

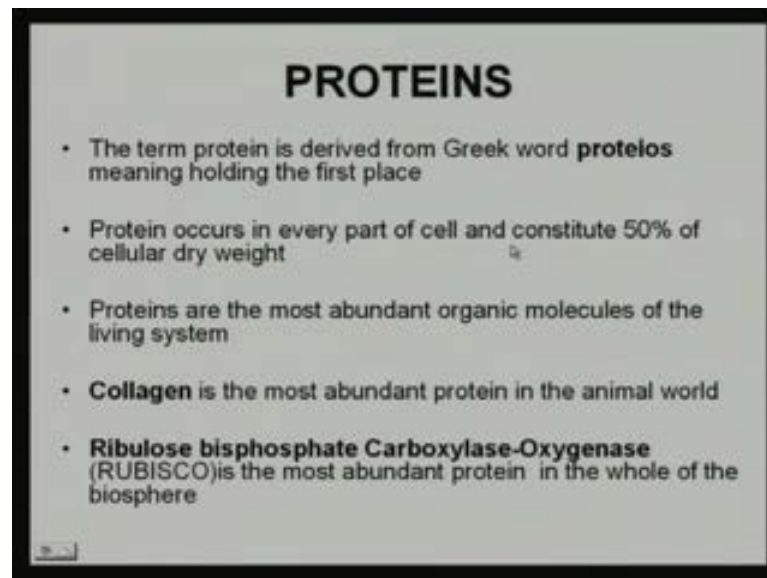
Biochemical Engineering
Prof. Dr. Rintu Banerjee
Department of Agricultural & Food Engineering
Asst. Prof. Dr. Saikat Chakraborty
Department of Chemical Engineering
Indian Institute of Technology, Kharagpur

Lecture No. # 07

Proteins

Good morning students. Today I will be talking to you about new macro a molecule which plays a very significant and important role in most of the cellular activities of the cell.

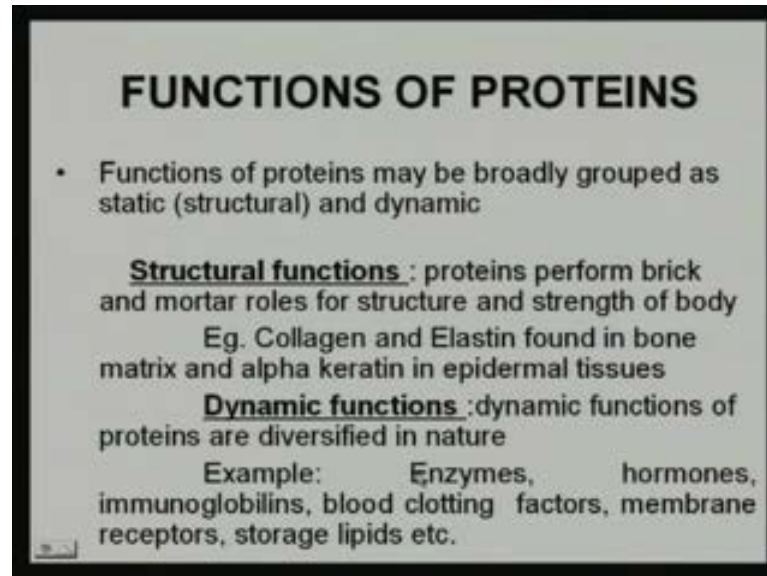
(Refer Slide Time: 00:30)



And that macro molecule is called protein. Now, the protein the term protein is derived from the Greek word, which is proteios means it holding it is holding the first place. Protein occurs in every part of the cell and it constitutes 50 percent of the cellular dry weight. Now, proteins are the most abundant organic molecule of the living system. Now, if we see the different protein molecules the most commonly found proteins in the animal system is the collagen. Collagen is the most abundant protein in the animal world

whereas, RUBISCO Ribulose bisphosphate Carboxylase-Oxygenase this is one of the protein molecule enzyme protein which is available plenty in the plant system.

(Refer Slide Time : 01:35)



FUNCTIONS OF PROTEINS

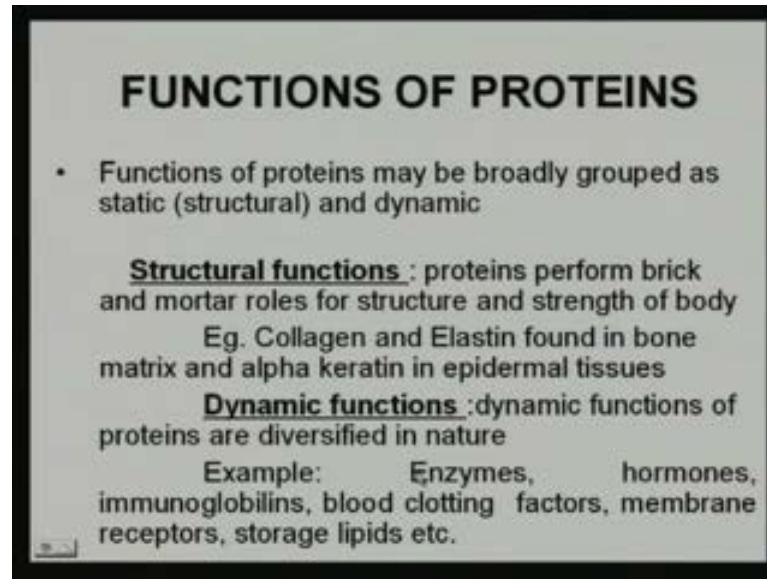
- Functions of proteins may be broadly grouped as static (structural) and dynamic

Structural functions : proteins perform brick and mortar roles for structure and strength of body
Eg. Collagen and Elastin found in bone matrix and alpha keratin in epidermal tissues

Dynamic functions :dynamic functions of proteins are diversified in nature
Example: Enzymes, hormones, immunoglobilins, blood clotting factors, membrane receptors, storage lipids etc.

So, if we see the function of a protein molecule we can broadly group the function of this protein into two basic structure divisions. One is the static or the structural function another is the dynamic function. Now, as far as the structural function is concerned the name itself indicates that it gives the structural and the strength of the body. Collagen Elastin etcetera are the protein molecules which are found in bone matrix and alpha keratin in the epidermal tissues of the cell.

(Refer Slide Time : 02:26)



FUNCTIONS OF PROTEINS

- Functions of proteins may be broadly grouped as static (structural) and dynamic

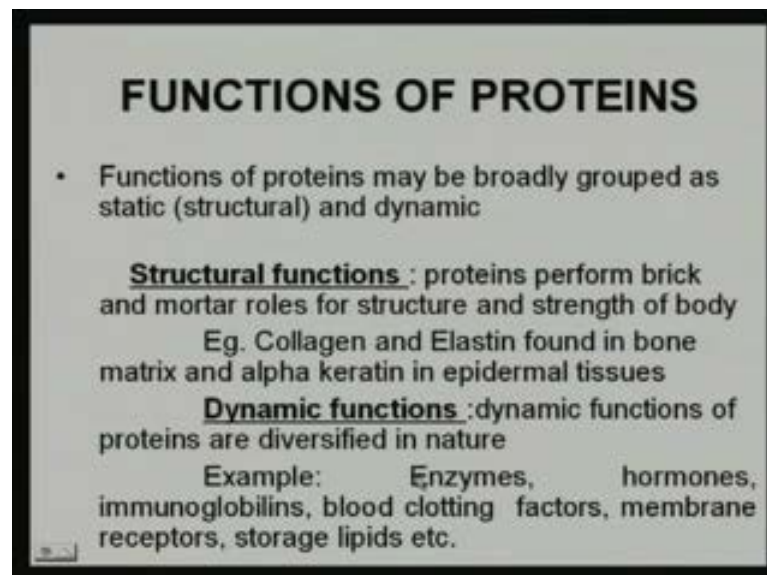
Structural functions : proteins perform brick and mortar roles for structure and strength of body
Eg. Collagen and Elastin found in bone matrix and alpha keratin in epidermal tissues

Dynamic functions :dynamic functions of proteins are diversified in nature

Example: Enzymes, hormones, immunoglobilins, blood clotting factors, membrane receptors, storage lipids etc.

Whereas, the dynamic function it is the protein that diversified which are diversified in nature and they play different catalytic role like enzymes. Enzymes are the biocatalyst and they play different catalytic role in the cell. Similar to that hormones, immunoglobulins, blood clotting factors, membrane receptor storage lipid etcetera, are coming under these categories.

(Refer Slide Time: 02:58)



FUNCTIONS OF PROTEINS

- Functions of proteins may be broadly grouped as static (structural) and dynamic

Structural functions : proteins perform brick and mortar roles for structure and strength of body
Eg. Collagen and Elastin found in bone matrix and alpha keratin in epidermal tissues

Dynamic functions :dynamic functions of proteins are diversified in nature

Example: Enzymes, hormones, immunoglobilins, blood clotting factors, membrane receptors, storage lipids etc.

Now, in my earlier lecture I have told you that there are certain molecules which constitute

(Refer Slide Time:03:08)

ELEMENTAL COMPOSITION OF PROTEINS	
• Carbon	: 50 – 55%
• Hydrogen	: 6 – 7.3%
• Oxygen	: 19 – 24%
• Nitrogen	: 13 – 19%
• Sulfur	: 0 – 4%
• Besides the above, proteins contain elements such as P, Fe, Cu, I, Mg, Mn, Zn etc.	
• The content of nitrogen, an essential component of proteins, on an average is 16%	

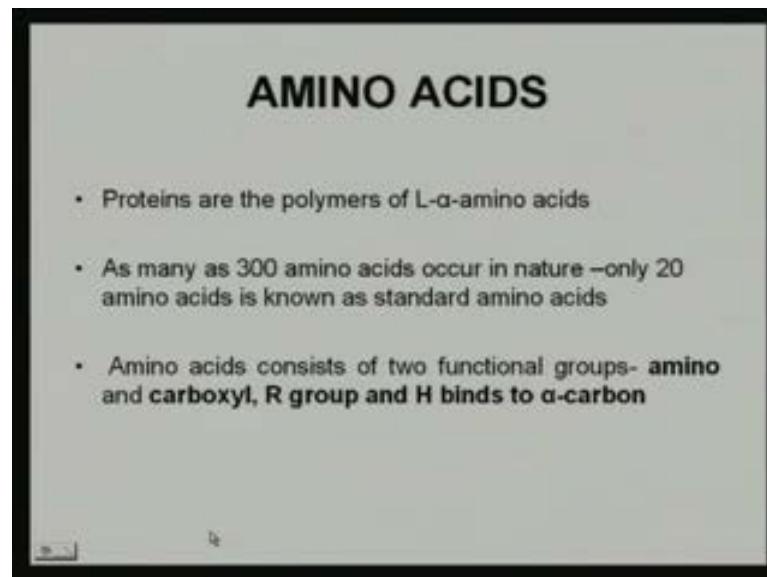
This macro molecular structure and if we see that carbon, hydrogen, oxygen, nitrogen and sulfur are the basic constituent of this protein molecule. If we see the percentage of carbon it varies from 50 to 55 percent whereas, nitrogen 6 to 7 percent, oxygen 19 to 24 percent, nitrogen 13 to 19 percent and sulfur 0 to 4 percent. That means if, we see the major constituent we can find that that carbon, hydrogen, oxygen, nitrogen and sulfur are the basic constituent of the protein molecule.

(Refer Slide Time : 04:01)

ELEMENTAL COMPOSITION OF PROTEINS	
• Carbon	: 50 – 55%
• Hydrogen	: 6 – 7.3%
• Oxygen	: 19 – 24%
• Nitrogen	: 13 – 19%
• Sulfur	: 0 – 4%
• Besides the above, proteins contain elements such as P, Fe, Cu, I, Mg, Mn, Zn etc.	
• The content of nitrogen, an essential component of proteins, on an average is 16%	

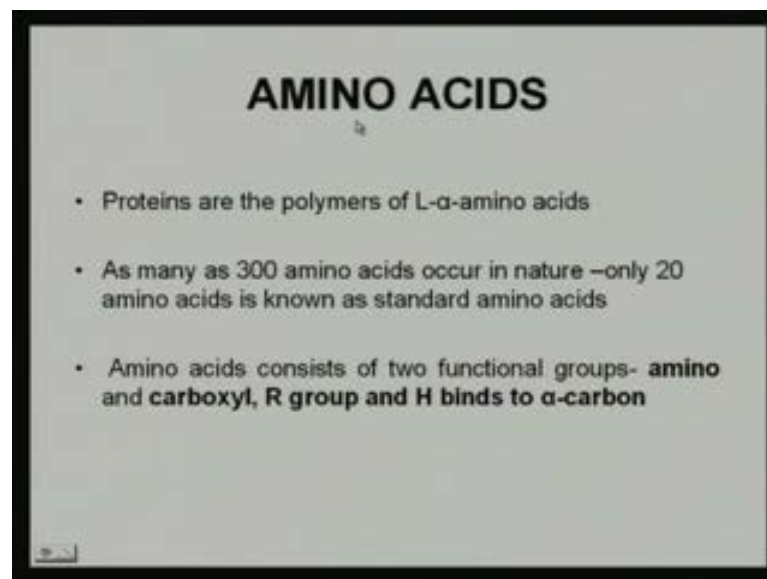
Besides these above molecules protein contains some elements such as phosphorous, ferrous, copper, iodine, magnesium, manganese, zinc etcetera. Within it the content of this nitrogen and essential component of the protein varies on an average to 16 percent.

