Biochemistry - I

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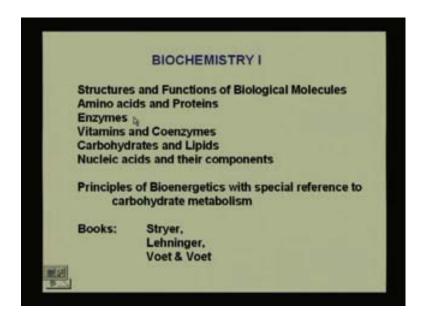
Indian Institute of Technology, Kharagpur

Lecture 1

Amino Acids I

Hello, welcome to the course Biochemistry 1 conducted by me Dr. S Dasgupta, Department of chemistry Indian institute of technology Kharagpur. In this course we will be studying certain aspects of Biochemistry starting from structures and functions of Biomolecules right on to Bioenergetics and Metabolism.

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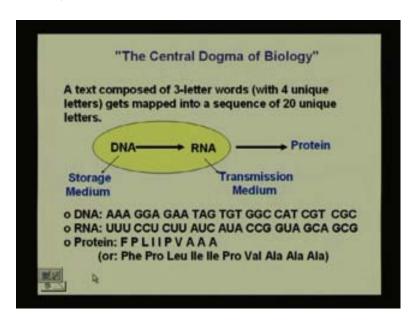


The topics we will be covering are structures and functions of Biological molecules. In that we will be considering Amino acids and Proteins, Enzymes. Under the Enzymes we will be considering the mechanisms of specific enzymes move to Vitamins and Coenzymes, Carbohydrates and Lipids, Nucleic acids and their components. The entire range of topics of this course will be covered in their detail as is relevant to this course.

Also we will be considering the Principles of Bioenergetics with special reference to carbohydrate metabolism. The books we will be covering are common Biochemistry books such as Stryer, Lehninger, Voet & Voet (Refer Slide Time 2:15 min).

When we consider "The Central Dogma of Biology" the first thing that comes to mind is DNA, the DNA is the storage medium. The Central Dogma of Biology goes like this DNA \rightarrow RNA \rightarrow Protein. We have a text that is comprised of DNA which is the four basis of DNA that is the storage medium and this is transcripted to RNA which is the transmission medium that also is comprised of four basis one of them being a bit different. The RNA is then translated to the Protein. The alphabet of DNA, RNA and Protein is slightly different. What is this alphabet? In DNA we have four letters to the alphabet the four letters are as you can here A, G, T and C. These are the four letters that comprise the alphabet of DNA.

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If you look at the corresponding alphabet of RNA you will see that we have U, C, A and G. When we go on to study the structure and contents of Nucleic acids the structures of each of this basis will be much clear but we have to know that DNA and RNA are comprised of these letters which actually represent nitrogenous basis.

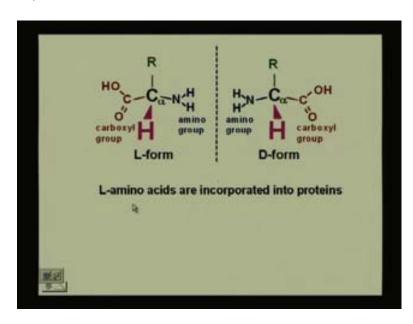
The Protein alphabet is a bit different the Protein alphabet is some times represented as a 3-letter code which we will see in a moment or by the 1-letter code which is another representation of the very same 3-letter code. The Protein alphabet is comprised of twenty unique letters that tell us what the Protein sequence is. We will understand what an Amino acid sequence is once we get into the details of what is a peptide bond and what is an Amino acid. The first thing that we know or we try to understand here is the carbon atom.

