Experimental Biochemistry
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Lecture – 08
Amino Acid Titrations

We continue our lecture on Amino Acid Titrations; we spoke about certain things in the last lecture, where we discussed the acid base properties of amino acids the ionizable groups that are present amino acid titrations and p I determination.

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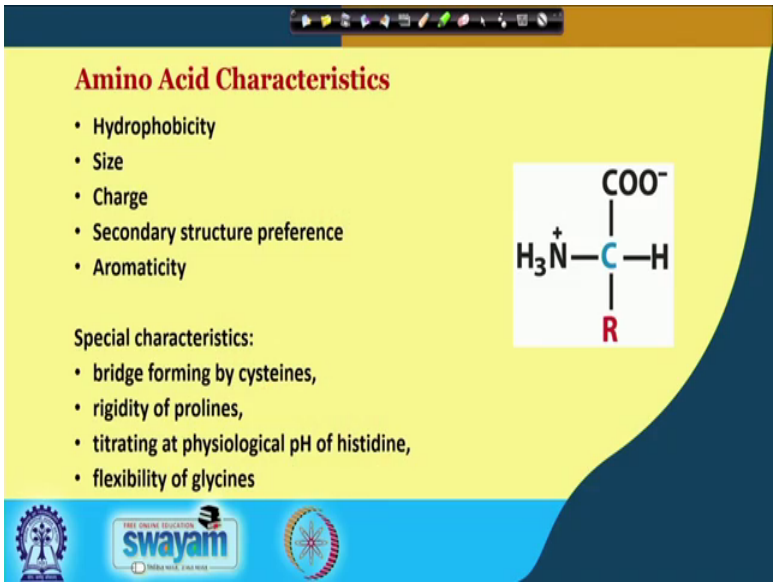

But we considered only the basic characteristics. So, if we look at amino acid characteristics, we see that we have this specific structure for the amino acid where we have what is called the alpha carbon a hydrogen and amino group and a carboxyl group attached which is common to all amino acids the difference we find is in the r group that actually determines the hydrophobicity, the size, the charge, the secondary structure, preference, the aromaticity and other special characteristics, which we will come to as we study more about proteins.

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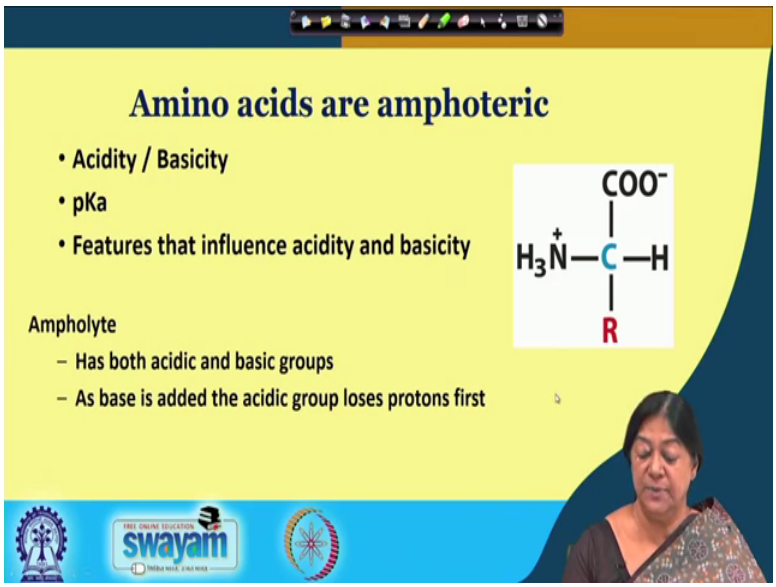

The amino acids we know amphoteric in nature, what does this mean? It has an acidic and basic property to it, it has specific pKa values and these features influence the acidity and the basicity of the specific amino acids that we talk about.

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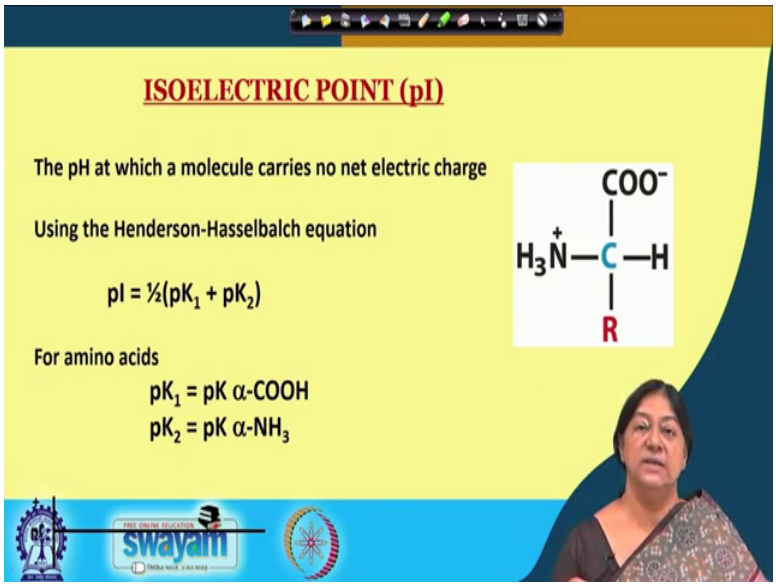

Now if we go and try and understand what the isoelectric point of an amino acid is we learnt in the last class, that we have something called the Henderson Hasselbalch equation and we learnt how to derive it based on the dissociation of acids and we can find what is called the isoelectric point, which is the pH at which the molecule does not carry any net electric charge.

And using the Henderson Hasselbalch equation in the last lecture, we learnt how to derive a quantity called the p I the isoelectric point from a knowledge of the pK 1 and the pK 2, which for amino acids we would consider the pK 1 to be the pK of the alpha carboxylic group, that is this group where all of it is protonated, but this is also an h, where we have pK 2 that is the pK of the alpha amino group positive charge here, where we consider this group this is what is called the zwitterionic form of the amino acid.