(Refer Slide Time: 04:32)



Now, this is the basic constituent of the molecules which constitute the protein.

(Refer Slide Time: 04:40)

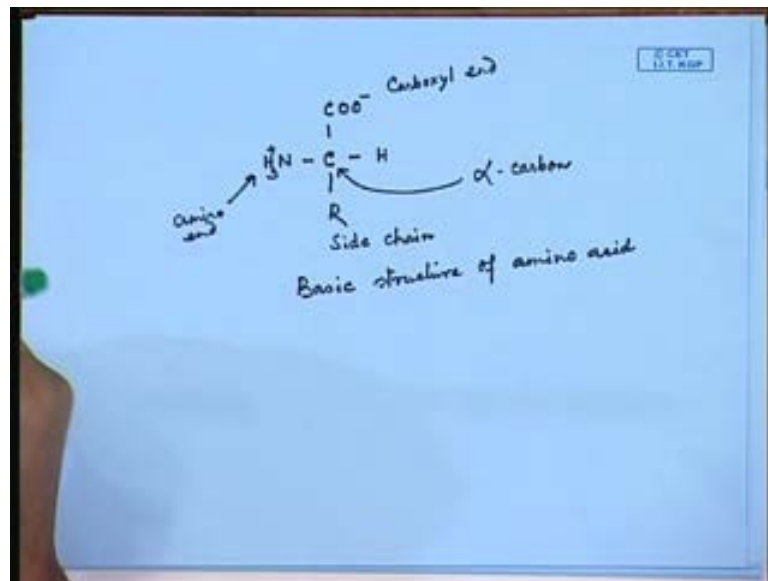


Now, when we are talking about these amino acids, amino acids are the basic building block of protein molecule. Now, proteins are the macro molecules which are polymers in

nature polymeric in nature. And the basic constituent of this polymers are the alpha amino acid there are more than 300 amino acids, which are there in this nature.

But, only 20 amino acids are considered to be the standard amino acid and with it is permutation and combinations protein structure is made amino acid consist of two fundamental groups. That means if we see the structure of amino acid we can find that this amino acid.

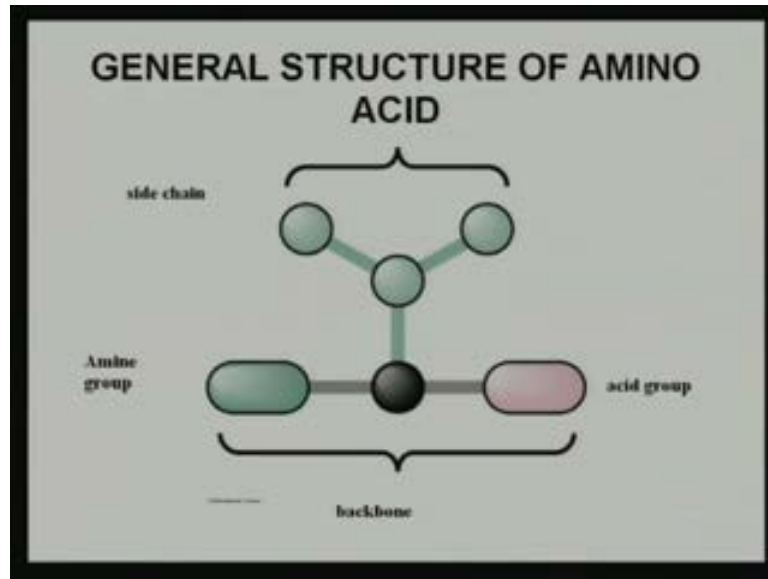
(Refer Slide Time: 05:39)



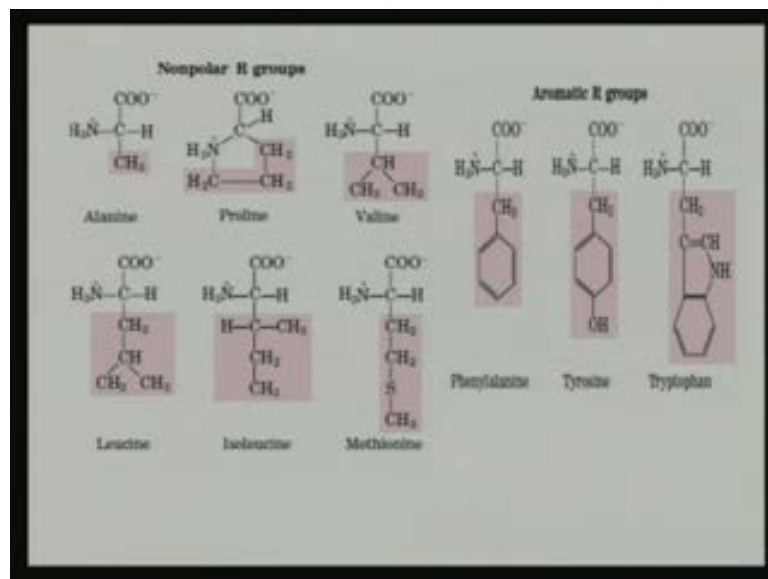
Has got one carboxyl end amino acid has got one amino end and one amino acid is different from the another amino acid with it is R group.

So, this is the side chain and this side chain varies from one amino acid to the another amino acid. So, this is the fundamental basic structure of any amino acid and as this is the first carbon then this functional carbon this is the alpha carbon and this is this is the basic structure of amino acid. So, if we see the structure one hydrogen is present one carboxyl end is there carboxylic acid and C O O H group is there, one amino group is there and the side chain R group is present in the basic core structure of amino acid.

(Refer Slide Time: 06:44)



(Refer Slide Time: 06:45).



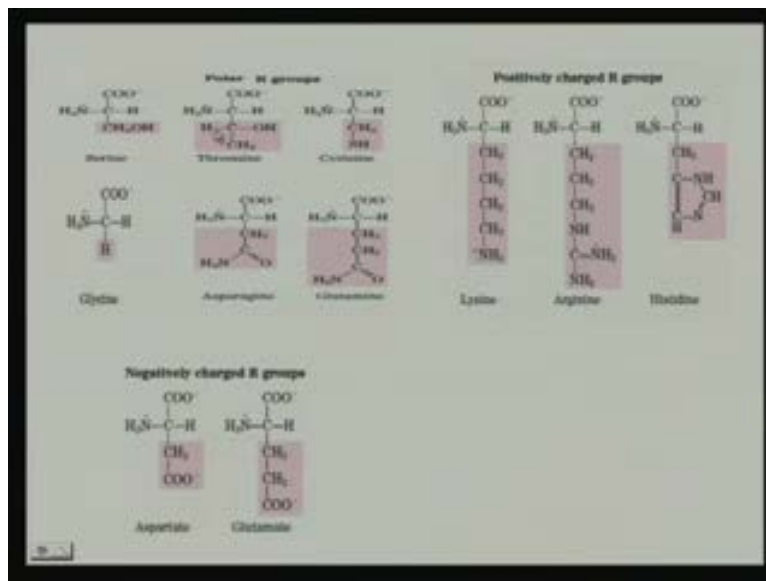
Now, if we divide this 20 standard amino acid based on its characteristics or based on the properties of this R group. It can be categorized into 4 different divisions. One is called the non polar or the hydrophobic amino acid, second group is the polar or hydrophilic amino acid, third is the acidic amino acid and fourth group is the basic amino acid.

Now, whatever is there the 20 standard amino acids has been divided into these 4 different categories. So, this 20 standard amino acid has been divided into 4 different

categories some are non polar, some are polar, some are acidic and some are basic in nature. Now, let us come to the non polar amino acid.

Alanine, proline, valine, phenylalanine, tyrosine, tryptophan, methionine, isoleucine and leucine are the non polar or the hydrophobic amino acid. What is the basic nature of this type of amino acid they are insoluble mostly or sparingly soluble in water. Now, these are the R group so, when I am talking to you this is the R group and this is the core moieties of the amino acid, these core moieties are same in most of the amino acids.

(Refer Slide Time: 08:41)

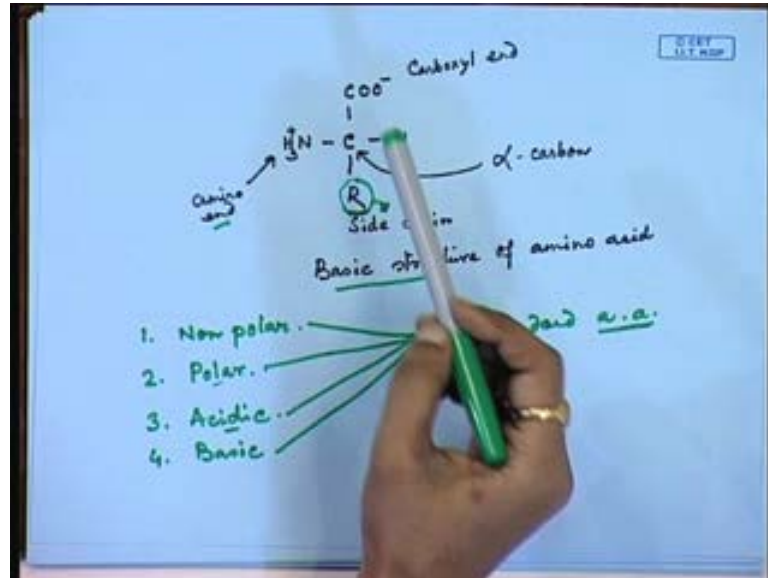


Now, when we are coming to the polar R group then we can find that serine threonine, cysteine, glycine, asparagine, glutamine are the polar amino acid, where glycine is the amino acid which is placed on the transition phase of polar and non polar amino acid. Now, this is the only amino acid where this carbon is not an asymmetric carbon atom that means here this R group is also another hydrogen H. So, as this is H this glycine is not having this chiral carbon within it when we are coming to the basic amino acid. It is otherwise known as positively charged amino acid and here.

We can find that in case of lysine here that additional amino containing groups are there arginine has got guanidini group and histidine has got imidazolium group within it. And here they have this additional amino containing group within it and that is the reason why it is called the basic amino acid, whereas the negatively charged amino acids are the aspartic acid and the glutamic acid.

Now, here I would like to tell you that amino acid if, we see the core structure of this amino acid as I have already discussed.

(Refer Slide Time: 10:22)



That it has got the carboxyl end and another is that amino end. So, besides this carboxylic group and the within the core structure of this particular amino acid and additional C O O H group is present in both these amino acid. That aspartic acid and glutamic acid resulting the nature of this amino acid acidic in nature. So, these are the broad classification of this amino acid amino acid can also be classified based on this aromatic ring some groups can be also once again taken which contain the aromatic amino acid such as this phenylalanine tyrosine tryptophan and so on so based on the it is structure the twenty standard amino acid has been classified.

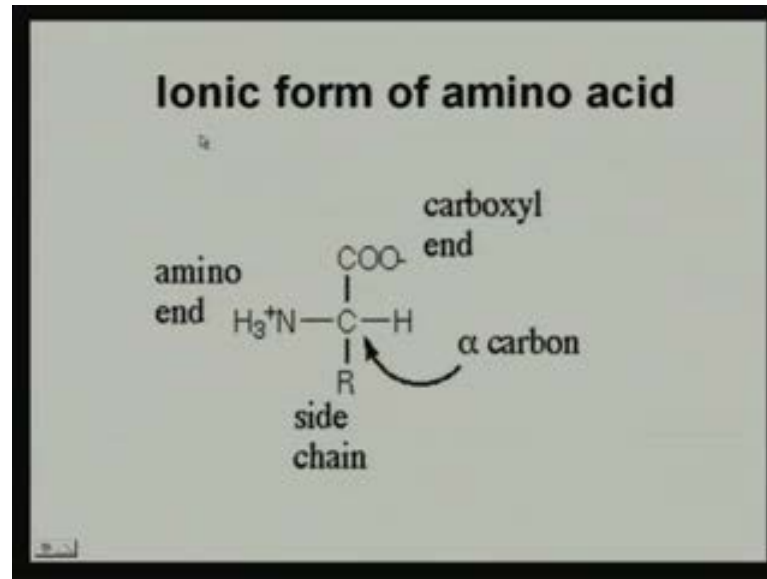
(Refer Slide Time: 11:26)

Amino Acids and their three letter and one letter Symbol	
• Glycine – Gly – G	• Threonine-Thr-T
• Alanine-Ala – A	• Asparagine-Asn-N
• Valine-Val- V	• Glutamine-Gln-Q
• Leucine-Leu-L	• Tyrosine-Tyr-Y
• Isoleucine-Ile-I	• Cysteine-Cys-C
• Methionine-Met-M	• Lysine-Lys-K
• Proline-Pro-P	• Arginine-Arg-R
• Phenylalanine-Phe-F	• Histidine-His-H
• Tryptophan-Trp-W	• Aspartic acid-Asp-D
• Serine-Ser-S	• Glutamic acid-Glu-E

Now these amino acids are playing a very important role as far as the mechanism of the cell functionality is concerned and that is the reason these amino acids have been classified or symbolized with either three letter digit or with one letter digit. For example, now glycine if we considered glycine as one of the amino acids it can be denoted by Gly that is the three letter symbol and in single letter it is G. Alanine is A or Ala and Valine is Val or V. Leucine is Leu or L.

