Experimental Biochemistry
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Lecture – 07
Amino Acids and Their Properties

We begin lecture 7 of experimental biochemistry, where the discussion is on Amino Acids and Their Specific Properties.

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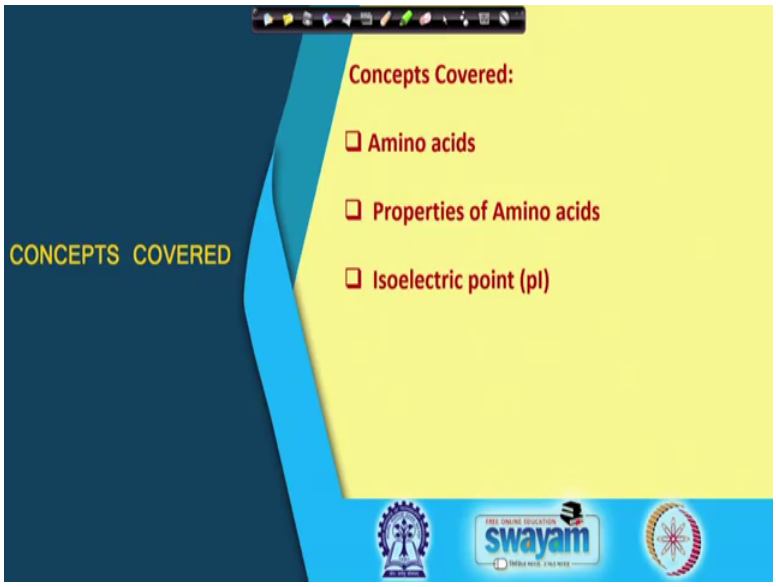

The concepts that will be covered in this lecture relate to amino acids and their properties and what we mean by the isoelectric point. In the next lecture we will look at amino acid determination of the isoelectric point of an amino acid.

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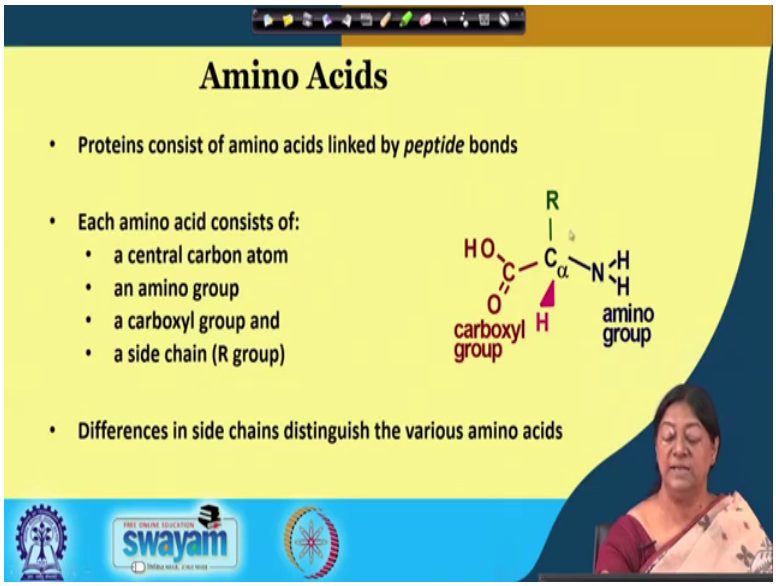

We all know that these amino acids are the building blocks of proteins, where the amino acids are linked by peptide bonds. Each amino acid consists of a central carbon atom, a carboxyl group, an amino group and a side chain. Now, the differences in this R group that is the side chain results in the different types of amino acids known.

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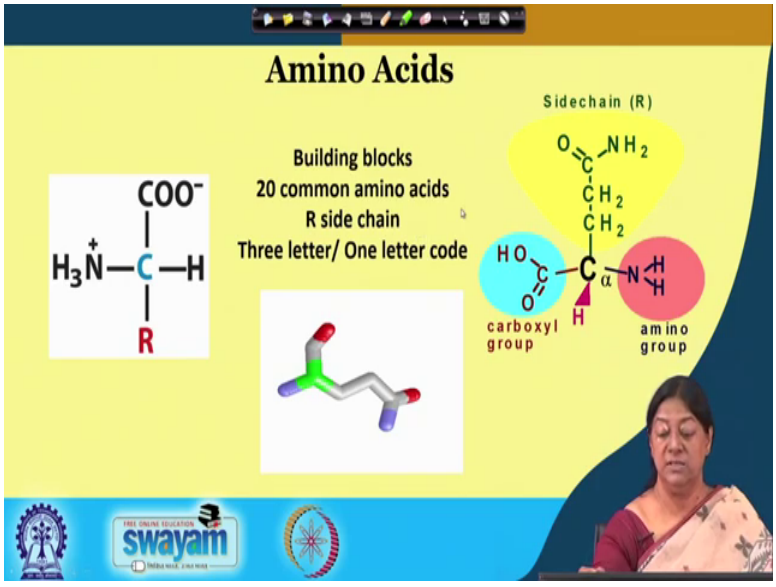

So, these amino acids are the building blocks of proteins, we have 20 common amino acids. The R side chain given here is in this yellow shape structure is a specific side chain, this is the carboxyl group and this is the amino group.

Each such amino acid is referred to by a 3 letter and a 1 letter code, there are different representations of the amino acids and the common reference method used is red for oxygen, blue for nitrogen and these correspond to the carbon atoms.