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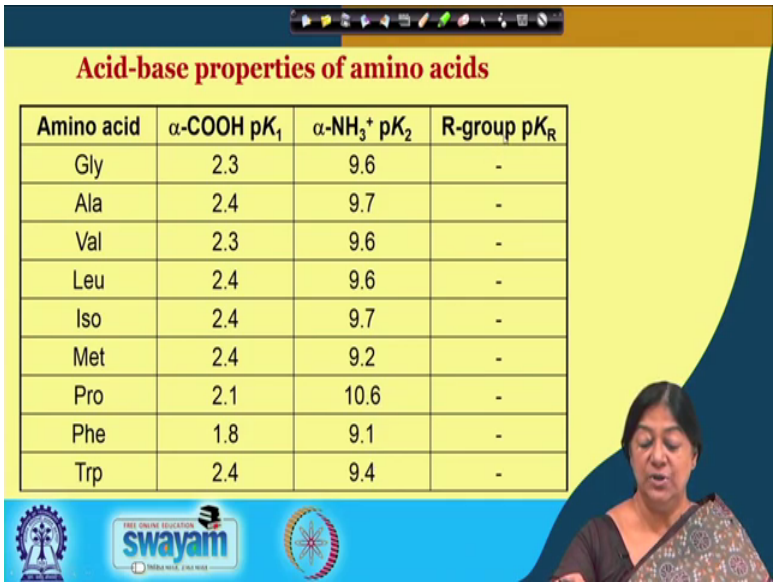

Now, if we look at the acid base properties of the amino acid, we have a series of amino acids listed here, we have the alpha COOH values you can see the pK ones are very low, because they this is a carboxylic acid on the other hand the amino group has relatively high pK values all the pK 2 values.

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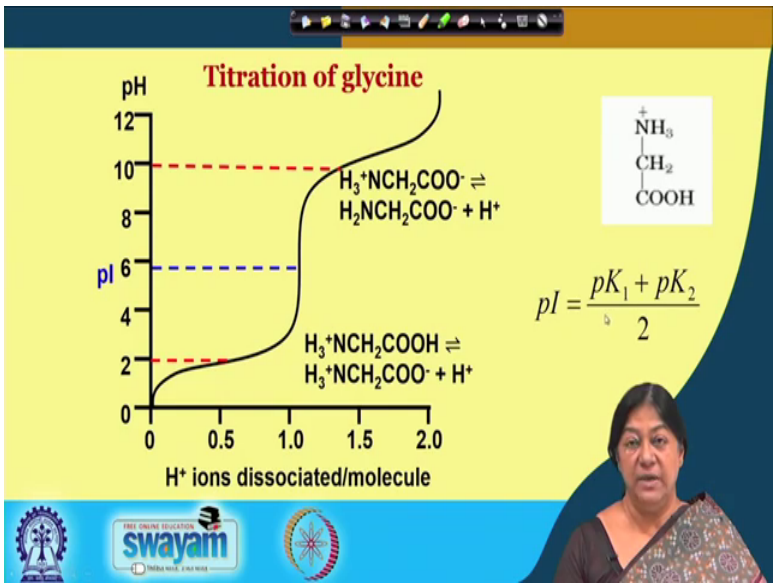

Interestingly we do not have any pK R value here; as soon as we come to amino acids with PK r values we will see how our titration curves will change. For the example that we have here and we discussed in the previous lecture, we are looking at glycine, the

titration of glycine that has a pK 1 for the loss of this hydrogen this proton from the carboxyl group.

The next PK 2 value is the loss of this hydrogen from the amino group rendering this to be a deep protonated form complete form, where this corresponds to the pK 2, this corresponds to the pK 1 and the p I can be obtained by using this relation where we have pK 1 plus pK 2 by 2.

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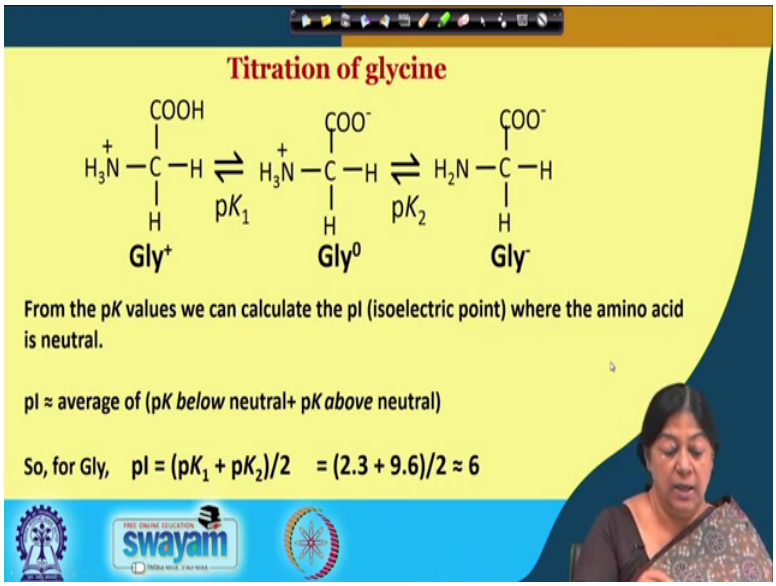

So, the structure that we saw in the first case, where we have a glycine that is positive, where we just have a positive charge pK 1 means the loss of this proton. So, it renders this neutral then we have pK 2, which is the loss of this proton rendering this as the gly negative. So, from the pK values we can calculate the pI that is the isoelectric point, where the amino acid is neutral this is the neutral form of this amino acid. So, the pI becomes the average and with the knowledge of the pK 1 and the pK 2 values we determine that the pI for glycine is approximately 6. This experiment would be demonstrated to you, when with the experimental part is shown.