So, in this way these 20 standard amino acids are classified with the 3 letter and single letter symbol. The significance of this single letter and 3 digit symbol will be understood gradually when we will be going a little bit in depth and you will be learning the role of other macromolecular structure and functionality of that particular macromolecule in the cell system. So, these are the 3 letters and single letter symbol of amino acid.

(Refer Slide Time: 12:54)



Now, as I have told you this is the basic ionic form of amino acid this is the carboxyl end and this is the amino end and this R group. So, as I have already told you that R group of each standard 20 standard amino acid varies from each other and which makes one amino acid different from another amino acid

(Refer Slide Time: 13:24)

CLASSIFICATION OF AMINO ACIDS

- Amino acids are classified based on the structure and chemical nature, nutritional requirement, metabolic fate.

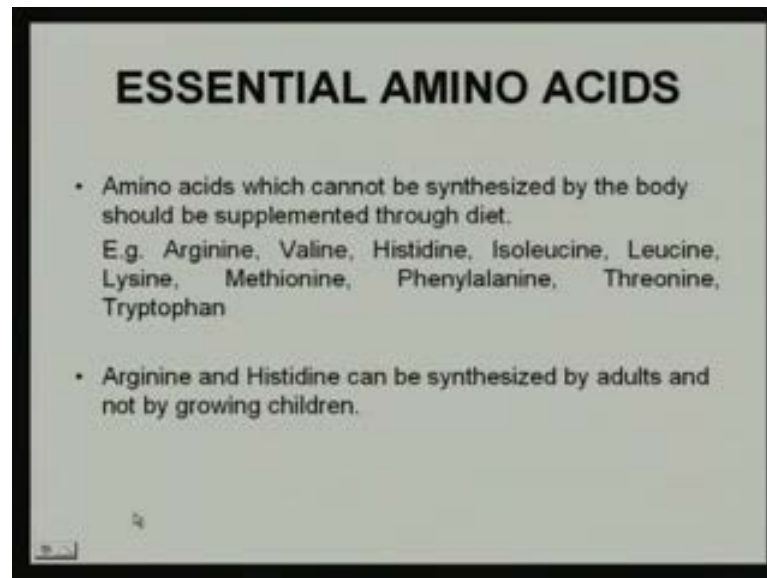
NUTRITIONAL REQUIREMENT

- Based on nutritional requirements amino acids is classified into two types
 - 1)Essential amino acids
 - 2)Non- essential amino acids

Now, if we go for this classification of amino acid, then amino acids are classified based on the structure and the chemical nature. That is the nutritional requirement and the metabolic activities of any living cell.

Now, based on the nutritional requirement then this amino acids has been classified into two types. One is called the essential amino acid another is the non essential amino acid. Now, what is essential amino acid this essential amino acids.

(Refer Slide Time: 14:02)



Which cannot be synthesized by our body system? That means for the supplementation of these amino acids, we have to supplement these amino acids through diet and what are those amino acids, which are which cannot be synthesized by the body. They are arginine, Valine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, threonine and tryptophan. So, these are the amino acids which cannot be synthesized within the body system. And that is the reason it these amino acids are replaced or it is the demand is meet through diet or food. Arginine and histidines are the amino acids which are synthesized by the adult. But, it cannot be it is not present in the growing children.

(Refer Slide Time: 15:18)

Non-essential amino acids

- Can be synthesized by the body

Glycine, alanine, serine, cysteine, aspartate, asparagine, glutamate, glutamine, tyrosine, and proline.

In case of non essential amino acid that means those amino acids which can be synthesized by the body system itself. That means glycine, alanine, serine, cysteine, aspartic acid, asparagine glutamic acid, glutamine, tyrosine and proline these are the amino acid body can synthesize these amino acid by itself. So, supplementations of these amino acids are not that essential.

(Refer Slide Time: 15:55)

PROPERTIES OF AMINO ACIDS

- Amino acids can donate proton or accept proton due to (COOH) and (-NH₂) groups
- Zwitter ion : A hybrid molecule containing positive and negative ionic groups
- Isoelectric pH : the pH at which a molecule exists as a Zwitter ion carries no net charge

$\text{NH}_2\text{—CH}_2\text{—COOH}$
Glycine

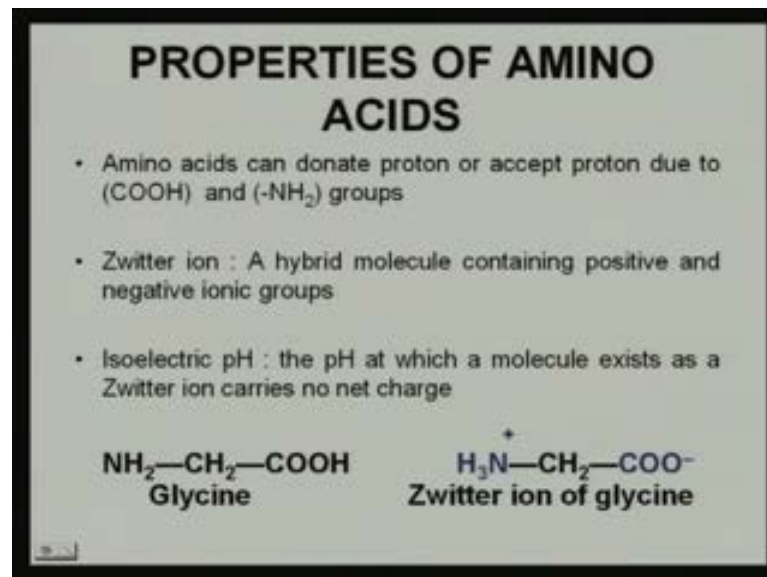
$\text{H}_3\text{N}^+\text{—CH}_2\text{—COO}^-$
Zwitter ion of glycine

Now, when we are coming to the properties of this amino acid, now amino acid can be denoted by a proton or it can donate proton or accept proton due to the presence of the C

O O H group. And amino group present within it Zwitterionic form it is a hybrid molecule containing a positively charged as well as a negatively charged ionic group within it. That means if we see this structure it has got positive charge as well as negative charge resulting charge in the Zwitterionic condition is 0. That means no net charge is present on the surface of the amino acid.

Isoelectric point it is isoelectric p H is that p H where a molecule exist as a Zwitterionic condition that means no net charge is there. So, positive charge is equal to the negative charge that means when we are passing this protein molecule or this amino acid in a electrical field. There will be no movement because of the net charge as it is 0 or neutral.

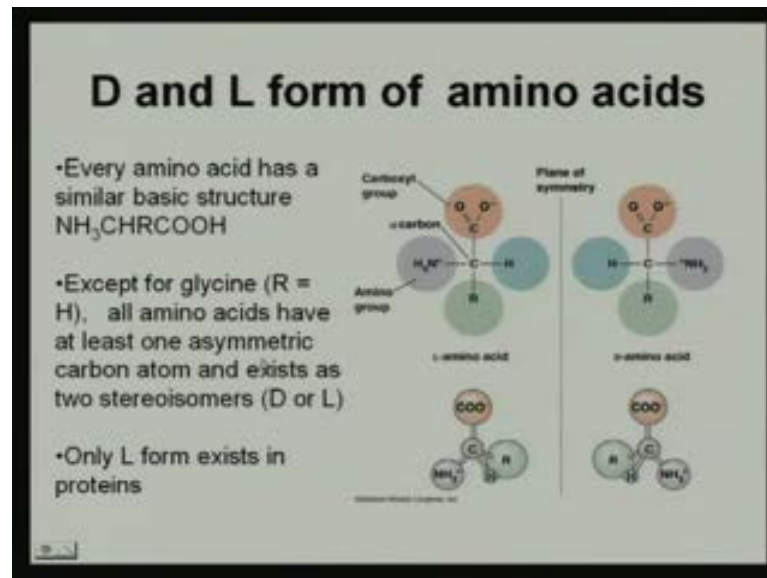
(Refer Slide Time: 17:24)



Now, this amino acid can be seen by the Fischer projection all amino acids except glycine. I have already mentioned that in glycine the R group is simply H and that is the reason. It is not the chiral molecule amino acids have stereoisomer and in biological system only L form of amino acids are used in the protein molecule and not the D form this is the L form and this is the D form.

So, this L form of amino acid is present in the protein molecule but, in D form this is found in the larvae and pupae of the insect. So, these are not playing any role as far as the protein is concerned now.

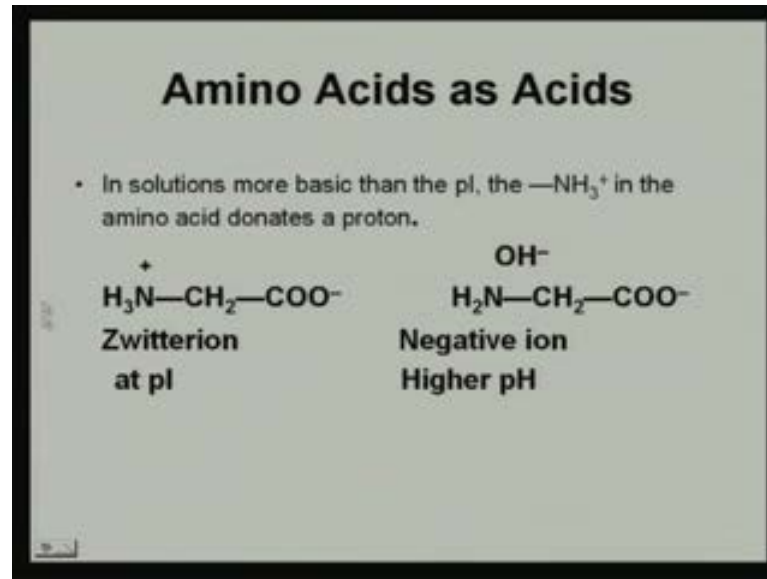
(Refer Slide Time: 18:27)



When we are talking about the D and L form of amino acid, we can find that every amino acid has a similar basic structure that means one amino terminal another carboxy terminals are there except for glycine. Where R is H all amino acids have at least one asymmetric carbon atom and exist as two stereoisomer. That is either the D form or the L form this is the D form or the L form. So, why this is the chiral carbon and it is the amino group is present here and in the D form the amino group is present on the right hand side.

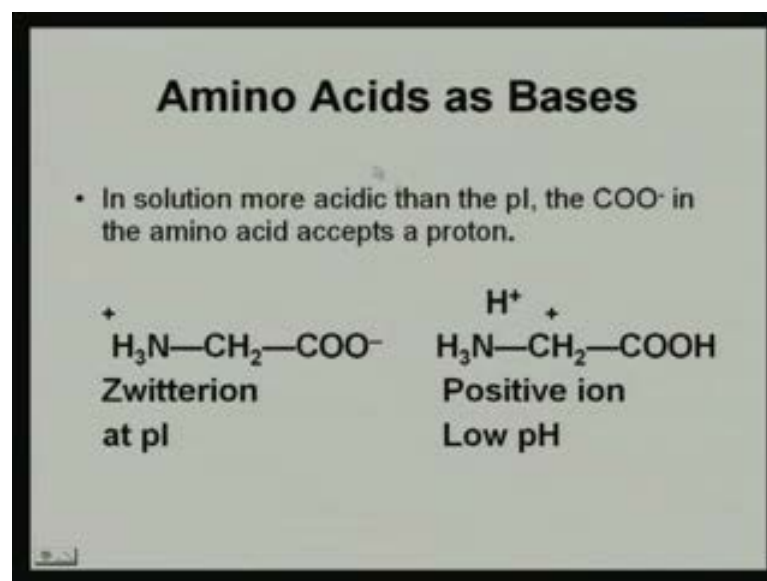
So, this is the D form and L form of these amino acid. So, stereoisomeric character is one of the very important properties which determine the L form or D form of this amino acid.

