Introduction to Proteomics
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Lecture – 01
Introduction to Amino Acids

Welcome to the proteomic course. Before we move on to the proteomic and discuss about what are all different techniques and concepts in the modern proteomics. Let us first start the basic concepts on proteins.

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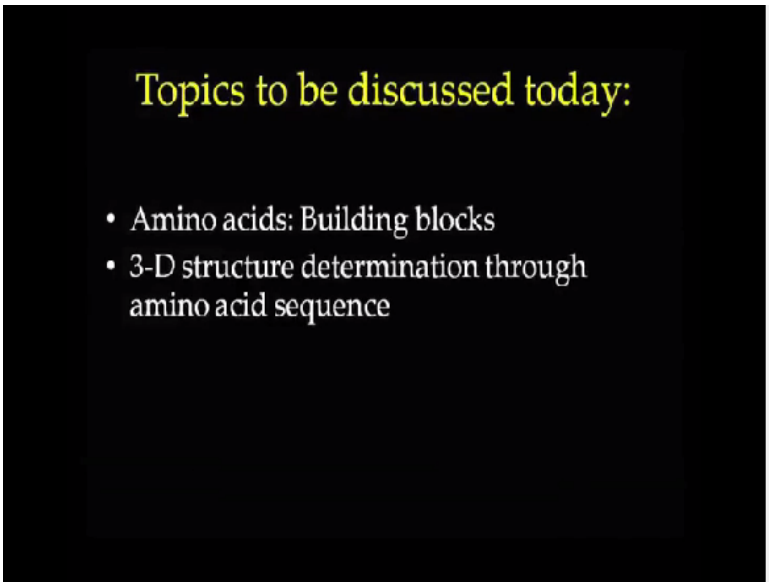

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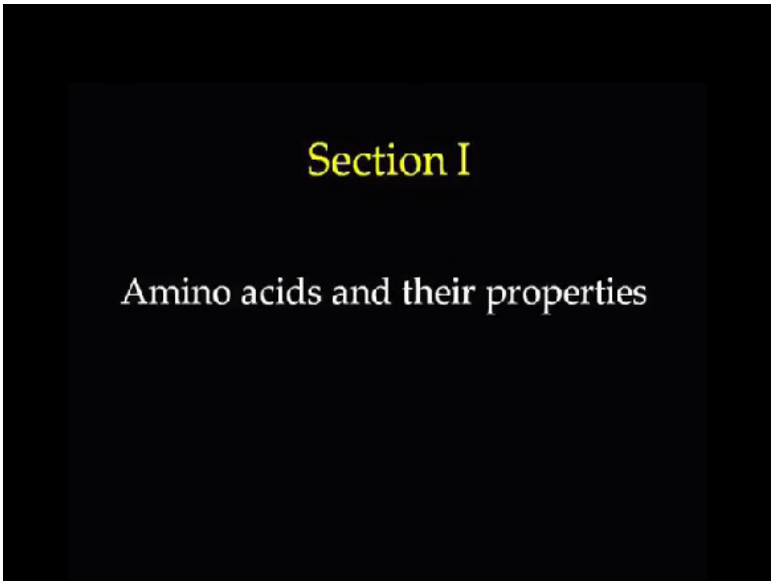

Amino acids, the building block of proteins.

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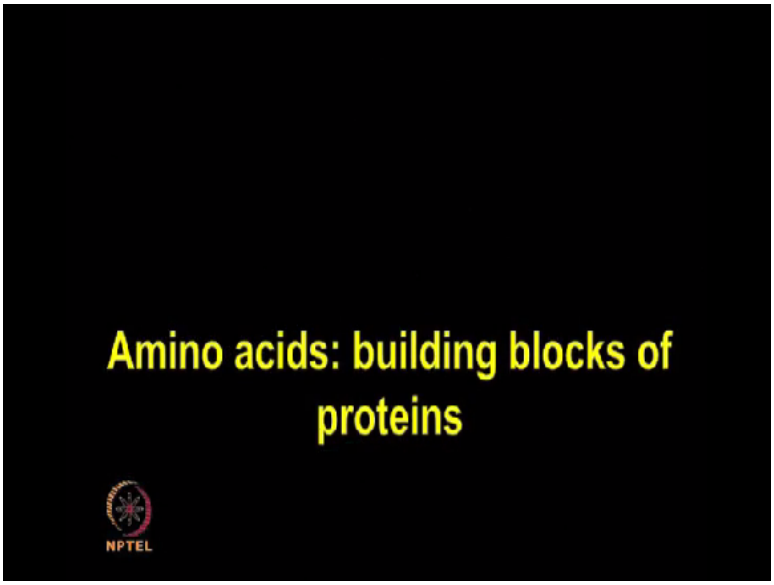

Amino acids constitute the basic monomeric unit of proteins which are joined by the peptide bonds. The 20 standard amino acids which can be arranged in several ways giving rise to numerous proteins having different structural and functional properties. The diversity and versatility of 20 amino acids enables a range of protein functions. Due to the side chains which can vary in size, shape, hydrogen bonding capacity, hydrophobic characters, charge and chemical reactivity, proteins perform much diverse function as compared to the DNA.

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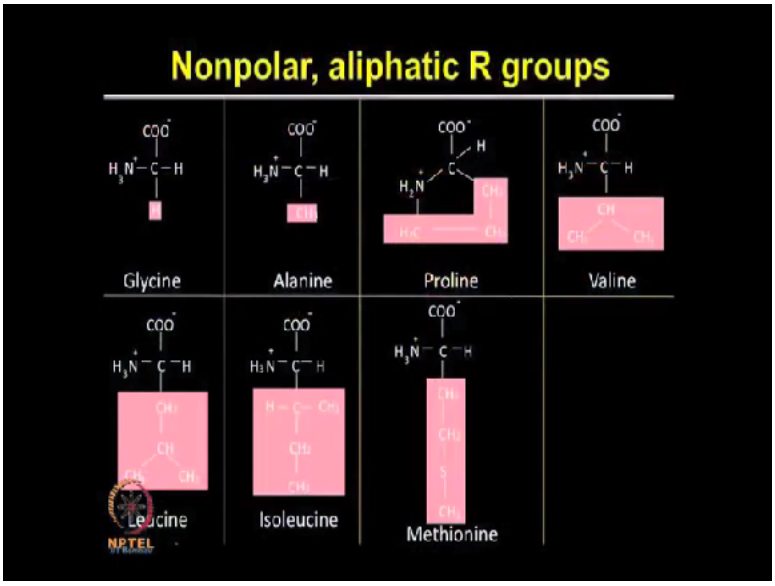

We have already studied about different amino acids in your undergraduate education. I will again try to refresh you on some of those concepts but in more nutshell. So here I have shown various amino acids which are nonpolar and aliphatic R groups starting with the glycine if you see on the left-hand side, the topmost which is the simplest and achiral. Now next is alanine which contains a methyl group. Proline which has aliphatic side chains.

Proline has very unique feature which has no free amino group and the side chain is bonded to the N and alpha carbon of the alpha carbon atoms. The ring structure, it provides more conformational restrictions and therefore proline plays very crucial role and unique properties in many fashions. Valine, it is branched-chain amino acid. Leucine on the left-hand side bottom panel that is hydrophobic amino acid with isobutyl R group.

Isoleucine, it also has the hydrophobic amino acid characteristic and it contains chiral side chain. It is one of the essential amino acids. The last in the group is methionine which includes a thioether group. Again there are only 2 amino acids which contain sulphur and they play some very critical role.

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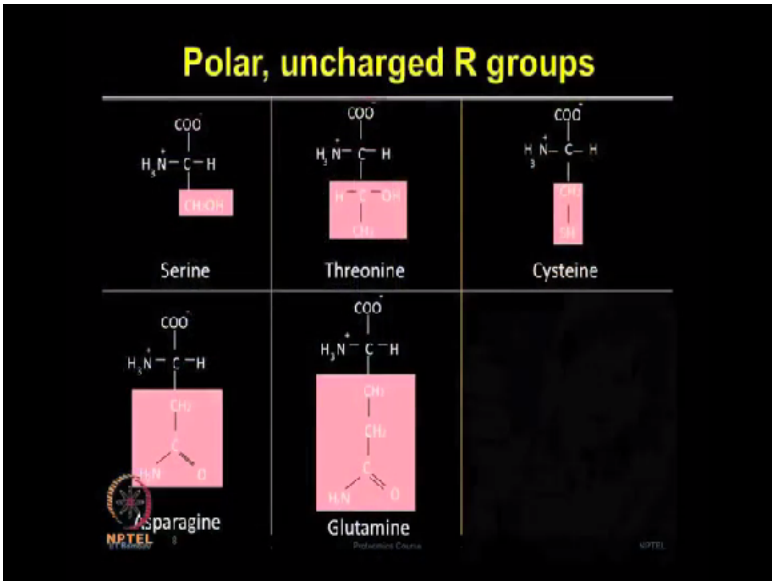

Now next category is polar, uncharged R groups. Just start with the serine which resembles in the structure like alanine but it contains hydroxyl group. Threonine, it resembles in the structure like valine and it contains hydroxyl group. It has an additional asymmetric center. Cysteine, it is similar to serine but it contains sulfhydryl or thiol group.

Two cysteine molecules form cysteine. Let us talks about asparagine which is shown in the left-side lower panel. It contains carboxamide side chains as a functional group. Glutamine, the side chain called as amide of glutamic acid which is formed by replacing the side chain hydroxyl group glutamic acid with an amino functional group.

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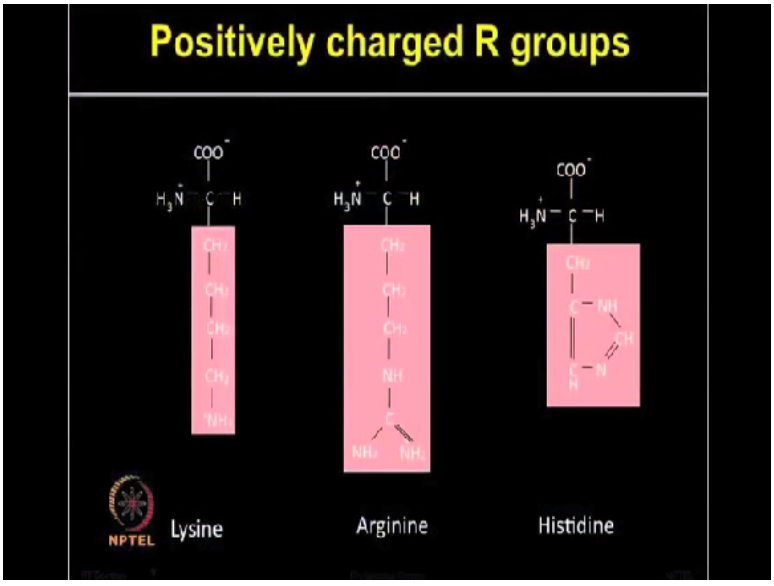

Next category is positively charged R groups. Three amino acid are here, lysine, arginine and histidine. Lysine is a base. It contains the capped primary amino group where as arginine contains guanidine group. Histidine, it has a functional imidazole group which is aromatic ring that can be positively charged. Histidine plays very critical role in many enzymatic activities.

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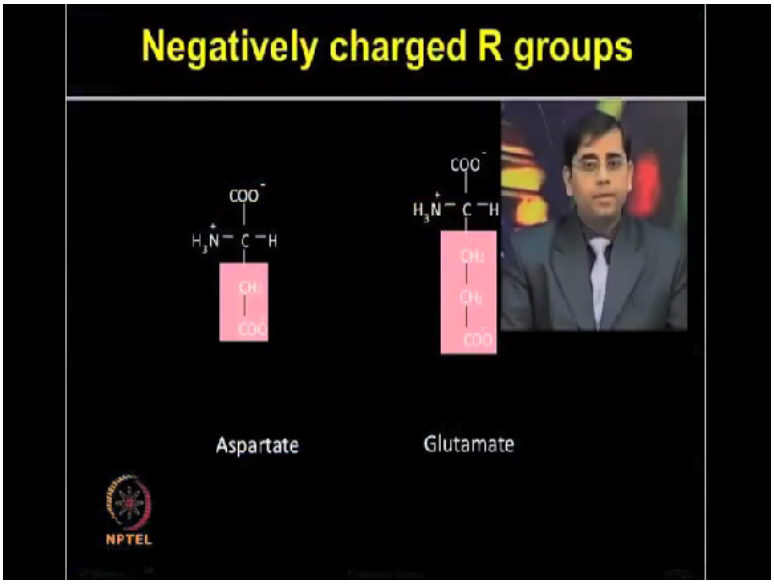

Next group is negatively charged R groups, aspartate or glutamate or aspartic acid or glutamic acid. The name aspartate or glutamate is because at the physiological pH, the side chain of these amino acids lacks a proton present in the basic form; therefore, these amino acids are negatively charged. Aspartate is a carboxylate anion of aspartic acid known as aspartate. whereas the carboxylate anions and salts of glutamic acid are known as glutamate.

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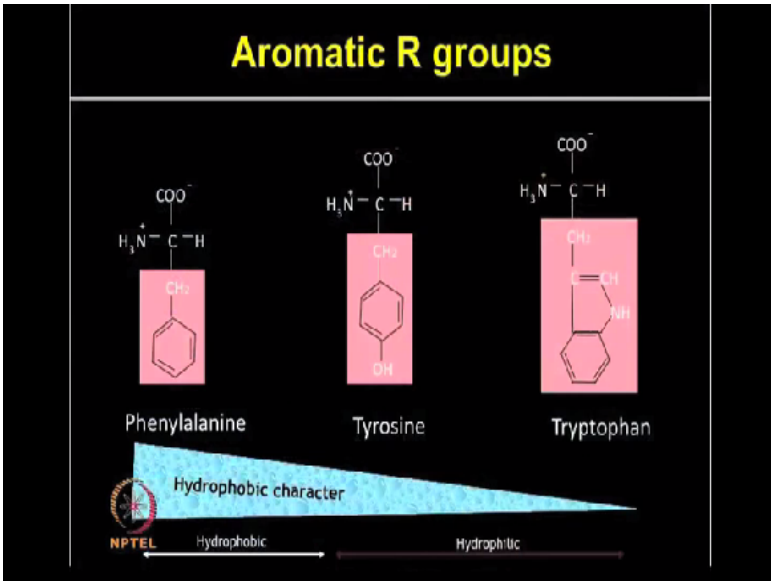

Next category is aromatic R groups. In this one, there are 3 amino acids, phenylalanine, tyrosine and tryptophan. Phenylalanine contains phenyl ring. Tyrosine has one reactive hydroxyl group and tryptophan contains indole ring, 2 rings which are fused. Now if you look at the hydrophobic or hydrophilic characteristics, phenylalanine is hydrophobic whereas tyrosine and tryptophan, they are hydrophilic due to the side chain containing hydroxyl and NH reactive groups.