The Proteins are actually made of the Amino acids that are linked by peptide bonds. We will also see how a peptide bond is actually formed and how these letters were linked together to form like a sentence. Each amino acid consists of: a central carbon atom C_{α} so we have the central carbon atom

that is marked as C_{α} and we have an amino group NH_2 group, and we have the carboxylic acid group which is -COOH and we also have what is called an R group. This R group is the side chain of the amino acid. And we also have the hydrogen atom. (Refer Slide Time 7:14 min)

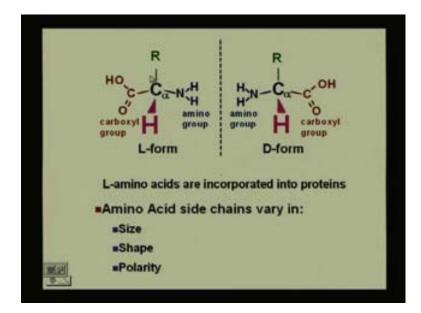
This part is common to all Amino acids because it has an amino group, it has an acid group, it also has an H a hydrogen attached to it. So we see this central carbon atom is actually a chiral carbon which means it is asymmetric which again means that there are four different groups attached to this central carbon atom. And since all amino acids have a common set of groups here in the amino group, the hydrogen atom and the carboxylic acid group. The side chain differs from one amino acid to another amino acid which can be different atoms, different groups of atoms and this is what actually distinguishes the various amino acids.

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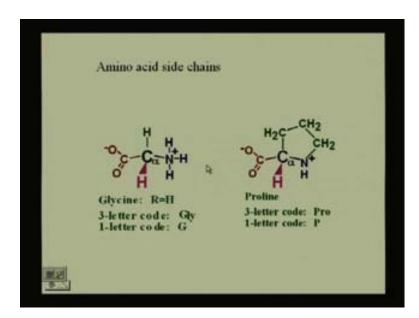
Now we are going to consider the types of R groups we can actually have. If we look at the different forms of amino acids that could be incorporated into proteins as we mentioned earlier we have an amino group, an carboxyl group, an hydrogen atom and we also have an R group attached to it. Now because of its chirality it can have an L -form or a D -form. Usually L -amino acids are incorporated into proteins. (Refer Slide Time 9:03 min). Now you understand that these side chains the R group can differ in its size, it can differ in its shape, it can differ in its polarity. There are twenty common amino acids which have distinctive R groups with distinct properties of size, shape and its polarity.

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We will consider the amino acid side chains by group in each case and you have to remember the 3-letter code of the amino acid along with their 1-letter code as well and obviously you have to remember what the side chain comprises. We have listed here is Glycine and Proline which are unique amino acids. The Glycine is the simplest amino acid because the R group is just a hydrogen atom.

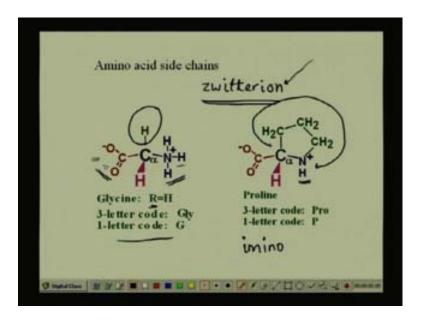
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This hydrogen atom makes the central carbon atom of Glycine symmetric because it does not have four different groups attached to it. It was attached with two hydrogen atoms which do not make it chiral anymore and this is the only such amino acid. (Refer Slide Time 10:42 min). So, if we look at

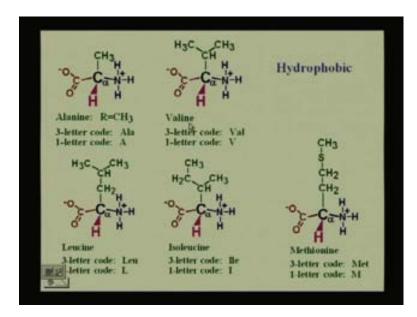
the Glycine the side chain the R group is attached to central carbon atom and we have an amino group and also a carboxylic acid group attached to it. We have all the amino acids are in the form of NH_3^+ and a COO^- because at physiological p^H the –OH group looses its hydrogen atom due to p^k value of the carboxylic acid group and this amino group is protonated which means that it has an additional hydrogen atom making this nitrogen positively charged and this is called the zwitterion form of the amino acid and it is represented in this fashion because we would like to represent the amino acids as what they would be at physiological p^H . (Refer Slide Time 12:22 min). The next unique amino acid is Proline. The Proline does not have a distinct R group attached to it but the R group is actually linked up to the amino group here.