(Refer Slide Time: 19:29)

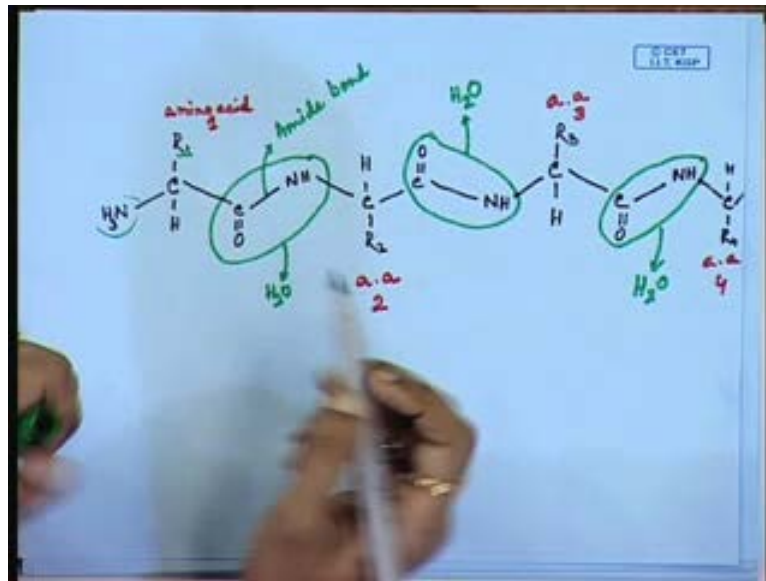


Now, amino acid when it is acting as acid in solution more basic than the pI. That pI what is the pI it is the isoelectric point where there is no net charge, which is present on this amino acid the amino NH_3^+ in the amino acid donate a proton to this and it forms. This ionic structure so, this is the Zwitterionic at the neutral pH, pI and here under the basic condition where hydroxyl group are there. So, in higher pH this pI is having this it can donate a proton to these and making this structure $\text{NH}_2\text{—CH}_2\text{—COO}^-$ and COO^- minus.

(Refer Slide Time: 20:32)



(Refer Slide Time: 21:42)

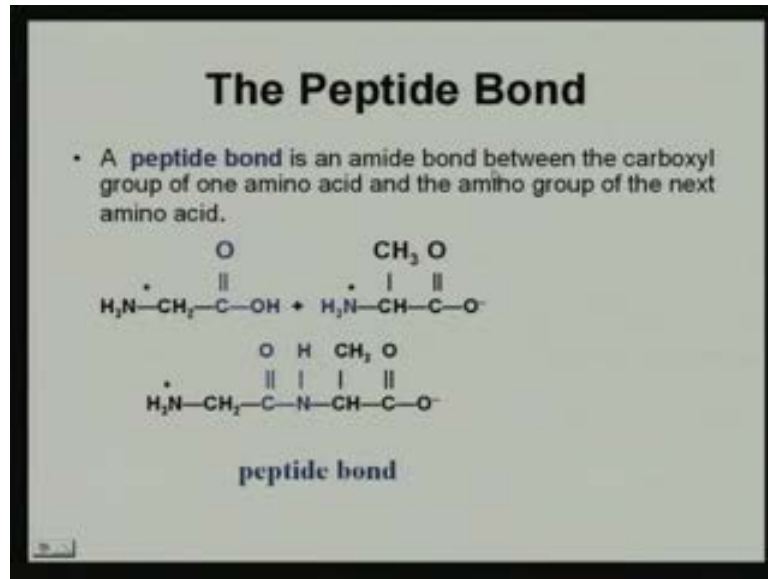


The structure of one amino acid now see this is the structure of one amino acid this is the R 1 this is C H this is the amino terminal and this is the carboxy terminal.

Now, when one amino acid is joining with the another amino acid that means I have already mention you that amino acid has got two terminal one is the amino end another is the carboxyl end. So, one carboxyl end of one amino acid when it joins with the amino terminal of the second amino acid they form the amide bond.

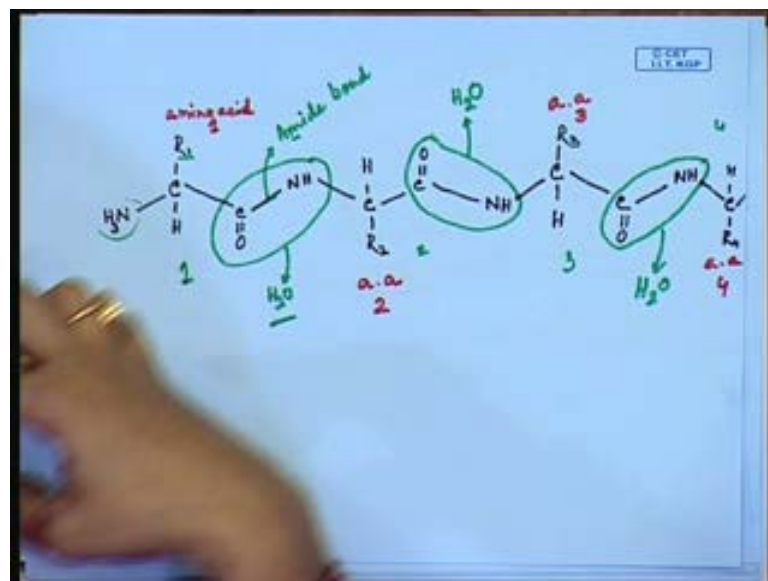
Now, when C O O H is coming in contact with this N H 2 they form they release a molecule of water from these and this bond is called the amide bond. Now, in this way when it forms a chain like structure it is the called the peptide bonding. So, peptide bond is the amide bond.

(Refer Slide Time: 22:50)



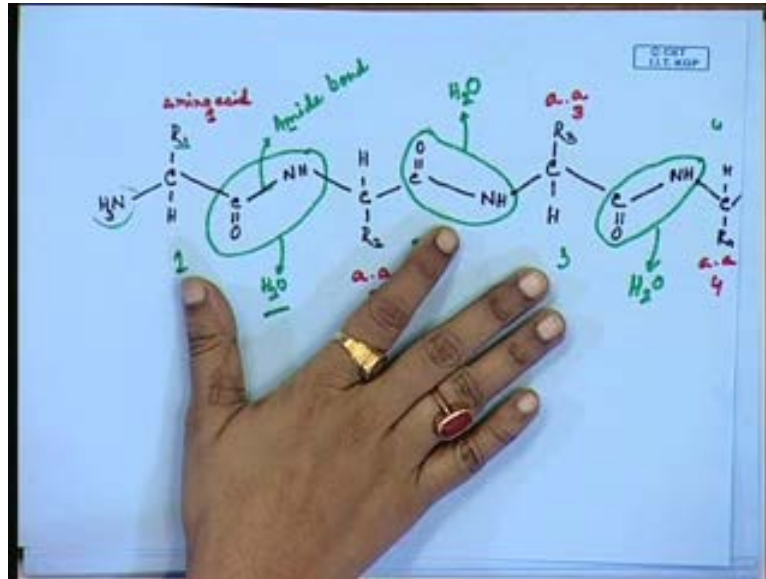
Between the carboxyl group of one amino acid this is the first amino acid, this is the

(Refer Slide Time: 22:57)



Second amino acid, this is the third amino acid and this is the fourth amino acid, so this when this amino acids are joining together and making a chain like structure.

(Refer Slide Time: 23:19)



Now, classification and they form. The protein structure now, when we are coming to this protein. Now, let us come to this classification of protein. Now, based on the amino acid composition structure shape and solubility properties of the protein are broadly classified into 3 major groups. First group is called the simple protein they are composed of only the amino acid residues. It consists of the globular and the fibrous protein example is albumin, globulin, histone, collagen etcetera.

(Refer Slide Time: 24:00)

Globular proteins

- Usually water soluble, compact, roughly spherical
- Hydrophobic interior, hydrophilic surface
- Globular proteins include enzymes, carrier and regulatory proteins

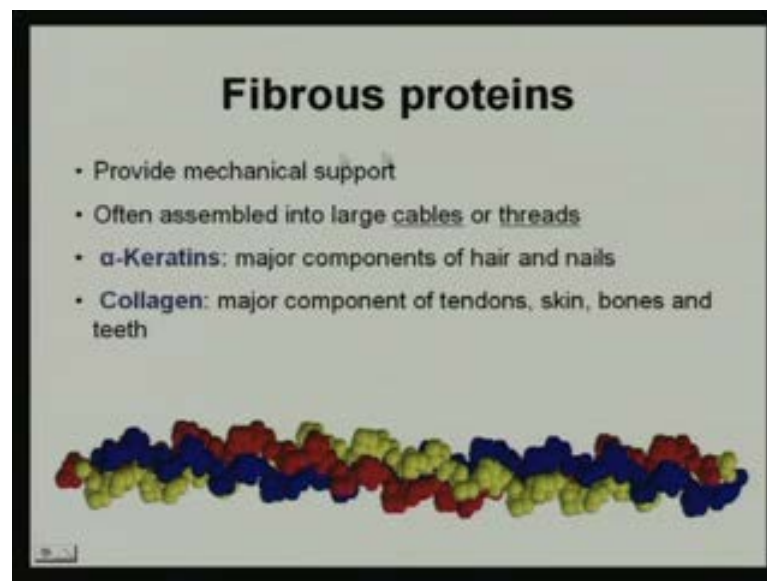
A 3D ball-and-stick model of a globular protein, showing a compact, roughly spherical structure. The atoms are represented by spheres of different colors: red for oxygen, white for hydrogen, and blue for nitrogen. The model is set against a light gray background.

Now, when we are coming to this globular protein it is usually water soluble compact and roughly spherical in nature hydrophobic interior and hydrophilic surface. Now, globular protein includes the enzyme carrier and the regulatory protein. That means if we see the nature of this amino acid I told based on the characteristics of this amino acids. That means hydrophilic amino acids are present on the surface of the protein whereas, hydrophobic moieties because hydrophobic are water heating.

Water is not a very suitable environment for them they try to put it inside where is which is covered by the hydrophilic moieties.

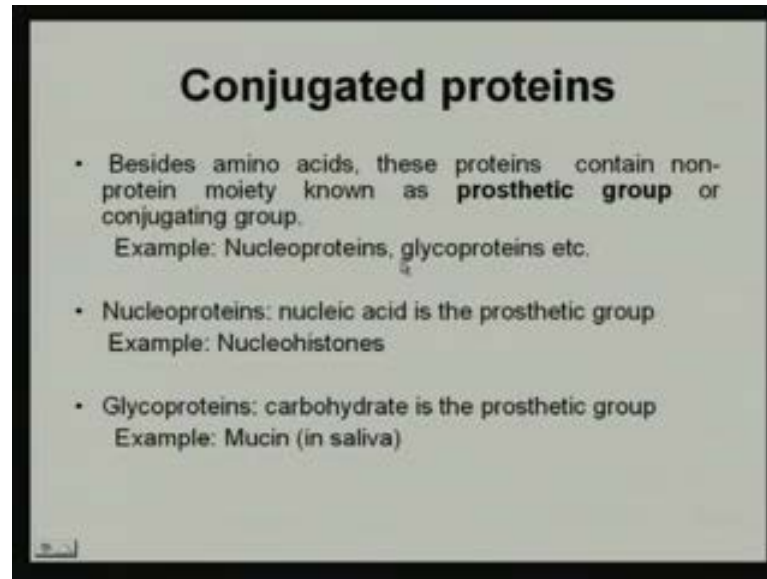
So, these type of protein this combinations it forms the protein structure and it is forming the globular protein.

(Refer Slide Time: 25:03)



When we are coming to this fibrous protein it provides the mechanical support of an assembled in a large cable or thread like structure alpha keratin, which is a major component of hairs nails etcetera are the example of alpha keratin, which are insoluble in water. Collagens are the major component of the tendon skin, bones, teeth etcetera. Where these collagen proteins are there so you can understand that all this biomolecules, which we are carrying, are insoluble proteins that fibrous protein molecule.

(Refer Slide Time: 25:50)



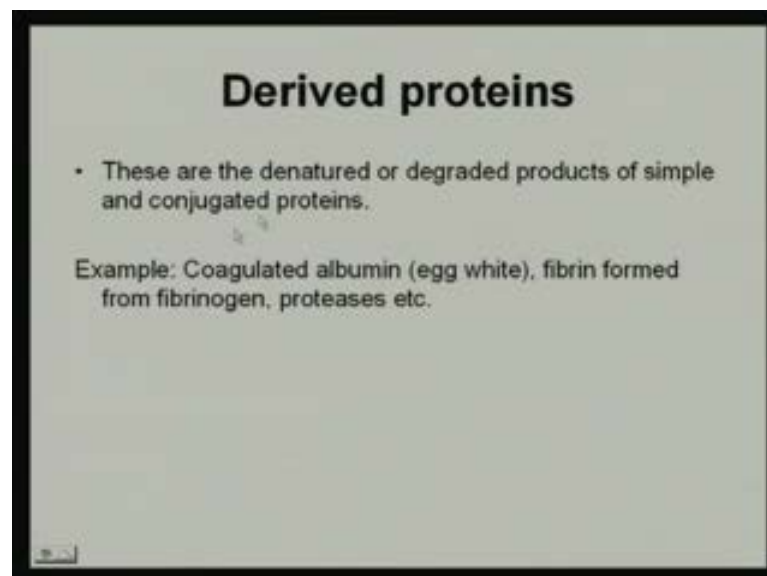
Conjugated proteins

- Besides amino acids, these proteins contain non-protein moiety known as **prosthetic group** or conjugating group.
Example: Nucleoproteins, glycoproteins etc.
- Nucleoproteins: nucleic acid is the prosthetic group
Example: Nucleohistones
- Glycoproteins: carbohydrate is the prosthetic group
Example: Mucin (in saliva)

The conjugated proteins are besides this amino acid these proteins contain non protein moieties along with it which is called the prosthetic group. Nucleoprotein, glycoprotein are some of the example of this conjugated protein.