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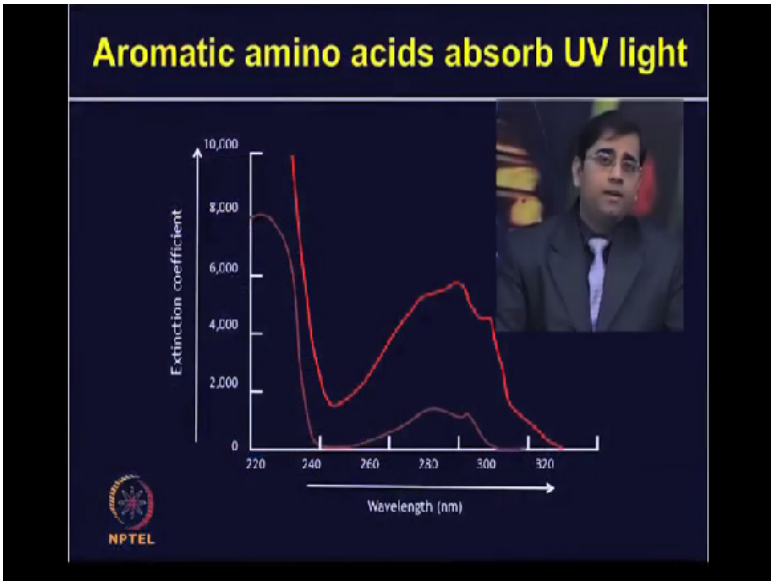

Aromatic amino acids have unique property. They can absorb UV light. In all the 3 amino acid which we just discussed, tryptophan, tyrosine and phenylalanine, they can absorb UV light. Tryptophan absorption maxis at 280 nanometers, tyrosine at 276 nanometer, phenylalanine, it absorbs light less strongly and at the shorter wavelength. The light absorption at 280 nanometers

is used for the protein concentration determination.

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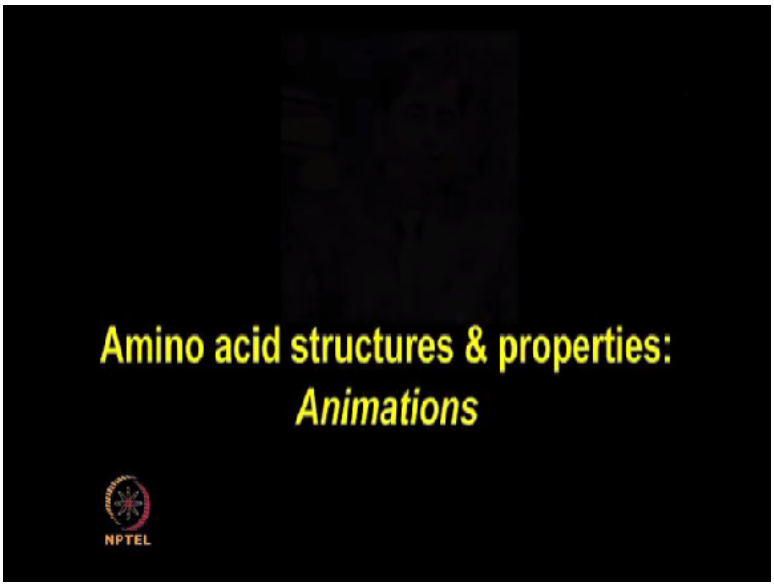

I will refresh some of the concepts discussed in the amino acid structures and properties in following animation.

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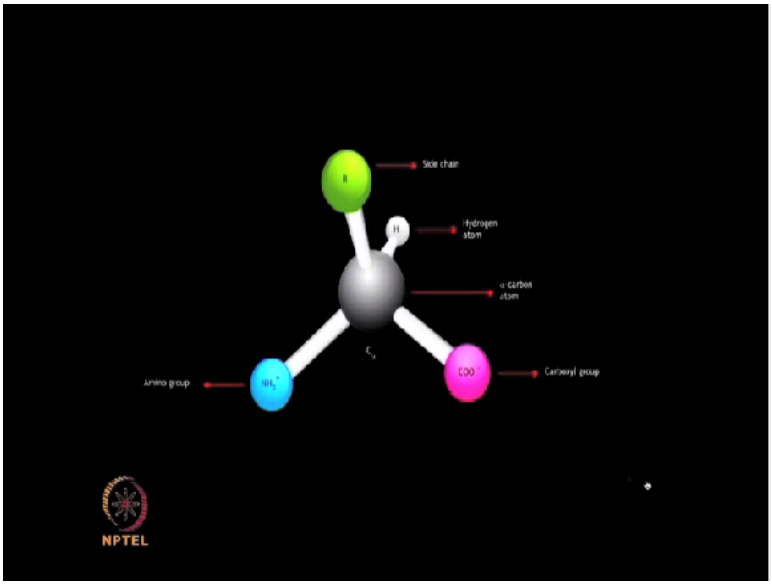

Amino acids are the building blocks or monomers that make up proteins. They consist of a central alpha carbon atom bonded covalently to an amino group, a carboxyl group, a hydrogen atom and a variable side chain called as R group.

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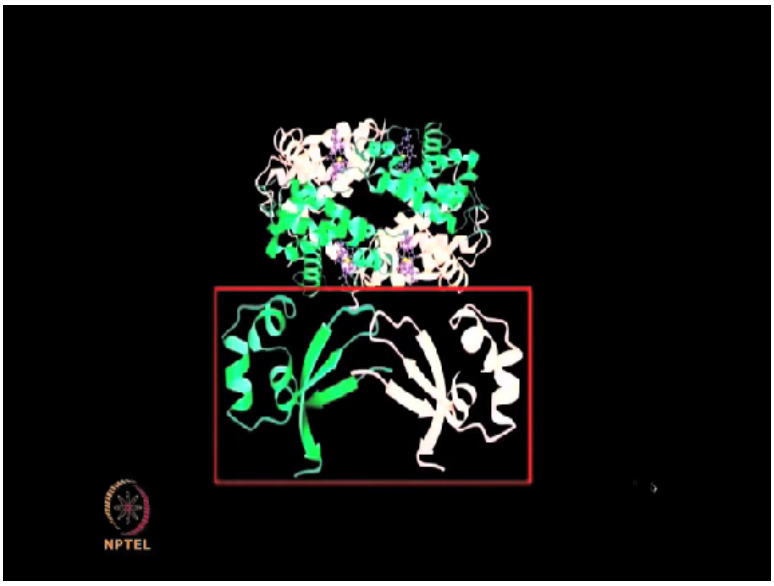

Amino acids are the basic monomeric constituents of proteins found in varying amount depending upon the type of protein. They are classified based on the properties of their side chains or R groups which vary in size, structure and charge.

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Polarity of side chain is one of the main basis for the classification.

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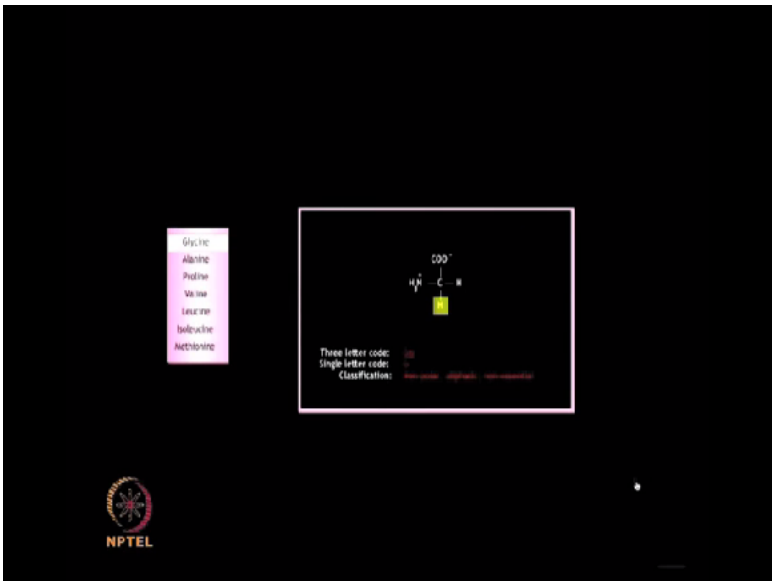

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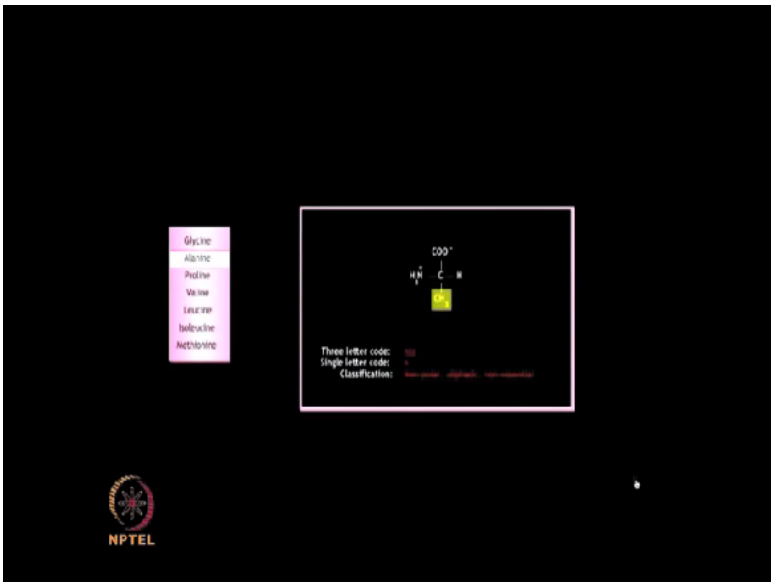

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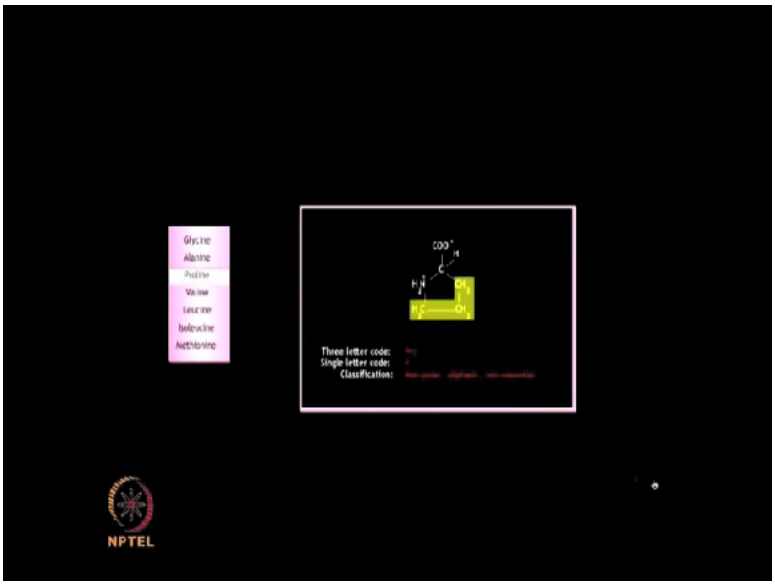

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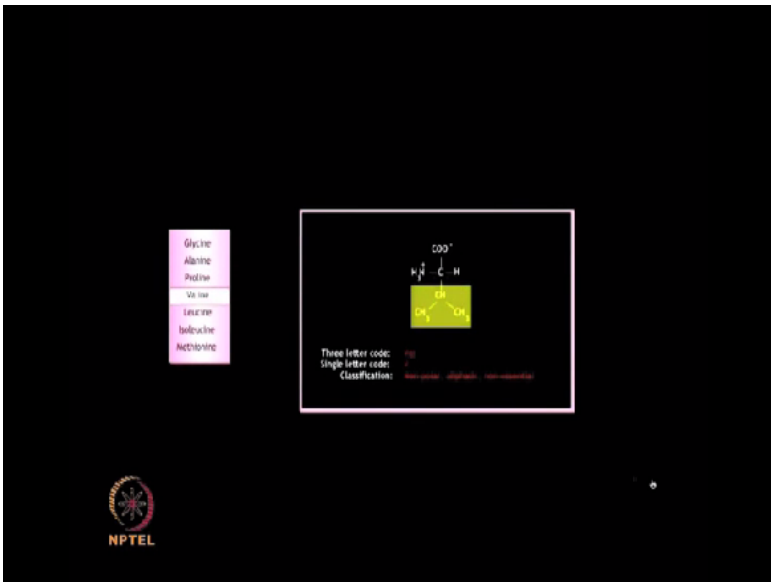