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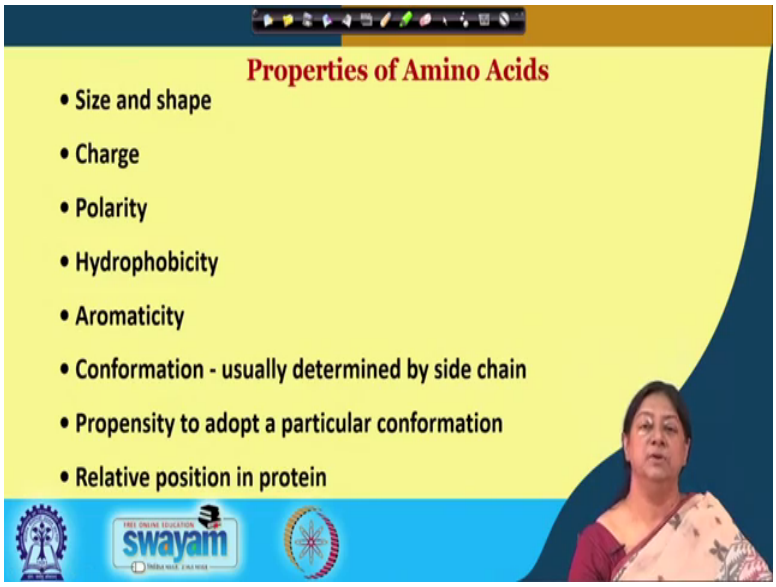

The specific properties of amino acids that are extremely important in their formation of the proteins or in the formation on an understanding of how proteins fold, we will look at all of this nature when later when we consider the folding and the denaturation of proteins, where we will be doing specific experiments to understand the amino acids nature.

But first of all we need to understand the amino acid acid base behavior. So, we will get a knowledge of why they behave as they do. So, the size and shape is an important feature of the amino acid, the charge, the polarity, its hydrophobicity, its aromaticity they have confirmation that it usually adopts the propensity or why it would adopt a particular conformation and the position in proteins.

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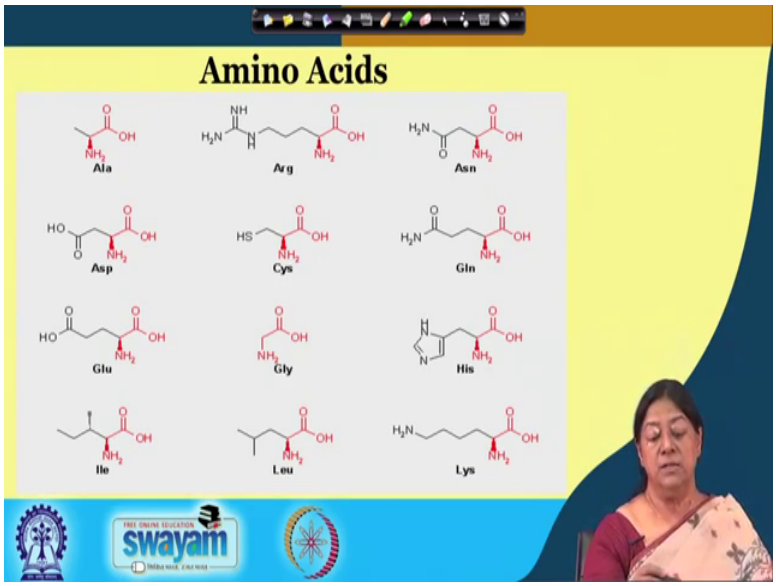

These are some amino acids that have been given here, when we look at the portion given in black that is: what is the side chain that was referred to as the R group. So, this is the carboxylic group, this is the amino group as you will notice each of these amino acids have a common carboxyl group and an amino group and what differs is the portion in black, that is the side chain and that gives it the different amino acid perspectives or the characteristic.

So, this is alanine, this is arginine asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine. What you will notice here is that some of the amino acids have nitrogen and oxygen in their side chains, some of them have only carbon and hydrogen, isoleucine, leucine, alanine, if we go on to the next page we will see valine.

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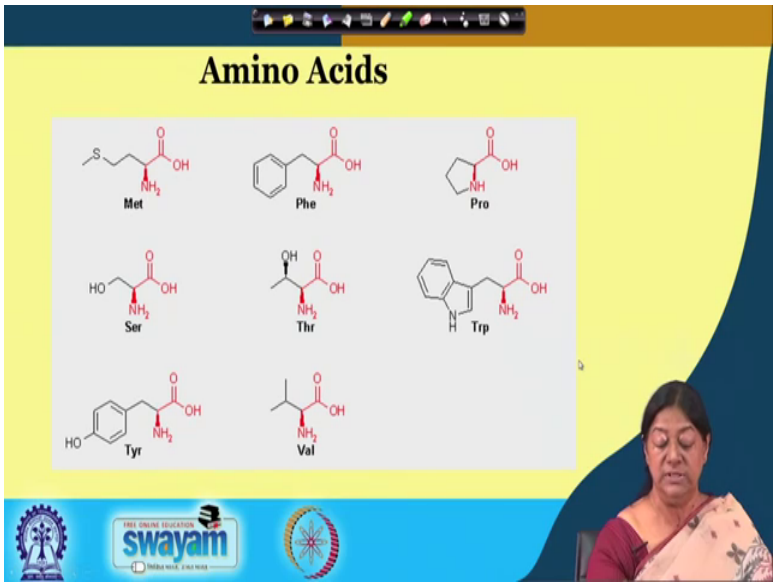

These are ones therefore, that have carbon and hydrogen in their side chain making them hydrophobic in nature. The ones that have aromatic side chains are aromatic in nature they have specific properties that we are extremely useful in looking at the spectroscopic details of proteins. So, if we have the ones with nitrogen and oxygen they would be the polar ones, they would be the ones that would be involved in hydrogen bonding in electrostatic interactions, which we will look at later on in much more detail.