Now, in case of nucleoprotein nucleic acid is there as the prosthetic group for the functionality of that particular protein, whereas carbohydrate moieties are present sometimes as a prosthetic group in the protein molecule for it functionality like mucin which is present in the saliva.

(Refer Slide Time: 26:32)



Derived proteins

- These are the denatured or degraded products of simple and conjugated proteins.

Example: Coagulated albumin (egg white), fibrin formed from fibrinogen, proteases etc.

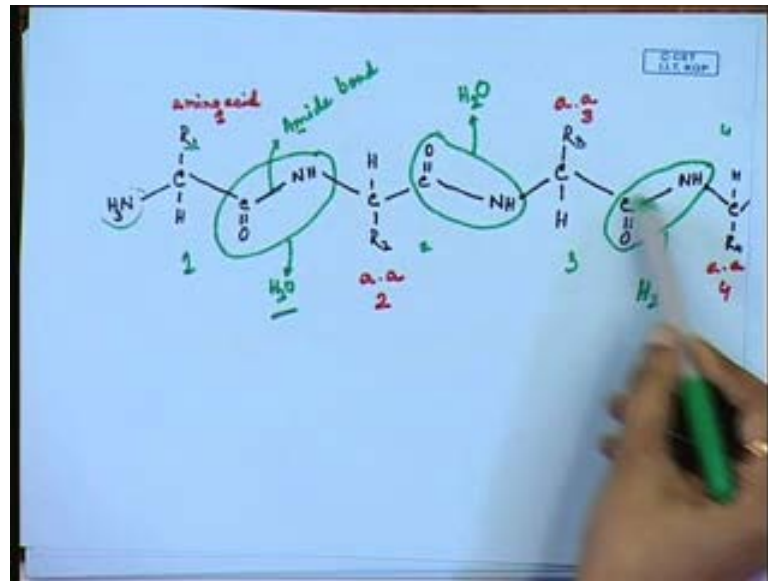
Sometimes a and it is categorized as a derived protein these are the denatured or degraded products of the simple and conjugated protein. Examples are the coagulated albumin that is the egg white fibrin which is formed from the fibrinogen and proteases etcetera are the example.

(Refer Slide Time: 26:58)

<i>Level of structure</i>	<i>Basis of Structure</i>	<i>Bonds involved</i>
Primary	Amino acid sequence	Covalent bonds
Secondary	Folding into α helix, β sheet or random coil	Hydrogen bonds
Tertiary	3D folding of a single polypeptide	Hydrogen and disulfide bonds, electrostatic and hydrophobic interactions
Quaternary	Association of 2 or more folded subunits	Same as tertiary

Of the derived protein now, when we are I have given you enough example of this protein. Now, if we see the organization level of this protein then we can categorize the structure of this protein molecule into different groups, which is called the primary structure, secondary structure of the protein, tertiary structure of the protein and the quaternary structure of the protein molecule. Now, if we see the basis of this structure primary structure is the amino acid sequencing and it is the simple amino acid what I have told you.

(Refer Slide Time: 26:58)

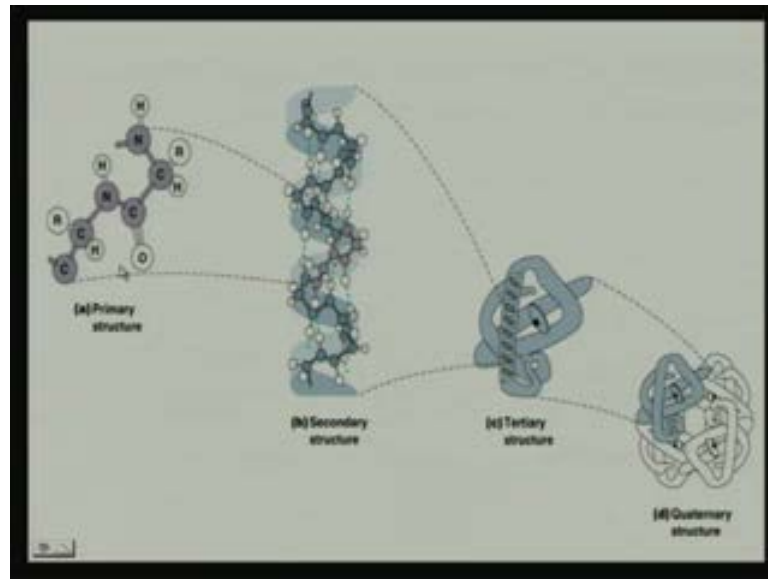


I have shown you here that one amino acid one carboxy terminal of this amino acid is joining with the amino terminal of another amino acid. And in this way they are forming a big chain resulting one amino terminal free and another carboxy terminal free of this chain.

When we are coming to the secondary structure it is the folding into the alpha helix and beta sheet or random coil. That means here besides this primary structure there are additional bonding, which is called the hydrogen bonding is present. When we are talking about the tertiary structure here 3d folding of a single polypeptides are there which may be hydrogen or disulfide bond electrostatic or hydrophobic interactions are also present within this tertiary structure.

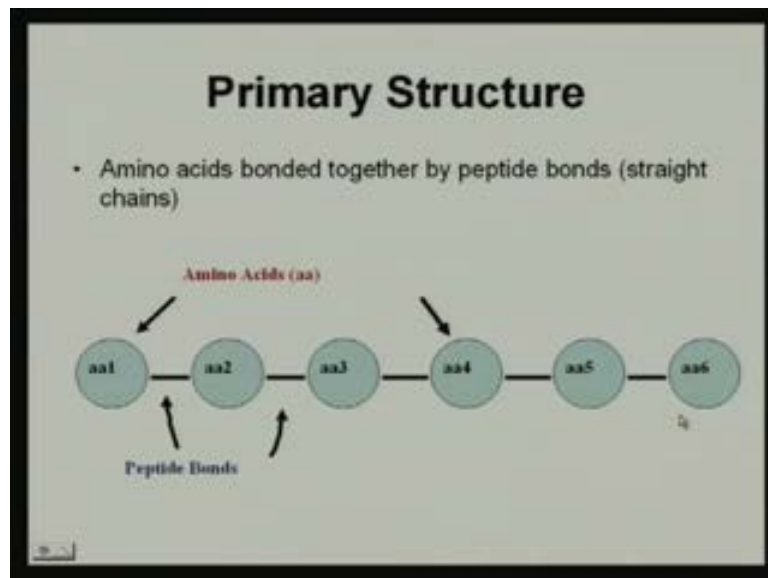
And when we are talking about this quaternary structure it is considered to be the more complex and here the association of different peptide bonds sub units is there and they are folded within it. And it is having the more complexity and bonding pattern is same as that of the tertiary structure.

(Refer Slide Time: 29:08)



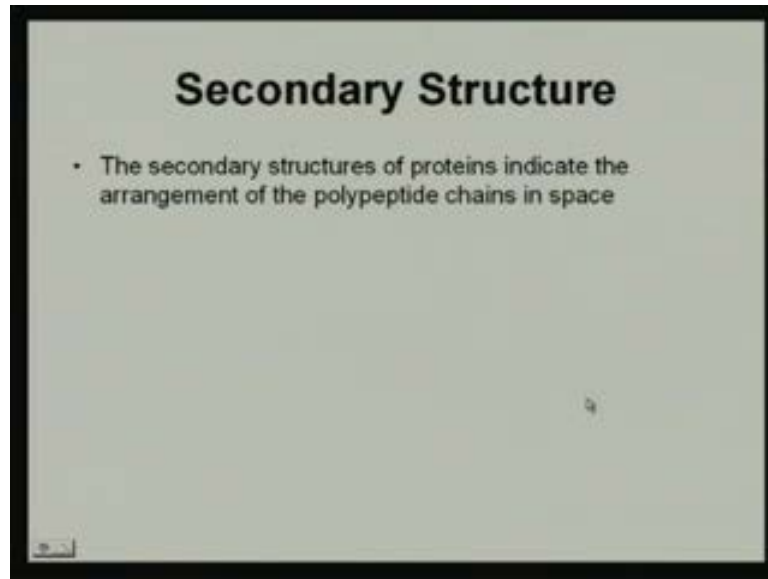
So, if we see the primary structure this is the simple structure secondary structure it is trying to form a coil, then tertiary structure it is the globular protein and trying to make more complex structure. And quaternary is the most complex structure which is there within the protein molecule.

(Refer Slide Time: 29:33)



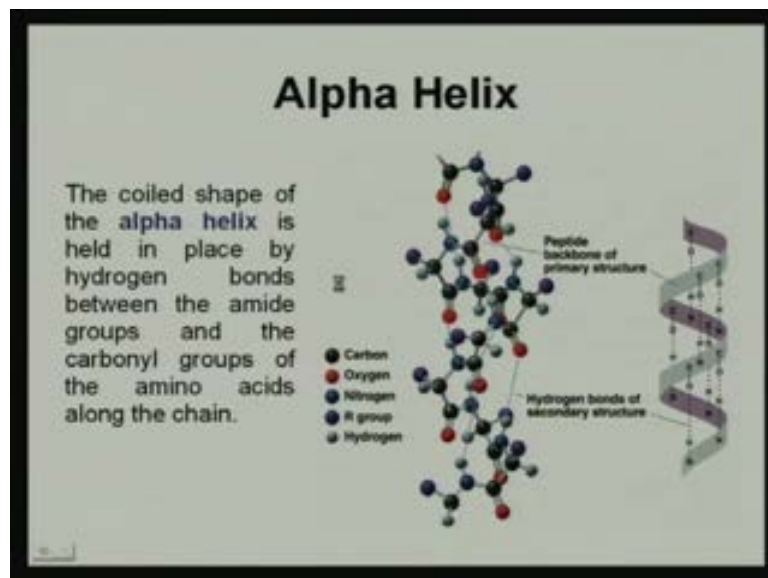
Now, coming to this primary structure I have already discussed this primary structure. It is the simple peptide bond which is having a very straight chain.

(Refer Slide Time: 29:48)



Coming to this secondary structure, Secondary structure of protein indicates the arrangement of the peptide chain in a space.

(Refer Slide Time: 29:56)

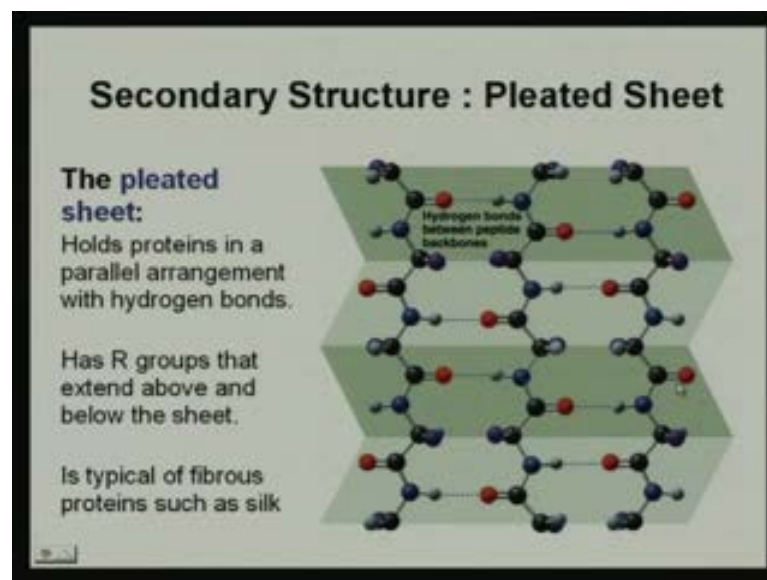


Now, see here this is the chain peptide chain and here it tries to be a coil like structure, when it is trying to be a coil like structure additional this amine containing group has got some hydrogen bonding with the carboxy end of any another amino acid. And they are trying to form little bit complex structure and they try to associate the coil within this

confirmation. And that is this coil shape of this alpha helix is held in its place by hydrogen bonds between the amide group and the carbonyl group of the amino acid.

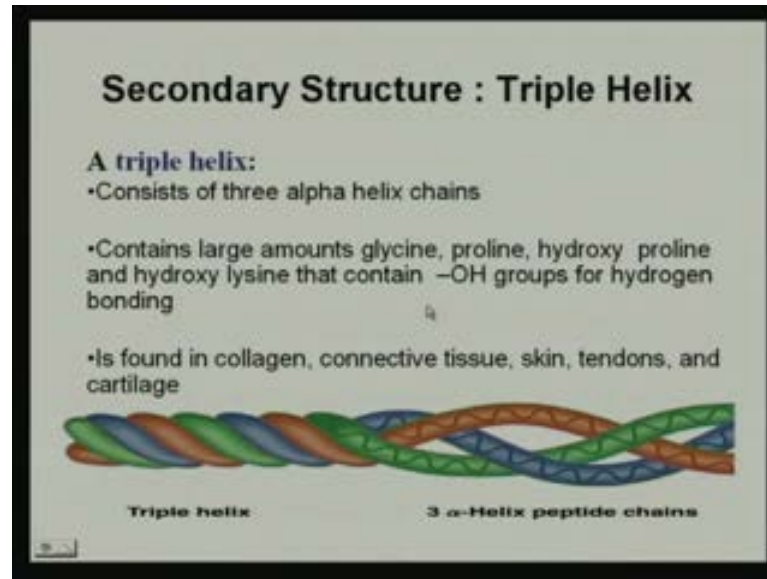
Now, if this is the black is the carbon, red is the oxygen, nitrogen is the violet R group is also the dark violet and pale blue is the hydrogen. Then we can find that say this bonding has got this carboxyl this oxygen is getting bonded with hydrogen. And they are associated with each other and these amino containing group has got linked with the carboxyl end with the amide bonding and this way.