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So the side chain $-CH_2$ $-CH_2$ is actually linked to the NH⁺ in this case. So the α - carbon have attached with a hydrogen atom, with a carboxylic acid. But the Proline is being as an imino acid instead of being an amino acid because it has an imine group instead of an amine group. So we have an imino acid where the side chain bends on to itself to form Proline. (Refer Slide Time 13:29 min). So these are the two amino acids that are unique in their features. The Glycine is being just because it has an hydrogen atom as its side chain and it is achiral. And the Proline is being as an imino acid because the side chain bends back upon itself. (Refer Slide Time 13:48 min). When we represent the amino acids we represent them in Zwitterionic form which is NH_3^+ and COO^- . Because this is how they would remain at a physiological p^H in normal solution. (Refer Slide Time 14:11 min).

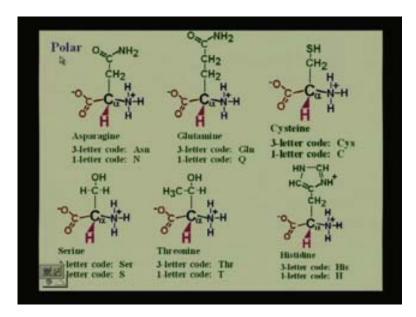
The next group of Amino acids is hydrophobic amino acids. The Hydrophobic amino acids are comprised of mostly of carbon atoms and hydrogen atoms in their side chains. So they would tend to be away from the solvent. Usually the solvent being water or water based. They would be away from water, not liking to be in water so they would be Hydrophobic. (Refer Slide Time 14:59 min). Here the simplest side chain having amino acid is Alanine. In which the 3-letter code is Ala and the 1-letter code is A. The side chain is a methyl group so this is what we would say the R group. Here again you recognize the Zwitterionic representation of amino acid.



Then we come to Valine. The Valine is a β -branched amino acid. Its side chain is -CH(CH₃)₂. The next one we have is Leucine in which the R-group is -CH₂ -CH(CH₃)₂. So it is branched at the γ -atom. The way these are represented is that if this is the C_{α} the next atom is the C_{β} beta which is connected to 2 C_{γ} atoms which would be a unique representation of the amino acid Valine. (Refer Slide Time 16:16 min). If we look at Leucine we would again have a unique representation. We will consider this as C_{α} , the next one which is attached to this is C_{β} and then C_{γ} . It is attached to the 2 C_{δ} atoms in which one is represented as $C_{\delta 1}$ and the other as $C_{\delta 2}$. The Alanine side chain has only a C_{β} -carbon. So this code actually be represented very clearly in a unique manner where each amino acid has this zwitterionic part being common. The chain could be represented by the types of atoms that are attached to the C_{α} . (Refer Slide Time 17:14 min)

If you look at the Isoleucine we have the side chain $-CH(CH_3)$ $-CH_2$ $-CH_3$. So we have a C_β atom attached by one methyl group and one ethyl group. All these side chains are comprised of C and H which makes them Hydrophobic in nature. In the same way the Methionine can fall into this category is well. But it has a Sulphur atom and a methyl group attached to the Sulphur atom in its side chain. So we have a C_α attached with C_β which is C_γ . And this C_γ is attached to the Sulphur atom and then we have a methyl group attached to the Sulphur.

The Methionine along with another amino acid Cysteine is the Sulphur containing amino acids. They could be grouped together in a group of their own or they could be considered in this group as well. (Refer Slide Time 18:35 min). The next group that we will be considering is the Polar amino acids. The Polar Amino acids have an oxygen atom or a nitrogen atom in their side chain. And by virtue of having the hetero atoms like the oxygen or the nitrogen in the side chain is they can participate in polar interactions not only among themselves but also with solvent molecules. So they can participate in Hydrogen bonding which is extremely important in non-covalent interactions in Proteins which holds a Protein fold it together in the protein chain the amino acid chain.



But the Polar amino acids are likely to interact with the solvent. In this interaction they can allow the oxygen and nitrogen atoms to interact with the solvent molecules or within themselves to form a network and remain in solvent. In contrast the hydrophobic amino acids are unlikely to be on the surface of the protein. So when we have an globally structure we will see that there are certain side chains are preferred to be on the surface of the protein and there are certain side chains that are preferred to be away from the solvent which we have seen earlier would be the Hydrophobic in nature. (Refer Slide Time 20:26 min).