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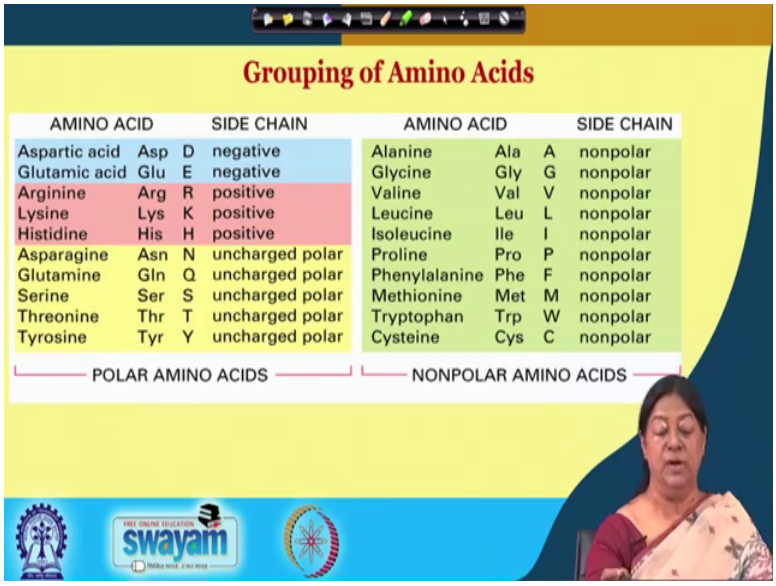

So, if you just grouped the amino acids we have aspartic acid glutamic acid that render or have a side chain that is negatively charged. So, these are the acidic amino acids, the basic amino acids are one that render the side chain positively charged then we have uncharged polar amino acid residues that do not have a charge themselves, but because of the presence of a nitrogen and oxygen would have a polar side chain that could be involved say in hydrogen bonding or some such interaction. These are the non polar side chains that would rather be involved in hydrophobic type interactions, which we will look at later.

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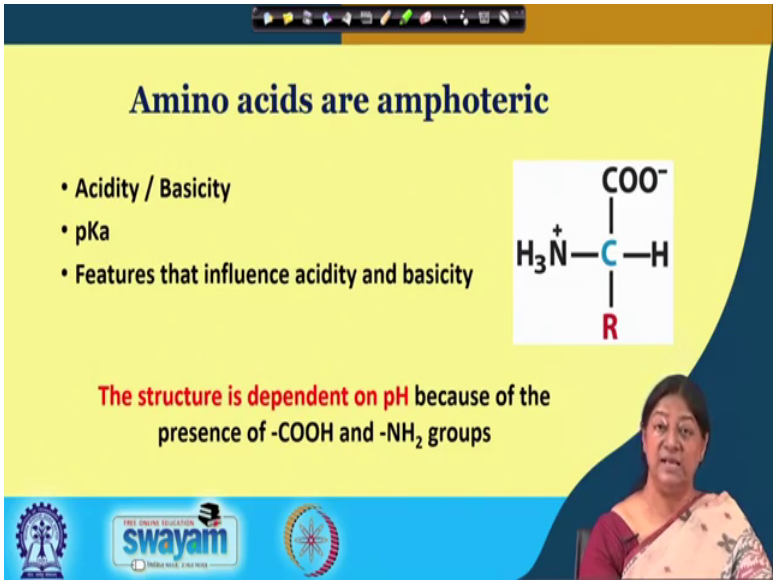

Now, if we look at the amino acids the basic structure is this, where we have the alpha carbon, the R group, the amino group and the carboxyl group. Now it is important to see the way this has been written as a neutral form, where we have a specific if we were to consider this as COO H NH 2, we would be considering again a neutral type of amino acid, but as we have protons that can be dissociated from the amino acids we have to remember there is a pH dependence. So, the structure is dependent on the pH, because of the presence of the COOH group and the NH 2 group.

So, there are specific pKa values associated with the acidic and the basic groups. So, the acidity and the basicity is important.

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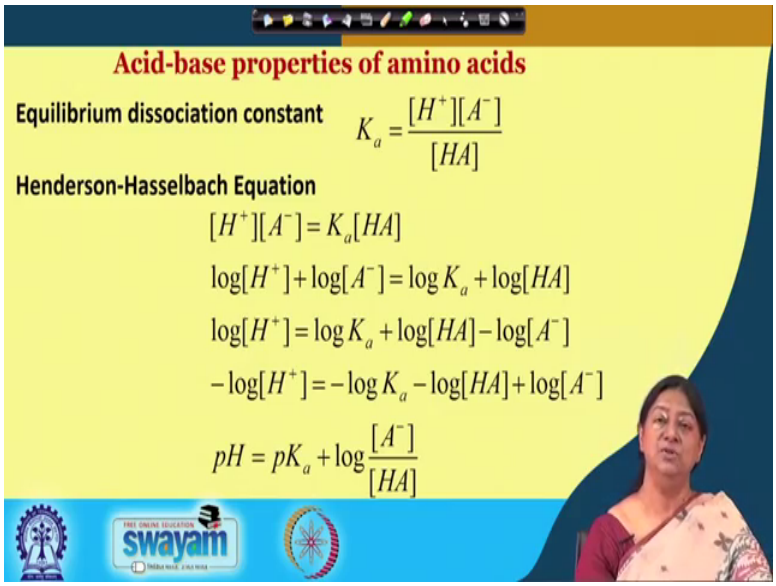

If we go back to the Henderson Hasselbach equation, which we did in the last lectures we have the K a value formed from a dissociation of the acid HA H plus A minus, we now consider the acid base properties of the amino acid. So, going back to recapitulation of the Henderson Hasselbach equation, if we rearrange this form we get the form of the Henderson Hasselbach equation that tells us that the pH is equal to the pKa plus the log salt by acid.