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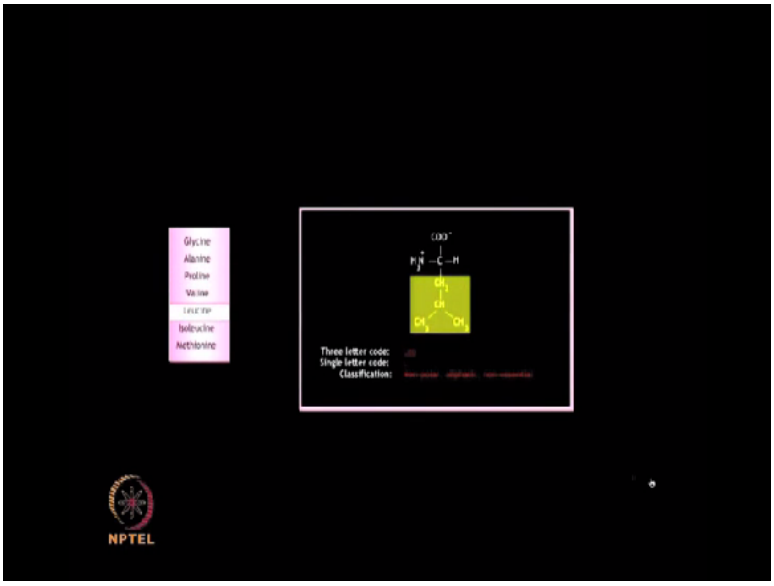

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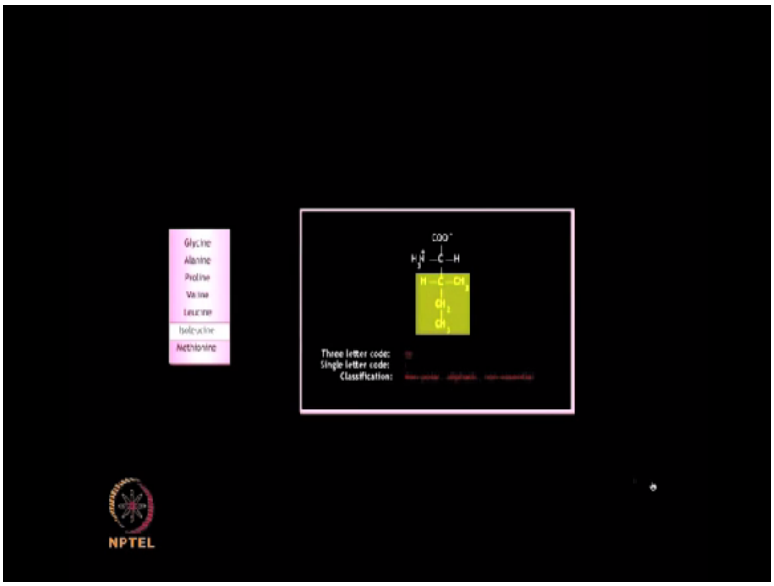

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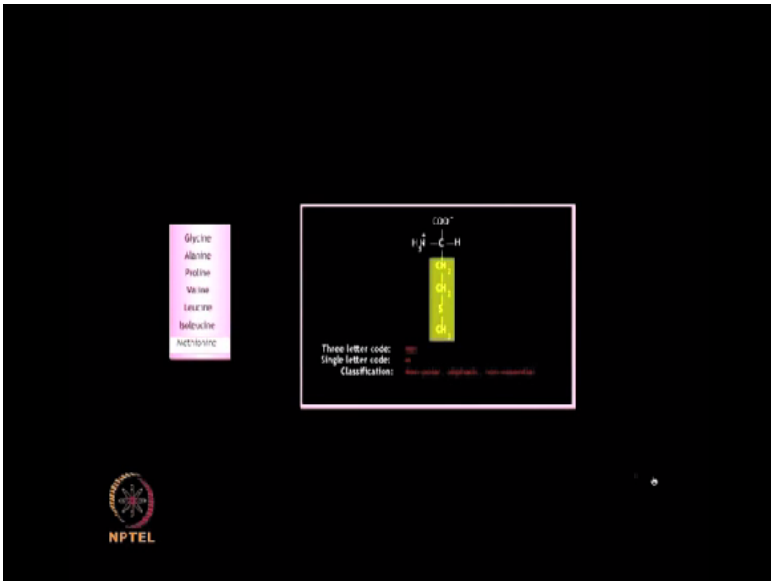

Amino acids having nonpolar aliphatic side chains including glycine, alanine, proline, valine, leucine, isoleucine and methionine.

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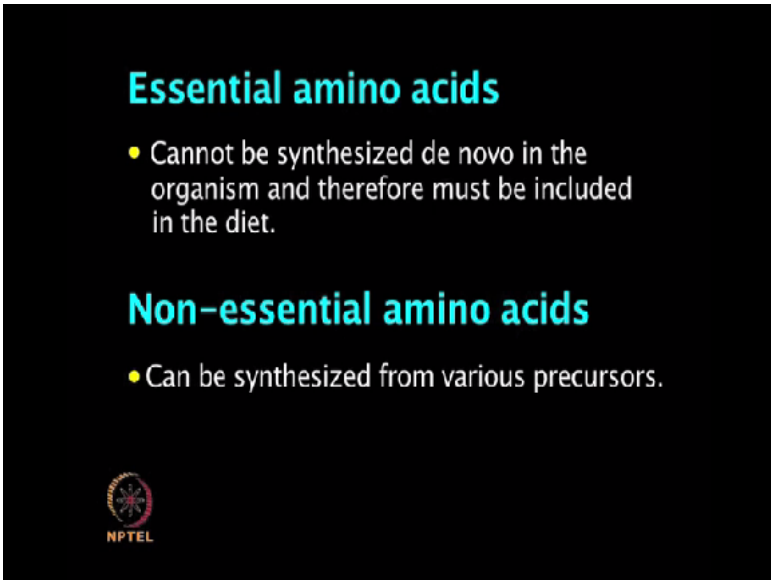

Essential amino acids are those that cannot be synthesised de novo in the organism and therefore must be included in the diet. Non-essential amino acids on the other hand can be synthesised from various precursors.

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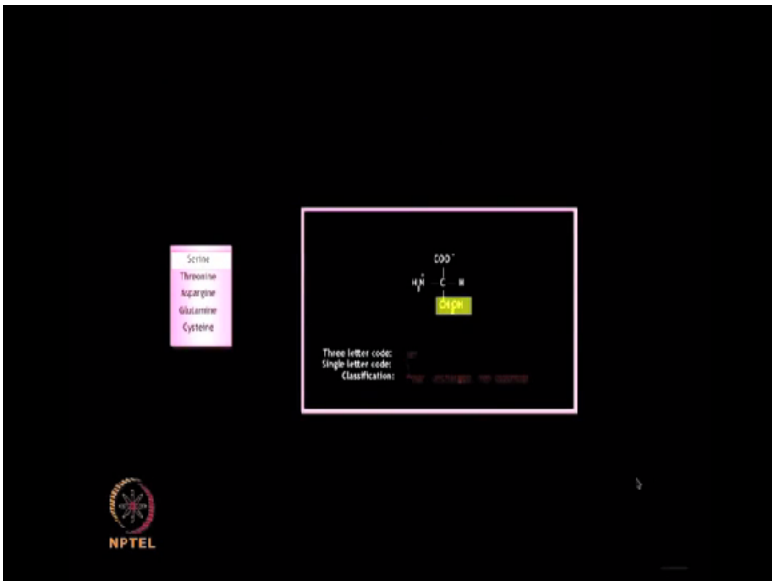

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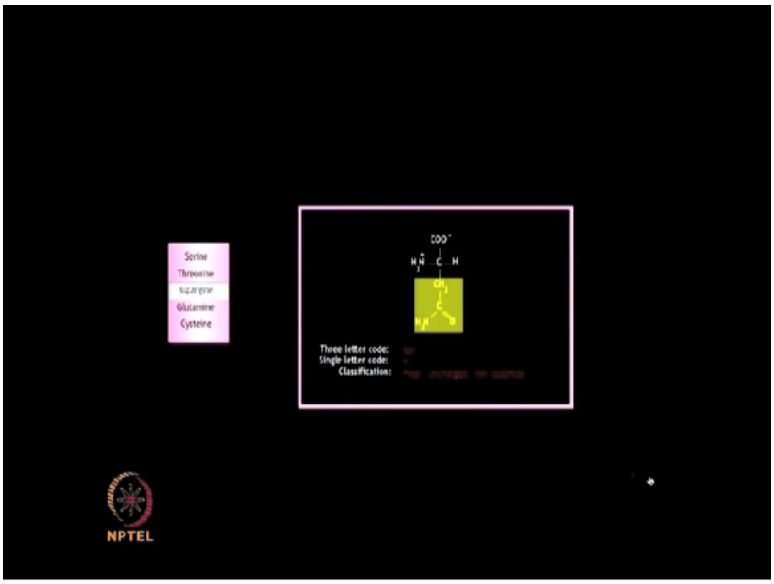

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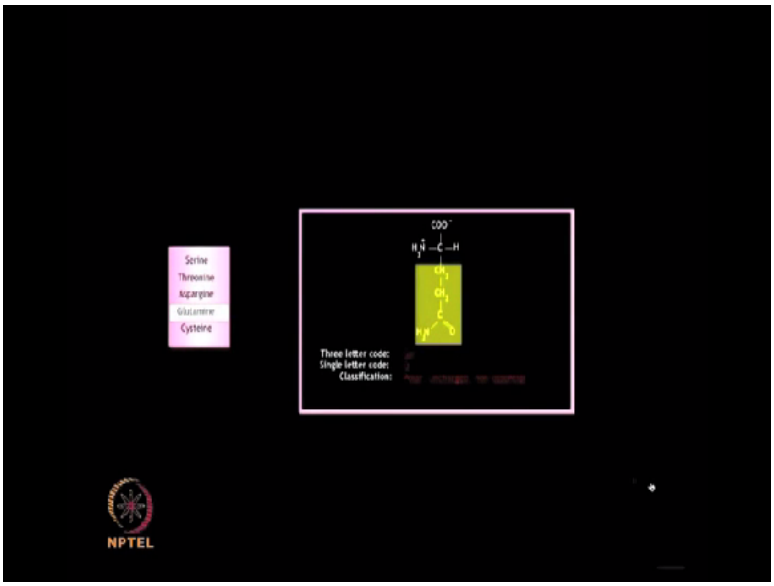

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Serine, threonine, asparagine, glutamine and cysteine consist of polar but uncharged side chains.

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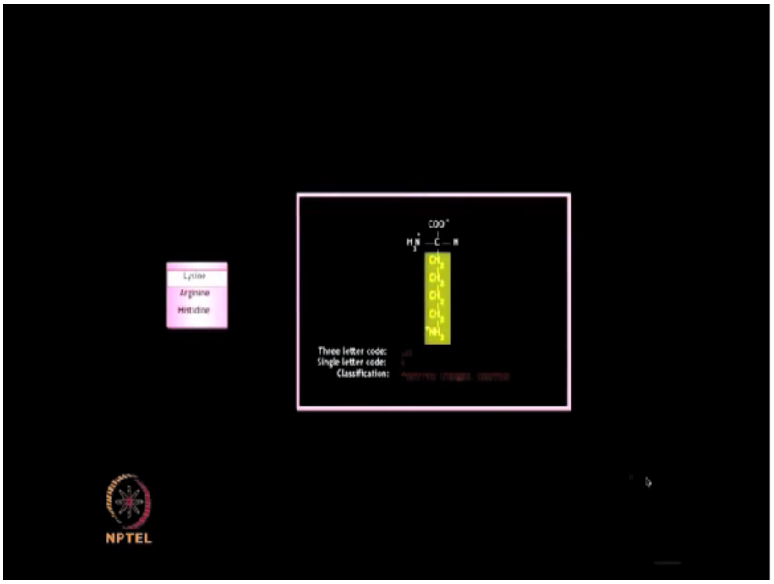

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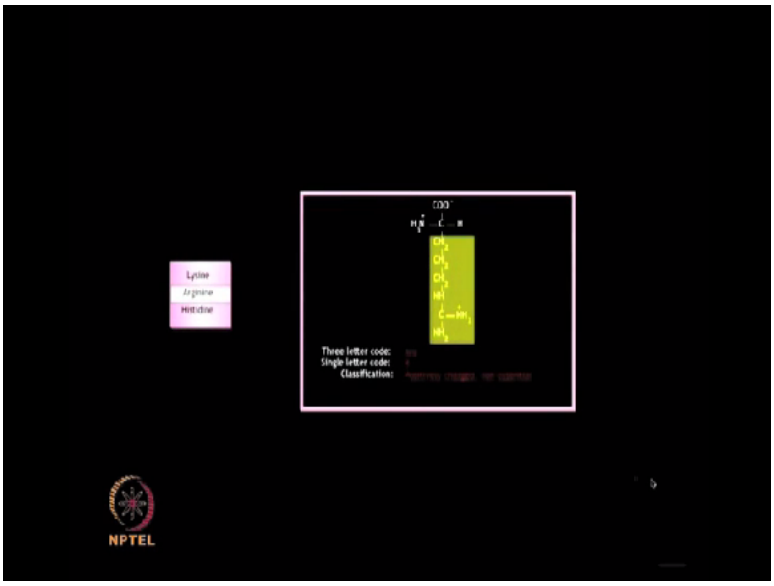

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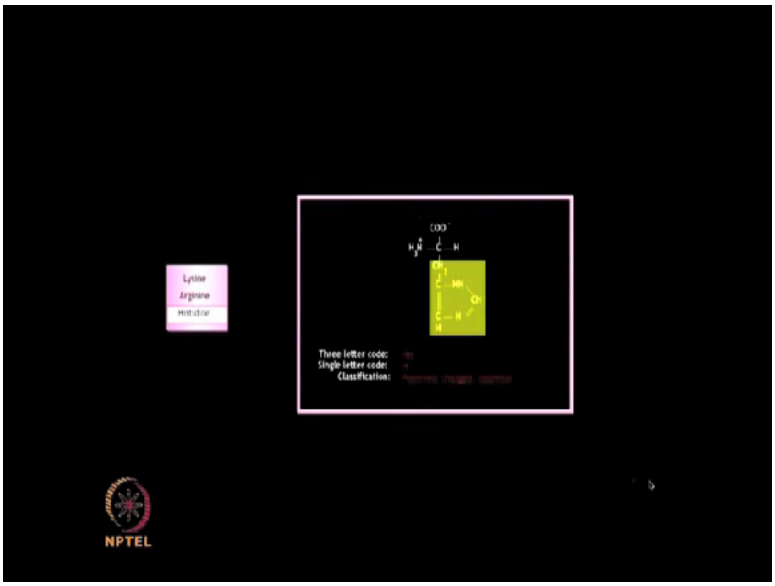

Lysine, arginine and histidine, these have positively charged side chains.