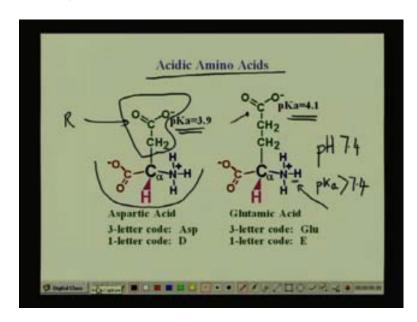
Now, if you to look at the side chains that are comprised this polar group of amino acids. Each of these have an oxygen or nitrogen attached to it. We have of course the common part of amino acid in the Asparagine side chain and in the glutamine side. (Refer Slide Time 21:00 min). These two amino acids have amide group in its side chain. The amide groups are $-C(O)NH_2$ groups. So this $-CH_2-C(O)-NH_2$ group comprises the amide of Asparagine. This $-CH_2-CH_2-C(O)-NH_2$ group comprises the amide of Glutamine. Here the only difference is the Glutamine chain is one carbon longer than the Asparagine chain. So here we have a $-CH_2$ group that is the β -carbon attached to the α -carbon followed by a γ -carbon that has attached with an oxygen atom and then $-NH_2$ group. So in Aspargine the amide group has a single β -carbon attached to the α -carbon.

In Glutamine we have two -CH₂ moieties in the side chain. We have C_{α} , C_{β} , C_{γ} and C_{δ} carbons. And this C_{δ} is attached with oxygen by a double bond and with a -NH₂ group. So in Aspargine and similarly in Glutamine the oxygen, nitrogen can participate in Hydrogen bonding which means if we have a specific donor or an acceptor then this could participate in Hydrogen bonding not only with other amino acids but also with the solvent. (Refer Slide Time 22:38 min). In this group the next amino acid is Serine. The Serine is a small amino acid but a polar amino acid. The group it has is -CH₂OH and this -OH can participate in the Hydrogen bonding. In this series Threonine is the next amino acid. It has a -CH₃ group and a -OH group attached to the β -carbon so again it differs from Serine.

The next amino acid in this group is Cysteine. The Cysteine is another type of the amino acid which has a Sulphur atom same as in Methionine which also has a Sulphur atom but the Sulphur was attached by a methyl group. Here we have a hydrogen atom making this ethyl so we have -CH₂SH. The Histidine is a very important amino acid. Enzymes and the Enzyme mechanisms will be covered in detail about the Histidine because of its specific polarity or specific properties of this side chain that is an amidazole group. So again Histidine have a common amino acid part. In the side chain of Histidine have two nitrogen atoms which are in part of the amidazole protein. (Refer Slide Time 24:34 min). So the side chains of all these polar group of amino acids have contained a hetero

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atom.

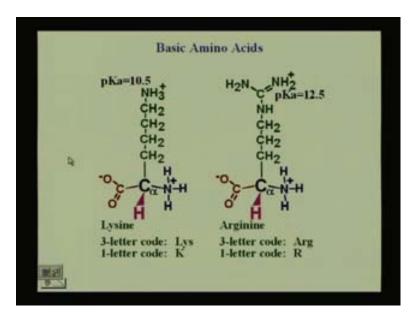


We will be considering the next group of side chains are Acidic Amino Acids. Earlier we looked at Asparagine and Glutamine. The Asparagine has the -C(O)NH₂group. We know that an amide comes from a carboxylic acid. So the Asparagine comes from a specific carboxylic acid. Similarly the Glutamine also comes from a carboxylic acid. So we group them into Acidic Amino Acids and we call these specific acids as the Aspartic acid which gives rise to a Asparagine and Glutamic acid which gives rise to Glutamine.

Now we have here is a -COO¯ group. This -COO¯ group is apart from the actual carboxylic acid that is common to all amino acids. This is part of the R group the side chain. So the side chain in Aspartic acid also contains a carboxylic acid group. Similarly the side chain in Glutamic acid also has the carboxylic acid group but it has an additional -CH₂ just as like in Glutamine. Here in addition we have a p^{Ka} value. If the p^{Ka} value is less than p^H in a solution then carboxylic acid is going to loose its proton similarly the carboxylic acid looses its proton but this amino group has not. Because the p^{Ka} value of this amino group is actually higher than physiological p^H which is why it has still kept its proton attached to it. But if we consider the physiological p^H to be 7.4 it means the p^{Ka} of this group is greater than 7.4 and we will see how it is actually something close to 9 or between 9 and 10. So if we have the p^{Ka} >7.4 this is going to remain protonated but these carboxylic acid cannot remain protonated. So these are comprised as Acidic Amino Acids. (Refer Slide Time 28:02 min). If

there are Acidic Amino Acids it means that there are also be Basic amino acids. These are Lysine and Arginine.

