ENZYME SCIENCE AND ENGINEERING

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LECTURE – 2

CHARACTERISTIC FEATURES OF ENZYMES

In our previous lecture we had discussed some issues regarding the enzyme science particularly the sciences that are associated with the study of enzymes, the nomenclature of enzymes and key issues that relate to the importance of enzymes in the over all perspective of biotechnology. Today we will be discussing some of the characteristic features of enzymes particularly as proteins and their chemical nature.

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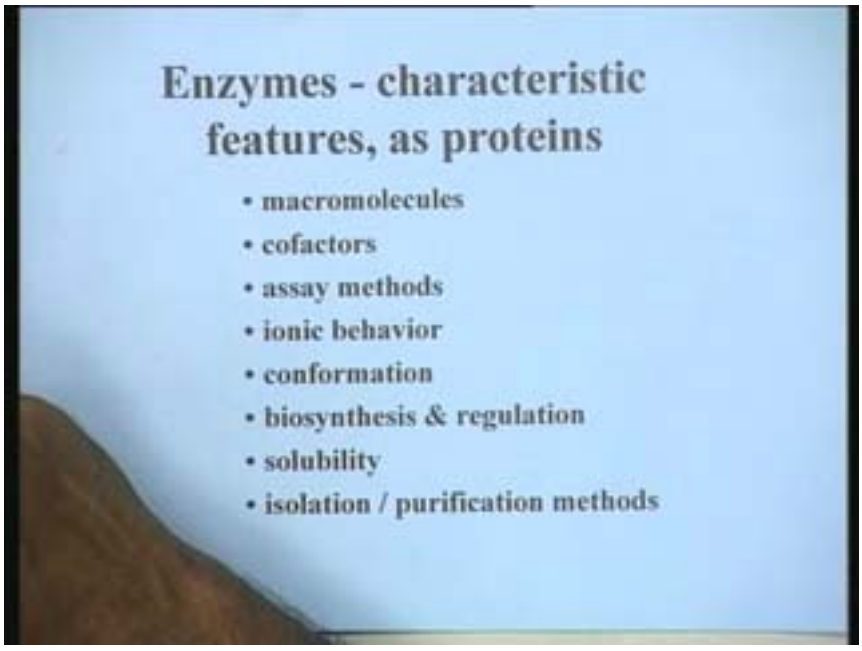

All of you may be aware that all the enzymes that are known so far are proteins in chemical nature. When I say proteins we immediately understand a chain of amino acids. Twenty different amino acids that are linked through peptide bonds and the size of the chain may extend some where from hundred amino acids to three hundred amino acids in different enzymes.

They are macromolecules of different amino acids in a sense they are hetero macromolecules, hetero polymers that means the monomer units are not identical in nature. They vary among the twenty different amino acids and their sequence and the number of amino acids or the relative concentration of each of the amino acid may determine the particular type of enzymes, its characteristic properties as well as its functional aspect. As a macro molecule we will notice that the properties of a macro molecule will largely depend upon the constituent monomers.

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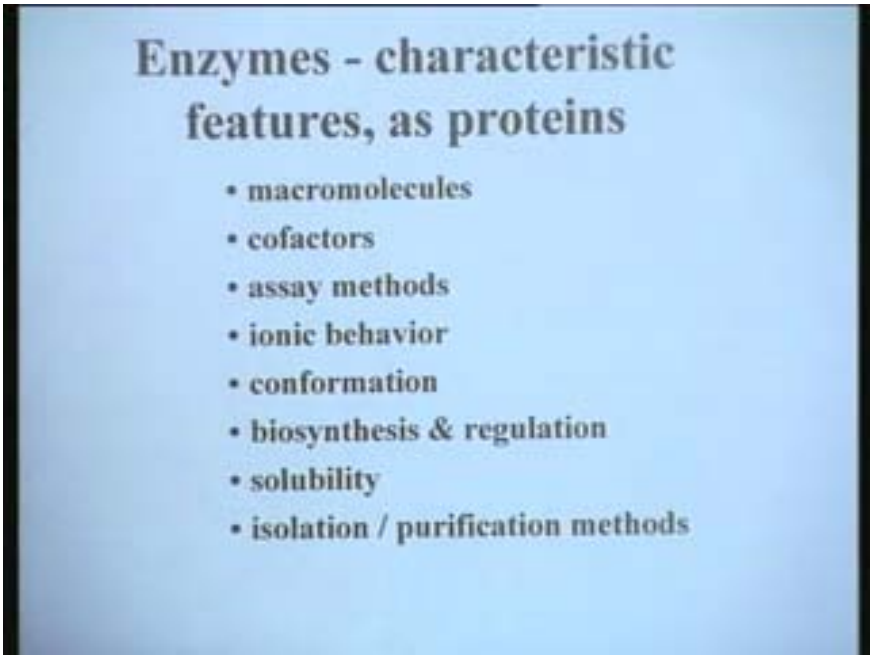

In the case of amino acids, they vary in terms of their various properties particularly ionic nature, their hydrophobic nature and their size. There is large variation and therefore the relative proportion of these amino acids also influence the properties of the consequent macromolecule what we know as proteins.

Besides a macromolecule of various combinations of amino acids, the enzymes may also be considered as a catalyst.

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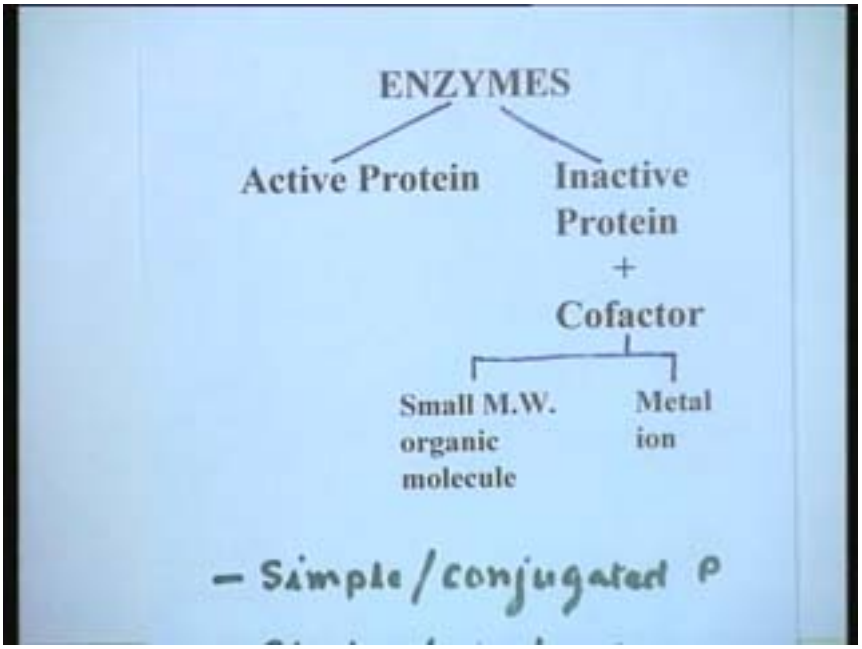

When I say catalyst that means not all proteins will qualify to be called enzymes. Only those proteins which have catalytic function, that means they can accelerate the rate of a particular reaction, can be termed as enzymes. Proteins as you all may be aware have diverse functions in a living cell like structure, transport molecules, example- haemoglobin or myoglobin, defense molecules like antibodies and the fact remains that their more significant function in a living cell is catalytic.

Enzymes therefore can be termed as active proteins which have catalytic function in a living cell. Alternatively some of the protein molecules which are known to be enzymes but may not be intrinsically catalytically active may require the role of an associated molecule what we term as cofactor for them to be catalytically active. That means the protein molecule itself is not catalytically functional but in the presence of the cofactor the molecule may become a catalyst and may be considered as an enzyme.

These cofactors again could range from a small molecular weight organic molecule. This may be required to be externally added like for example NAD, ATP, FAD or a variety of cofactor that you are familiar with in many of the bio chemical reactions or they could be or they could be as simple as metal ions and the fact that because in the absence of these cofactors the protein is not catalytically active, it implies that these cofactors have a very significant role in the catalytic mechanism which these molecules display. Also these proteins may be very simple proteins for example a simple amino acid polymeric chain. They could also be conjugated proteins. By conjugated proteins we mean in addition to the amino acids the proteins chain will also be associated with some other non-amino acid molecules. Very commonly encountered proteins are glycoproteins, many of the enzymes, particularly those which are isolated from eukaryotes, a glycoproteins which means that in addition to the amino acids the protein molecule is associated with an oligosaccharide, monosaccharide or an oligosaccharide, they could lipo proteins a lipid molecule, they could be nucleoproteins and a large number of proteins which are

metalloproteins containing metal enzymes where the metal ions acts as a cofactor are known.