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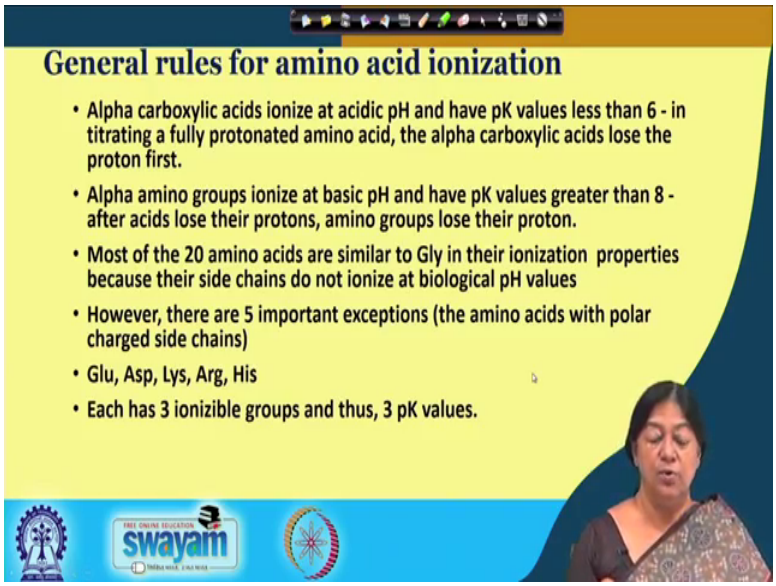

So, if we look at general rules for amino acid ionization, we have the alpha carboxylic acids that ionize at acidic pH and they have pK values that are less than 6 and where we are looking at, if we look at a fully protonated amino acids, the alpha carboxylic acid will be lost first the proton belonging to the alpha carboxylic acid will be lost first. Then if we do not have an ionizable group present in the r that is the r side chain then what is going to happen? The amino group is going to finally, get the proton associated with the amino group is going to dissociate and we are going to have then a negatively charged protein amino acid.

Now, most of the 20 amino acids are similar to glycine just like the table I showed you where we have a pK 1 and a pK 2 value and we can determine the p I value, which is just going to be the average of the pK 1 and the pK 2, however, there are specific exceptions to these we will look at these exceptions, where we have the r group that is the side chain that is also ionizable.

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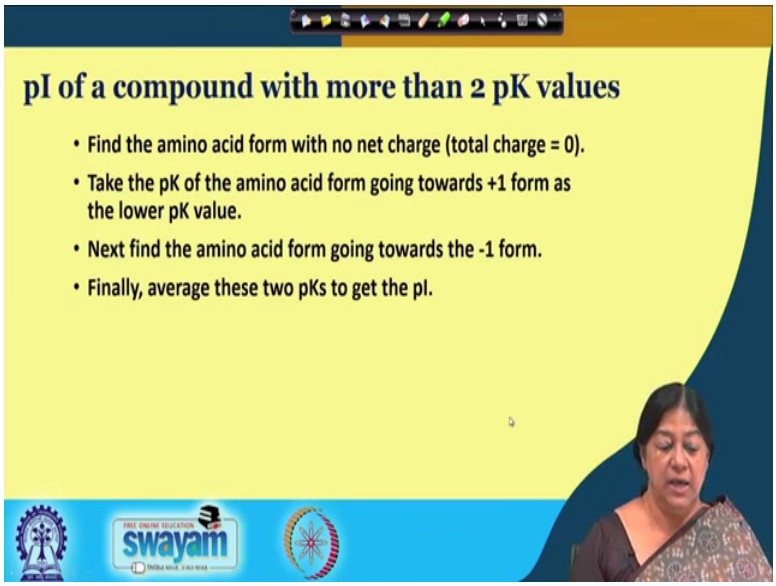

So, if we have a pI of a compound with more than 2 pK values how do we determine the p I value? The first thing is we have to find the amino acid form with no net charge that is a total charge of 0.

We take the pK of the amino acid form going towards plus 1, the plus 1 form as the lower PK value and then the amino acid form going to the minus 1 form and we average these 2 pKs this will be demonstrated to you in the following slides. So, what are we looking for? We are looking for the charges on the amino acid residue, but we have to remember that we do not have only the pK 1 and the pK 2 anymore in addition we have what is called a pKr which is due to the side chain.

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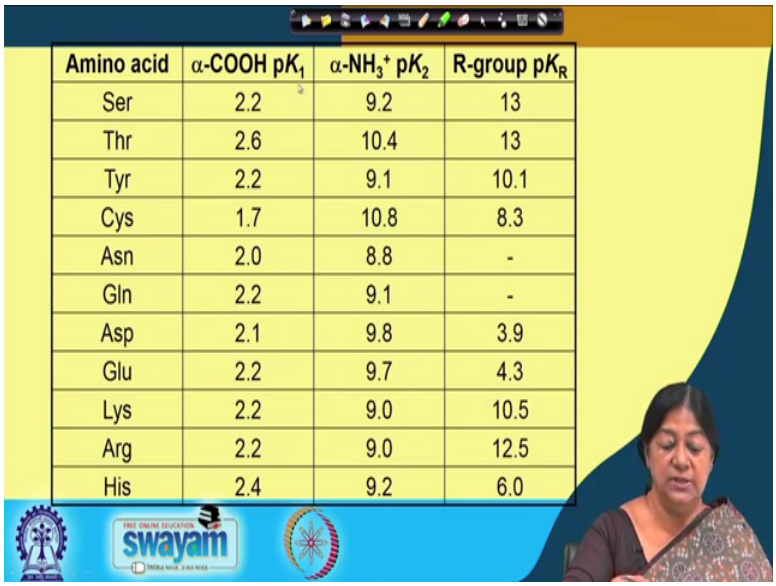

So, this is the pK 1 value of these amino acids the pK 2 values of the amino acids and in addition we have the pKR groups. We are mostly concerned with these 5 amino acid residues because, we will see how they affect or their effect is actually more prominent are more important in their presence in proteins.