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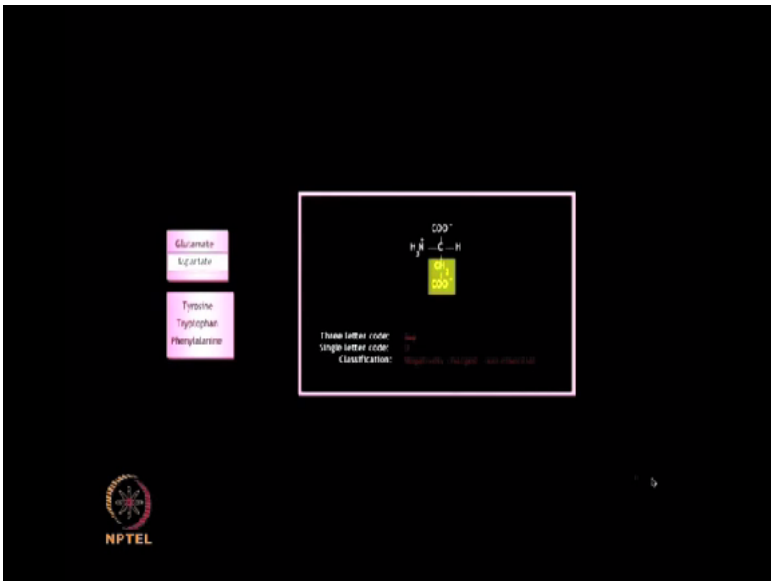

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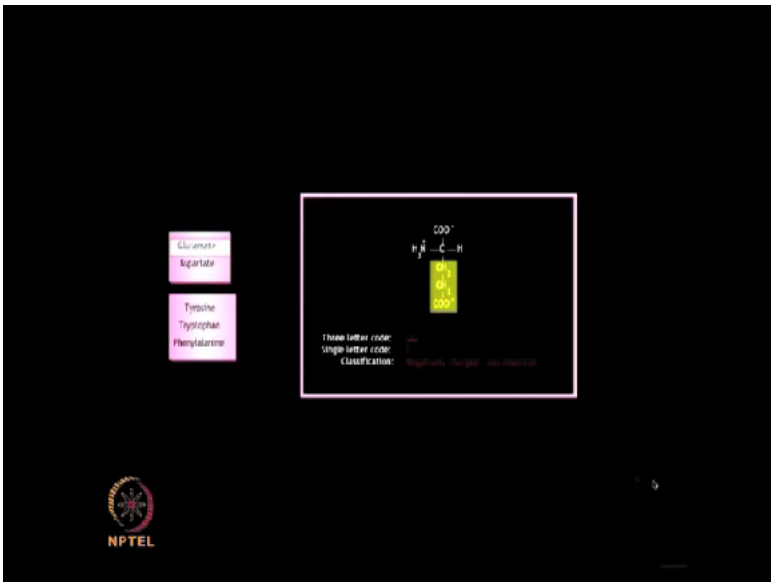

Aspartic acid and glutamic acid are polar and negatively charged amino acids.

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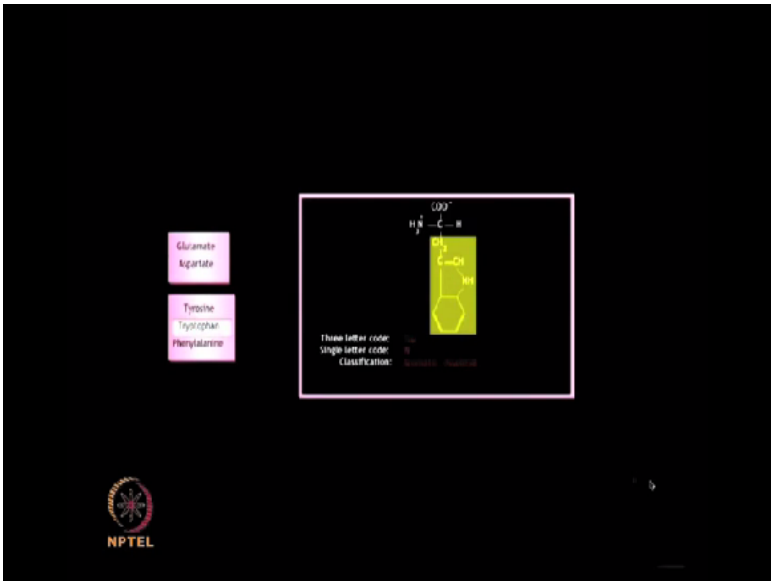

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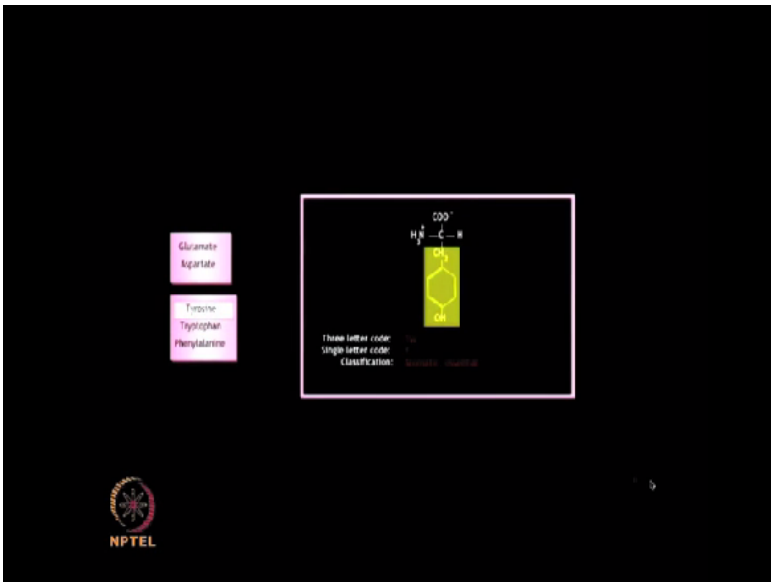

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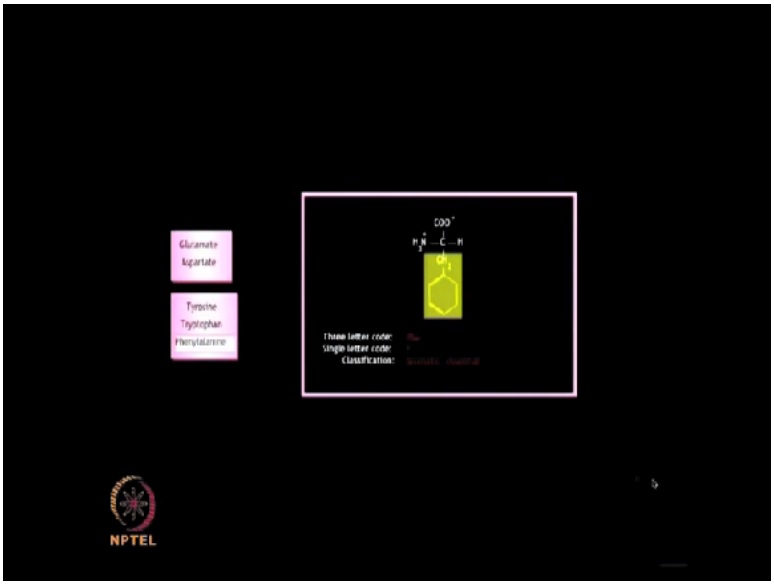

Tryptophan, tyrosine and phenylalanine are all essential amino acids having an aromatic side chains. After having discussed the different type of group of amino acids, let us look at the basic constituents of amino acids and different isoforms which it can form.

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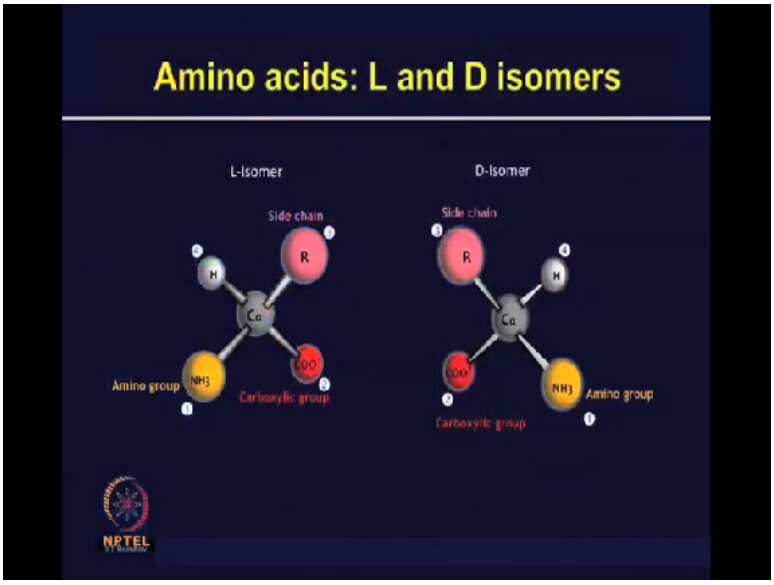

Amino acids effect having the 4 different groups which are connected to the alpha carbon atom. It can form 2 mirror images which can exist in L or the D isomers which are shown in the slide here. The alpha amino acids are chiral. There could be R or S configurations in the amino acid depending upon the priority groups but only L amino acids are present in the proteins.

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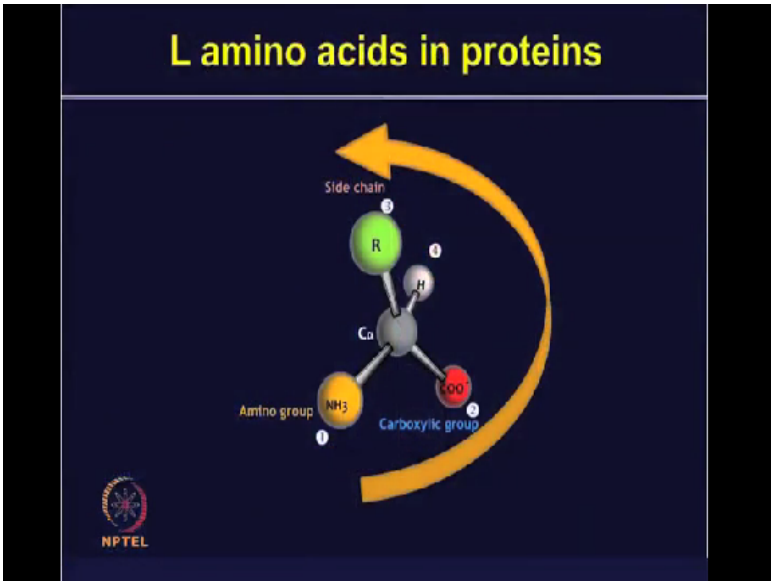

All the L amino acids have S configuration which describes the counterclockwise direction as shown here from highest to the lowest priority groups which is an indicative of chiral centre with the S configurations.

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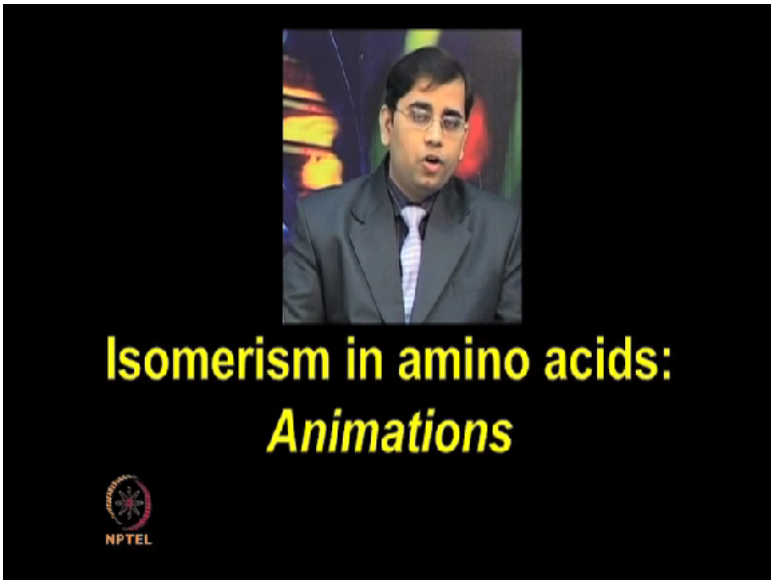

Some of the isomerism properties of amino acids will be discussed in the following animation.

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Before learning about the isomerism, let us first know what is chirality. The term chirality arises from the Greek term kheir meaning handedness just like the 2 hands are non-superimposable mirror images of each other. Amino acid molecules are also non-superimposable due to their chiral alpha carbon centre.