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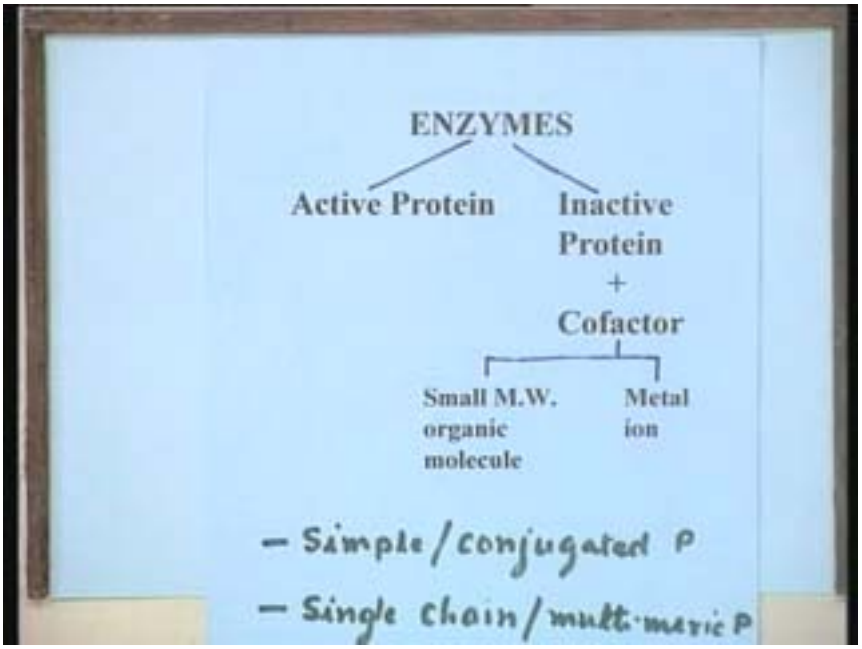

Another characteristic feature of these proteins as a constituent or as a chemical body of the enzyme is that in many cases there may be single chain of amino acids, a single chain protein and many of the industrial enzymes particularly hydrolytic enzymes are single chains which means that the backbone of the protein consists of only one polymeric chain and in many cases they could be even multimeric proteins that means more than one chain constitute the whole enzyme molecule. A single chain is not functionally active.

The arrangement or the organization of the multiple chains could be two chains; there could be three chains or four chains. The organization of those chains provides certain natures which are responsible for the catalytic function and these multimeric chains or the multimeric proteins as some of you are familiar have a distinct role in regulatory aspect of the cellular metabolism.

As I was talking about the synthesis of the macromolecule the basic backbone of the protein chain in the case of a protein lies in the condensation of two different amino acids. Here R_1 and R_2 indicate the distinction between two amino acids and the carboxylic group of one and amino group of other amino acid condense and form a -CONH bond which is known as peptide bond. These peptide bonds have a resonance structure. It has a very peculiar property which is really carried forward in the function and structure of the enzyme molecules.

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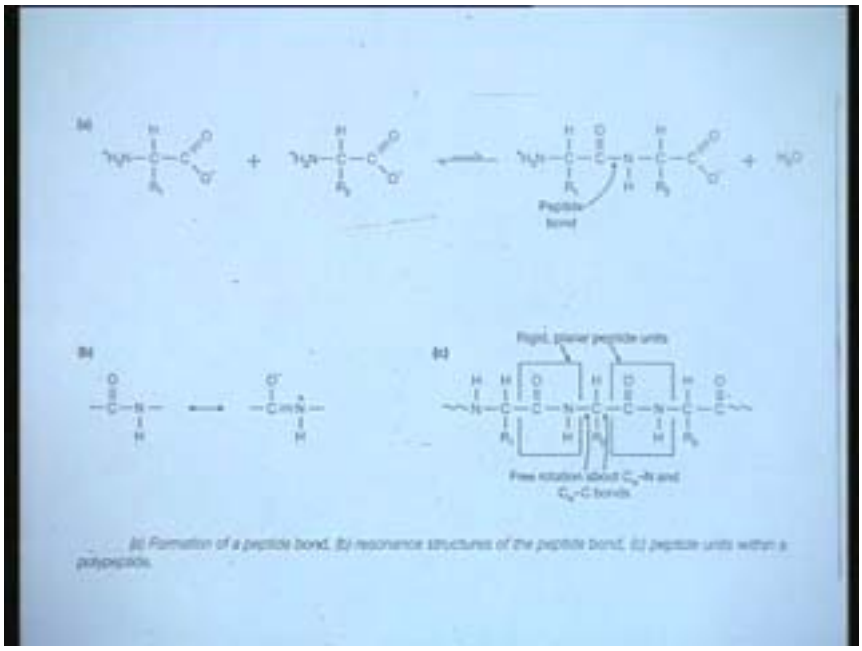

(a) Formation of a peptide bond. (b) resonance structures of the peptide bond. (c) peptide units within a polypeptide.

This is the resonance structure and also the peptide units are rigid and planar in nature and the most important property which contributes to its secondary structure is the capability of free rotation about the alpha carbon and nitrogen and alpha carbon and carbon. At these two sites they can freely rotate. The bond can freely rotate and thereby can give rise to hydrogen bonding between the different amino acid residues in the chain and constitute the subsequent structural organization of the macromolecule.

The two major attributes of an enzyme molecule is the chemical nature of the protein and their catalytic function. When we look at quantification of an enzyme molecule or enzyme sample or what we know as assay methods they also take into account these two major characteristics.

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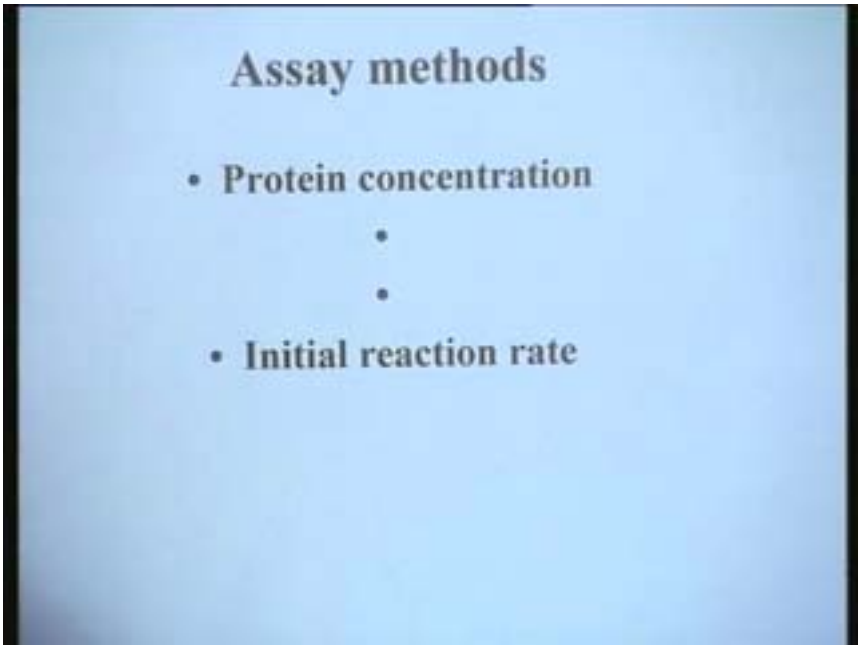

The quantification methods could be classified into two categories mainly or the two constituents could form the basis of assay methods. One is based on the protein concentration that means an enzyme sample can be quantified on the basis of a protein sample. That means what is the concentration of protein in a given sample and in terms of say milligram per liter. One could determine by any method that is known to us and I will talk about it a little later. The other way could be and which is probably more important for assay of an enzyme is its catalytic function. That means you assay it or you determine its capability to carry out a biochemical reaction and represent its concentration in the form of initial reaction rate.

Any catalyst or any chemical reaction if you look at the reaction profile it can be indicated as between time and product formed and ultimately the rate of the reaction at duration dp/dt at the zero time will give you the initial reaction rate.

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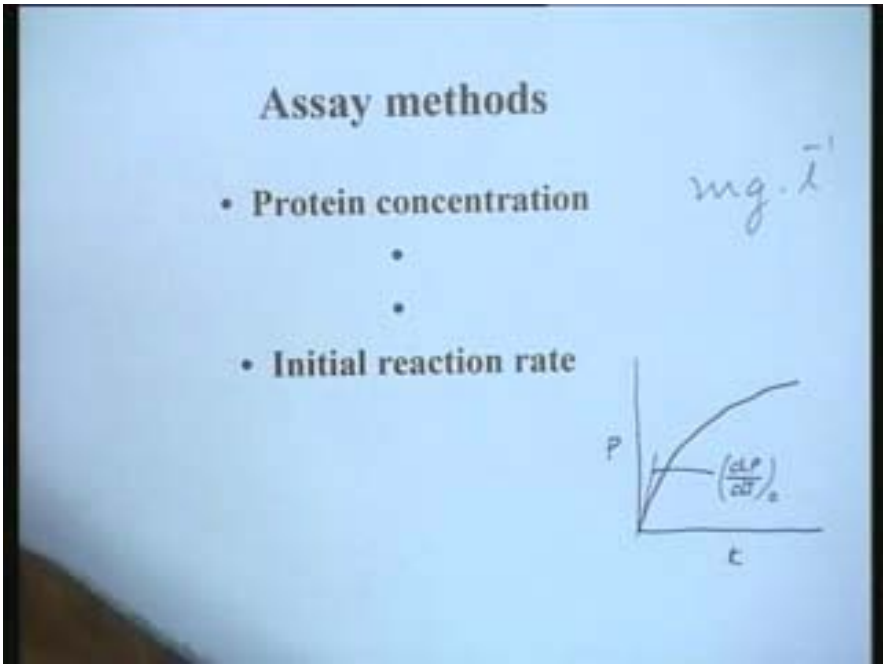

This initial reaction rate is considered one of the parameters for determining the concentration of any enzyme sample. As a convention the quantity of enzyme which converts one micro mole of substrate into product per minute is termed as the rate. Quantity of enzyme which can exhibit this kind of a rate, initial rate is termed as one international unit.