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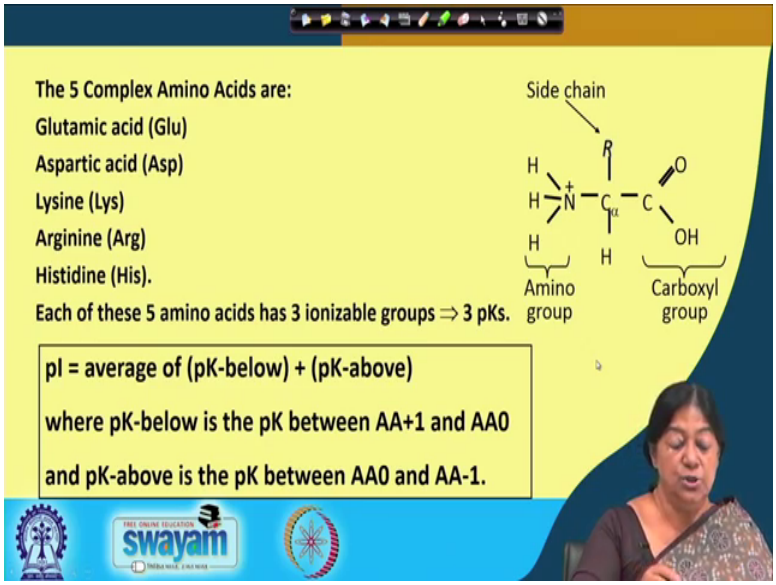

So, what do we have as the five complex amino acids, if we call them? We have glutamic acid, aspartic acid, lysine, arginine, histidine.

So, what do these amino acids have in common? They have these 5 amino acids have 3 ionizable groups. The common part for each amino acids is the amino group, where it has a proton the carboxylic group, where it has the proton, in addition we are not only going to look at the pK 1 and the pK 2, but we are also going to have a pK R.

So, we have 3 pK values, what are we looking for? We are looking as to how to calculate the p I value of the amino acid. So, we have a p I that is going to be the average of the pK below that's the minus 1 the pK above that's the plus 1. So, we are looking at the charge of the amino acid between AA plus 1 and amino acid 0. So, there is a pK that is going to be responsible for the charge going from plus 1 to 0. Similarly, there is going to be a pK responsible for the charge going from amino acid charge 0 to amino acid charge minus 1, it is these 2 pKa values that we have to find out and the average of that is going to be the pI of the particular amino acid.

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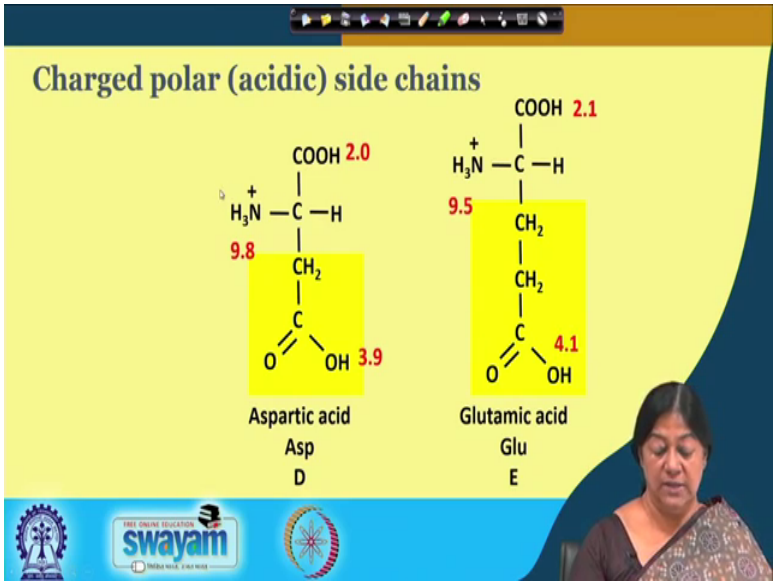

For example, let us look at the charged amino acids that we have here, these are the acidic amino acids we have aspartic acid, we have glutamic acid. Now if this is the completely protonated form what do we mean by that? We mean by when we are looking at the amino acid, what do we have to observe? The alpha carbon, the amino part, the carboxylic acid part and in this case what is blocked in yellow here is the R group, this R group belongs to aspartic acid the 3 letter code is Asp and the 1 letter code is D, when we

look at glutamic acid, we see that if we look at the top portion here it is identical because, these are all amino acids.

But what do they differ in? They differ in their R group this R group has an additional CH2 associated which is glutamic acid 3 letter code Glu and 1 letter code E. So, when we look at these 2 amino acids, we see that there are specific pKa values associated with the protons. So, what is going to happen when we do a titration for example, if we look at aspartic acid as we do a titration, the first proton that is going to be lost is the one with the lowest pKa value this corresponds to the carboxylic acid group of the amino acid not of the side chain.

As we continue, the next pK the next lowest pKa is the one that corresponds to the side chain of aspartic acid that is the pK r value. So, it is the pK 1 value the pKR value and then as we he continue the titration, it will be the pK 2 value similarly, when we consider it here it will be the pK 1 value the pK R value and the pK 2 value. So, this is how we would consider it for acidic amino acids.