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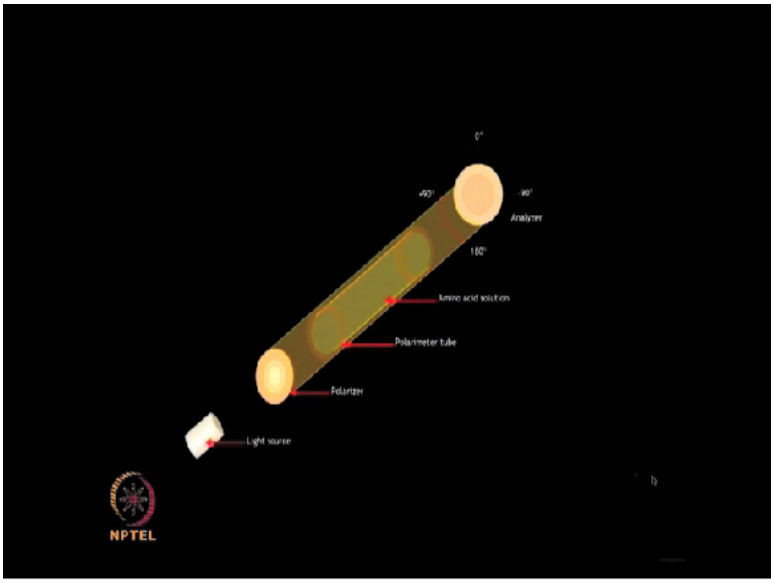

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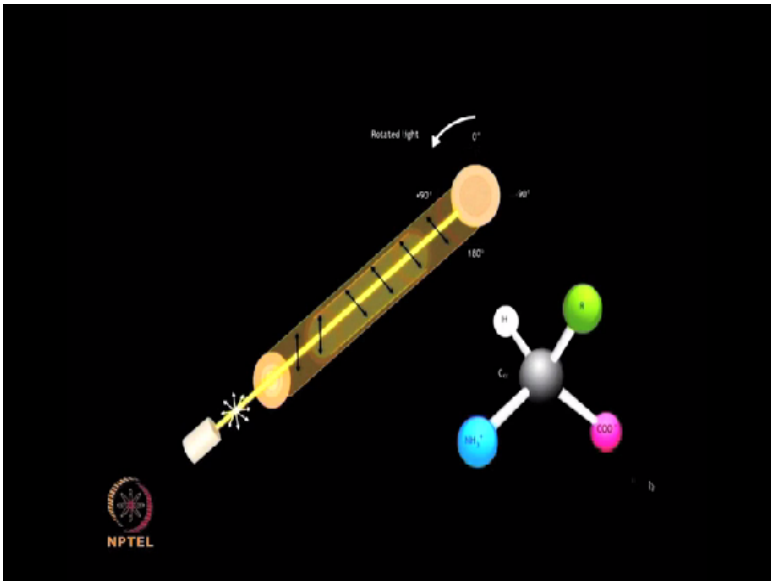

All amino acids except glycine contain an asymmetric centre that makes them chiral in nature due to which they can rotate the plane of polarised light.

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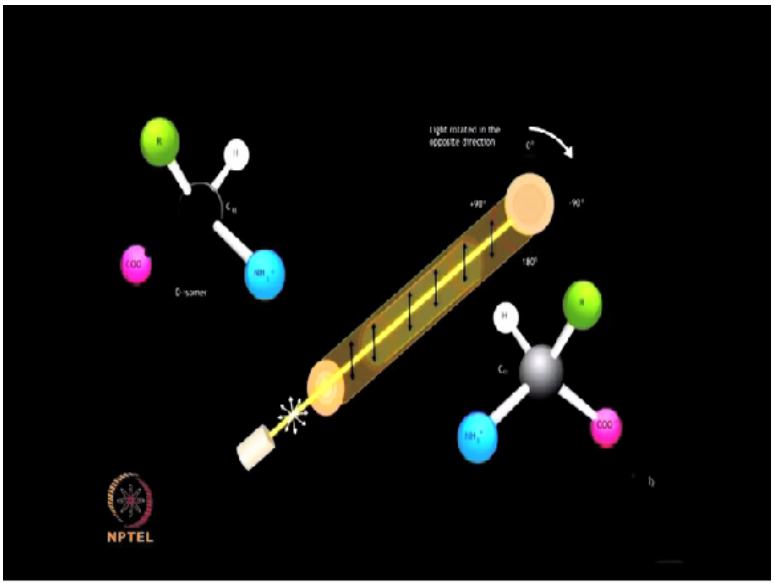

The 2 anatiomers designated as D and L rotate the plane of polarisation in opposite directions.

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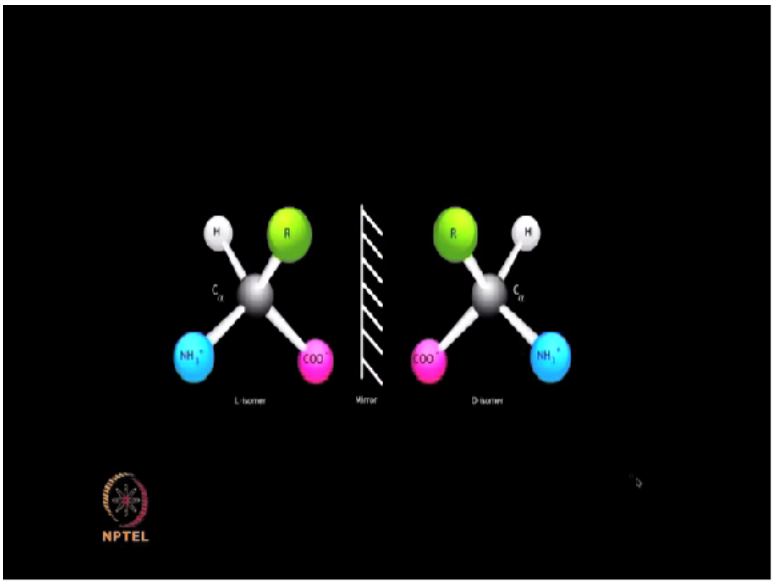

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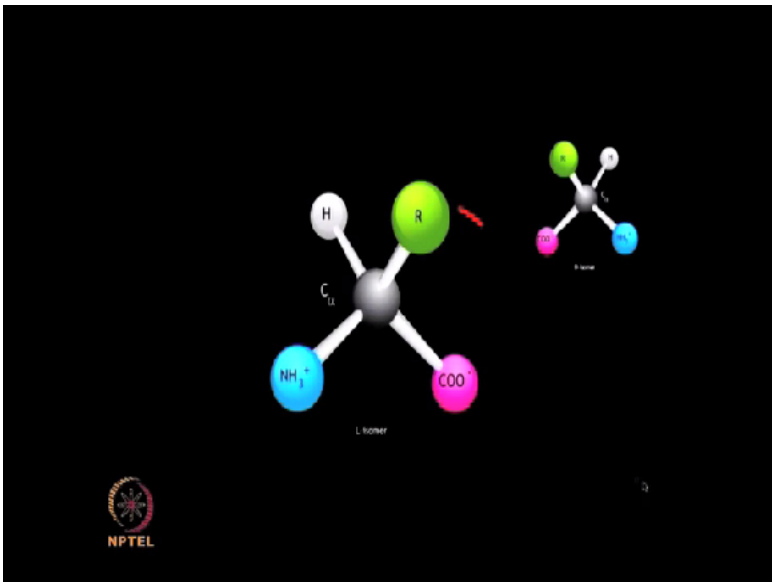

The 2 anatiomers of amino acid are non-superimposable mirror images due to the spatial arrangements of 4 different groups about the chiral carbon atom.

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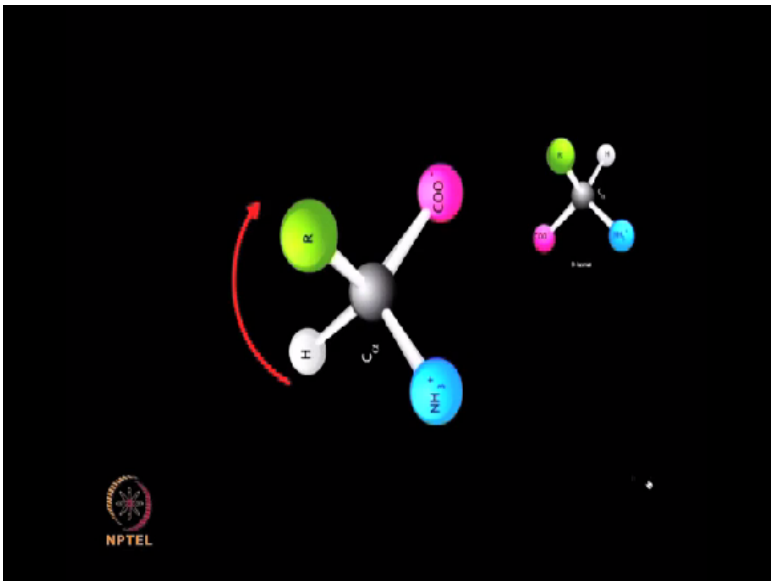

Rotation of either isomer about its central axis will never give rise to the other isomeric structure. Let us now talk about iraration state of amino acids. The iraration state of amino acids varies with its pH.

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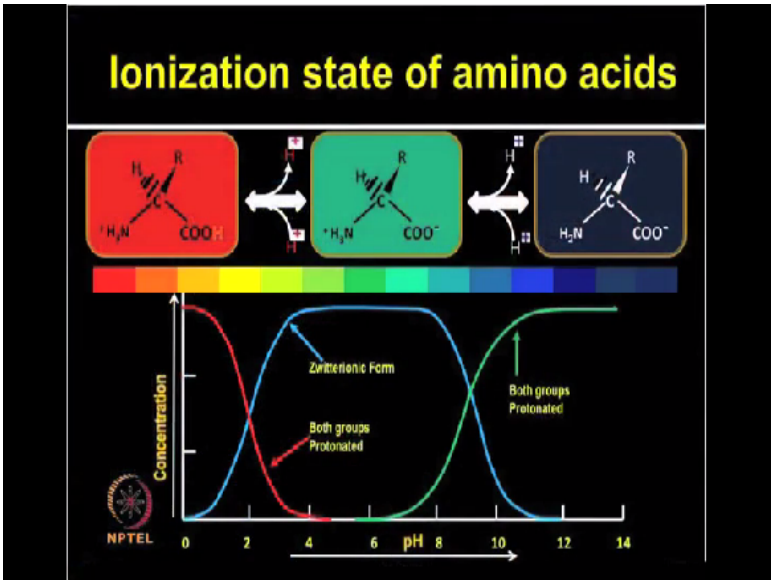

In the acidic solution, if you follow the slide from the left to right. The amino group is protonated NH3+, carboxyl group of undissociated, COOH and the neutral ph, amino acids exist as dipolar ions or zwitterions. Amino group is protonated NH3+ and carboxyl group is D protonated, COO-. Now this dipolar form can exist till pH 9. Now when you move to the basic pH, the protonated amino group loses its proton and forms NH2. Let us now talk about peptide bonds.

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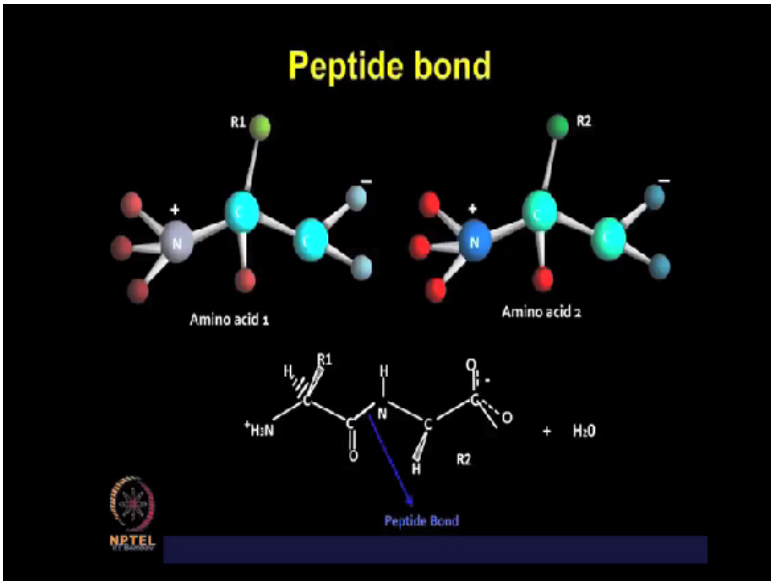

The alpha carboxyl group of one amino acid link to the alpha amino group of another amino acids. As you can see here, these 2 amino acids are forming a bond and peptide bond is formed with accompanying loss of water molecule.

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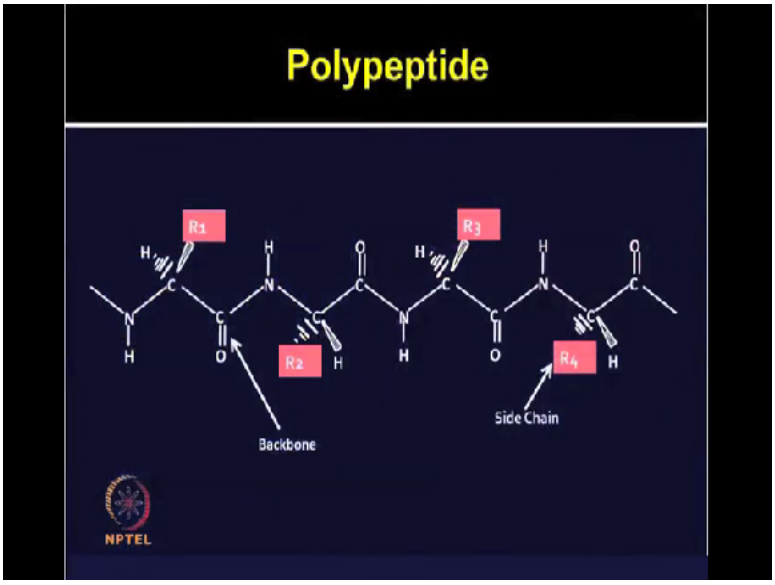

Now when meaning amino acids are linked together, they form a polypeptide as you can see in this slide, the multiple peptide bonds are present.

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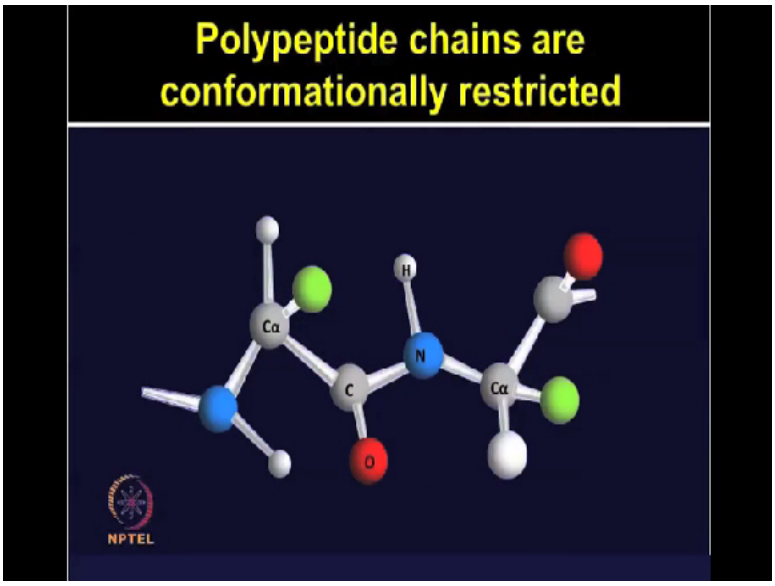

The polypeptide chains are conformationally restricted; therefore, peptide bond is planer. Amino acid players, they are linked by the peptide bonds and all the 6 atoms lie in the same plane as you can see here, alpha carbon, carbon, oxygen, nitrogen, hydrogen and another alpha carbon. Peptide bonds can be stabilised by the resonance structure.