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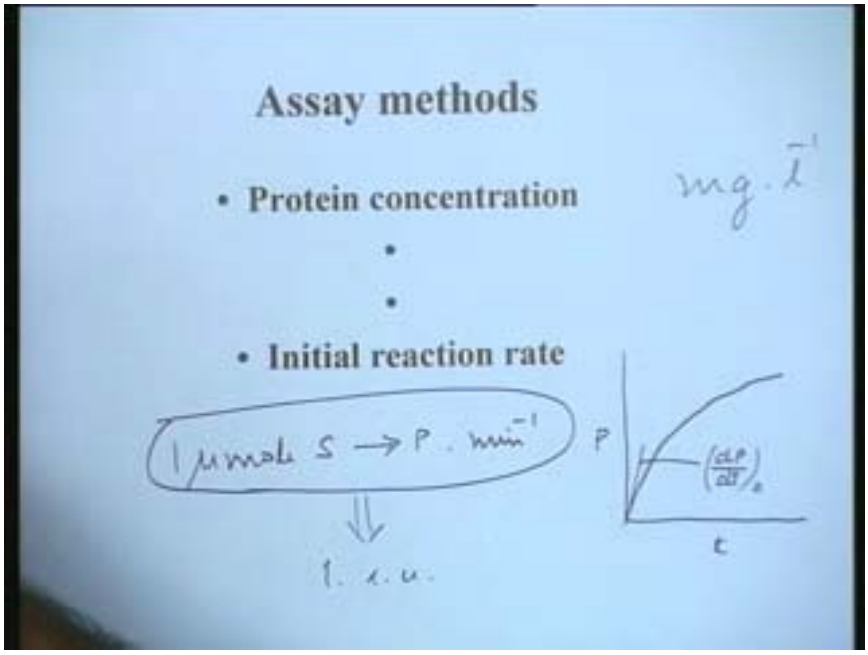

That is the conventional, internationally accepted unit of an enzyme activity. One international unit of enzyme is the quantity of that enzyme or the concentration of that enzyme which can convert one micro mole of substrate into product in unit time per minute. One macro mole of product formed per minute of the reaction can be considered as one international unit.

One of the limitations is that this is not a SI unit which is an internationally accepted system of units. In the case of enzyme activity the SI unit is katal.

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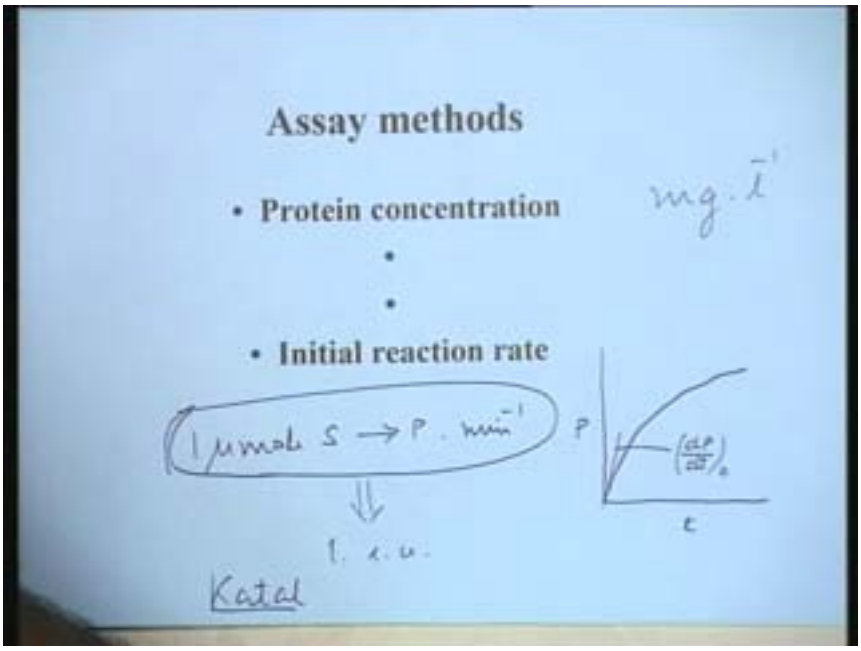

If we look into basic units of the international SI system, a katal in terms of rate terms will be the quantity of enzyme that produce one mole of product per second. One mole of product per second is a very large unit. If you make a comparison between the international unit and katal, katal is a very large unit and one katal could correspond to 10^6 x 60 international units because convert moles into macro moles and minutes into seconds. A unit of katal is much larger and one can interconvert. The initial reaction rate is the basic philosophy in determining the enzyme concentration.

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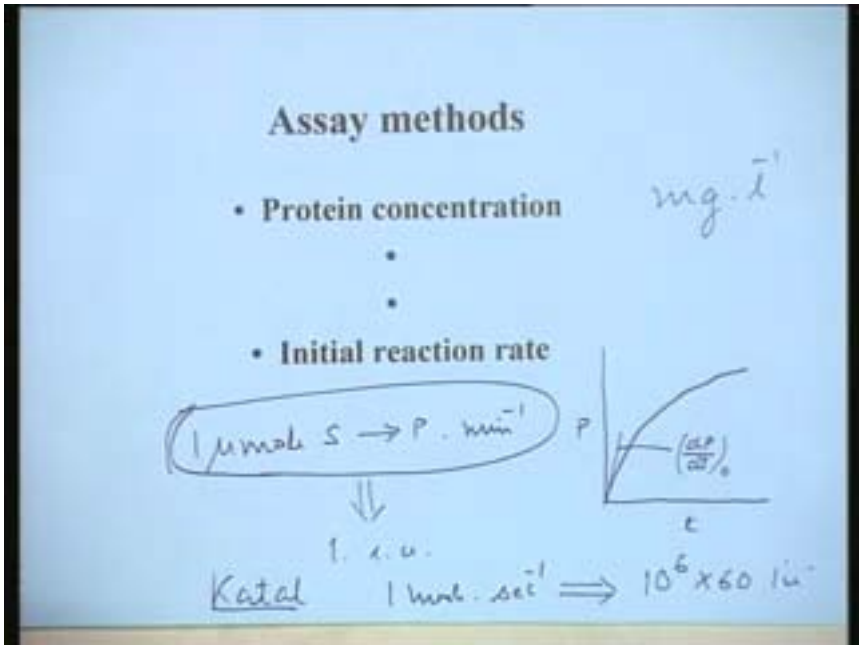

On the other hand the protein concentration also gives us an idea of the amount of an enzyme present in a given sample. Although it may not always be true if the protein is not in its functional form, it will loose its status of the enzyme. It may remain as the protein. But the importance of the protein concentration lies in the fact that it gives you an idea about the purity of the sample. In case of mixture of proteins you can identify what proportion of the total protein is the enzyme molecule or what proportion of the total protein is the catalytically functional enzyme molecule and therefore protein concentration determination is important.

Whenever we analyze any given enzyme sample, we usually resort to both the basis: its initial reaction rate as well as protein concentration. A large number of methods are used to determine protein concentration, as you may be familiar with, and all of them are linked or based on the native structure of protein, the constituent amino acids of a protein. The most simple system probably you are familiar is by ultra violent absorption. We determine the absorbance at 280 which gives you very precise information about the concentration of protein in the sample and this absorbance is based on the content of amino acid residues tyrosine and tryptophan in the protein which are more or less universally present in most of the enzyme samples.

One of the major limitations of this method is that it will have the interference or some kind of error involved if the impurities contain molecules that also absorb near this wave length. Major contaminant in biological samples are nucleic acids which also have absorbance at about 260 nm and therefore sometime how to make an account of protein concentration taking in view the absorbance as a result of nucleic acid and very often the λ_{280} - λ_{260} with some appropriate factors are used to determine the absolute protein concentration in a given sample.

Another method which is very popular is Loury's method which is based on the production of a blue purple color which absorbs maximum at 660nm with a sodium tungstate molybdenum phosphate reagent what we call as fallacy of call to the reagent which is usually a standard which is available and reagent is very accurate in the sense that you can monitor the protein concentration as low as ten milligram per litre.

A very accurate method which also has many other merits is in terms of lack of interference. Another method is biuret method which is based on the reduction with alkaline Copper sulphate solution which contains sodium potassium tartrate and the cupric ions form a co-ordination complex with the amino groups or the -NH group present in the peptide bond. The basic advantage here is that the method is based on the peptide bond which is very unique as far as the proteins are concerned. The other polymers or other molecules present as contaminant will not contribute to error in the case of a biuret method.

Biuret method is based on the development of a blue color with an alkaline copper sulphate solution containing sodium potassium tartrate. This cupric ions after reduction forms a co-ordination complex with the –NH group present in the peptide bond. The specificity of this method is based on co-ordination complex formation with the peptide bond which is a unique property or a basic property in all proteins. Unlike in the case of other methods which are based on tyrosine or tryptophan as residues, the relative concentration of these amino acids in the protein will give you a different reading. But biuret reagent will give you a very uniform method in case of all proteins.

You have other methods which are based on dye binding or silver binding method where the protein can bind certain molecules and the binding is assayed quantitatively and expressed in terms of protein content.

But all these methods have a unique linkage or they have been standardized right from beginning in a more basic fundamental method what we know as Kjeldhal method. The basis of Kjeldhal method is primarily estimation of nitrogen content. Just like peptide bond, the nitrogen content is also intrinsic component of any protein. You digest the protein sample with concentrated sulphuric acid in the presence of a catalyst. Ammonia is produced and the ammonia then is absorbed into dilute acid solution which is titrated and the quantity of amino generated can be calculated. There are minor modification in terms of automation and macronization of these methods which are available today commercially but the basis is to determine the nitrogen content in any given sample which gives you an estimate of crude protein in the sample.