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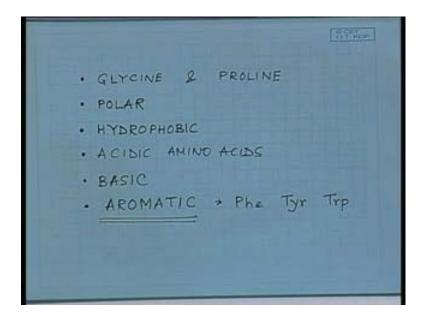


Now we will look at the side chain groups of the Lysine and the Arginine. This is the long side chain of Lysine and this is the side chain of Asparagine. (Refer Slide Time 28:37 min). Now each of these two amino acids has different p^{Ka} values. The amino groups are still protonated because of the pKa values are greater than the physiological. So we have protonated nitrogens because the physiological p^H is 7.4. And it did not reach the p^{Ka} value where this is going to loose its proton.

So, here we have an additional amino group apart from the common part of the amino group because it is a Basic amino acid. We have a guanidinium group in Arginine which is part of the side chain and it has nitrogen here, nitrogen here and nitrogen here (Refer Slide Time 29:47 min). So this is Lysine and this is Arginine. Especially these are the residues preferred to be on the surface of the protein especially because of their properties.

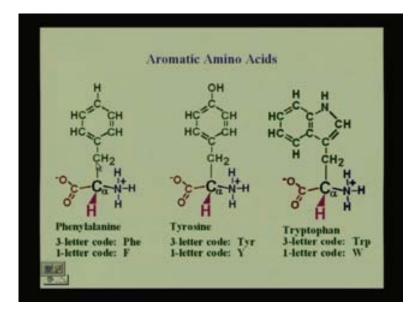
So, if we look at the different structures of the amino acids in which we have considered specific groupings. The different groupings are the GLYCINE and the PROLINE which forms a group by itself because of the uniqueness in their properties and we have the other group the POLAR AMINO ACIDS, we have HYDROPHOBIC AMINO ACIDS, we have ACIDIC AMINO ACIDS and we have BASIC AMINO ACIDS. There is another group of amino acids the AROMATIC AMINO ACIDS. The Aromatic amino acids are unique and Aromatic in nature, under these we have three amino acids namely Phenylalanine, Tyrosine and Tryptophan.

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Here we have Phenylalanine. As we have seen earlier the Alanine was just a methyl group attached to the α -carbon. But in this case one -H has been replaced by a phenyl group so its name is Phenylalanine. Of course it also has the common part of the amino acid. The 3-letter code for Phenylalanine is Phe and the 1-letter code is F.

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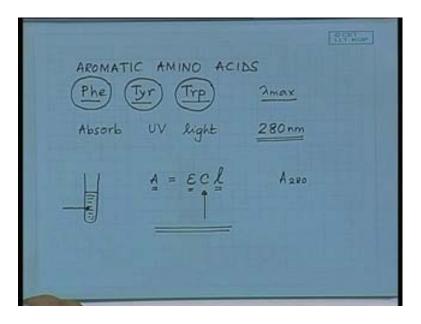


So we will get Phenylalanine by replacing one -H with phenyl group in Alanine side chain. So this is Aromatic in nature (Refer slide time 32:35 min). Then we have Tyrosine which is similar to Phenylalanine but the only difference in the side chain one hydrogen is replaced by an -OH group. So the Tyrosine can actually also be involved in hydrogen bonding. In the grouping of amino acids

this could also be put in a Polar group but it is already grouped under the Aromatic amino acids because of the presence of phenyl ring. So the Tyrosine have a -CH₂ and a phenyl and an -OH attached to this phenyl ring (Refer slide time 33:10 min). In this group the other amino acid is the Tryptophan. It has an Indole ring attached to this -CH₂. This is very bulky amino acid as you can see by the size of it and it is quite rare in Proteins. So it is not present in very large extent in many of the Proteins.