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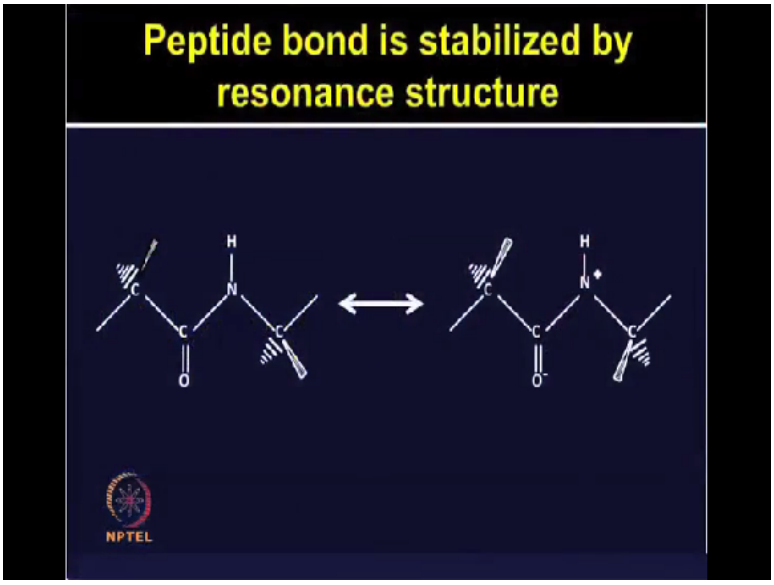

Peptide bond is rigid because of its partial double bond characters which arises due to the resonance structures present in peptide bond. Now there could be 2 forms, cis form and the trans form by but peptide bonds in protein exist in the trans form.

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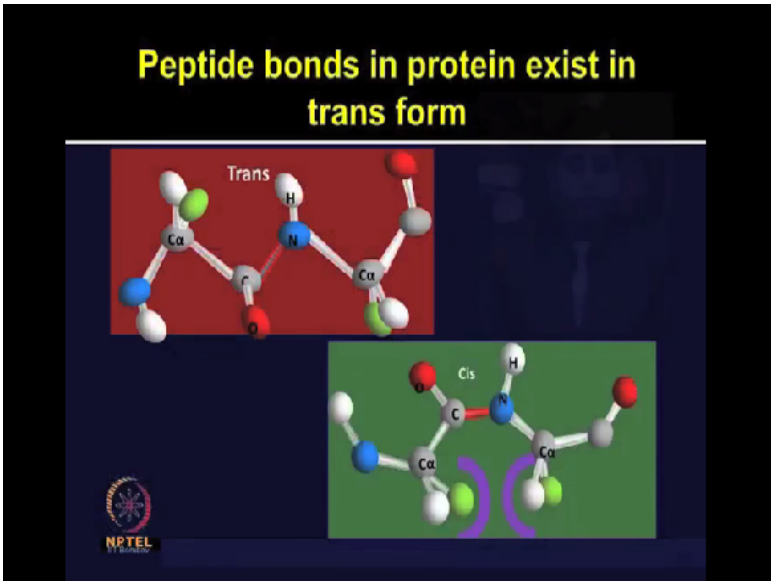

If you see the top panel, the trans configuration, there are 2 C alpha on the opposite side of the peptide bonds. This configuration is allowing less steric clashes whereas if you look at the bottom panel, the cis configuration, there are 2 alpha carbons on the same side of a peptide bond, so there is poor probability of having steric clashes; therefore, peptide bonds in protein, they exist in the transform. Now proline is a unique amino acid as we discussed earlier.

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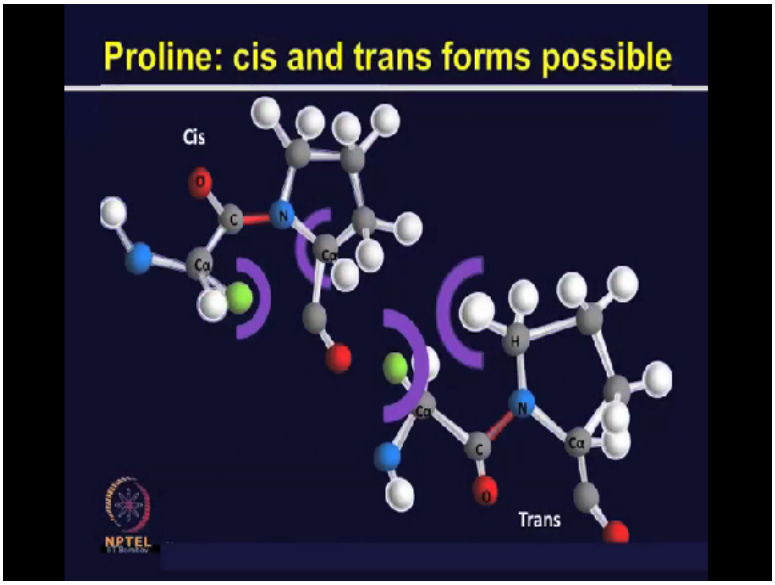

Proline with peptide bonds, it can form both cis and the trans forms. So as you can see here, it can avoid the steric clashes and both cis and trans configurations are possible.

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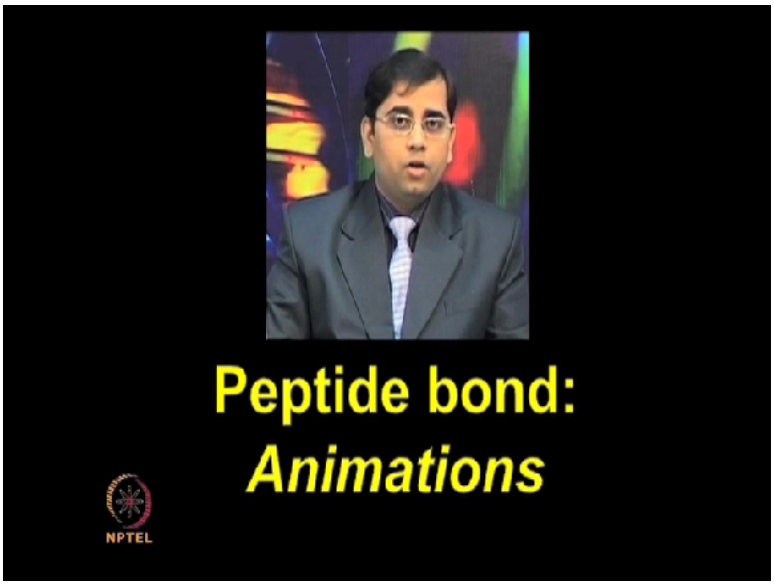

Some of the concepts of peptide bonds will be described in the following animation.

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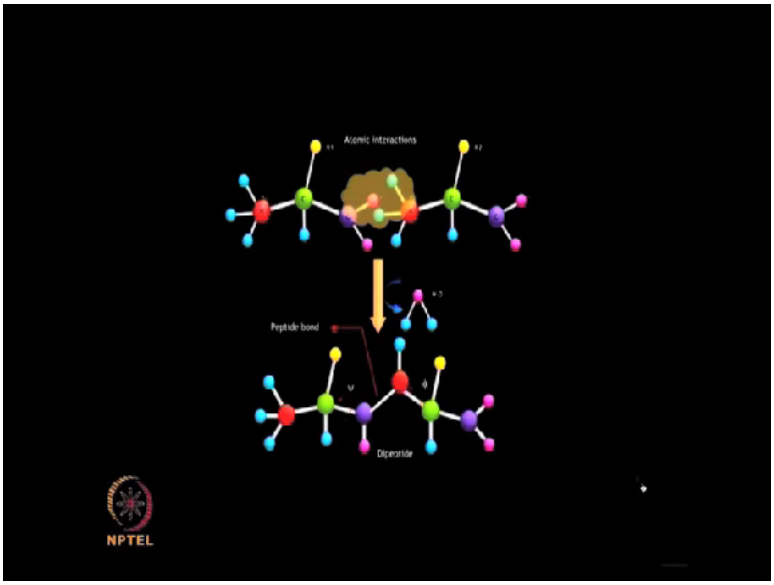

Amino acids are the building blocks or monomers that make up proteins. Amino acids are oriented in a head to tail fashion and linked together such that the carboxyl group of one amino acid combines with the amino group of another amino acid. Two amino acids joined together by means of such a condensation reaction with the loss of water molecule forms a dipeptide. Many such amino acids linked together and form polypeptide.

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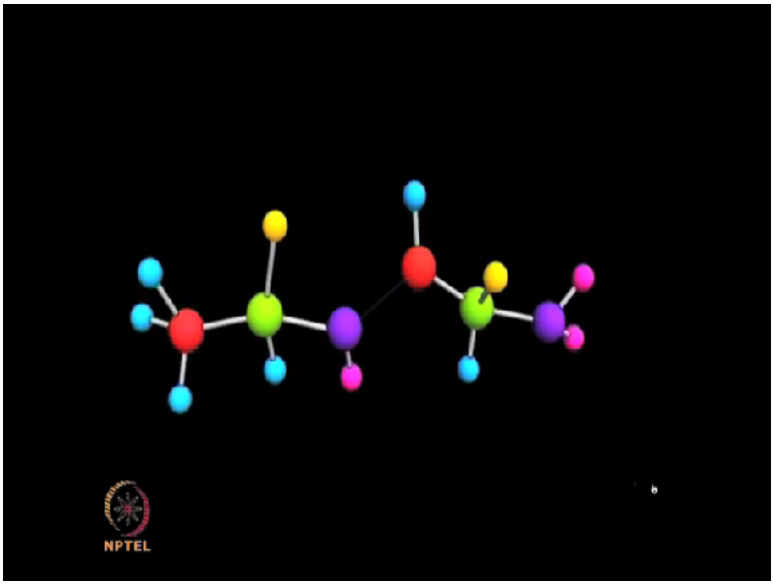

The peptide bond is rigid due to its partial double bond character which arises from the resonance structure; however, the bonds between the alpha carbon and amino and carboxyl groups are pure single bonds that are free to rotate.

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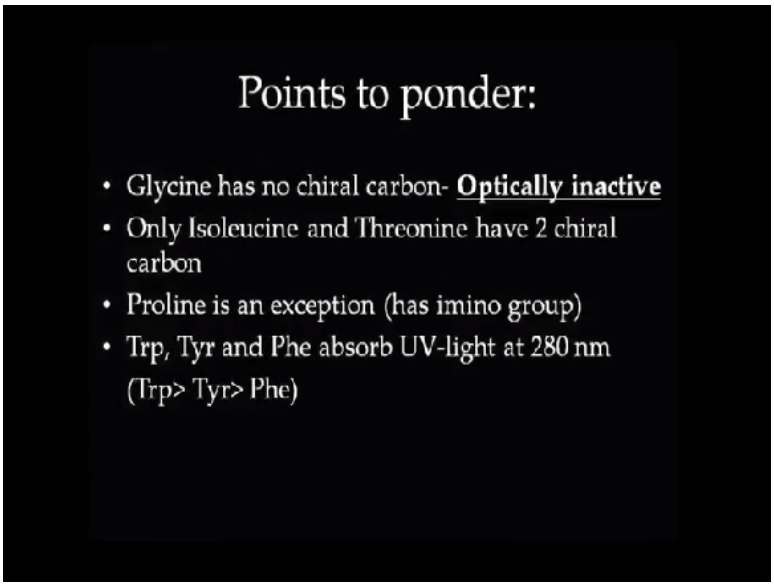

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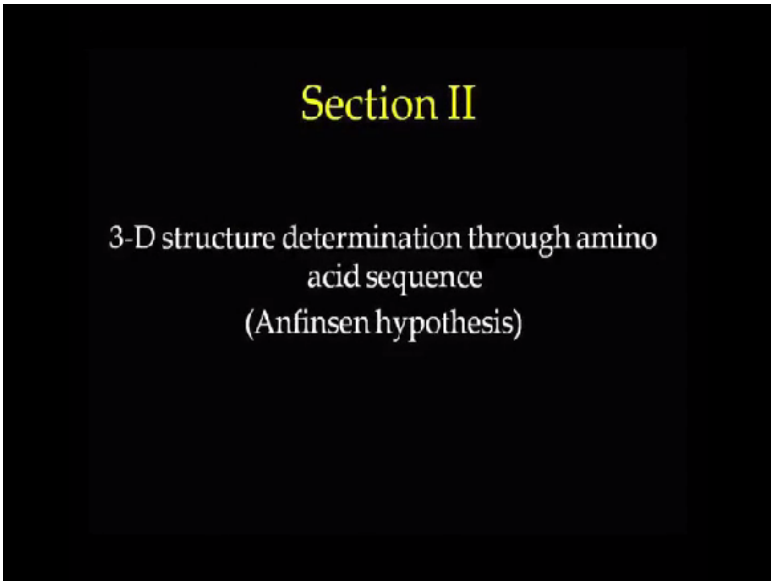

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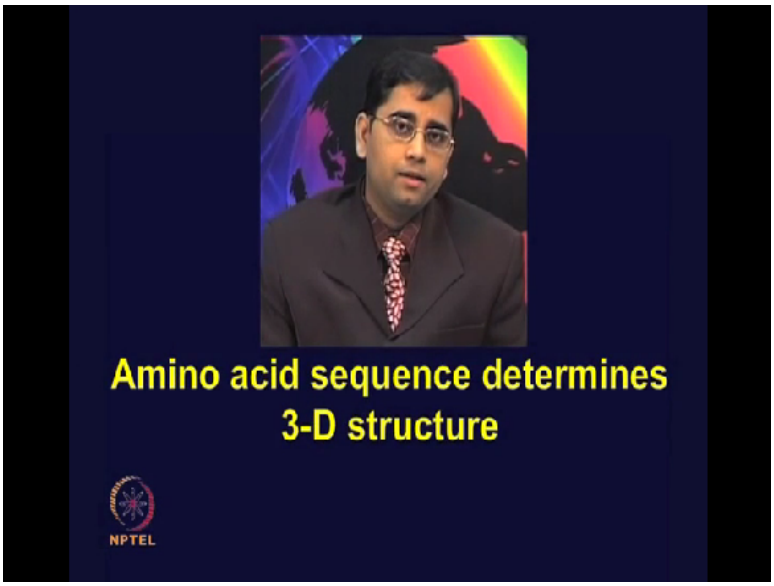

Amino acid sequences determine 3-dimensional structure of proteins. So there is very intricate sequence structure relationship. The amino acid sequence dictates the conformations which are adopted by the polypeptide chains at secondary and tertiary levels.