It is assumed that any given protein contains approximately 16% of nitrogen. So if you multiply nitrogen content by 6.8 you get the net protein. But it will be called crude protein in the sense that it will also measure all those molecules which contains nitrogen in addition to proteins. But still it is one of the earliest methods that we had known and had been used. So a very important aspect is assay which you should be very clear.

Another point which I like to highlight here before I proceed further is about the SI. When we measure the effect of enzyme concentration with respect to initial reaction rate or reaction velocity the profile is linear at the small concentration range and then it tapers of in the large enzyme concentration range.

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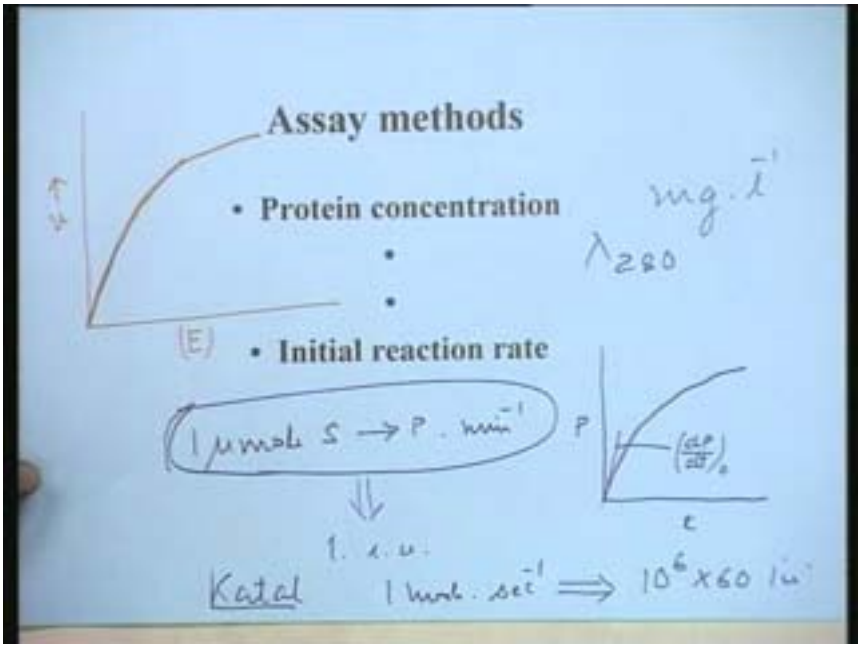

We must ensure that we are measuring our enzyme concentration in the linear range that means at low enzyme concentration level. The best way to ensure such clarity is that instead of taking one sample we take at least two or three dilutions of the same sample which are let us say two fold, four fold, six fold, known dilutions and the final reaction rate which has been obtained must be proportional to the dilution. Unless you get two different samples in the same linear range you cannot depend on the result because if you take a higher concentration of the enzyme you may be able to measure this reaction rate which is much lower than you would have got in a diluted sample.

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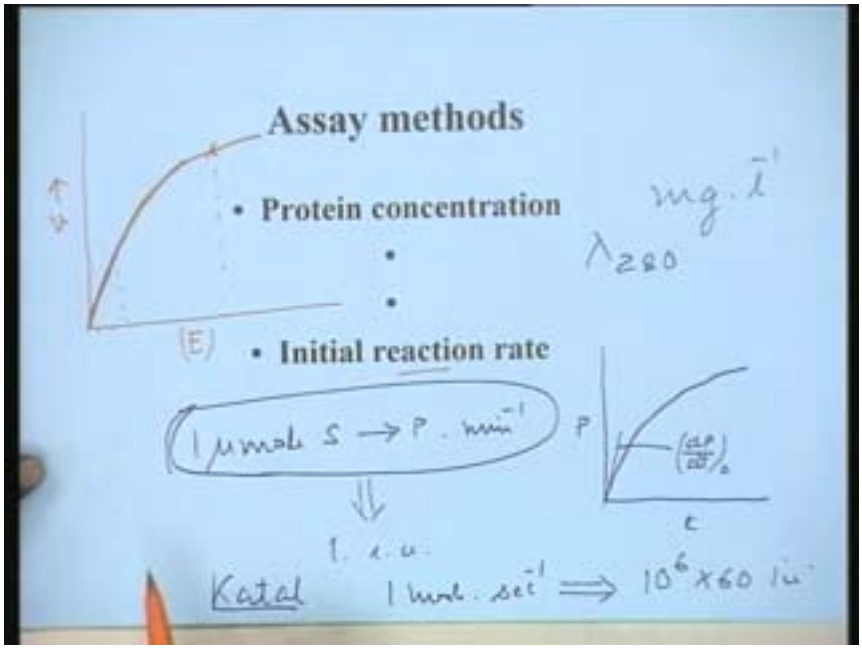

One of the conventional ways is that we try to ensure that the reaction rate measured in the low dilution region or low concentration region can be easily measured by taking more than two samples of known dilution.

The next important property of proteins is their ionic behavior.

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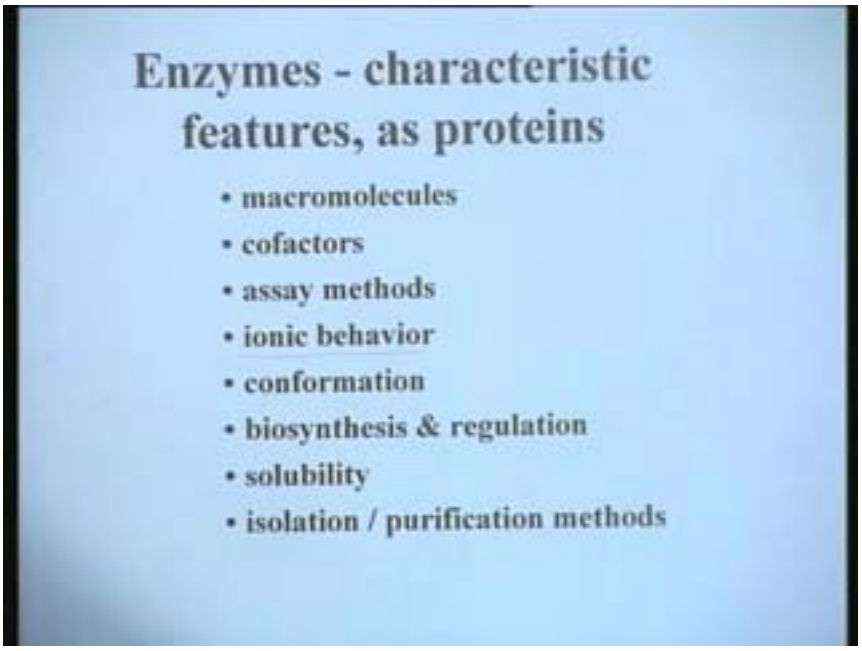

This ionic behavior results from the monomer units that are present in the amino acid which are present in the macro molecule and when we consider a long protein chain bulk of the amino groups and the carboxylic groups in the amino chain will be involved in the formation of peptide bond. It will be only the residual amino acids and the two ends one will have a carboxylic group and other will have an amino group free and both these groups will be free to ionize at any given pH. Having both positive and negative group at the end will ensure that at any given pH the molecule will have some charge.

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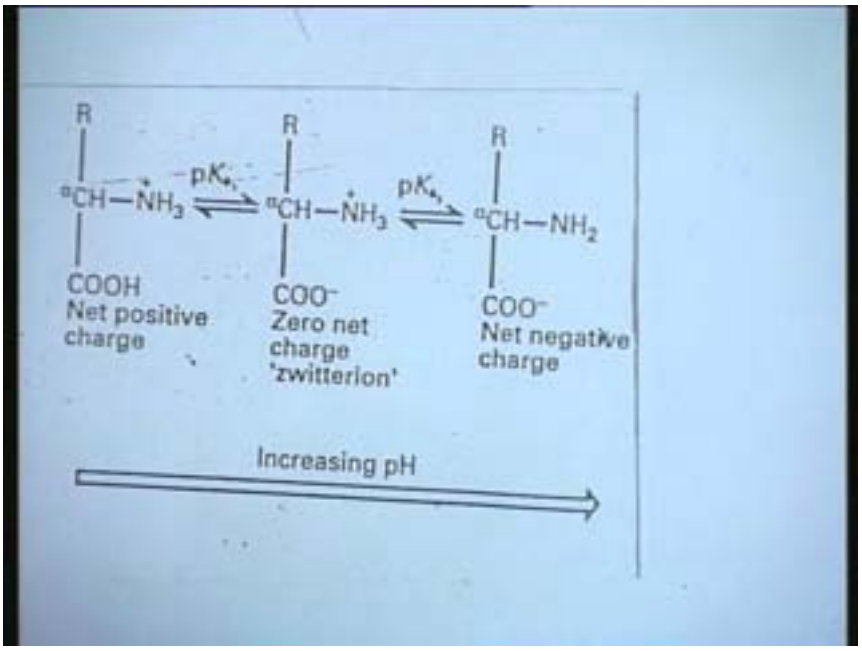

If you look at a very general system where I am assuming that all the R's that are mentioned here are non-ionisable. So we have only two groups with net positive charge. At a low pH the molecule will act like a cation and as we increase the pH it will be negatively charged and it will behave like anion and some where in between there will be a zero net charge that means it will have both positive and negative charge of equal magnitude, what we call as zwitterion in the case of amino acids.