The unique properties of these Aromatic amino acids are makes the Protein useful in an analytical way. All the Aromatic amino acids which are Phenylalanine, Tyrosine and Tryptophan are absorbs the Ultra violet (UV) light. So their presence in Proteins can actually be neutralized in this fashion. This means they absorb UV light in the range of 280nm. Even though they have different λ_{max} values but we usually look at 280nm to identify a Protein. If a solution has a certain amount of Protein in it so we can determine the amount of Protein present in the solution by a consideration of the number of Phenylalanine, Tyrosine and Tryptophan that are present in the Protein chain.

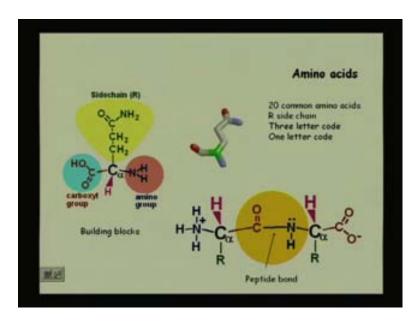
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So, if we monitor or if we find out the Absorbance at 280nm we know that the extension coefficient of the protein and we know the length of the cell and we also know the absorbance at 280nm which is also represented as A_{280} then we can determine the concentration of the protein. So the presence of these Aromatic amino acids can help in determining whether the solution is actually contained the protein or not. We can also find out the content of the concentration of the protein in solution by virtue of having Phenylalanine, Tyrosine and Tryptophan. In which the Ttryptophan has the highest extension coefficient which means if you have a large number of Tryptophan amino acids in the protein you are going to have a larger absorbance at 280nm. But the presence of the Aromatic amino acids themselves will give an absorbance at 280nm which is how proteins are monitored in Biochemistry laboratories (Refer Slide Time 37:12 min).

The next thing we are going to look at is a representation. As we have seen already we have a carboxylic acid group, we have an amino group, and we have a hydrogen atom which is common to all amino acids and we have a side chain, R. Here the side chain is an amide group.

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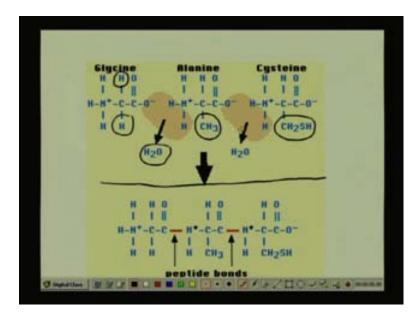
Asparagine and Glutamine are the amide group having amino acids. So the Glutamine side chain had two –CH₂ groups. The 2 –CH₂ groups attached here. This is a stick representation where the asymmetric carbon is in green, the other carbon atoms are in grey, the nitrogen atoms are in blue and the oxygen atoms are in red (Refer Slide Time 38:15 min). If we look at the linking of these amino acids, we know these amino acids are the building blocks in the formation of proteins. These building blocks have to be linked together to form a protein. They are linked together by a peptide bond.

Now the representation here is not zwitterionic representation because the proton of the carboxylic group is attached to it and this is the NH₂ group. In actual form it would remain as -NH₃⁺ and -COO⁻. Here we have two R groups and the first amino acid has an amino terminal, the other has a carboxylic terminal. So this is a di peptide because these two amino acids were linked by a peptide bond. This peptide linkage has a carbon with doubly bonded oxygen and a _NH group. But as we have here is the two R groups that in the first amino acid which is on the left hand side has an amino terminal. so this is a di-peptide because the two amino acids linked were by a peptide bond. The original amino acids were missing an -OH from the carboxylic acid side and missing -H from the amino side to form a peptide bond. These were makes an H₂O molecule. So the linking to two amino acids by the elimination of H₂O can form a peptide bond. (Refer Slide Time 40:02 min)

We will look into the features of the peptide bond once we considered the protein structure in general and the amino acid sequence. But when these amino acids are linked together on the left hand side you always have the N terminus and on the right hand side you always have the C terminus because this is the way the proteins are formed where this is the way they are synthesized. So we have an amino terminal and we have a carboxylic acid terminal. The first amino acid is

always has the $\mathrm{NH_3}^+$ group attached to it and the last amino has the -COO $^-$ attached to it acid in a protein sequence or in a protein chain. So this is a di peptide linked by a peptide bond. There are certain features of the peptide bond unique to protein structure.