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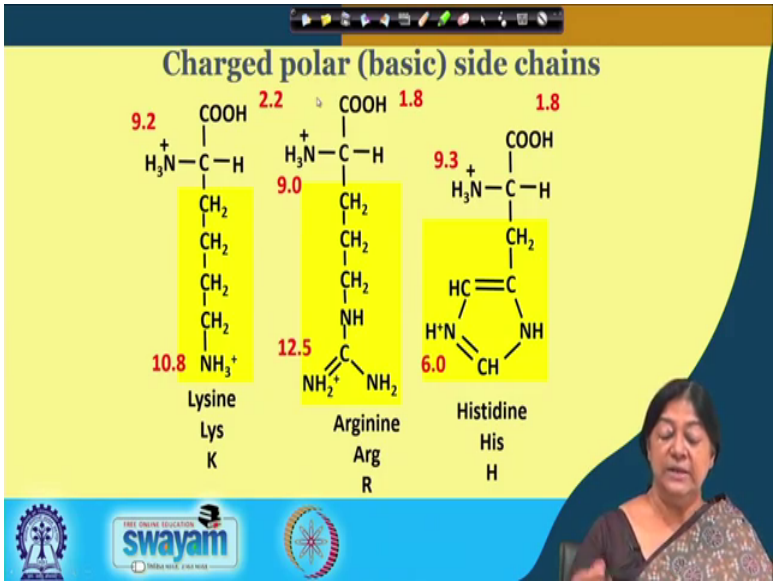

For basic amino acids, we would look at the so, we have again the same amino acid characteristic the amino part, the carboxylic part, the amino part, carboxylate part for lysine, for arginine, for histidine again, we have the 3 letter and the 1 letter codes given here and these are the specific side chains.

What we observe here in case of lysine and arginine is that the protons that are going to be lost are lost in a different order, the first proton to be lost is going to be the carboxylic acid 1. The next proton, if we look at the pK values now of the side chain and the amino group of the amino acid connected to the alpha carbon then we see that this value is lower, which means that this proton is going to be lost before this proton, the same with arginine it is this proton that is going to be lost before this proton is lost.

If we come to histidine, we see that the first proton to be lost is COH that the carboxylic acid then is the side chain proton then is the pK 2. So, we have a pK 1, pK 2, pK R, pK 1, pK 2, pK R, pK 1, pK2, pK R and depending upon their values you will have the loss of the protons.

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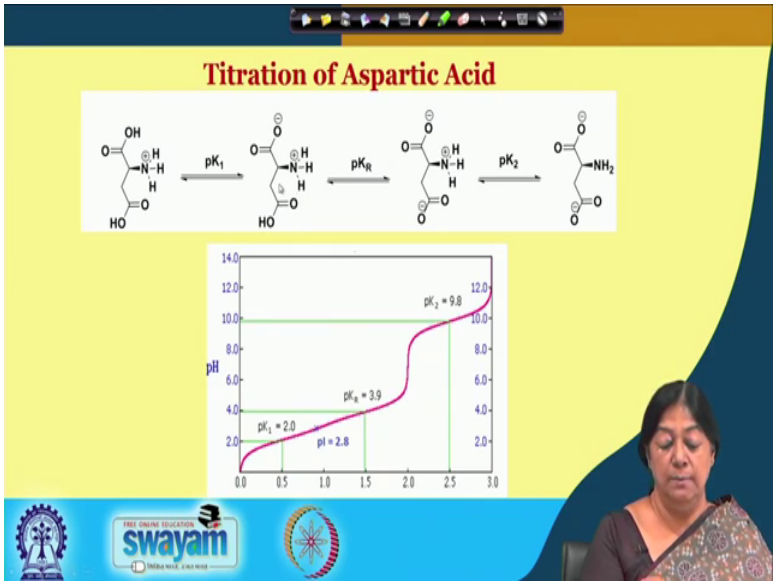

Let us look at aspartic acid, when we look at the first completely protonated form, what we observe is that there is a side chain. So, this is the alpha carbon, where we have associated the amino group the carboxyl group and this is the CH 2 COO OH. So, what is this is a completely protonated form.

The pK 1 corresponds to the loss of this proton. So, this renders this COO minus then we have pK R because, this is the next one. So, this is what renders this minus. Now let us look at the charges, what do we have here? The total charge here is plus 1, the charge here is 0, the charge here is minus 1 and the charge here the pK 2 corresponds to what? It is going to correspond to the loss of this proton, which means that now we have NH 2

and the charge here is minus 2. What we learnt that in determining the p I, we have to look for the amino acid form that is plus 1 to 0 the pK responsible for going from plus 1 to 0 and the pK value responsible from going from 0 to minus 1.

These are the values that we have to consider. So, if we look at the 4 forms of the structure that we have here, we see that this is the 0 form, what does this mean? It means we have to take this pK value and this pK value, the average of those values is going to be the p I of aspartic acid, this is the titration. So, we have a pK 1 that is close to 2 we have a pK R close to 3.9 and the pK 2 9.8. So, what is the charge going from pK 1? It is going from plus 1 to 0. For pK R it is going from 0 to minus 1 and here it goes from minus 1 to minus 2, but we are interested to determine the peak I from this set. So, it is pK 1 plus pK R divided by 2.

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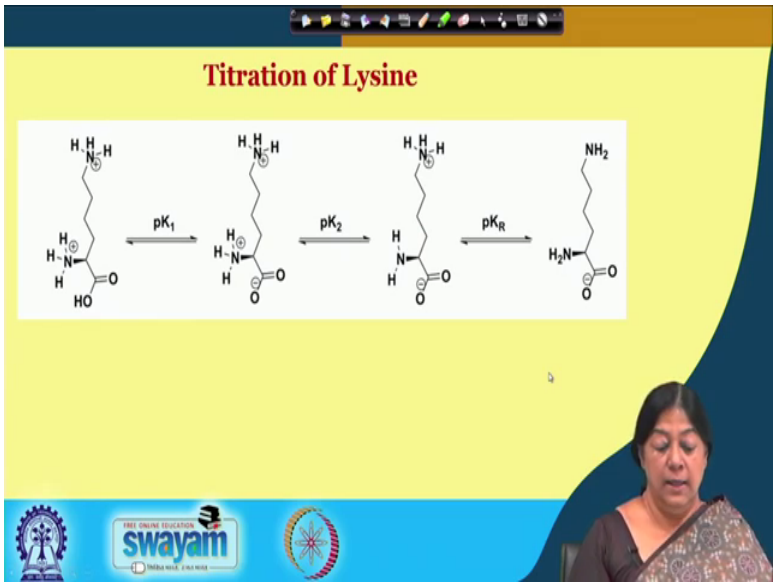

In case of lysine, what are we going to see? We are going to look at the charges again, in this case we have the alpha carbon atom here, the amino group the carboxylic group and the side chain, which is CH 2 CH 2 CH 2 CH 2 NH 3 plus. Now when we look at this, we see that this charge now at the present state is plus 2.