That will entirely depend on the pk_a value of the two charges. It will not be at pH 7. It will depend upon the pk_a value of the two. That means the negative logarithm of the dissociation constant of the two charges. The situation is a simplified situation where we are assuming that only two terminal amino acids are ionized. It will always happen that it will have residues like for an example these R's which are also ionisable and the system might become a little more complex. For example consider a protein chain in which you have amino acid like aspartic acid.

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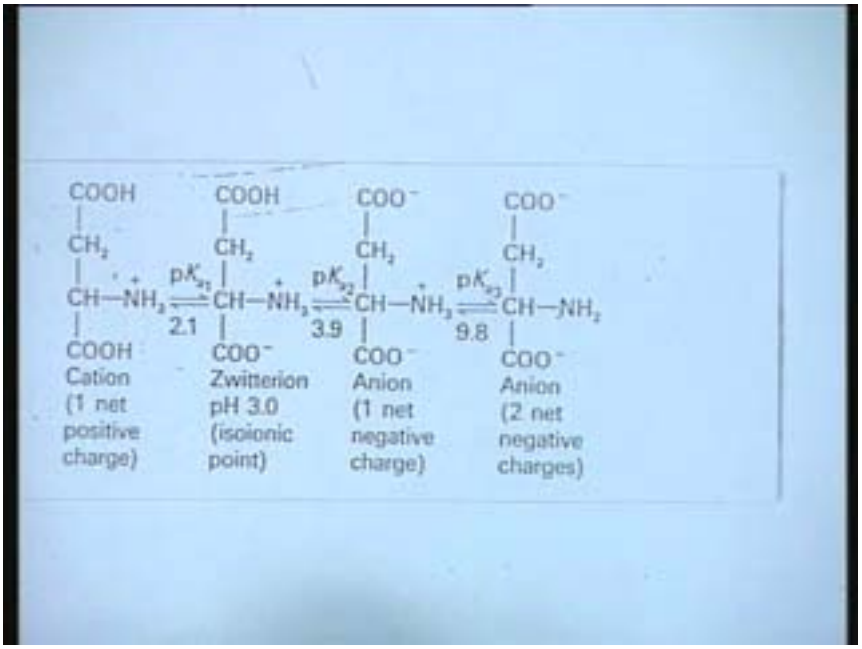

It has two carboxylic groups instead of only an amino group. It has an additional carboxylic group. At low pH, again similarly here it will have only one net positive charge, amino group will be ionized. As we move further, increase the pH it will have one carboxylic and one amino group ionized. When the pH is increased to a level where both the negative ions are ionized and the amino group is not ionized and ultimately you will also meet here a situation where you have zwitterions where one amino group and one carboxylic group are ionized, the other carboxylic group is non-ionized and that is a status. This carboxylic group may be different than your amino group or the carboxylic group known.

A similar situation will be in the case of lysine for example when you have more than one basic amino group. Some of the ionisable groups found in proteins are not only aspartic acid and lysine or glutamic acid that contribute to the ionization state of a protein. There are other amino acids particularly like histidine or cystine or tyrosine. They also play a role in ionization and they also have ionization behavior and their approximate pKa values are indicated here.

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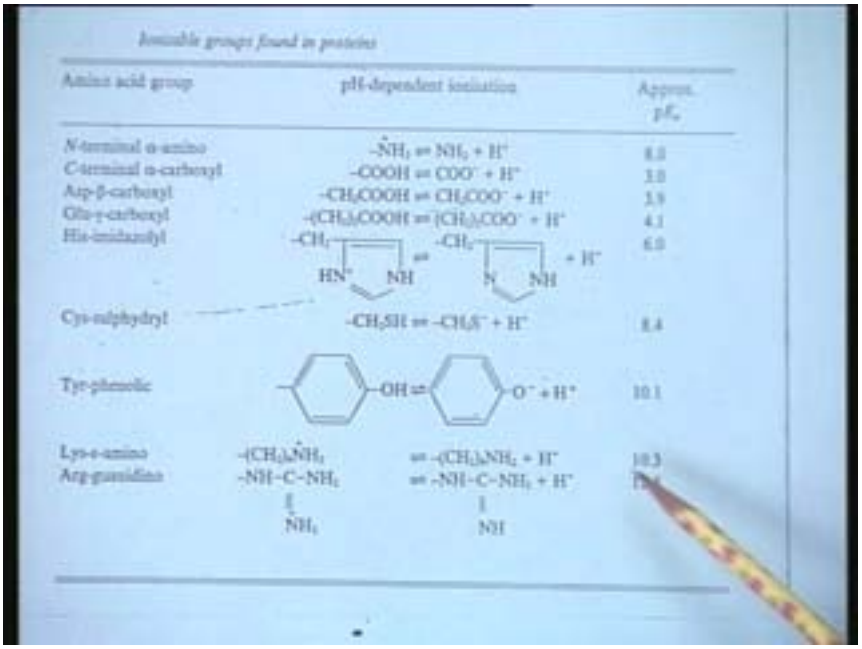

The net charge in the protein will depend on ionization state at a given pH of all the contributing proteins in the system. That is a very important property which we must keep in mind when we look at enzymes for a variety of functions. Whether it is a catalytic function, whether it is a purification of enzyme protein the ionic behavior plays a very key role.

That will depend entirely on pH. For example you look at let us see the approximate pKa values. For the C-terminal alpha carboxylic group the pKa is three and for the aspartic acid beta carboxylic group, the pKa is 3.9. It is because it is linked to another carbonate which is not in the basic chain. The pKa is different and ionization will totally depend on the pKa value of the functional group. Usually yes but it cannot be cited as a rule.

The nature of amino acid has a very important role to play in the conformation.

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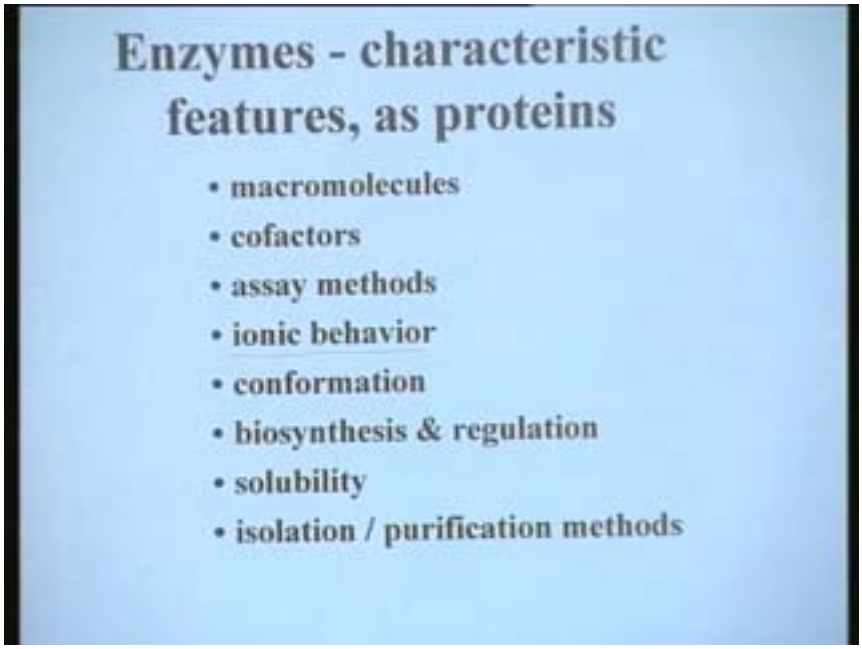

One of the important aspects is that unlike synthetic macromolecules which we are familiar in our day to day life say polythene or styrene they are all linear chain molecules, long linear chain molecules, hydrocarbon residue of monomers. In the case of proteins the beauty lies in that it is a very highly folded molecule. That means its volume is very much conserved. The molecule is very compact. It is not extended like a rod. Not that there are no proteins which are not in the extended form. Many of the structural proteins will be in the extended form but most of the proteins which constitute enzymes are globular proteins and they form almost a spherical shape under ideal condition. But usually they will be in the ellipsoid kind of the situation where you can determine the conformation by their minor and major axis. In fact there are relationships available where many of the hydrodynamic and transport properties of these polymers solutions can be related to the major and minor axis of the ellipsoid particle and these are broadly the methods which are used in general to determine the dimensions of the protein molecule or the nature of conformation, if suppose we know about the native conformation, we know the dimensions and after certain treatment if the dimensions are altered we tend to understand that they have under gone a conformational change. These conformations are the result of the nature of the amino acids, the side chains of the amino acids, the principle functional group.

The principle interactions that are present in the case of any given protein or that applies to an enzyme are peptide bonds. They form the backbone of the total protein chain but the conformation is a result of a number of non covalent interactions. Most important non covalent interaction which involves in the conformation of the protein molecule is hydrogen bonds, very large number of hydrogen bonds.