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Scientist Anfinsen created a classical experimental where he tested the ability of reduced and unfolded proteins to spontaneously fold into native state by using a protein ribonuclease A. This experiment established that the primary amino acid sequence of a protein contains all the information which is required for the proper protein folding into its native form. The fundamental discovery of Anfinsen led him to receive the Nobel Prize in chemistry in 1972. So let us explain you how this experiment work.

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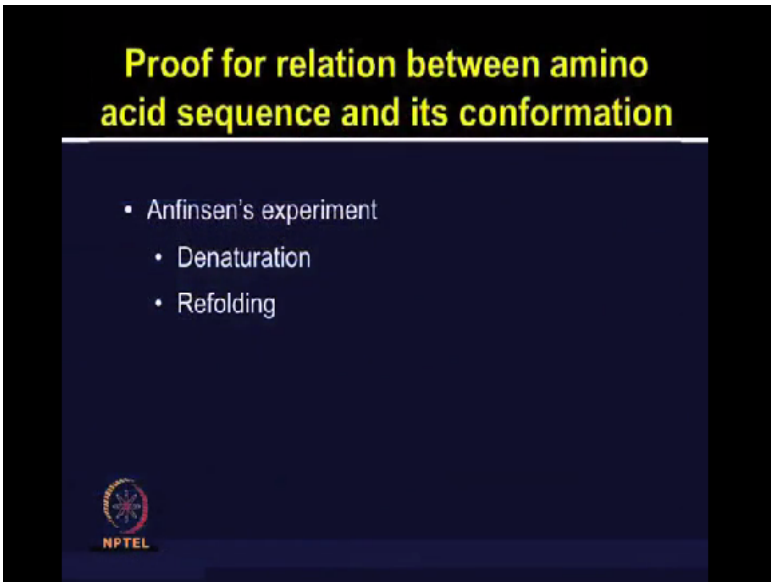

To establish a proof for relationship between amino acid sequence and its conformation, Christian Anfinsen in 1950s performed an experiment where he performed 2 steps, denaturing and refolding. So how denaturation and refolding works.

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So in this classical experiment, Anfinsen used protein ribonuclease A, he used few denaturants such as urea or guanidine hydrochloride and beta-mercaptoethanol which breaks the disulphide bonds.

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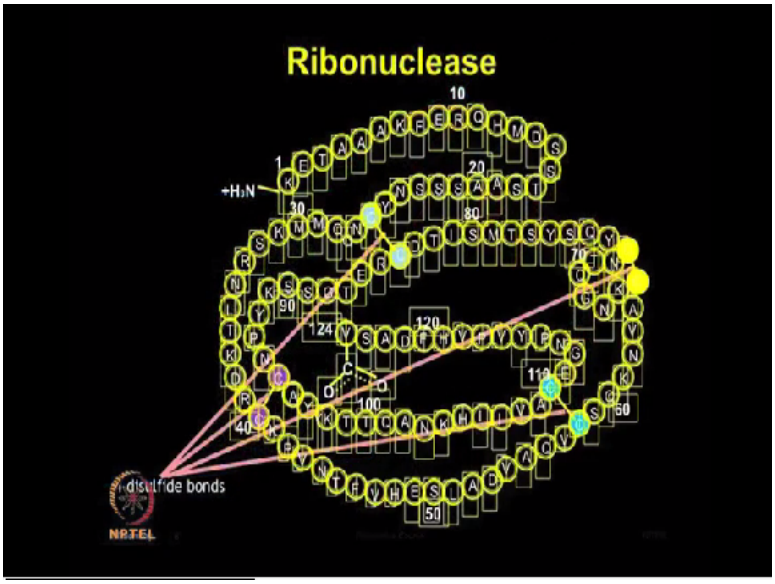

So let us look at each one of these component a little bit more detail. First talk about ribonuclease A protein. So this protein has contributed greatly to our understanding of protein folding in vitro from the landmark experiment of Anfinsen. As you can see in the structure, ribonuclease has 124 amino acid residues and it forms 4 disulphide bridges which are located between the cysteine residues of 26 and 84, 40 and 95, 58 and 110, and 65 and 72. This protein

catalyses the hydrolysis of RNA.

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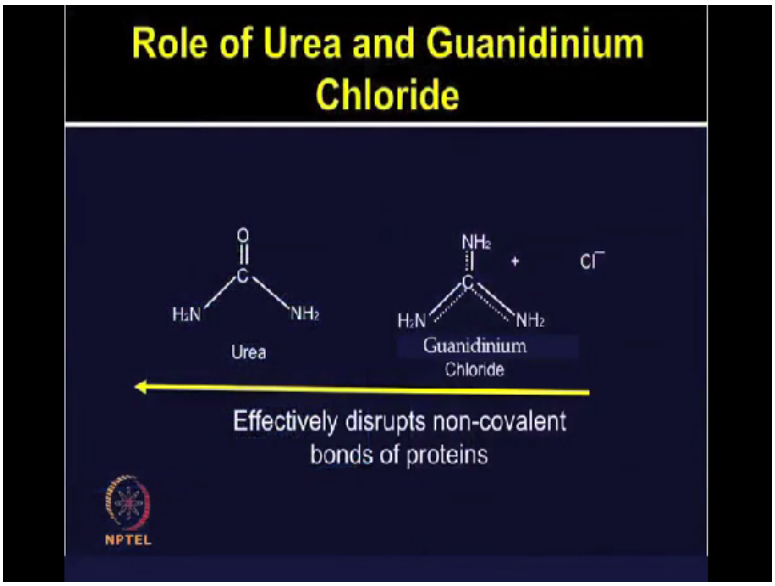

So what is the role of urea and guanidinium chloride. Urea is an organic compound which has 2 amino groups joined by a carbonyl group and used at a concentration of around 6 molar for denaturing the proteins by breaking a non-covalent interactions. Both urea and guanidinium chloride can effectively disrupt the proteins non-covalent interactions.

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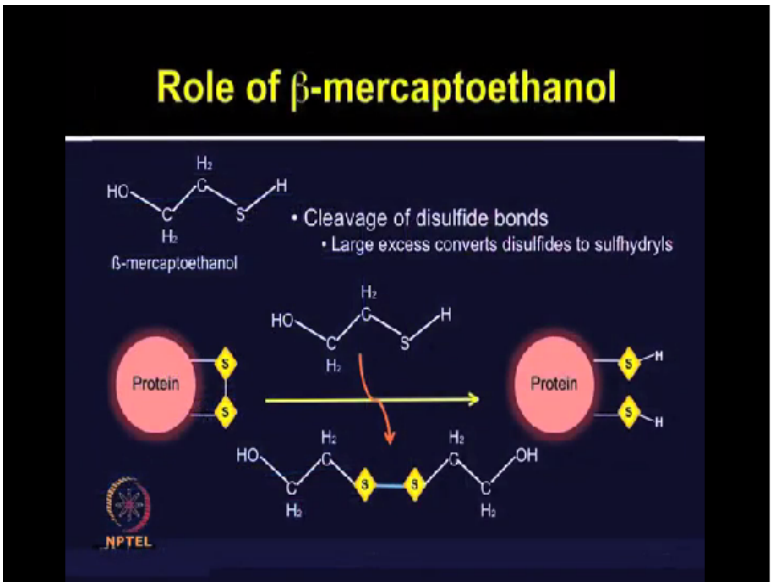

What is the role of beta-mercaptoethanol? The beta-mercaptoethanol is commonly used to reduce the disulphide linkages in proteins and thereby it disrupts the tertiary and quaternary structures. As you can see in the structure here in the slide in presence of excess of beta-mercaptoethanol,

the disulphide or cysteine can be fully converted into sulfhydryls or cysteines.

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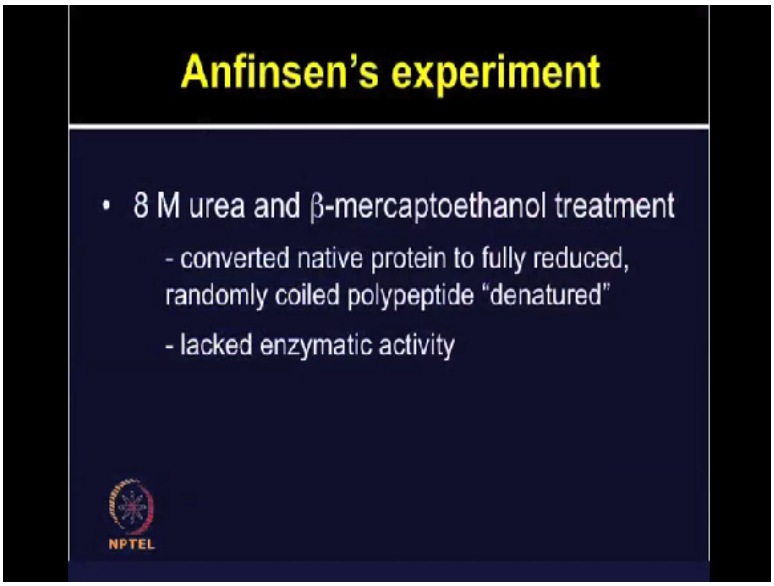

Anfinsen's experiment, he used 8 molar of urea and beta-mercaptoethanol treatment which converted the native proteins to fully reduced state into the randomly coiled polypeptides known as denatured structure. The denatured polypeptide lacked enzymatic activity.

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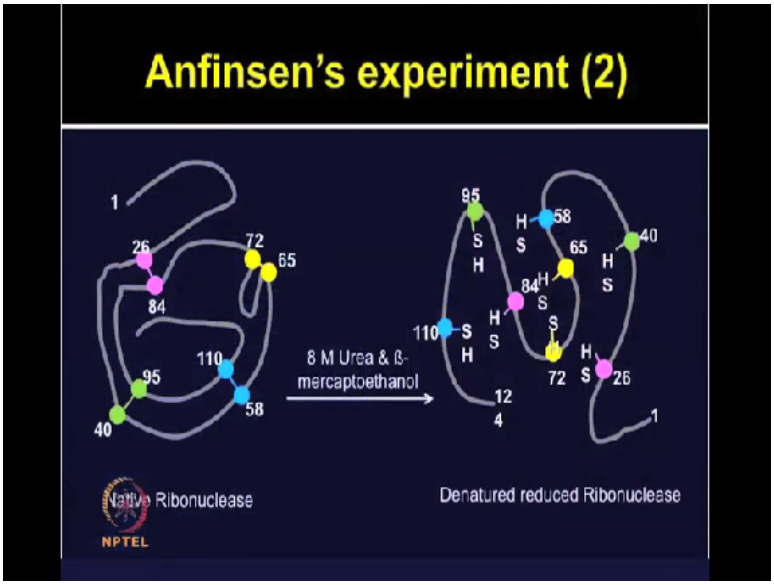

(()) (22:36) discussed the ribonuclease protein, it contains 124 amino acid residues and forms 4 disulphide linkages. These linkages are formed between the cysteines as shown here of 26-84, 40 and 95, 58 and 110, and 65 and 72. The ribonuclease native conformation is lost when it was treated with 8 molar urea and beta-mercaptoethanol. As you can see here, the native ribonuclease

has formed denatured reduced ribonuclease due to the breaking of disulphide and non-covalent interactions.

Anfinsen noticed that when the ribonuclease was oxidised in air and urea was removed by the process of dialysis, the enzyme activity slowly recovered as a result of the protein folding.

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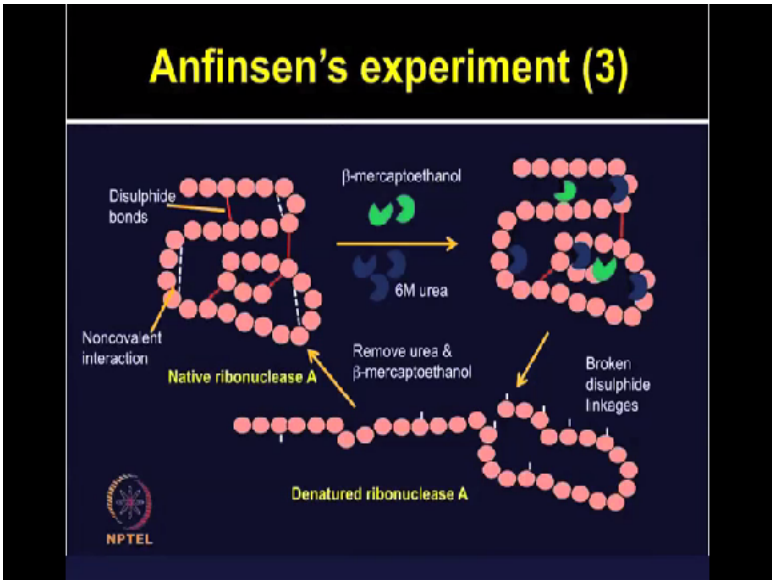

As you can see here, if you have used beta-mercaptoethanol at 6 molar urea, all the disulphide and covalent bonds are breaking. Once urea is removed, then slowly protein folding occurs. It results into the re-formation of tertiary structure and active site. But Anfinsen repeated this experiment in presence of denaturant urea that led to regeneration of less than 1% of enzyme activity.