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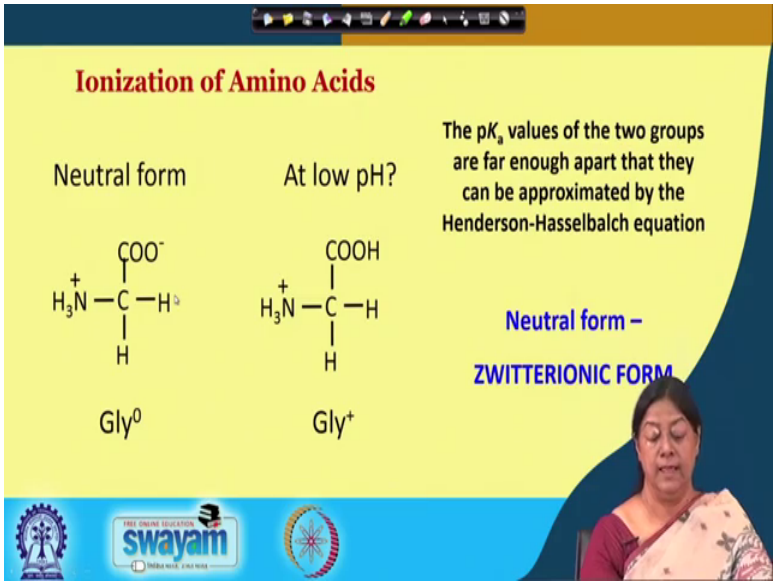

Now, when we consider this as the neutral form say of glycine, which is the simplest amino acid the side chain being just hydrogen. So, this is one of these is the R group. So, if this is our neutral form then, if I were to the ask the question what is the structure of glycine at low pH? If we look at the structure of at low pH, it means that the NH 2 group is also protonated and the carboxyl group is also protonated, the neutral form that we have here is called this zwitterionic form.

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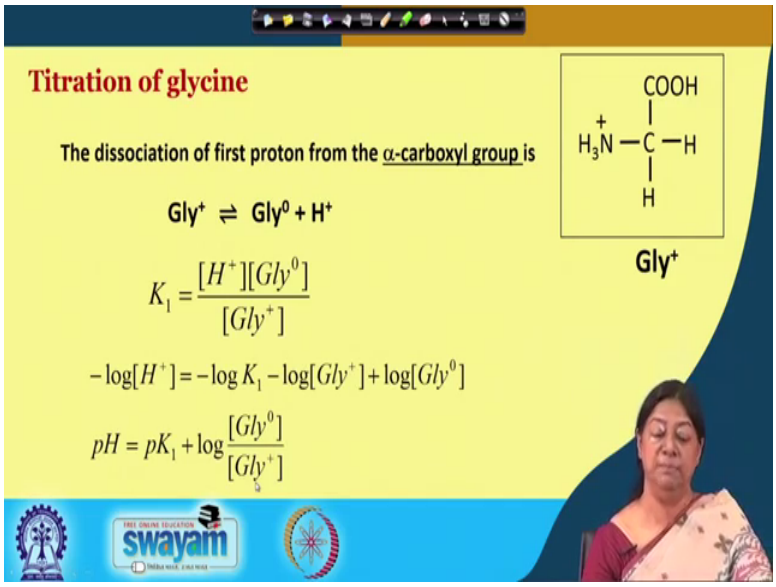

Now, if we are to consider a titration of glycine what do we mean by a titration of an amino acid? We understand that the amino acid is amphoteric in nature, there is a specific structure that we have here for glycine.

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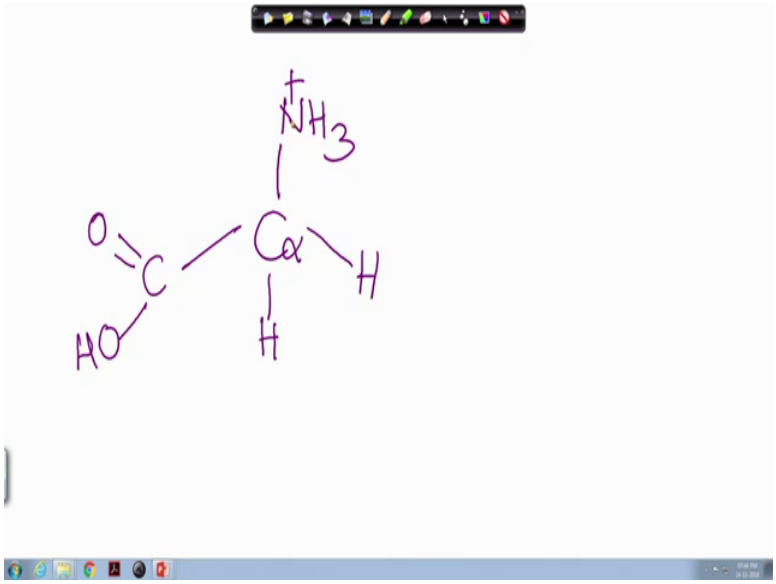

If now we were to consider a titration and acid a base titration, we would want to look at this in a manner where we could think of the amino acid being as the alpha carbon, the hydrogen, the hydrogen forming the side chain or we could consider the C double bond O and the OH and the NH 3 plus.