The pK 1 we now corresponds to the loss of the proton of the carboxylic acid group. So, we have a COO minus the charge here now is plus 1 the loss of pK 2, now we remember that it is going to be the amino group of attached to the alpha carbon. So, this renders this NH 3 plus 2 NH 2 the charge now is 0, when we go to pK R now the loss of this proton,

the side chain proton is going to make this minus 1 what are we interested in? We are interested in charge changes from plus 1 to 0 to minus 1.

So, what is the charge here? Plus 1, what is the charge here? 0, what is the charge here? Minus 1, what are the 2 pK values responsible for this? PK 2 pK R. So what will be the p I of lysine? It will be pK 2 plus pK R divided by 2. So, that is how we will be able to determine the p I values of these specific amino acids.

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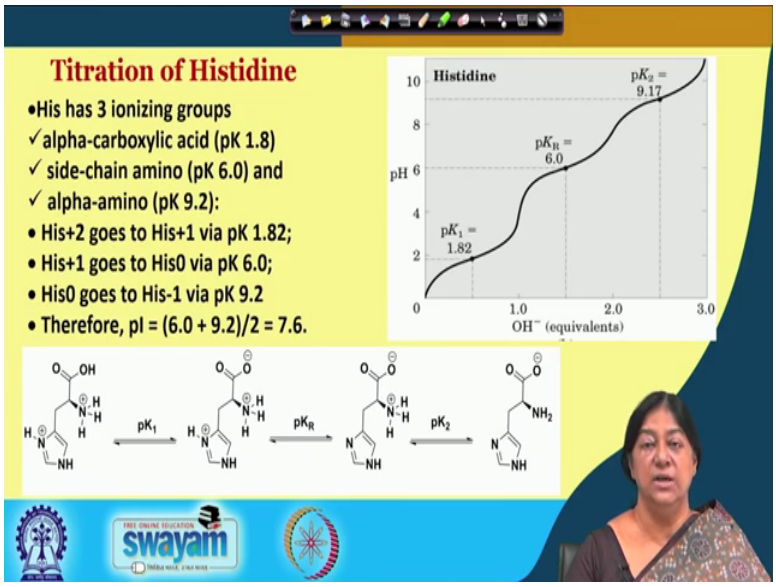

We look at the titration of histidine now, in case of histidine again we have 3 ionizing groups, it is an ionizable amino acid and the most interesting point about histidine is that the pK R value is close to 6 and this can vary depending upon where histidine is positioned in the protein and because it can vary from around 5.82 more than 7.5.

It is extremely important in physiological processes and as enzymes in proteins, where we will we see a lot of histidine residues present at the catalytic site of proteins. We have the 3 ionizable groups, what are these? The alpha carboxylic acid, the side chain amino acid and the alpha carbon. Now if we look at the charges again, these are the structures of the histidine molecules, when we have the pK 1 value we know that we have 2 positive charges being a basic amino acid. So, the total charge here is plus 2, the pK 1 again renders the loss of the carboxylic acid proton making this a charge of plus 1.

We come to a pK R value here where we are now rendering this as a 0 charge because, we are now losing the proton on the side chain then, we go to the pK 2 value and we look and see that the loss is going to now be the amino group attached to the alpha carbon. So, we have now NH 3 plus going to NH2. So, what are the charges? The charges are plus 2 plus 1 0 minus 1. What are we interested in? We are interested at the specific point, where the charge is going from plus 1 through 0 to minus 1 and the pK value is responsible for that. So, what are the pK value is responsible for it?

It is the pK R which is 6, the pK 2 which is 9.2 the average of that gives us the p I of histidine, we have here the histidine titration curve. So, based on these types of titration curves, based on an understanding of the titration curves and what forms of the molecule are present, we can actually determine the p I values of the amino acid residues. So, we can consider the epi of a normal I mean, what do we mean by a normal amino acid? One that does not have any ionizable group.

In this case, we would call it normal because it would have a pK 1 and a pK 2 without a pKR associated with it; however, when we consider the ionizable ones, the specific ones, the acidic ones and the basic ones. It is important to remember that the pK R values also are extremely important and there is a way in which we can determined the p I value of an amino acid that has an ionizable side chain, this will also be demonstrated to you in the laboratory.

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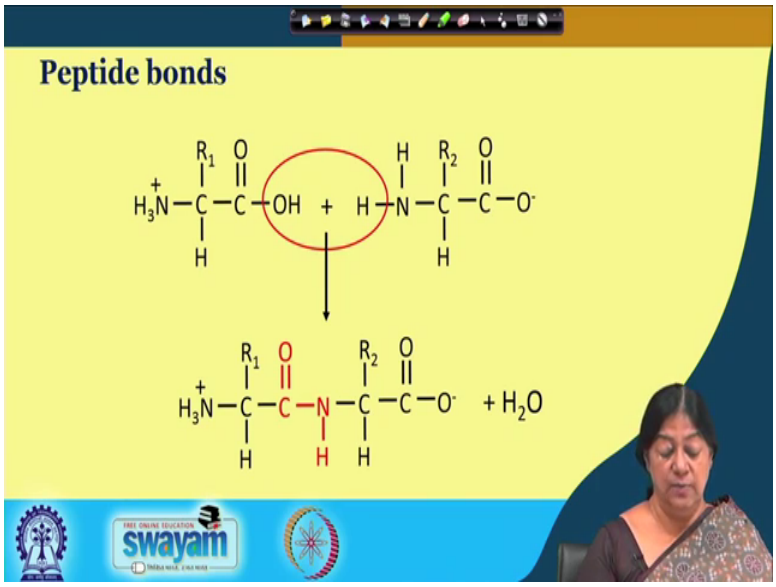

The next thing that we are going to consider, now if what happens, if we have 2 amino acids linked to one another? If I have a dipeptide is it possible to determine the p I value the way we consider it for a single amino acid and the answer is yes.