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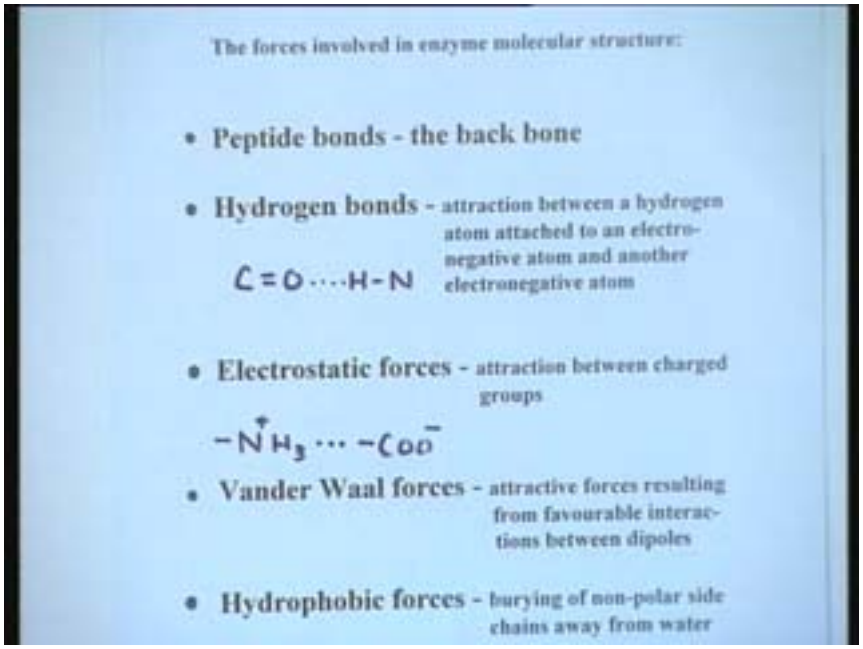

Individually they may not be very strong in terms of bond energy but collectively in terms of number of bonds present they provide a very stable structure to a molecule. The hydrogen bonds are attractive forces between a hydrogen atom attached to an electro negative atom. That means the electro negative oxygen atom of the carbonyl group is attached to the hydrogen of -NH group. This kind of a bond particularly between different peptide bonds can create a hydrogen bond and a large number of these bonds are present in any given molecule.

The next important interaction, non-covalent interaction is electrostatic force. I mentioned that the molecule will consist of a number of ionisable amino acid residues and these ionisable amino acid residues might have molecules at a distance having positive and negative charges and when they interact they might exhibit the an electro static interactions and provide stability to the molecule.

Vander Waal forces the attractive forces resulting from favorable interaction between dipoles is a very important component in the conformation of a protein. Hydrophobic forces are very often considered to be a major force which is involved in the globular structure.

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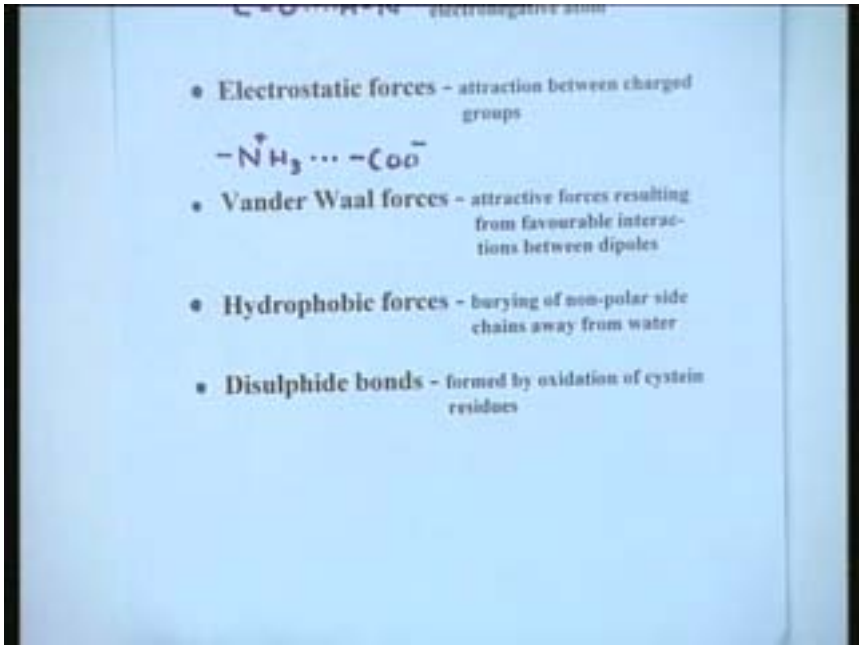

Because of the nature of certain non-polar side chains to drift away from the water molecule, which in the case of an enzyme solution is usually water, it causes the non polar side chains to burry inside the core. That means when you consider the wet side, on the surface there will be most of the charged groups and most of the non polar side chains will be located inside the three dimensional molecules. It doesn't mean that on the surface there are no hydro phobic patches. Most enzymes will have certain hydrophobic patches on the surface which are required for many functional purposes because many of the interfacial actions will involve the hydrophobic patches on the surface and have an important role on the functional aspect. Another covalent bond, disulphide bonds which have an importance in the case of stability of these conformation are the oxidation of cystein residues. Two cystein residues oxidize to give S-S bond and that can come at different points in the chain. After the folding is over, two cystein residues can join together to form a sulphydryl and thereby provide a stability to the conformation.

The conformation resulting from the forces of these interactions that take place are usually classified into four categories. The simplest and the primary structure is the sequence and the content of different amino acids present in an enzyme sample.

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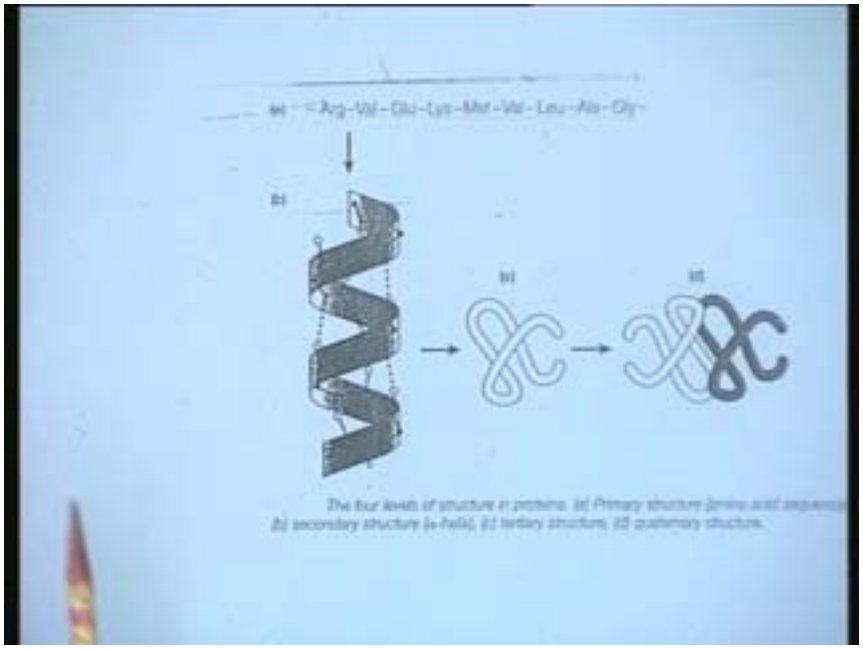

Both the content and the sequence will vary from enzyme to enzyme. There are no similarities except that there will be some homologies of the sequence of different amino acids in same nature of functional enzymes. Then this chain can fold itself locally in the shape of either an alpha helix or a pleated sheet so as to give a secondary structure. If you look at secondary structure in the form of a right handed alpha helix, the helix moves upwards and basically their structure results from the formation of hydrogen bonding between the peptide bonds. A hydrogen bond is formed between the -NH group and the -C0 group in the chain at a distance by four amino acid residues. That means the folding of the polypeptide chain into an alpha helix results with hydrogen bonding between the –NH and -CO residues located at the distance and this interaction results in a very smooth and regular dimensions of the chain in the case of alpha helix. That mean the distances between these two turns is about 0.54 nm per turn 3.6 amino acid residues going to 0.5 and these dimensions have been extended over a large number of proteins and are found to be uniform.

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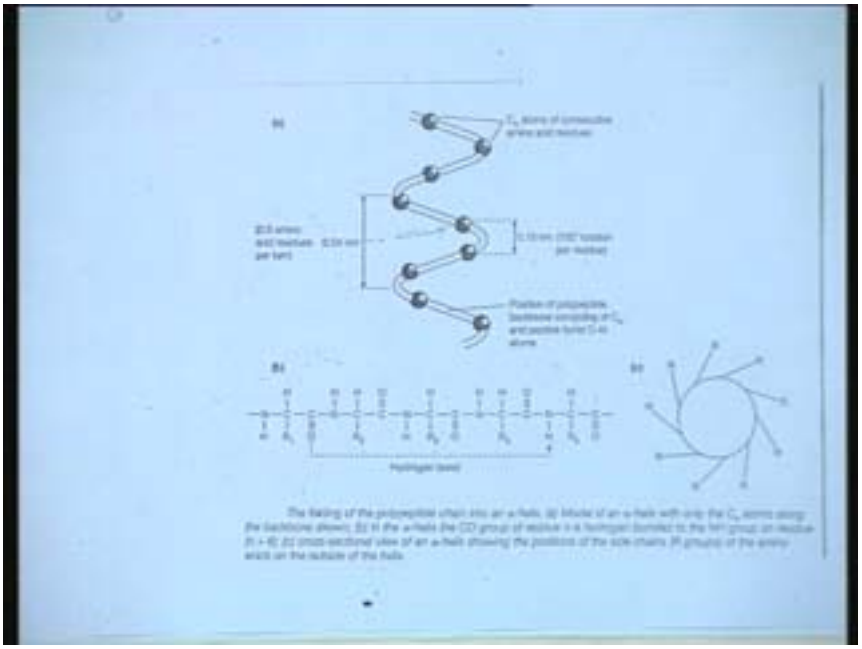

When the protein molecule gets folded into a secondary structure of alpha helix, the side chains of the amino acids are projected outwards like a rotatory form. The side chains are also free to interact with each other and provide you electrostatic interactions and other features. This alpha helix and other type of conformation which many proteins will have will be again as the result of hydrogen bonding will be in the form of beta pleated sheet. They can be parallel or anti parallel.