(Refer Slide Time: 31:30)



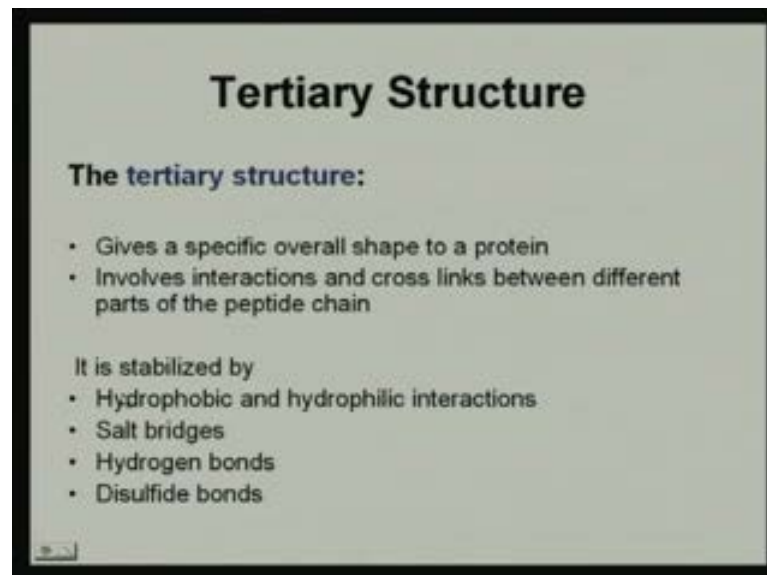
They are associating they are making the link when we are talking about the secondary structure it is called the beta pleated sheet. Now, here the it holds the protein in a parallel arrangement. So, these are the peptide linking and they are just trying to hold themselves in the parallel arrangement and it looks like a matrix like structure it has a R group that extend above and below the sheet. This above and below the sheet it is a typical fibrous protein such as silk. So, this is one of the examples of the beta pleated protein.

(Refer Slide Time: 32:19)



The secondary structure but, we can also get the triple helix it consists of 3 alpha helical chain it contains a large amount of glycine, proline, hydroxy proline this is a derivatized product of proline hydroxy lysine. That contain the O H group for hydrogen bonding it is found in collagen connective tissues skin tendon and the cartilage of the body. Now,

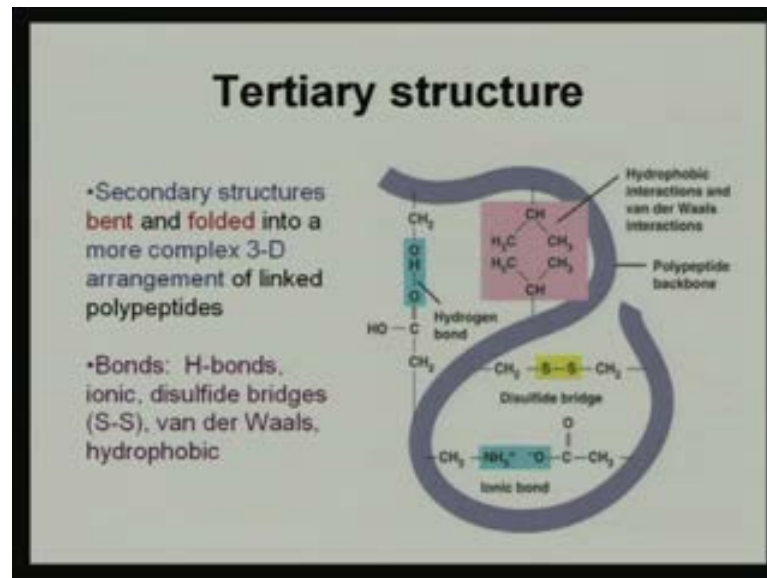
(Refer Slide Time: 32:57)



this is the tertiary structure of the protein molecule when we are talking about the tertiary structure it it gives the specific or overall shape to a protein that involves the interaction and cross links between the different part of the peptide chain. Hydrophobic and

hydrophilic interactions are there salt bridges are there hydrogen bonding and disulfide bonding that is the system molecule. When system is the amino acid which is which contains sulphur, methionine is also another example of sulphur containing amino acid. So, disulfides bridges when such type of amino acids is present they form the disulfide bridges.

(Refer Slide Time: 33:45)



So, when we are talking about this tertiary structure, the secondary structure when it is getting bent or folded into a more complex 3d structure. We call it as the tertiary structure here besides this peptide and the hydrogen bonding. We can expect all possible of other bonding like ionic disulfide Van der Waal hydrophobic and so on. Every reaction every bonding are possible because it is more and more folded together to form this structure.

(Refer Slide Time: 34:29)

Tertiary Structure: (Aggregation of individual protein)

- **Hydrophobic interaction:** the close association interaction of hydrocarbon side-chains
- **Ionic bond:** between positively charged groups and negatively charged groups
- **Hydrogen bonds**
- **Disulfide bonds**

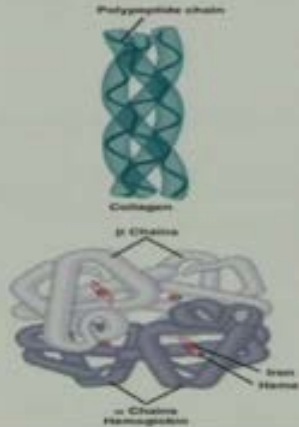
A protein has size and shape as well as unique arrangement through hydrogen, ionic, hydrophobic and disulfide bonds.

Now, here hydrophobic ionic hydrogen bond, disulfide bond etcetera are present for proper aggregation of the individual protein molecule.

(Refer Slide Time: 34:42)

Quaternary Structure

Quaternary structure is the interaction between adjacent polypeptides that make up a single protein E.g. Hemoglobin



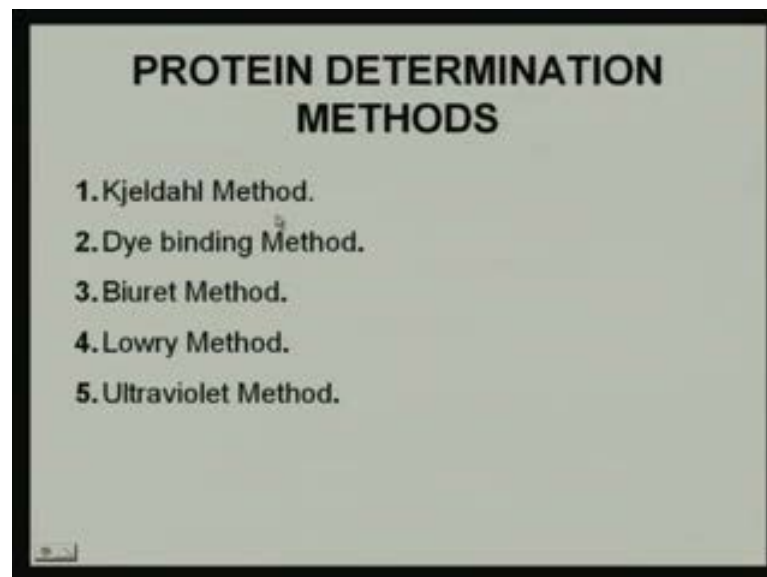
The diagram illustrates the quaternary structure of a protein. It shows a single polypeptide chain (labeled 'Polypeptide chain') and a complex structure of multiple polypeptide chains (labeled 'Polypeptide chains') interacting to form a single protein molecule (labeled 'Hemoglobin'). The diagram also shows 'Collagen' and 'α-Chains Hemoglobin'.

And it forms the tertiary structure. Now, when we are talking about this type of chain the they are called the peptide chain. Now, in this tertiary structure it is the peptide chain which is folded bent and they form the complex molecule. Now, in case of quaternary structure it is more and more complex I have already told. Now, when such peptide more than one peptide chains are getting associated with each other now say for example,

hemoglobin is one of the example of quaternary structure of the protein here. 2 beta chain and 2 alpha chains are associated with each other and they are forming the very complex structure. And when we are talking about this association obviously whatever bonding, whatever complexities are there so all the peptides are linked together and they form a jumbled like structure. And here this type of structure is complexities are found in quaternary structure of the protein.

So, quaternary structure is the interaction between the adjacent polypeptide that makeup the single protein that is hemoglobin collagen etcetera are.

(Refer Slide Time: 36:10)



The example of the quaternary structure of the protein molecule. So, I wanted to give you some idea about the structure and function of different protein. Now, how to quantify how to know that what is the protein concentration in that particular environment now, we can measure directly or indirectly. Now, kjeldahl method is one of the technique through which we can quantify the total organic, nitrogen present in the system and then when we are multiplying it with 6.25. We are getting the approximate quantification of this protein molecule which is present in that particular solution.

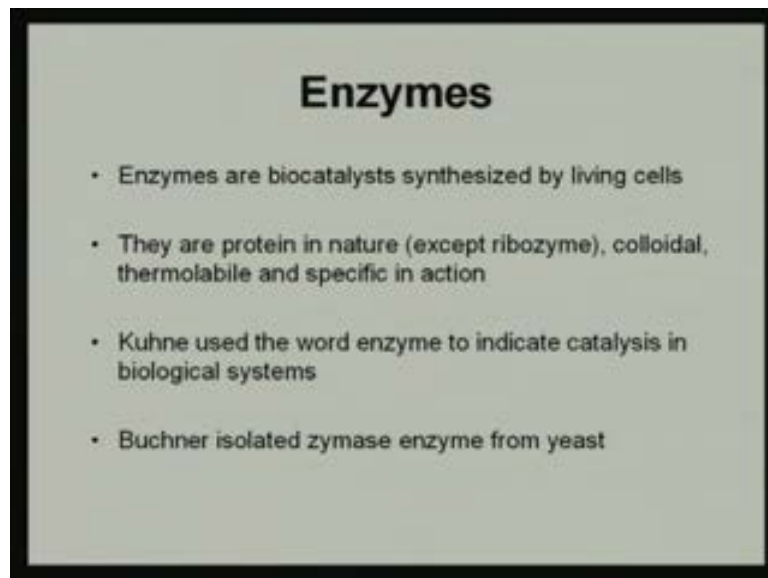
When this is a dye binding method is also another technique where we are using some specific dye. Suppose quasi brilliant blue is one of the dye, which is otherwise known as Bradford method of protein quantification, which has got a direct link with this protein

more the concentration of protein more will be the binding of this dye to that particular protein more will be the color intensity.

And through this quantification standard quantification method, we can determine the protein content of that unknown solution similarly, Biuret method, Lowry's methods are some of the standard quantification method of protein estimation.

And protein concentration determination ultraviolet method is also one of the techniques where without using any dye any reaction medium. If we are taking the protein solution colorless protein solution and if we are measuring the optical density at 280 nano meter. Then we can find the we can quantify that what is the protein concentration present in that particular solution. It is worth mentioning that 260 nano meter is the optical density for nucleic acid and 280 nano meter is the wave length. Where we can quantify the protein solution in any solution, unknown solution colorless protein if it is presents we can quantify that.

(Refer Slide Time: 38:47)



Now, coming to the another important aspect of the protein molecule now, enzyme is one of the important biocatalyst which is present in every living system. It is worth mentioning that all enzymes are protein but, all proteins are not enzymes.

Now, here we can find that enzymes are the biocatalyst, which synthesize by the living cell they are proteinaceous in nature some exception is there except ribozyme. That is the

one enzyme which is non proteinaceous in nature colloidal thermoastable and specific in action kuhne used the word enzyme to indicate the catalysis of the biological system and Buchner isolated the zymase enzyme from the yeast.