Now, how do we, first of all we will be understanding more about peptide bonds, when we consider a little bit about protein structure to understand, what we mean by protein denaturation in subsequent lectures. When we are talking about a peptide bond we have one amino acid that has the R1 side chain another amino acid that is the R2 side chain, this is written or demonstrated or displayed in a very very simplistic manner and what we say is in a condensation reaction, what happens is these 2 connect to form, what is a peptide bond and now we have a dipeptide.

So, we have an NH 3 plus a COO minus and this CO NH does not have any charge associated with it, this is the peptide bond. So, if we were to have to determine the pI of this particular dipeptide, what is the information that I need? The information that I first need is the pK 2 value of the first amino acid because, we are looking at the NH 3 plus the pK 1 value of the second amino acid because, we are looking at the COO H because, it is going if we consider a complete protonated form this is COO H. So, we are looking at the pK 1 value of R 2, the pK 2 value of R 1 amino acid and what other information do we need? We have to see whether R 1 and R 2 themselves have any ionizable groups or not. If not then we know that it is going to be a pK 1 plus pK 2 divided by 2.

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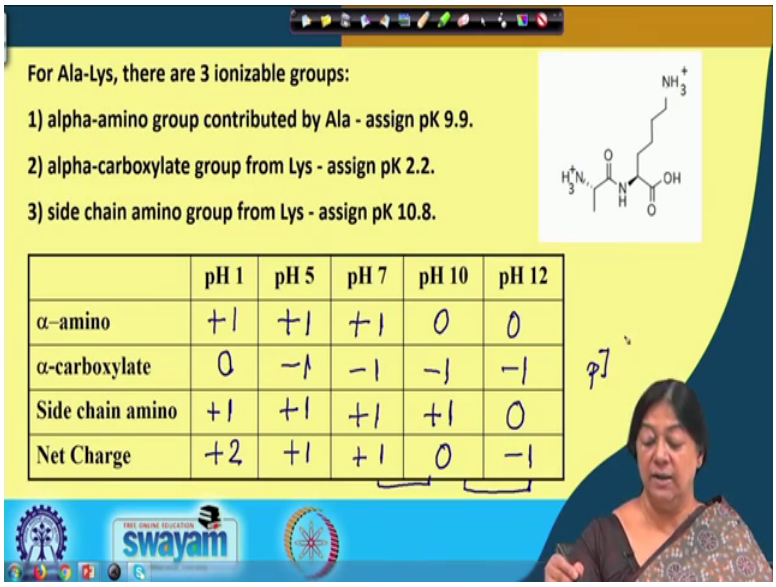

So, for example, if we look at Ala-Lys, this is the amino acid alanine, this is the amino acid lysine and it has been drawn at low pH, what does it mean? It means that everything is protonated, what do we have here? This is the R 1, this is the R 2 and we have the amino group that is the pK 2 value is associated with this alanine the pK 1 value is associated with the lysine and we see that one of the groups does have an ionizable side chain. So, what we do is we calculate the charges of each of these possible, how do we do that? We say that if we want to look at this, we say that the amino group here now has a charge correspond to plus 1.

The alpha carboxylate is 0 because, it still has not lost it and the side chain is plus 1 from the lysine because, the alanine does not have any charge. So, what is the net charge? The net charge here is plus 2, if we look at now pH 5 all the values are given to here. The amino group contributed by alanine is 9.9. So, when we are at pH 5 we do not need to worry about that. The alpha carboxyl group from lysine as assigned that pK 2.2, what does it mean? It means that when I have reached pH 5 this proton is lost.

So, this proton is now lost. So, the amino group will remain at plus 1 the carboxylate now is minus 1 the side chain is still plus 1 because, we have not lost the side chain proton. So, what is the overall charge? It is plus 1 we go to pH 7, but do we have any changes at pH 7? No. So, it remains in the same condition, where we have the same charges, we can write the same charges out and it is still plus 1. So, there is no change here, as soon as we come to pH 10, what happens is we lose this. So, what is this? This is the alpha amino group. So, this becomes now 0, because it's lost the proton this remains at minus 1, this is plus 1 it is now 0.

When we reach 12 we lose this. So, this becomes 0 this remains at minus 1, this is at 0 and this becomes minus 1. So, what are we interested in? We are interested at the pKa value that form goes from plus 1 to 0, we are interested in the pKa value that takes from 0 to minus 1 and the average of those 2 is going to give us the p I of the peptide Ala- Lys, we want to go now to the next idea and we want to consider that if we have now a greater longer other peptide then what do we have seen.

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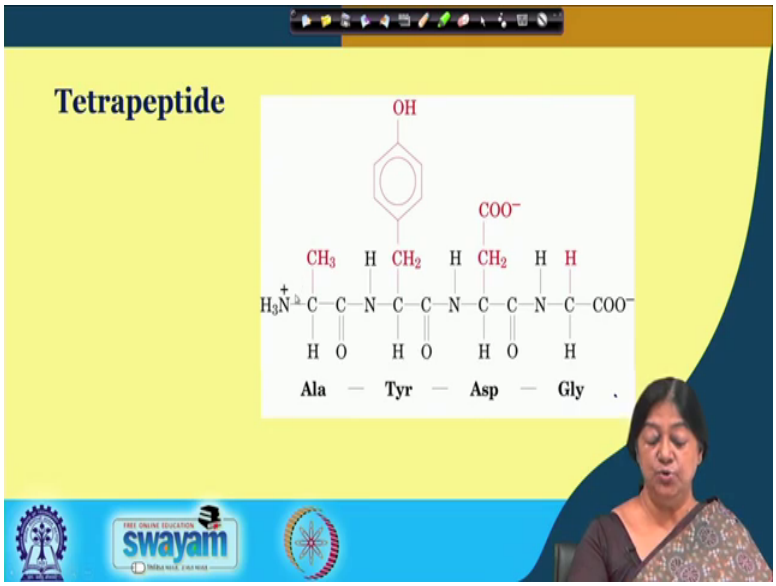

So, if we have a tetrapeptide like this, we can do the same thing Ala tyrosine aspartic acid glycine, we just need to know how we are going to go about doing it.