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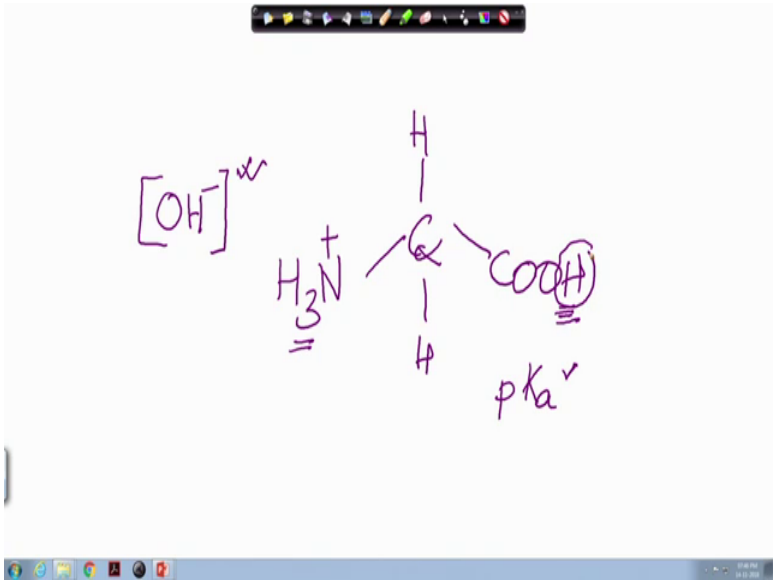

So, when we consider this we think of our we think of our structure as something like this is the alpha carbon, this is the R group, this is the. So now, we have this proton and we have this proton. Now depending upon the pKa values, the protons are going to be

lost; what do we mean by that? If an acid base titration is being done which, would mean that a certain amount of OH minus is being added to the solution, the solution of glycine. So, if we take the solution of glycine and we add OH minus then this proton is going to be lost first, because the pKa is less. So, what does our structure now become? A structure now loses this proton.

So, once it loses this proton then we have lost the proton associated with the COOH which means that the Gly plus becomes Gly 0 plus H plus this means that there is associated with this specific pKa value if we call this K 1, the equilibrium would be H plus Gly 0, what do we mean by Gly 0? It is COO minus NH 3 plus just like we had in the previous slide. So, this is what is Gly 0, which means that it does not have any charge it is a neutral form. At low pH, we say everything is protonated as we do the titration we are losing the H plus, which H plus are we losing? We are losing the one connected to the carboxyl group.

So, this is the K 1 value if again we rearrange this into a form like the Henderson Hasselbach equation, we get that the pH is equal to pK 1 plus log Gly 0 that is the base the conjugate base and our acid Gly plus. If we continue the titration, we will reach a point where this proton associated with the NH 3 plus will now be lost.

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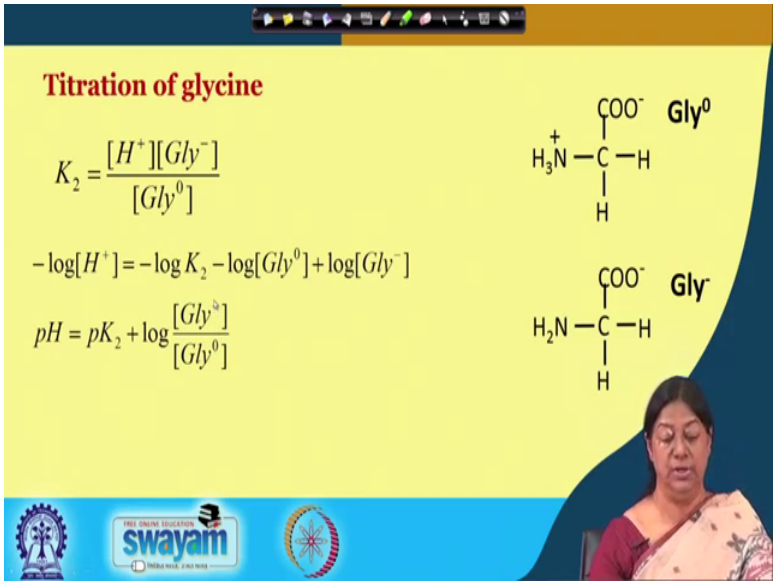

So now, we have Gly 0 as we continue the titration we reach a point when all of the protons associated with the carboxyl group have been lost and now an addition of OH

minus or the increase in N OH minus concentration is going to result in a loss of the proton from the NH 3 plus, which will go to NH 2. So, what do we have? We have Gly 0, now going to gly minus because the overall charge on this is minus, this is associated with a K 2, what is the K 2? In K2 this becomes the conjugate base and this is the acid because, this is providing the proton. So, we can do the same set of equations or the same algebra associated as we saw with the Henderson Hasselbach, we get the pH is equal to the pK 2 plus log of Gly minus by Gly 0 because, Gly 0 is now the acid and this is the conjugate base.

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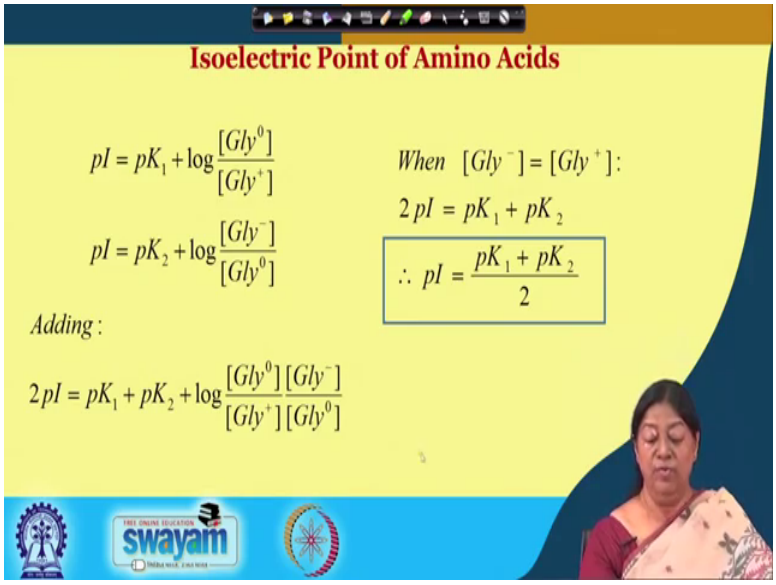

In our next step we look at what is called an isoelectric point of amino acids, what do we mean by an isoelectric point?