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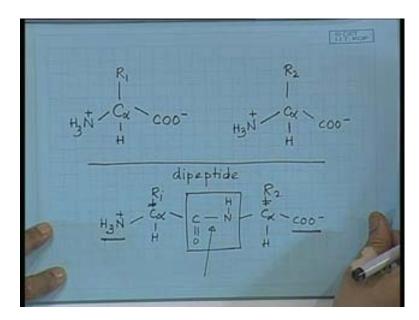


Here we have an amino acid the Glycine which is having a Zwitterionic representation. The Glycine is a unique amino acid where the R group is H where this R is the side chain representation of the amino acid. Here now the R group is CH₃ in case of the Alanine and the R group is -CH₂SH for Cysteine.

For Glycine we actually cannot distinguish which is the R group because the hydrogen is present and the hydrogen is also the side chain. So we have an -NH₃⁺ and a -COO⁻ and by eliminating water into forming a peptide -C(O)-NH-. So we have two peptide bonds a -C(O)-NH- and a -C(O)-NH-. So we have Gly -Ala -Cys in the formation of a tri peptide. And this can continue to form other peptide linkages (Refer Slide Time 43:00 min). So we have a C_{α} and we have -NH₃⁺ group and we have -COO⁻ group, we have H atom and an R₁ group is attached to this in the basic structure of the amino acid.

If we look at other amino acid you would have another $-NH_3^+$ group, we have $-COO^-$ and we have H and we have R_2 group. So now when we combine these two amino acids to form a di peptide we would have linked these by a peptide bond. So we have a -C(O) connected to -NH which is coming from the second amino acid, we have an α -carbon, we have R_1 and R_2 , the two hydrogen atoms and the COO^- group and $-NH_3^+$ group in this di peptide. So this particular linkage is known as the peptide linkage. Now we have linked the R_1 and the R_2 . In a representation of a protein it is not very convenient to keep on writing all the atoms together. We already knew that the amino acids are the building blocks in the protein sequence in the primary amino acid sequence which are linked together by the peptide bonds.

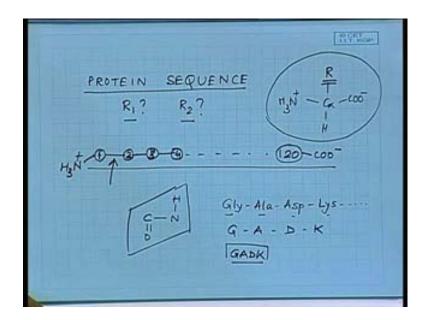
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Now since they are just linked by the peptide bonds. Then it is not necessary to write the common part of all the amino acids because these are certain features that we already know. We know that the first amino acid is going to be linked with the $-NH_3^+$ terminal and we know that the last one is going to be linked with the $-COO^-$. So it is sufficient to write instead of writing $-NH_3^+$ and $-COO^-$ in each case in a laboratory fashion. When we write a protein sequence all the information we actually need is R_1 and R_2 . Because we know each amino acid looks the same but it differs only in R group. So if we just know the R_1 and R_2 we need to know about how they are linked together in the protein sequence.

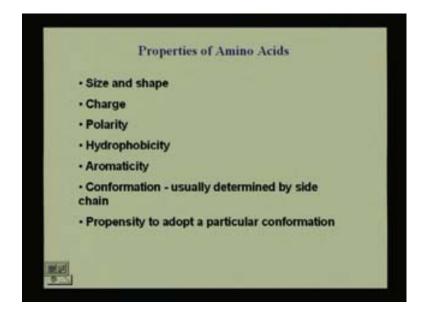
Suppose in this protein sequence if this is one amino acid and this is the other amino acid. Then this first amino acid has to have the $-NH_3^+$ attached to it and therefore3, 4 so on and say to 120 will have the $-COO^-$ attached to it. And also we know that these linkages are nothing but peptide bonds. So here we need to know what R_1 is, what R_2 is, what R_3 is and what R_4 is so on up to R_{120} in this sequence (Refer Slide Time 46:54 min). Here we just write either the 3-letter code or the 1-letter code. In the 3-letter code, if this first one were a Glycine then we would write as Gly and it is linked with for say Alanine which is represented as Ala linked with acidic amino acid Aspartic acid linked with a basic amino acid Lysine and so on and so fourth the sequence represented as Gly -Ala -Asp -Lys ------ because when we have the Glycine and the Alanine and the Aspartic acid so we know what are the rest of the atoms are because we know the side chain of the Glycine and we know the side chain of the Alanine and so on and so fourth. If we write this in a one letter code it would be G -A -D -K so just wrote as GADK you could write those structure of this tetra peptide.