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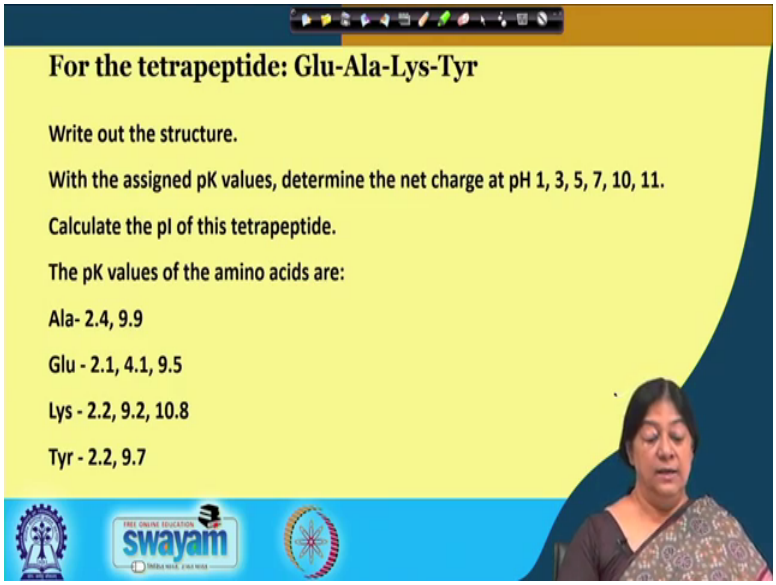

So, for an example or something that you can try for a tetraploid Glu-Ala-Lys tyrosine you write out the structure consider the assigned pK values and calculate the pI of this tetrapeptide as you can try that out as a homework.

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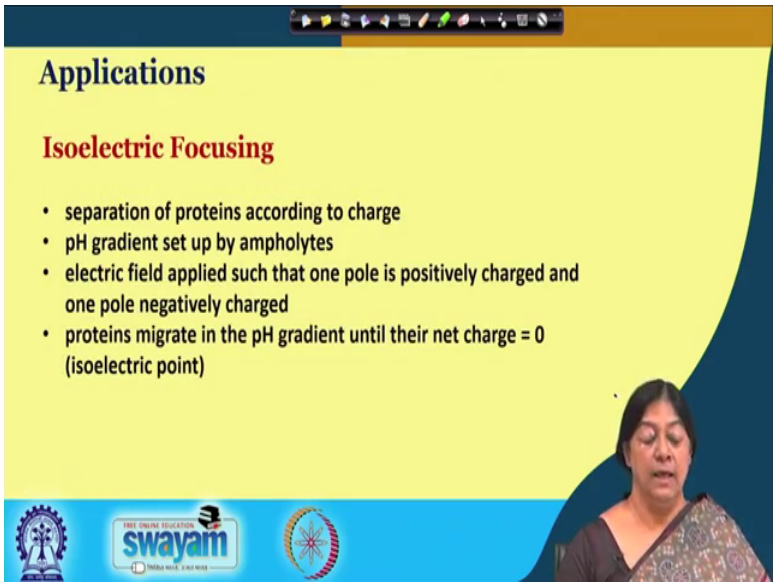

The applications are many, we want to know for separation of proteins for specific process called isoelectric focusing, where you can actually isolate proteins because, we look at protein migration in gel electrophoresis. Suppose, we do not want the protein to migrate, if we know what the pI of the protein is we know that the electric charge is neutral. So, the protein will not migrate further.

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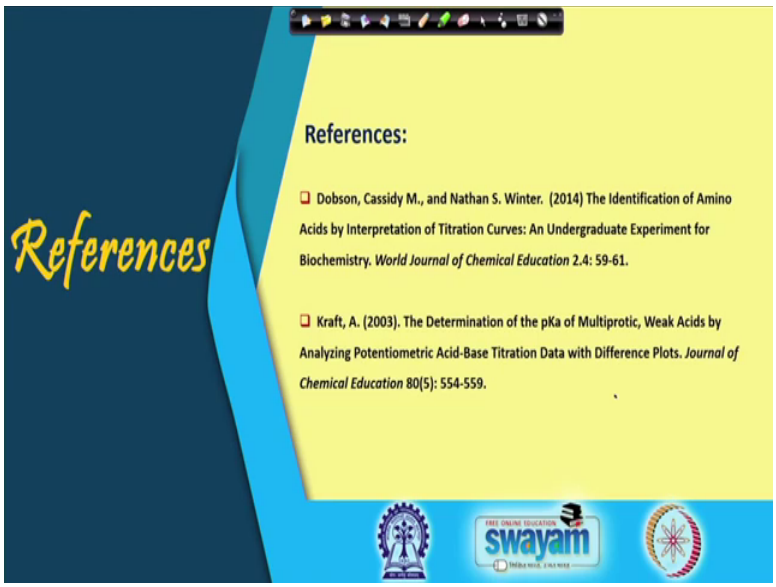

The references are called they are, some of them are old references that I will be giving you so that you have an idea of what to read and some of them will be relatively newer references, which give you a different perspective.

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So, what we learnt is a how amino acids are amphoteric in nature, the acid base properties, the amino acids will ionizable side chains and how we can actually determine the pI of the different peptides and further on for proteins.

Thank you.