We have an isoelectric point, where the charge on the amino acid is 0, it is neutral which means that it does not move in an electric field we found out that this p I has to be equal to the pH then the p I is equal to p K 1 plus log Gly 0 Gly plus, when Gly plus was the acid and this was the conjugate base. In the second step, when we had p K2 K 2, we have Gly 0 as the acid and Gly minus as the conjugate base. If we add these 2 together, we find out that if we look at a specific condition, where Gly minus is equal to Gly plus, this means that we find out that when Gly minus is equal to Gly plus this part becomes 0.

And p I is equal to pK 1 plus pK 2 divided by 2, what does this mean? It means that if we have a knowledge of the pK 1 value and the pK 2 value of an amino acid, we can determine what the isoelectric point is.

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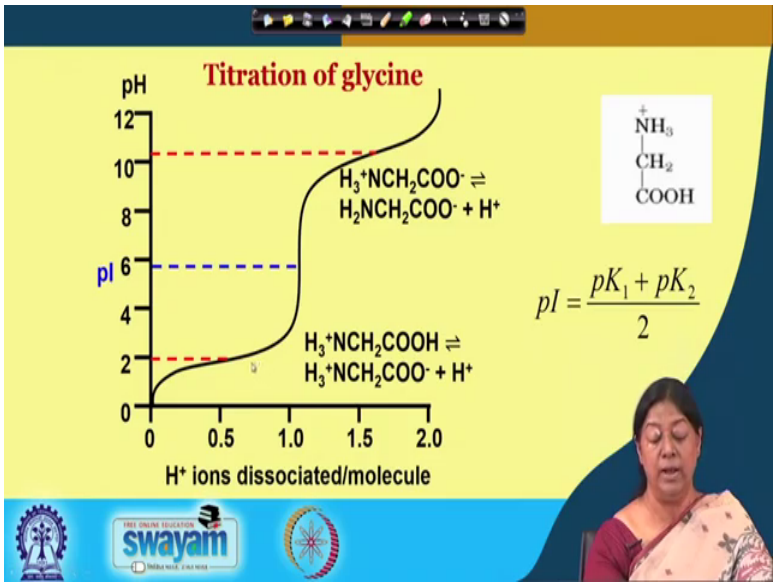

If we go further into looking at an exact experiment as to how this is conducted. So, at the initial point where the pH is very low it means that our glycine looks like this, the NH 3 plus the NH 2 is protonated to NH 3 plus, the COO minus is COO H, when the H plus ions dissociated per molecule, why are they this being dissociated because, we are adding alkali.

So, as the first drop of alkali is added we see that this is where the H plus is removed to form CO minus plus H plus. So, this corresponds to our K 1. So, here we would have a variation along this graph at this point, when we come to this point here we have now converted all the protonated glycine the completely protonated glycine into this form. So now, we have reached the K 2 value where now the proton being lost is going to be from the amino group. So, we have the NH 3 plus CH 2 CO minus go to NH 2 CH 2 CO minus.

Though so, this was our Gly plus, this is our Gly 0 Gly 0 going to Gly minus and the p I we found out is the pK 1 plus the pK 2 divided by 2.

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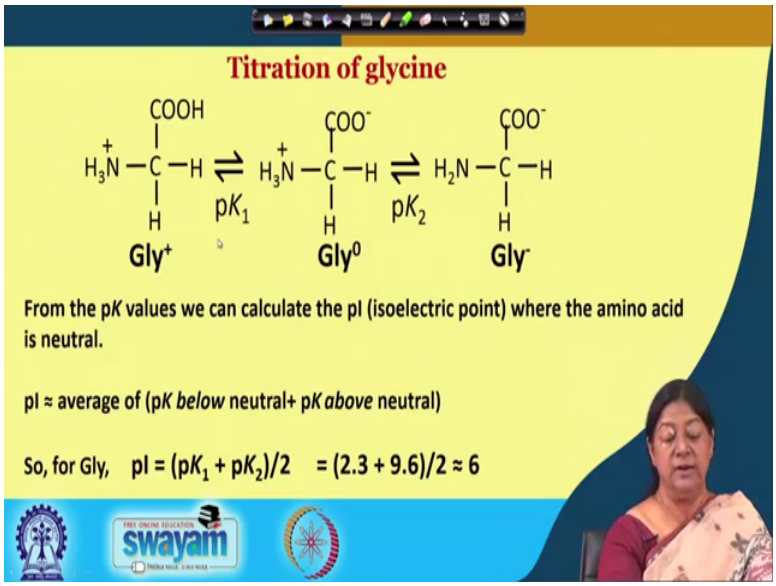

So, here is our Gly plus this is the pK 1, what do we do? We lose the first proton, when we consider pK 1, because this is lower a lower pKa value. So, it becomes CO minus which is Gly 0, because there is no charge on this particular species, we go to pK 2, where we lose the proton from the NH 3 plus making it NH 2 CO minus Gly minus. So, we are going from Gly plus to Gly 0 to Gly minus.

So, what is the charge? The charge is from plus 1 to 0 to minus 1. Now from the pKa values that render the amino acid charge from plus 1 to 0 to minus 1, the pK value is responsible for the change over from plus 2 0 to the minus 1, the average of those pK values determines the pI of the amino acid for example, for glycine the pI is going to be the average of the pK below neutral and the pK above neutral. So, 0 is neutral the pKa below neutral the pKa above neutral the average of these is what is going to give you the p I. So, for glycine we were I will just show you a table of the values, we have 2.3 plus 9.6 divided by 2, which gives you a pI of approximately 6.

This will be experimentally demonstrated to you as to how you can actually determine the p I of glycine in the experimental section that will be shown to you.