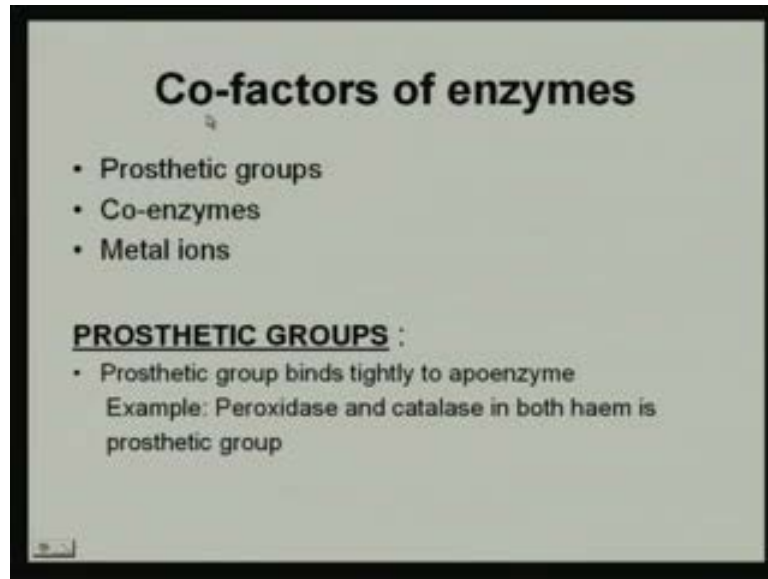
(Refer Slide Time: 39:48)

Chemical nature of enzymes

- All the enzymes are invariably proteins (except ribozymes)
- Functional unit of enzyme is known as holoenzyme
- Holoenzyme is made up of apoenzyme (protein part) and a coenzyme(non-protein organic part)
- Holoenzyme (active enzyme) — Apoenzyme (protein part) + Coenzyme (non protein part)

Now, when we see the chemical nature of this enzyme, we can find that all the enzymes are invariably proteinaceous in nature I have already told that except that ribozyme that functional unit of enzyme is known as the holoenzyme. That means the functional unit is called holoenzyme. Holoenzyme is made up of apoenzyme what is apoenzyme this is the proteinaceous part of the enzyme and the coenzyme is the non proteinaceous part which is it may be the organic other part of this body which is not proteinaceous in nature.

(Refer Slide Time: 40:35)



Co-factors of enzymes

- Prosthetic groups
- Co-enzymes
- Metal ions

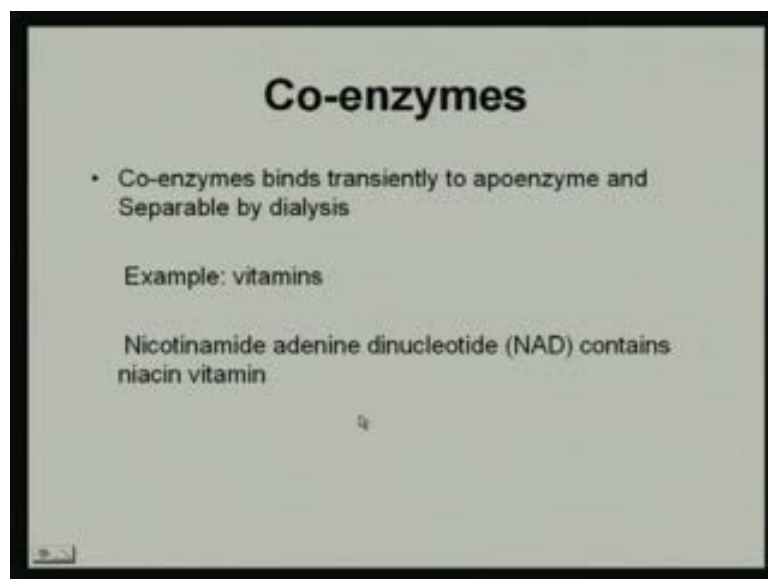
PROSTHETIC GROUPS :

- Prosthetic group binds tightly to apoenzyme
Example: Peroxidase and catalase in both haem is prosthetic group

Besides this some co factor like prosthetic group coenzyme and metal ions are also playing a significant role as far as the functionality or the characteristics of protein is concerned.

Now, when we are talking about the prosthetic group, prosthetic groups are the groups that are tightly bound with the apoenzyme. Apoenzyme is that proteinaceous part of the enzyme.

(Refer Slide Time: 41:06)



Co-enzymes

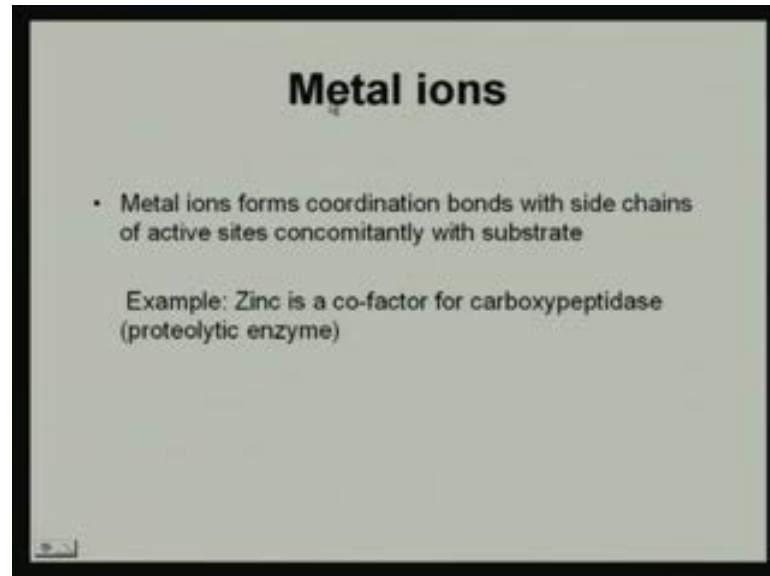
- Co-enzymes binds transiently to apoenzyme and Separable by dialysis

Example: vitamins

Nicotinamide adenine dinucleotide (NAD) contains niacin vitamin

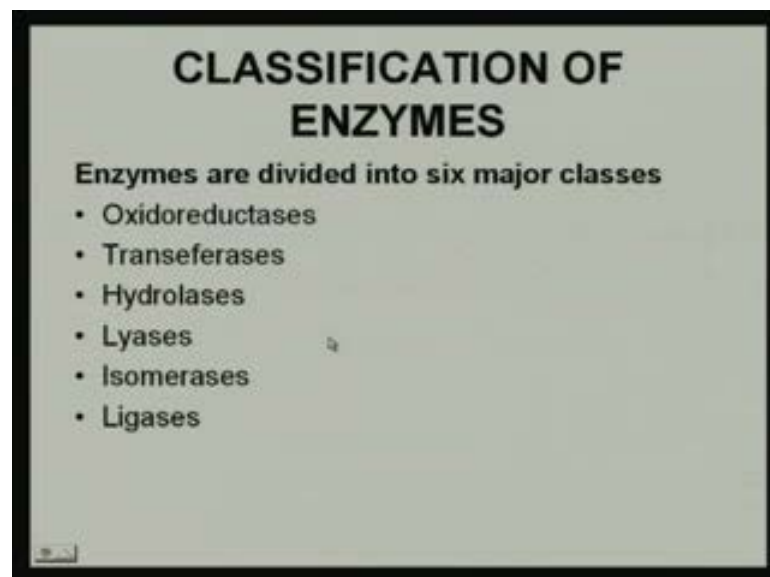
Now, when we are talking about this coenzyme, coenzyme bound transiently to apoenzyme and they are separable by dialysis example is vitamin Nicotinamide adenine dinucleotide that is N A D contains niacin as the vitamin.

(Refer Slide Time: 41:28)



Metal ion forms a coordination bond with the side chain of active side concomitantly with the substrate zinc is a co factor for carboxypeptidase that is the proteolytic enzyme.

(Refer Slide Time: 41:48)



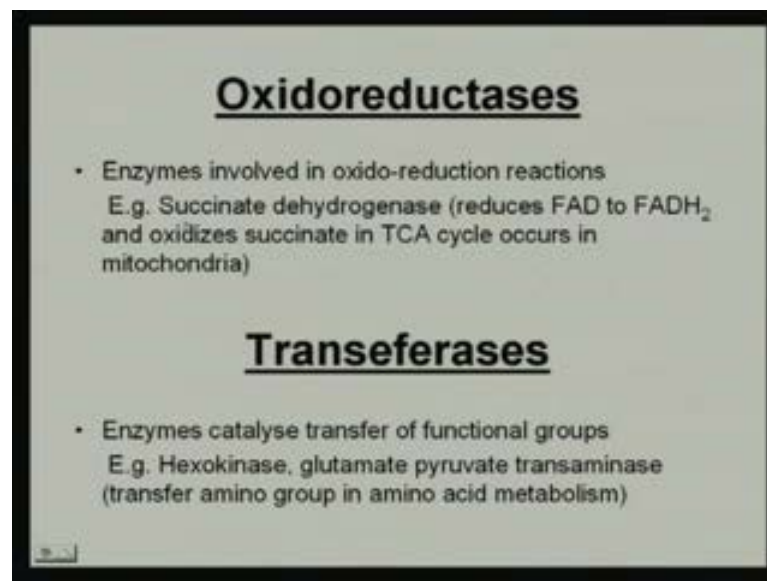
Now, if we classify the enzymes based on it is catalytic behavior the entire enzyme can be divided into 6 major classes. First group is called the oxidoreductases group of

enzyme, second group is the transferases, third group is the hydrolases, fourth group is the lyases, fifth group is the isomerases, sixth group is the ligases.

Now, suppose if anyone is talking to you about a particular number suppose 3.2.1.1 E C 3.2.1.1 what does it mean E C means enzyme commission. Now, three means this is the third group of this enzyme that means that particular (()) is talking about the enzyme which belongs to this category of hydrolases. Then it is further classified this enzyme hydrolases is further classified based on its characteristics and the behavior.

And that is the reason this sequence is so important when someone is talking about one point something that means it is he is talking about the first group of enzyme that is the oxidoreductases group of enzyme.

(Refer Slide Time: 43:23)



Oxidoreductases

- Enzymes involved in oxido-reduction reactions
E.g. Succinate dehydrogenase (reduces FAD to FADH₂ and oxidizes succinate in TCA cycle occurs in mitochondria)

Transferases

- Enzymes catalyse transfer of functional groups
E.g. Hexokinase, glutamate pyruvate transaminase (transfer amino group in amino acid metabolism)

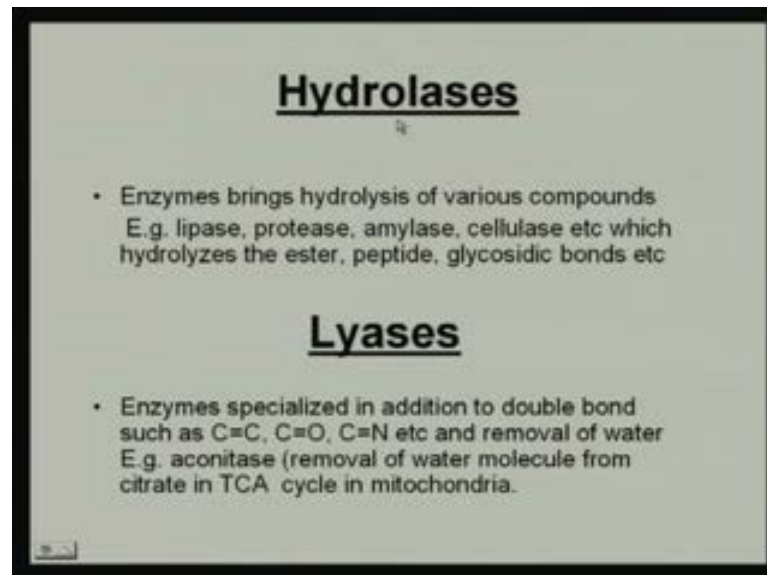
Now, let us come to that when we are talking about the oxidoreductases group of enzyme the name itself is indicating that different type of oxidation reduction reactions are carried out by this particular enzyme. Most of the intra cellular enzymes belong to this oxidoreductases group of enzyme. This enzyme involved in the oxidation reduction reaction example is the succinate dehydrogenase which reduces F A D to F A D H 2 oxidizes succinate in T C A cycle.

I will be coming to this particular step in some subsequent classes when I will be talking to you about citric acid cycle. So, this is one of the examples of this oxidoreductases

group of enzyme. Now, as a whole if any dehydrogenase group of enzyme if any kinase group of enzyme are there they belongs to this oxidoreductases group of enzyme.

Now transferases is another group of enzyme this is the second group of enzyme where this enzyme catalyses the transfer of the functional group hexokinase. Now, hexokinase is one of the examples where glucose is getting converted to glucose 6 phosphates with the help of the enzyme hexokinase. What it is doing it is transferring that glucose that phosphate group from to the 6 position of this hexo. So, that and it is called the transferases group of enzyme.

(Refer Slide Time: 45:12)



Now, when we are coming to this hydrolyses this hydrolyses brings the hydrolytic reaction of the various compound say for example, the lipases proteases, amylases, cellulases etcetera are the example of hydrolytic enzyme.

Now, they breaks mainly this lipase they are very specific to this ester bond proteases mainly break the peptide bond amylases and cellulases. Mainly breaks the glycosidic linkages of the protein molecule. Mainly we are talking about the lyases enzymes which are specialized in addition to the in addition to double bond such as C double bond C, C double bond O, C double bond N etcetera. And it is also removes the water from this reaction say this aconitase group of enzyme I will be talking to you this group of enzyme when in the subsequent classes.