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Similarly when we consider a whole protein chain in this case say just one hundred and twenty amino acids so the difference is in the properties of the amino acids side chains. Here the first property is the Size and shape of the amino acid. It is extremely important in its accommodation in the protein. The next property is the Charge on the protein whether it is acidic or it is basic. Next we look at the polarity whether it can be involved in hydrogen bonding or is it a polar amino acid.

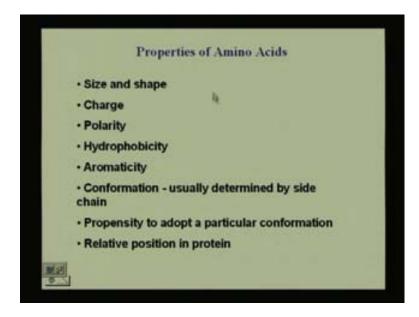
Then the Hydrophobicity, where this amino acid is likely to be located whether it is going to be located in the centre of the protein because its likes to be away from the solvent or whether it is going to be likely to be on the surface of the protein. But we know that any hydrophobic amino acid would prefer to be in the core of the protein. The next one is the Aromaticity, the aromatic amino acids that we are considered Phenylalanine, Tyrosine and Tryptophan are important in imparting UV properties to amino acids because these are the one set that absorb UV light. The proteins can be detected in solution due to the presence of the Aromatic amino acid which will absorb the UV light. And from the Beer Lambert's law we can find out the concentration of the proteins.



Now the confirmation, as we have already seen this is usually determined by the side chain. We will see the most of the side chains are linked by the single bonds. Also we will see how rotation about the side chains can actually bring about conformational changes to the amino acid orientations in the proteins. And this change in confirmation or the change in dihedral angles will allow us to look at different properties of amino acids in the way they interact with the other amino acids. We also look at the propensity to adopt a particular conformation. What does it mean? It means that if a protein were to have an amino acid that would likely form a helix or be part of a helix. We will see whether it is likely to be in a helix or it is likely to be in a sheet.

So, the summary of today's lecture is the different types of amino acids, the different groupings of amino acids and the important properties of the side chains of the amino acids. We were considered the central carbon atom is the asymmetric carbon atom which is also known as the α -carbon atom has linked to four different groups. These are hydrogen atom and amino group and carboxylic acid group and a side chain that is represented as R. We have twenty different common amino acids each having these various R groups. The twenty amino acids have twenty different R groups that differed largely in their properties. We have different types of amino acids which are grouped into the type of R group the type of side chain that they have attached to them. We have unique amino acids Glycine and Proline. we have Hydrophobic amino acids and we have Polar amino acids, we have Acidic and Basic amino acids and we have Aromatic amino acids.

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Methionine and Cysteine are Sulpher containing amino acids and there are other sets we have here is the hetero atoms oxygen atoms and nitrogen atoms in them and the side chains comprised entirely of carbon and hydrogen making them hydrophobic in nature.

And lastly we look at overall properties that we can consider together are the Size and shape, the Charge, the Polarity, the Hydrophobicity, the Aromaticity. All of these will actually determines the property of the protein in general. Because we know that these amino acids are linked by peptide bonds. These linkages of the peptide bonds bringing different types of amino acids together to form a protein sequence. We have seen the peptide bonds and we saw how the amino acids were linked together by the peptide bond and how we can actually represent the protein sequence by just writing either the 3-letter code one after the other or the1-letter code one after the other. Because we know that the first amino acid will have N terminus and the last amino acid will have C terminus which means that first amino acid is going to have the -NH₃⁺ attached to it and the last amino acid is going to have the -COO- attached to it to making up the protein chain.

We will learn in the later classes how the protein actually folds and how the hydrophobic amino acids tend to remain in the centre of the protein, thank you.