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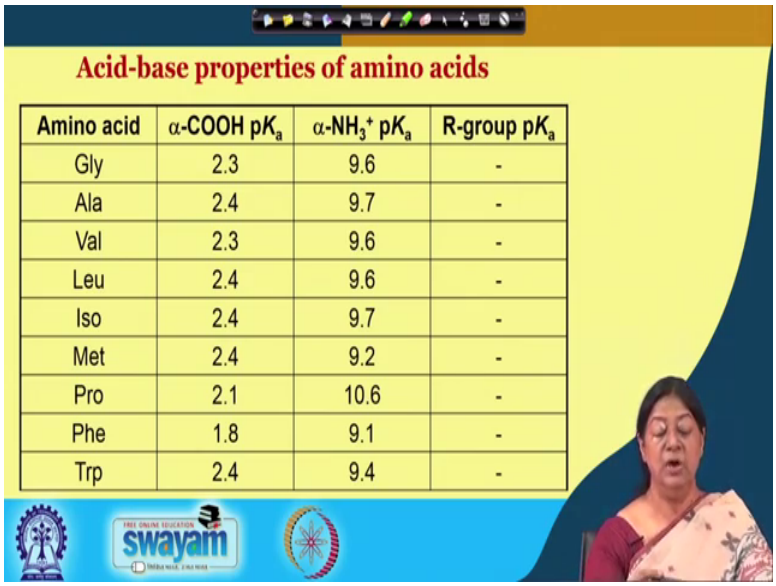

So, now if you look at the acid base properties of amino acids with the knowledge of what we have just learnt given that we have a pK 1 value, a pK 2 value and a pK R value then we say that if I were to determine the pI of these specific amino acids, I know what the pI of glycine is going to be what the pI of lysine is going to be and what the p I of methionine or any other amino acid is going to be.

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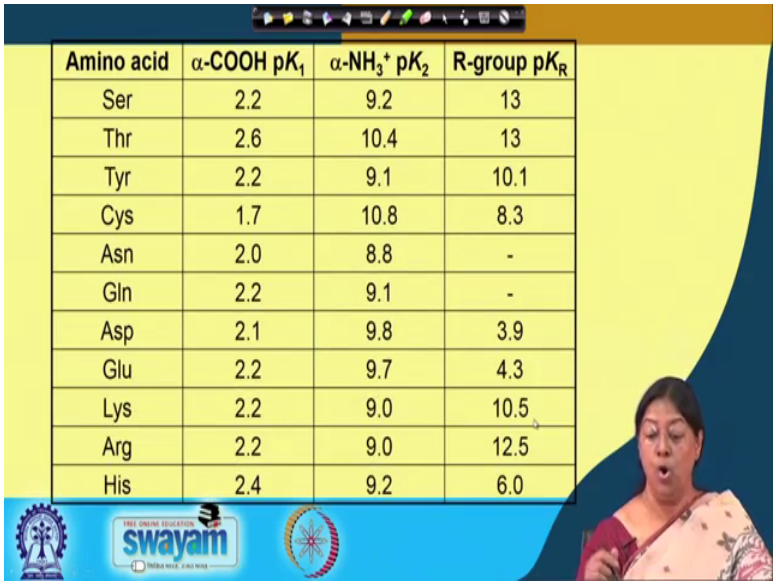

But if it so happens that associated with this the R group also has a pKa associated with this, what do we mean by this? If we have apart from the carbon and the hydrogen

present in the side chain, if we have a nitrogen, if we have an oxygen, if we have an acidic amino acid, if we have a basic amino acid then these are what are called ionizable amino acids. So, the which means that the side chains the R groups in addition to the pK 1 and pK 2 that is present for all amino acids because, the amino acid is always going to have an amino group associated with it is going to have a carboxylic acid associated with it.

So, this is what called the amino acid. So, these pK 1 pK 2 values are always going to be present, but in addition to that there are specific ionizable groups that are responsible for additional properties rendered to the amino acids because, they will lose additional protons, because of the side chain characteristics. For example, if we look at aspartic, acid aspartic acid has an additional COOH, what does this mean? It means that that proton can also be lost apart from the loss of COOH from the alpha carbon and the proton from the amino group the protonated amino group.

So in this case, there will be a p K 1, there will be a pK 2, there will be a pK R, we will demonstrate how the pK 1 and the pK 2 and the pK R, we understand how we are to determine the p I value, we will show you how you can determine or we can determine the p I value of a side chain that is ionizable. How do I determine the p I value of glutamic acid? We will see that in the next class.

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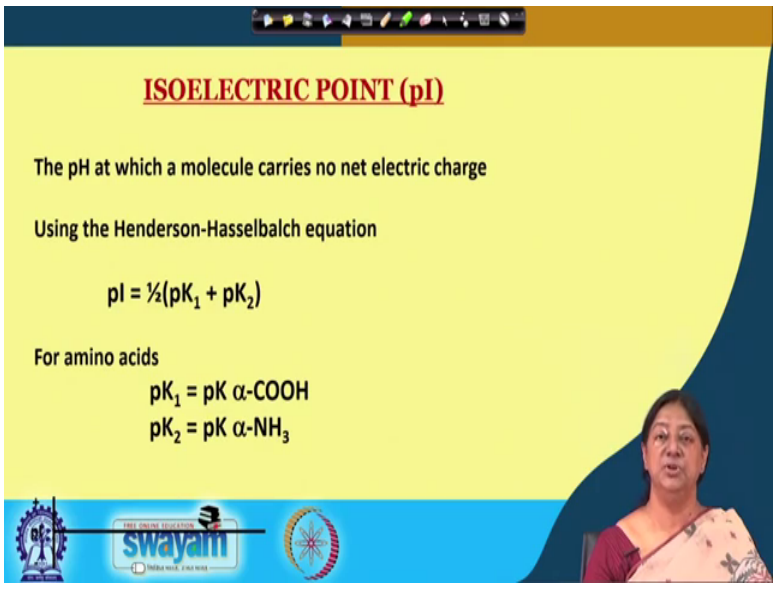

So, what we understand is that we have an isoelectric point of an amino acid. This is the pH at which the molecule in this case the amino acid carries no net charge. The important thing about this is particularly for proteins for biomolecules, there is a method where we can calculate the pI of a protein. Now calculating the pI of a protein, we will learn how to calculate the pI of a peptide, which can be extended to calculating the pI of a protein. What the knowledge that we require for this is the pK 1 value, the pK 2 value and the pK R value of the ionizable side chains. For amino acids without a side chain that is ionizable, we know that we have a pK 1 associated with the alpha carboxyl group, we have a pK 2 associated with the alpha NH 3 plus that should be a plus here associated with this group.