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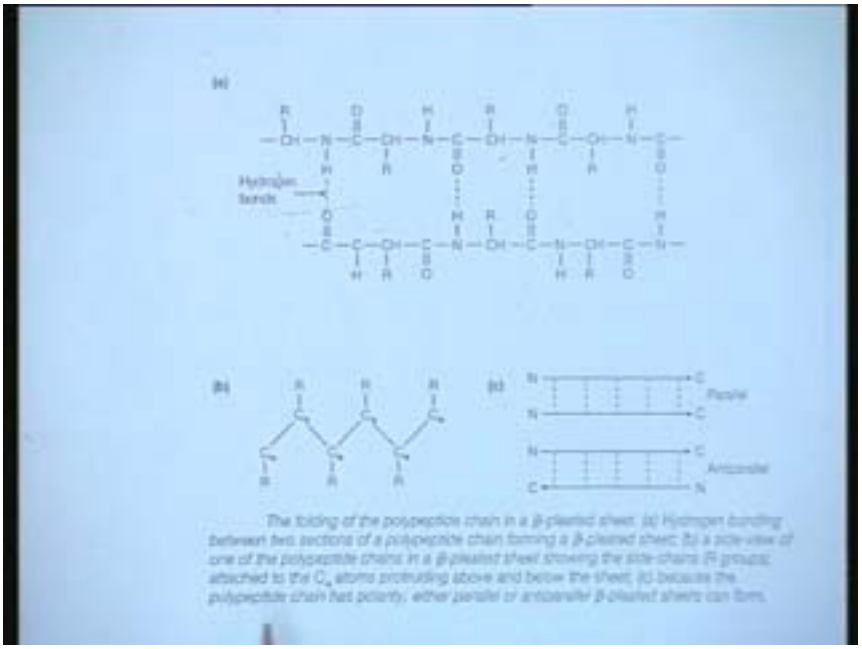

The hydrogen bonding between two sections of the polypeptide chain can form beta pleated sheet and because the poly peptide chain has polarity, either parallel or anti parallel, beta sheet can be formed and you come across in any given protein sample the alpha helix as well as beta pleated sheet structures very commonly. After these secondary structures are formed again the molecule as the result of Vander waal forces and electro static interactions force into a compact globular molecule.

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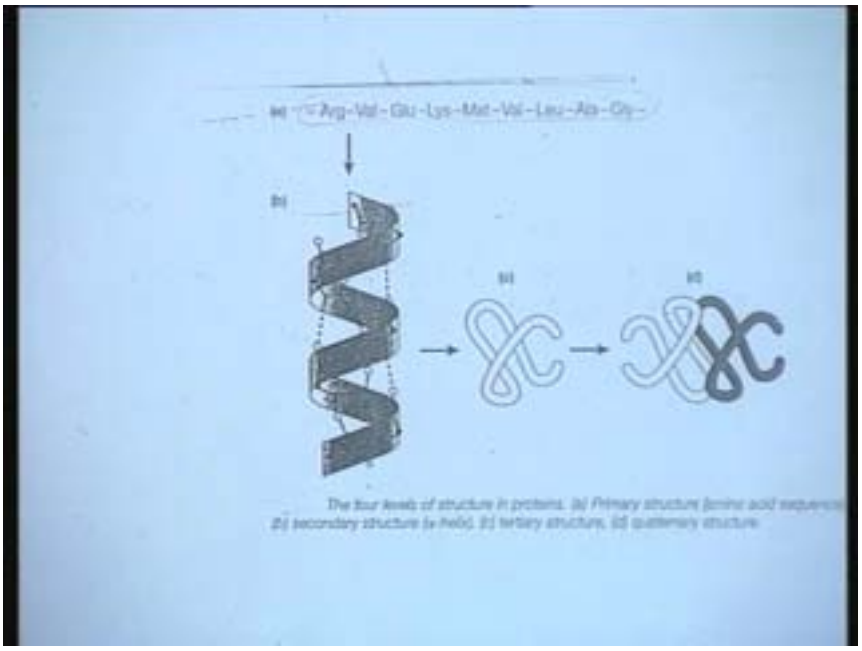

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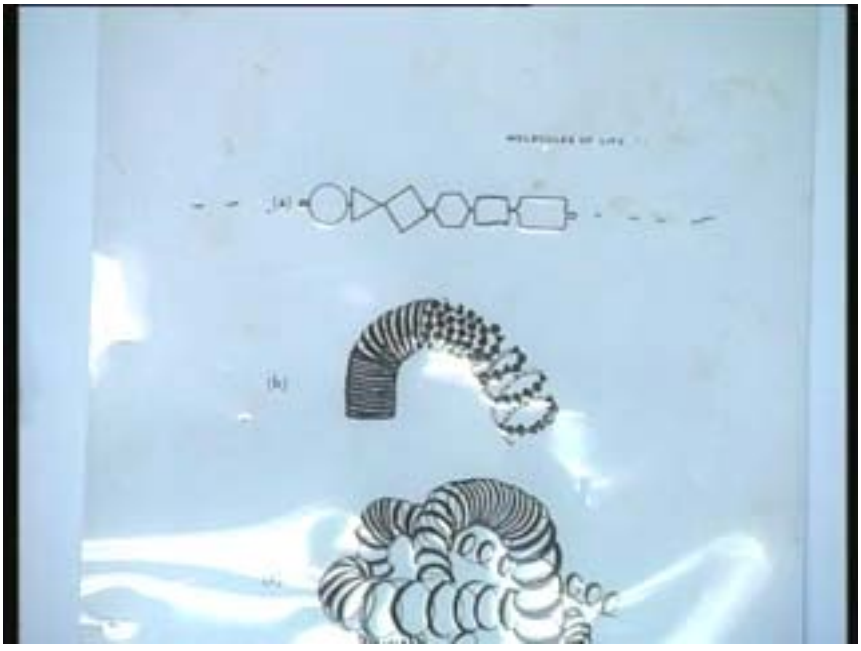

In the top figure different shapes indicate different amino acids and the linear chain of that is termed as primary structure. The folding of these polypeptide chains into alpha helix or the alternative could be the beta pleated sheets, the secondary structure. This structure again folds itself into a globular form as a result of disulphide bridges. In electro static interactions also hydrogen bonding and Vander Waal interactions forces into a globular mass and the basis of that ultimately leads to minimum energy level because at the minimum energy level it will be stable. Any deviation from the minimum energy level will lead to its denaturation or inactivation of the catalytic function of the enzyme sample. When I use the term denaturation, the term denaturation does not in any sway consider the break down of the covalent bonds. That is the basic backbone of the peptide bond. The disruption of the non covalent interaction is good enough to lead to denaturation. In a destructive way you can also break the peptide bond but then you will be destroying the protein itself. But the destruction of or disruption of non covalent bonds, non-covalent interactions will retain the protein molecule but we lose only the catalytic activity, catalytic function and that is what is the usual meaning of denaturation.

The globular form of the folded protein is termed tertiary structure. Many protein may be oligomeric proteins that means they may consist of more than one chain and the arrangement of these chains into a particular fashion so that they are able to bring about certain catalytically active sites is called quaternary structure. The quaternary structure is only applicable if the enzyme protein consists of more than one chain.

In case of a single chain enzyme the quaternary structure question doesn't arise but possible in the case of a multimeric enzyme or what we call as oligomeric enzyme. The quaternary structure could me a dimmer, it could be trimer, it could be a tetramer depending on the number of chains present and the organization will really lead to the final conformation.

I was talking about the formation of sulphydryl bridge. The two cysteine molecules can undergo oxidation to give you cystine molecule which acts as bridges and these bridges is very strong contributor to the stability of the protein molecule in its tertiary structure. Whenever we look into any method particularly the protein engineering methods we tend to increase the number of sulphydryl bridges.

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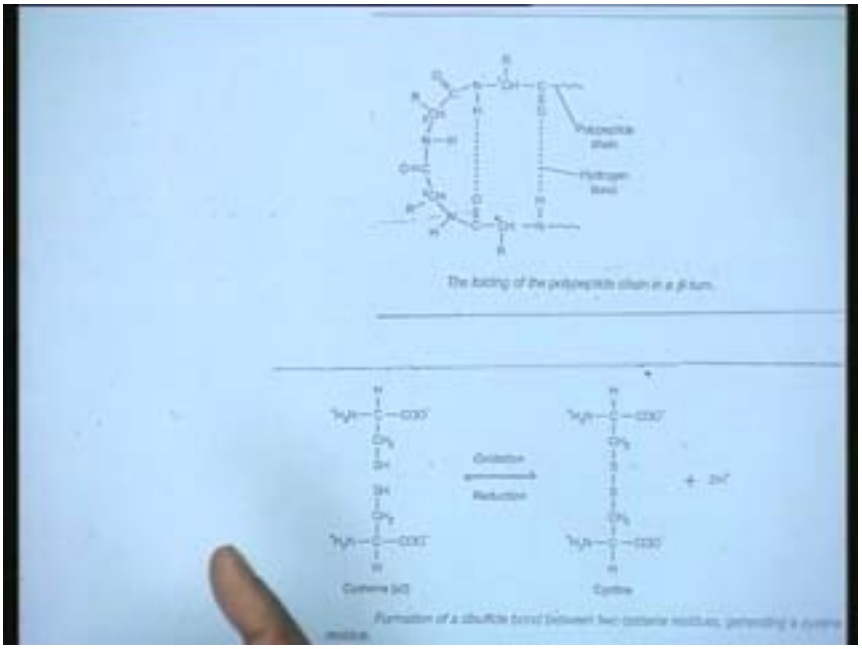

Because ultimately if we can have more sulphydryl bridges, it is likely to result into higher stability or in other words most of the enzyme samples which are obtained from thermophiles that means the organisms that can survive in the extreme temperatures will contain higher content of sulphydryl bridges and they are probably the features that results into the higher stability.