(Refer Slide Time: 46:19)

Isomerases

- Enzymes involved in all isomerisation reactions
E.g. Racemase, phosphohexose isomerase

Ligases

- Formation of bonds with ATP cleavage. Enzymes catalyzing synthetic reactions where two molecules are joined together and ATP is used
E.g. DNA ligase

Coming to this isomerases any type of isomerisation reactions are carried out by this group of enzyme say for example racemase, racemase is one of the example phosphohexose isomerase is another example of which belongs to this particular two group of enzyme. And ligases the formation of bond with A T P cleavage the enzymes catalyzing the synthetic reaction where two molecules are joined together and one A T P is used that is the D N A ligase.

(Refer Slide Time: 47:02)

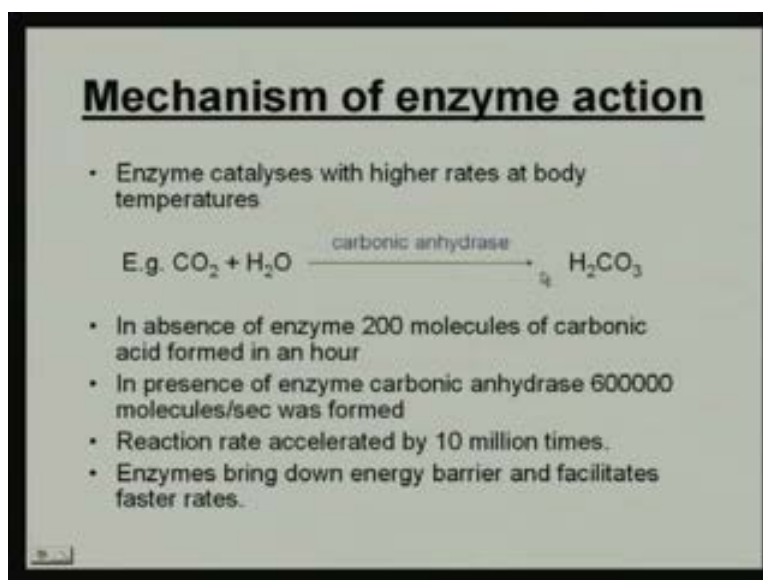
Enzyme vs catalyst

<u>Catalyst</u>	<u>Enzyme</u>
<ul style="list-style-type: none">• Works efficiently at high temperatures and high pressures• Slow catalysis compared enzyme mediate catalysis	<ul style="list-style-type: none">• Works efficiently at 37 °C• Catalysis with higher velocity compared to catalyst mediated catalysis

So, these are some of the example of the different classes and function of enzymes. Now, when we are comparing what is the importance of this biocatalyst now, when we are comparing this biocatalyst with any conventional catalyst then we can find that it works efficiently at higher temperature and higher pressure.

It that chemical any chemical catalyst can work under any high temperature and high pressure. But, in case of biocatalyst it cannot because it cannot cross the physiological condition of the living cell and living cell cannot work at very high temperature or else it will collapse. So, slow catalytic reactions are observed but, here the biocatalysts are highly specific towards it is targeted substrate molecule and that is the reason the velocity of reaction is much much higher.

(Refer Slide Time: 48:05)



Mechanism of enzyme action

- Enzyme catalyses with higher rates at body temperatures

E.g. $\text{CO}_2 + \text{H}_2\text{O} \xrightarrow{\text{carbonic anhydrase}} \text{H}_2\text{CO}_3$

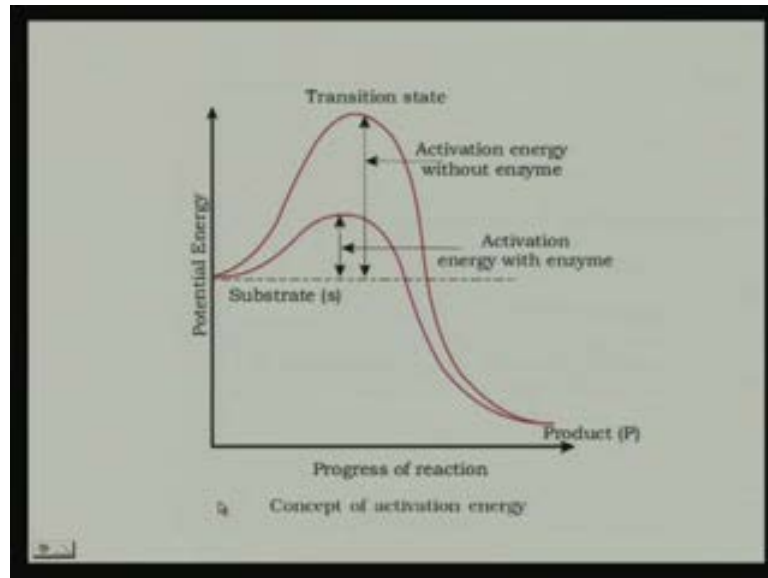
- In absence of enzyme 200 molecules of carbonic acid formed in an hour
- In presence of enzyme carbonic anhydrase 600000 molecules/sec was formed
- Reaction rate accelerated by 10 million times.
- Enzymes bring down energy barrier and facilitates faster rates.

compared to any chemical catalyst. Now, say for example, when enzyme catalyses any reaction at body normal body temperature say carbon dioxide and water in presence of carbonic anhydrase. It is producing the H_2CO_3 this in absence of the enzyme 200 molecules of carbonic acid was formed in an hour in presence of enzyme carbonic anhydrase. This is the enzyme when it catalysed this reaction 6 lakhs a molecule per second was formed within this.

You just compare and see how efficient this biocatalyst are and that is the reason why this biocatalysts are occupying such a significant position as far as the living system is

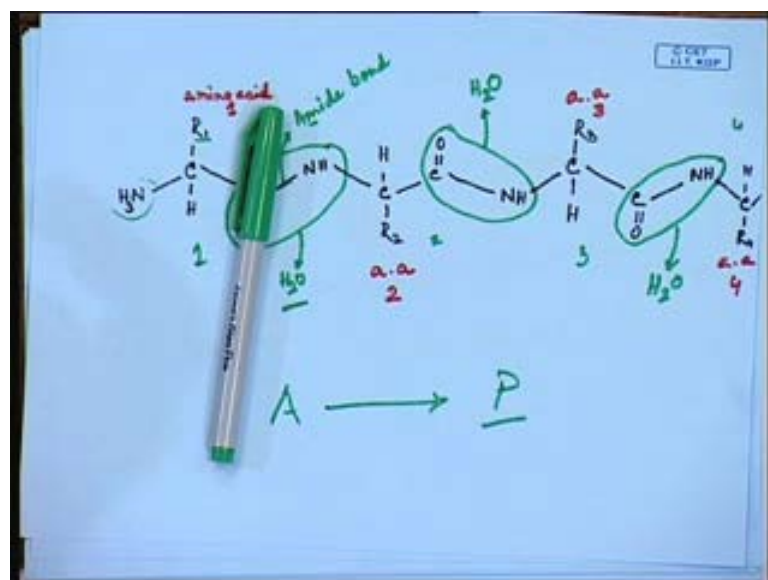
concerned reaction rate accelerates by ten million times enzyme brings down the energy barrier and facilitate the faster rates.

(Refer Slide Time: 49:23)



Now, what is that now a chemical reaction if we consider in any chemical reaction that is giving the product p so, any chemical reaction.

(Refer Slide Time: 49:23)



Suppose a is giving the product p which takes place because of certain fraction of the population of a molecules which gives the instant process enough energy to attain the activation condition called the transition state. And if we see this is the transition state of

substrate to product formation in absence of enzyme. Now, if we compare the transition state energy barrier without enzyme and with enzyme then energy barrier with enzyme is very high compared to the presence of catalyst for any product formation. This is the top of the energy barrier and separating the reactant that is to the product which is p.

Now, when we are talking about these then we are obviously coming to these that how a can produce this product this p. There are two ways in which this rate of chemical reactions can be accelerated. One is that by increasing the heat of this reaction by increasing the temperature to attain this transition state. Another is the addition of catalyst or biological catalyst which works mainly on the physiological conditions.

And it is mainly working under and it is converting this substrate or the reactant to the product molecule. Now, as this particular temperature which is there the transition state the energy barrier is so high and in compared to this particular enzyme mediated reaction the activation energy is so less obviously.

(Refer Slide Time: 52:02)

Mechanism of enzyme action

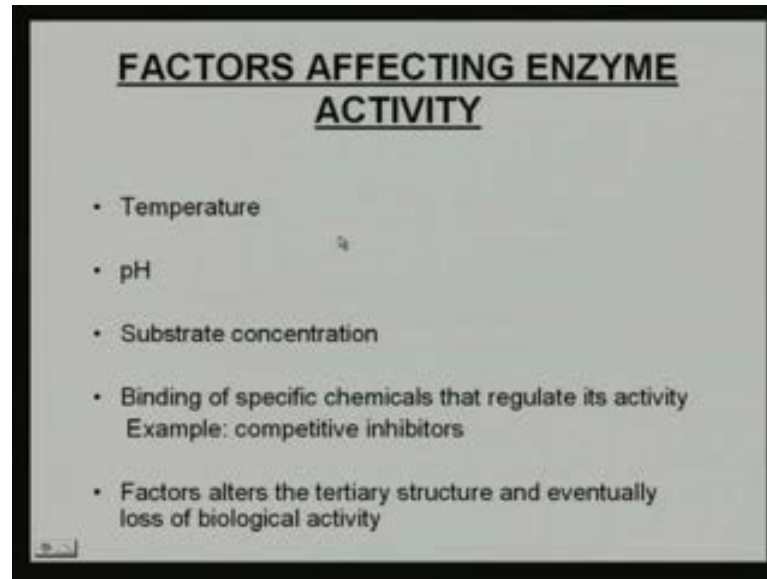
- Enzyme catalyses with higher rates at body temperatures

E.g. $\text{CO}_2 + \text{H}_2\text{O} \xrightarrow{\text{carbonic anhydrase}} \text{H}_2\text{CO}_3$

- In absence of enzyme 200 molecules of carbonic acid formed in an hour
- In presence of enzyme carbonic anhydrase 600000 molecules/sec was formed
- Reaction rate accelerated by 10 million times.
- Enzymes bring down energy barrier and facilitates faster rates.

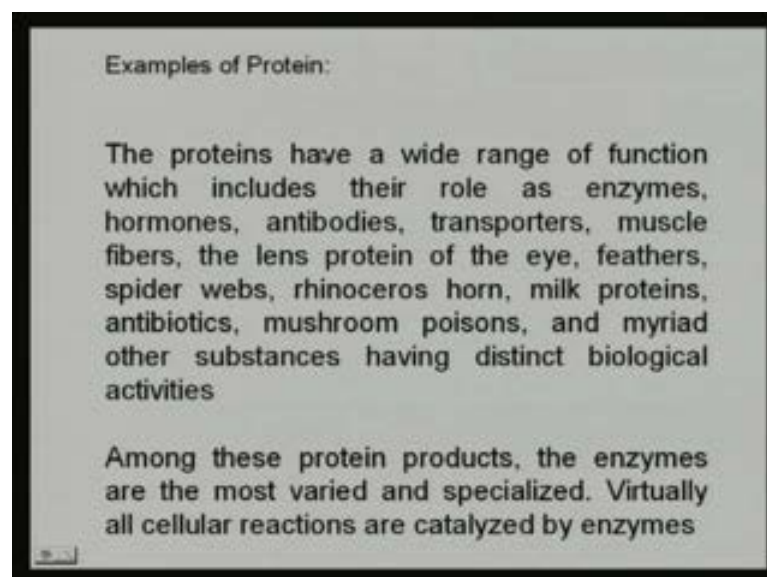
The Energy that is the energy barrier which is there for fascinating the energy this reaction is much favorable and the rate of reaction can be accelerated with the addition of the enzyme which lower down the activation energy of any process. And this is the reason why these enzymes are so important and this enzyme how the specificity is so important.

(Refer Slide Time: 52:31)



There are several factors which play a very significant role as far as the enzyme reactions are there are concerned that is the temperature, p H, substrate concentration, binding of specific chemical that regulate the activity of the enzymes. And the factor alternating alters the tertiary structure and eventually loss the biological activity of the particular reaction. So, these are some of the factors which plays a very significant role as I have told you that proteins are the biological molecules and sometimes depending upon the nature. It may be heat level; it may be cold levels so depending upon the nature of the protein catalytic reactions are designed.

(Refer Slide Time: 53:42)



Now, if we give some example of this protein, these proteins have a wide range of function, which includes their role as enzymes hormone antibodies transporters muscle fibers the lens protein of the eyes feather spider, web rhinoceros, horn, milk, proteins, antibiotics, mushroom, poisons and myriads other substances having distinct biological activities among these protein products the enzymes are the most varied.

And thus specialized by chemicals virtually all the cellular reactions are catalyzed by the enzyme molecule. And with these I think I have tried to give you the overview of this protein molecule how protein are proteins are formed what are the basic units of this building block of this protein what are the different characteristics of the amino acids.

So, I have and this primary, secondary, tertiary and quaternary structure of the protein molecule then I have also tried to give you some idea about this enzymes and how enzyme function. What are the different parameters which govern the enzyme activity I think you have got some idea about this protein and its structure. So, in my next class I will be talking to you about other macro molecules, which also play a very significant role in the cellular activities. Thank you very much.