So what could be the reason, in fact, urea prevented the correct disulphide pairing which resulted into the scrambled form, scrambled ribonuclease. Now if you mathematically calculate due to the presence of 4 disulphide bonds here and presence of 8 cysteine residues, it can actually give rise to 105 different forms in which these 4 disulphide bonds can be formed. So in the absence of urea, the correct disulphide bridge formation occurred and it allowed folded and thermodynamically stable state to be reached in ribonuclease protein.

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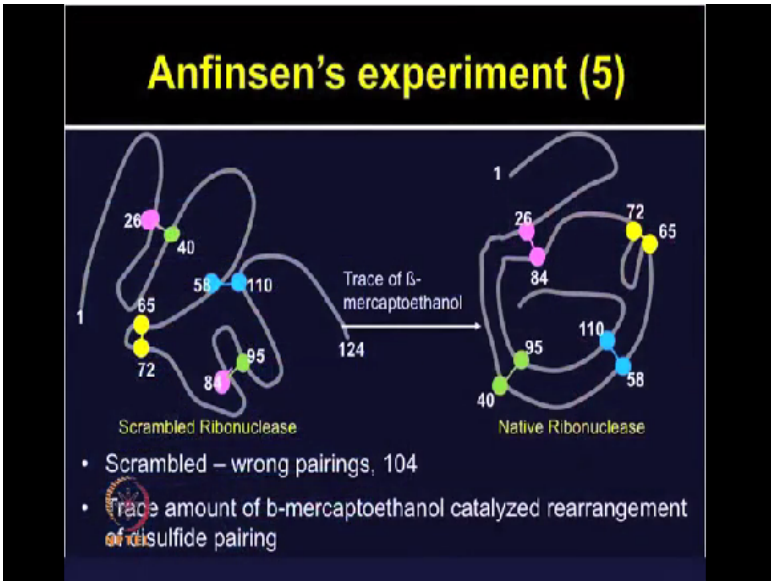

Now this figure shows here that in presence of trace amount of beta-mercaptoethanol and complete removal of denaturant urea, the refolding of ribonuclease was accurate and 4 intra-chain disulphide bonds were reformed in the same positions where they were expected in the native ribonuclease.

The random distribution of disulphide bonds was obtained when denaturants were used as you can see in the scrambled state which indicates that weak bonding interactions were required for the correct positioning of disulphide bonds and achieve the native conformation.

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So in Anfinsen's experiment, he removed urea and beta-mercaptoethanol by dialysis process. The

denatured ribonuclease regained its enzyme activity. The enzyme was refolded into the active form and the sulfhydryl groups became oxidised in presence of air. The experiment proved that information required for the specific catalytic active structure of ribonuclease is contained in its amino acid sequence. The classical study of Anfinsen proved that all the information which is crucial for protein folding resides in its primary sequence. Let me explain you this experiment in following animation.

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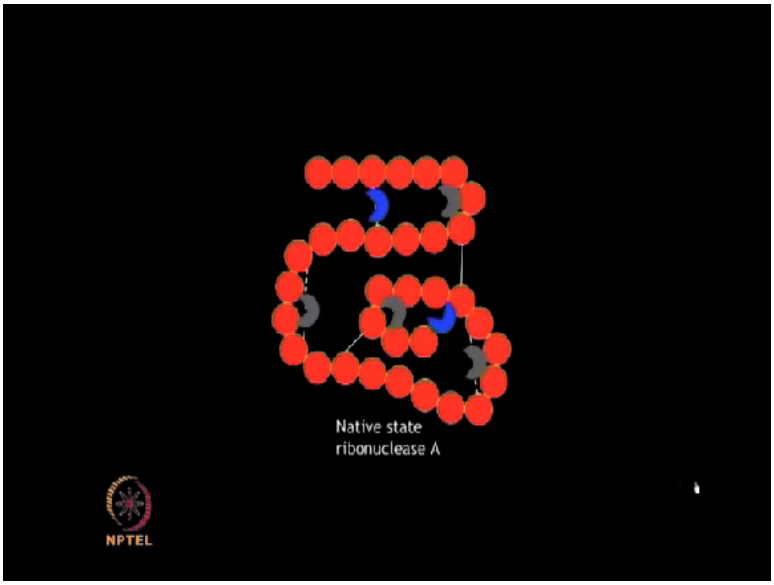

In Anfinsen's experiment, ribonuclease A in its native state has 4 disulphide bonds between its cysteine residues. When treated with beta-mercaptoethanol and 6 molar urea, the protein undergoes denaturation and the disulphide linkages are broken.

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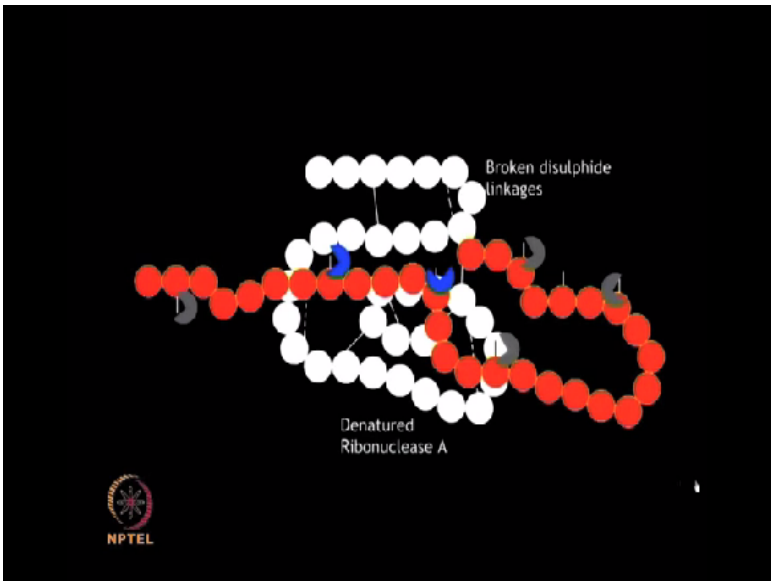

The enzyme activity is lost in its denatured state.

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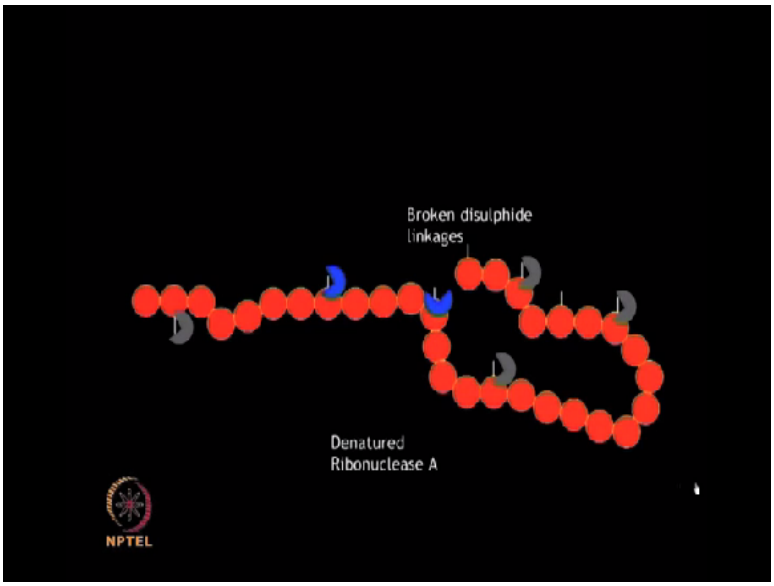

It was observed by Anfinsen that removal of urea and beta-mercaptoethanol let to the refolding of enzyme to assume its native state with more than 90% enzyme activity being intact.

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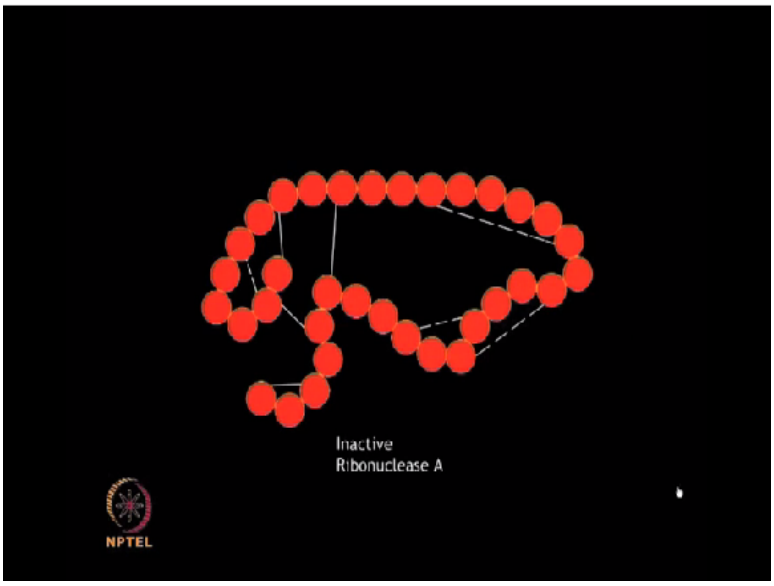

However, if only become ethanol was removed in presence of urea, the formation of disulphide bonds was random which led to enzyme with only around 1% activity.

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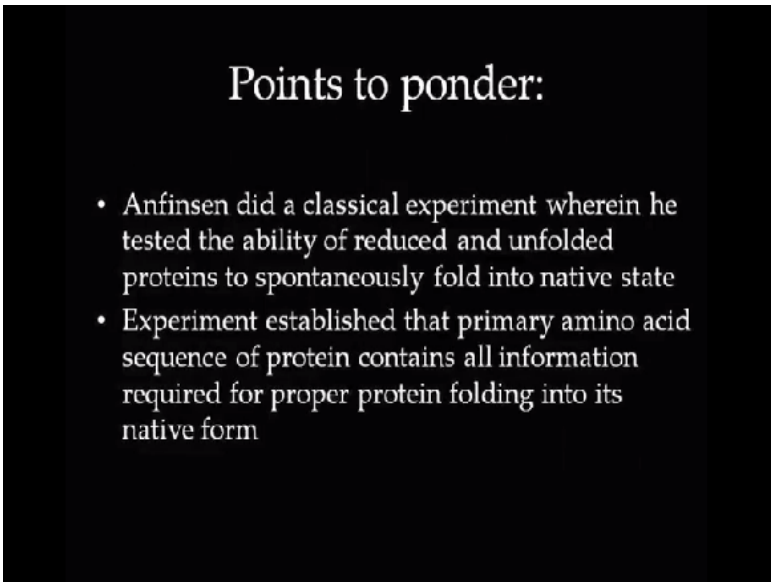

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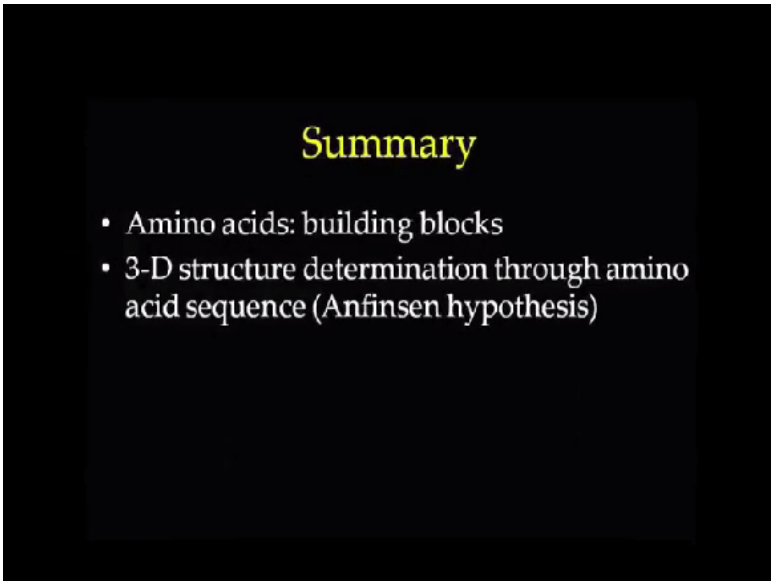

In summary, we refresh our concepts on amino acids which are the building blocks. We then talked about the classical experiment of Anfinsen which has proved that all the information which is crucial for protein folding resides within the primary amino acid sequence. We will continue our discussion on some basic concepts of protein in the next class. Thank you.

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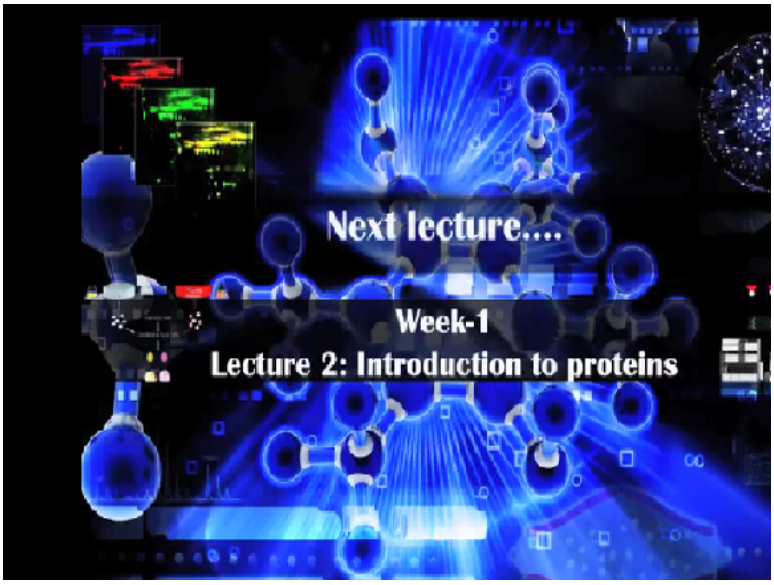

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