Now, this is true for all amino acids, but as I mentioned in addition to this we have the ionizable side chain. If we have the ionizable side chain this means that in our titration profile, which we saw for glycine, we would have an additional break due to the additional pK value that we see. For example, if we go back and just look at the titration curve here, here we have a pK 1 associated with this proton dissociation, we have a pK 2 associated with this proton dissociation.

Now, if we ask the question that if I have an acidic amino acid, how is this titration curve going to change. This means that apart from the carboxylic acid proton, there is going to be an additional proton lost near the acidic range. If it is a basic amino acid residue, it means that there is going to be an additional proton loss near the basic pK value. So, that would render a difference in the shape of the curve, it would give us a different pI value of the amino acid, which we will have to determine based on the charges as was shown the charge that is going to give us the specific pI value will be the pI is going to be the average of the pK below neutral and the pK above neutral.

So, what can be done is with the progress of the reaction, if we have an ionizable group present here, there will be an additional pK value associated with the pKR. So, what will happen is we will have to locate the neutral charge of the amino acid, check the pKa below neutral, check the pKa above neutral and the average of that will give us the pI of that specific amino acid, which we will see in the next class.

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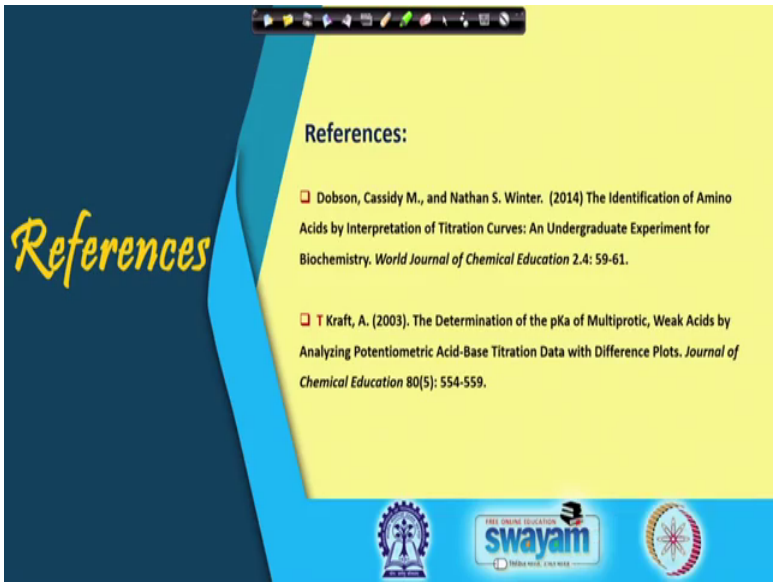

So, the references that we will follow are there are certain books and certain methodologies that can be used for the calculation of the isoelectric point.

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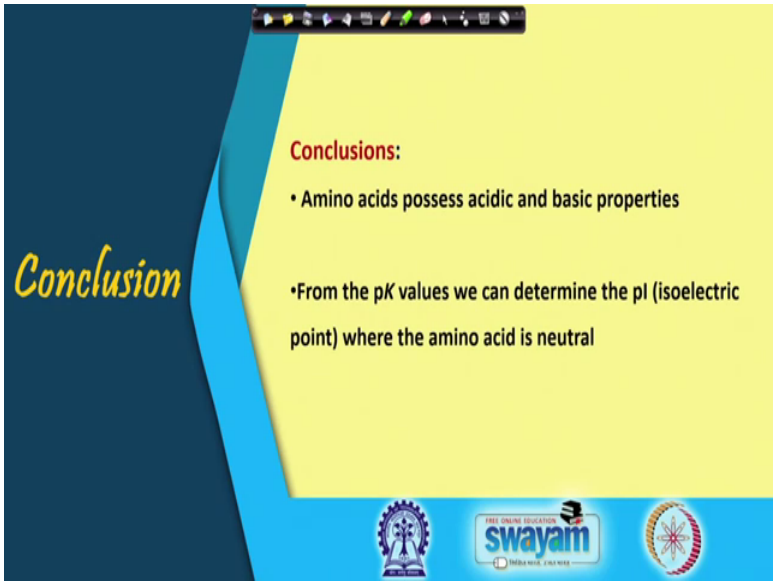

So, what we do know is the amino acids possess acidic and basic properties, which is extremely important in their final formation of peptides and proteins and how they are rendered in peptides and proteins in terms of their folding, their denaturation, their properties, their size, their charge, their polarity, their hydrophobicity, their aromaticity, what kind of structures they would form in proteins and what kind of properties we

would see, because of the folded structure. We will be looking at that during the course of experimental biochemistry. And, we found out that from the pKa values that we have from the amino part and the acid part we showed it for glycine that we can determine the isoelectric point, where the amino acid is neutral.

Given a knowledge of the pK 1 and the pK 2 values, we can find out the pI. In addition we will learn that if the amino acid has an ionizable side chain where that can also donate protons then we will see how the pI value can be determined. And, in an experimental demonstration glycine the calculation of the pI for glycine will be shown to you, in addition to the calculation of pI of an acidic amino acid and of a basic amino acid will be demonstrated to you experimentally using a pH meter.

Thank you.