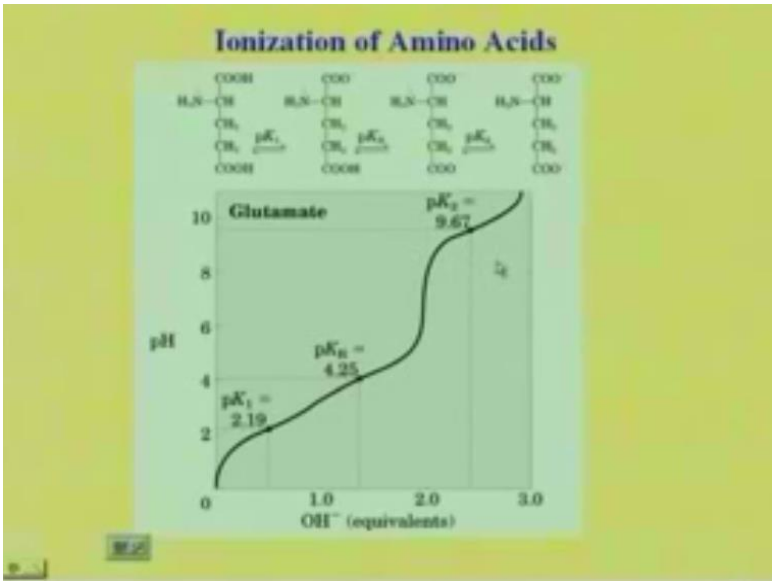

So, we have the first one lost, which is the carboxylic acid group attached to the C alpha of the amino acid. Not the side chain, because, why do we lose that first? Because, that is the most acidic proton. Then, we go on to the next one, which is the next most acidic proton the side chain one. So, we have the side chain one that we are going to lose. Then, we lose the amine one. What do I have in my curve here? I have a pK1 value, I have a pKR value. What is the pKR?

R is the side chain, so it corresponds to the side chain. Then, we have pK2 value, corresponding to the amine. Let us do Histidine now. When we see the Histidine pKR, remember, we showed the pK of 6, where we had the changes or where it could accept a proton or donate a proton. So, that is exactly what is happening here. What do we have? We have now at low pH, what do we have?

All the possible sides protonated, so we have the side chain protonated, we have the amine group of amino acid protonated, we have the COOH protonated. As we increase the OH- additions, then what is going to happen? Is I am going to lose this proton? So, as I lose this proton, what is the next one that I am going to lose? The one belonging to the side chain. The last one that I am going to lose is going to be the one belonging to the most basic group, which is the amine group.

Again, I have my profile or my titration curve rather look like this. So, I have a pK1, corresponding to the COOH, I have my pKR corresponding to the side chain and I have my pK2 corresponding to the amine group.

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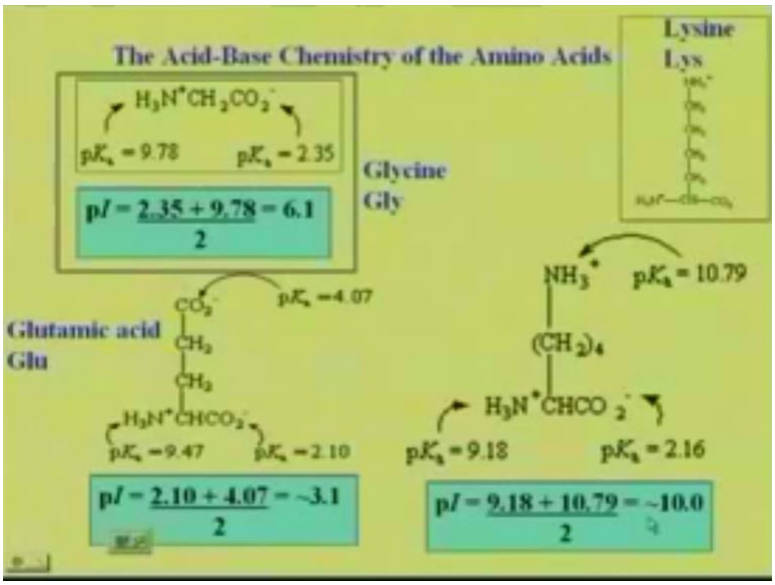

So, what do we have here? For the Glycine, I have two pK values. What is this form? It is this zwitterionic form. So, at a particular point, if I go back, Ok, what do I have here? Here I have a pKR value, but here I have something called a pI value. That is the isoelectric point. What do you mean by an isoelectric point?

An isoelectric point is the point at which you would have the zwitterionic form of this amino acids and the charge would be zero. Is the charge zero at this point? It is. So, what is my pI value, it is the sum of the pK of the carboxylic group, the pK of the amine group by 2. What happens if I have the glutamide? When is the charge zero for the glutamic acid? The charge is zero for the glutamic acid at what point? When it is between these two.

This pK2 for the amine, this is pKR, this is pKA. So, it first loses this one. So, in between these two, my charge is what? Zero. So, what is my pI value? 2.1+4.07 divided by 2. The net charge of the solution is always zero. It depends on the pH of the solution it is. If you have a pH corresponding to the pI, then the charge on the amino acids is zero. When we have Lysine, what does Lysine have? Lysine has an additional NH3+.

When you do a titration curve or if you study a titration curve of Lysine, it is going to behave differently. And what do we have? We have the pI now, at a point, where Lysine is uncharged. That is between the pK of amino group and the pK of the side chain this time. So, what is the pI of Lysine? It is very high.

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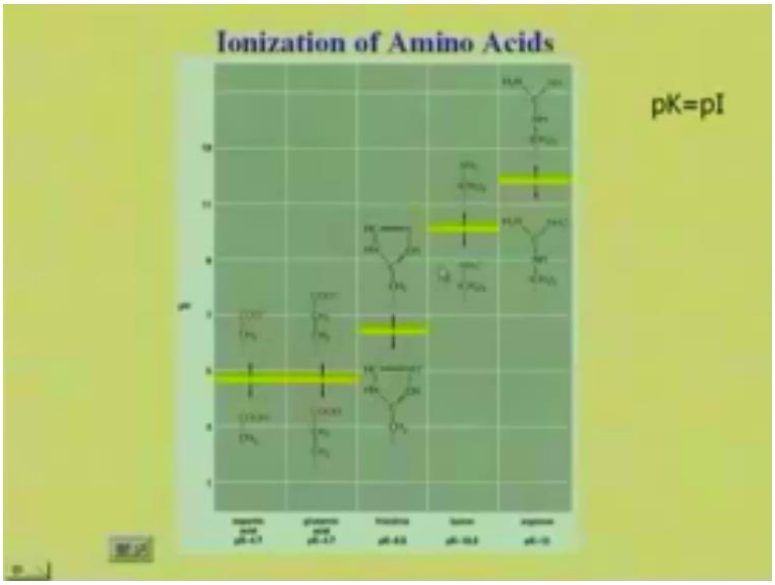

To summarize what we have is we have the Ionizations of the amino acids. These are the regions, low pH regions corresponding to aspartic. glutamic acid, the intermediate regions to Histidine, then we have Lysine and finally we have Arginine. These Lysine and Arginine are the two basic Amino acid, aspartic acid and glutamic acids are two acidic amino acids, histidine acts as the proton acceptor and proton donor, depending on what pH you are at.

We shall stop here for today and so, the discussion we had today was on understanding the properties of the amino acids, their size, their charge, their polarity and the titrations of the amino acids, which is extremely important when we consider the ionization that could occur based on how or what the pH of the solution is. Thank you.