The other issue a very general issue about the feature of enzymes and proteins is the bio synthesis and regulation.

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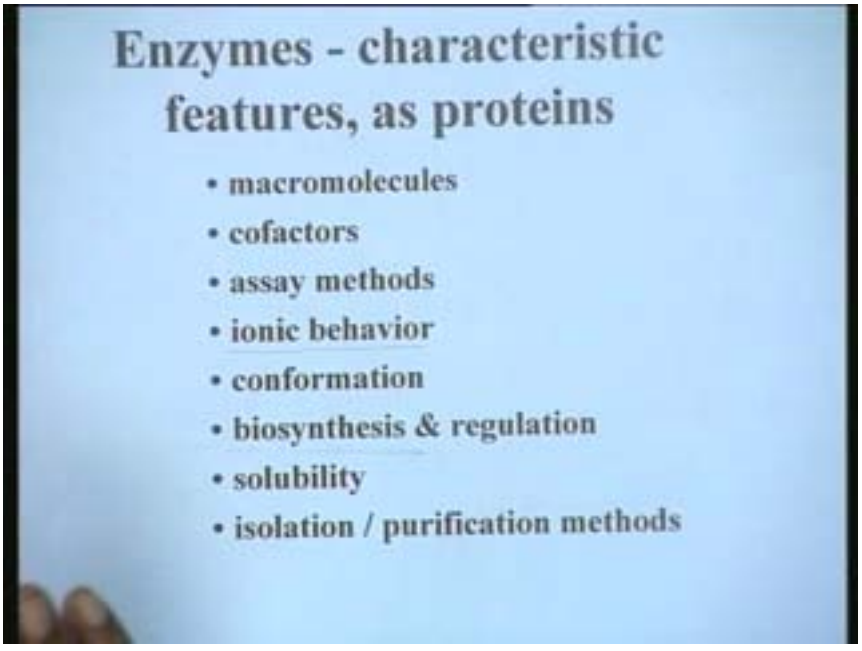

The biosynthesis of enzymes in any living system is exactly the same as biosynthesis of any protein. The central dogma of molecular biology covers the biosynthesis of enzymes and proteins. The enzyme in many cases, particularly the oligomeric enzymes have to undergo some modification. There are certain organizational changes that lead to enzymatic protein or enzymatic constituent but otherwise the synthesis of the protein that leads to enzyme synthesis is identical.

The whole set of information about the synthesis of a protein is coded in the DNA molecule. That means the sequence of nucleotides in a DNA determines the amino acid composition of a protein and that particular portion of DNA gets replicated, transcripted to mRNA and other forms of RNA and then translation of ribosomes to protein. These are the steps for protein synthesis and in case of enzyme synthesis also the same principle applies. As far as the regulation of enzyme synthesis is concerned most of the enzymes are regulated entities. Most of the hydrolytic enzymes are regulated by the mechanisms commonly known as induction or repression or catabolic repression. That means the structural gene of the particular protein is bound to a repressor molecule and unless an inducer is added it cannot be relieved. The famous Jacob-Monod model, the Lac-Operon model was the one earliest study as far as the regulation of proteins are concerned and induction is a very common tool for regulation of the enzyme synthesis. Most of the inducers for many enzymes very often are the substrate molecules themselves.

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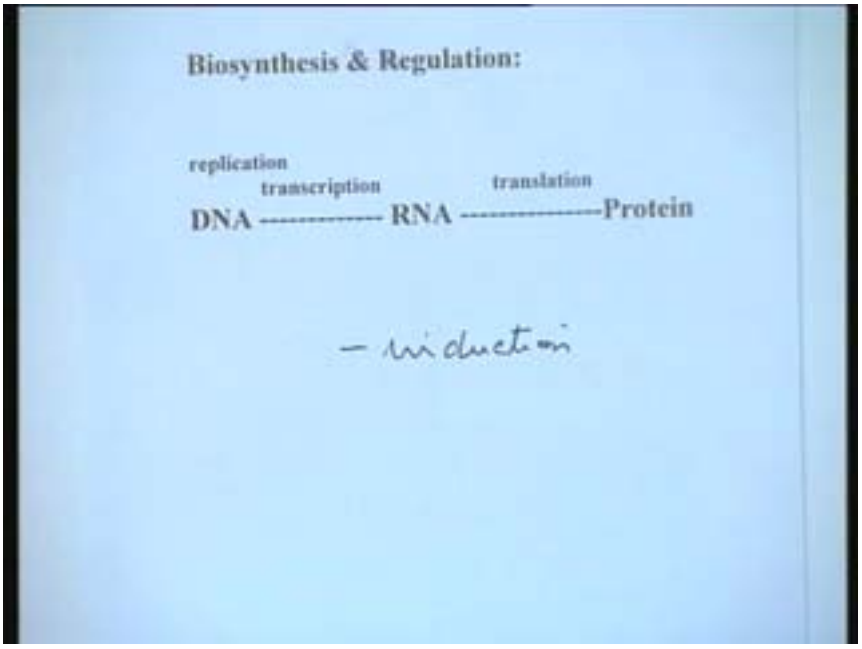

If you consider a glucose isomerase it is a xylose isomerase. There are a lot of structural similarities with xylose and glucose. Xylose is the inducer. In the case of cellulase for example the cellulose act as an inducer and for many of the hydrolytic enzymes which are regulated by induction the substrate itself act as an inducer. Then they also undergo catabolic repression that means the availability of an easily assimilable carbon source and

the most easily assimilable is glucose represses the synthesis of the enzyme. Various other mechanism of …… (48.54) and all that will also a play a role in the regulation of the enzyme synthesis. Another important feature of a regulation here besides biosynthesis, is regulation of the enzyme activity itself.

Most of the enzymes are known to have a mechanism by which even their activity can be regulated not only at the biosynthesis level but at the functional level. That means there are inhibitors and activator molecule which we normally know as effector molecules which can influence the initial reaction rate of the enzyme in the …… (49.31).

If you add along with the enzyme sample a known inhibitor molecule the reaction rate which is obtained will be much lower. Similarly an activator molecule will increase the reaction rate and that is a very common feature of the regulation of enzyme activity and which is of significance even for commercial applications.

Another property of the enzymes in terms of proteins is their solubility. All the globular proteins are known to be soluble in aqueous medium and so are the enzymes. The solubility is enhanced by the formation of weak ionic interactions and the hydrogen bonds between solute molecules and water and the protein molecule has a large number of charged amino acids which can contribute to the ionic interactions with the solvent and also formation of hydrogen bonds with the water molecule. Usually the solubility can be influenced by salt concentration that means ionic strength, the pH, the organic content in the solvent that means addition of a polymer, an organic polymer can affect the solubility and temperature is a universal parameter which influences solubility. But you will notice that at least the first three parameters are very commonly used parameters for even isolation of an enzyme sample from a crude protein sample.

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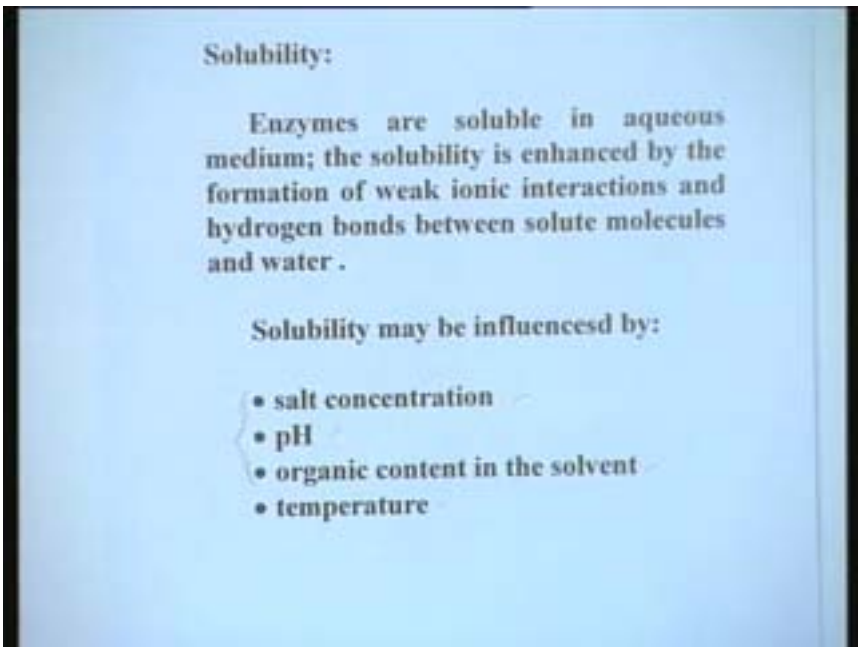

So finally to conclude I would like to say that today we have discussed the characteristic features of enzymes that are attributed by proteins nature, as a macromolecule in terms of assay method, their ionic behavior, their conformation, solubility and the role these properties play in the various techniques that are used for isolation, purification or application of these enzymes.

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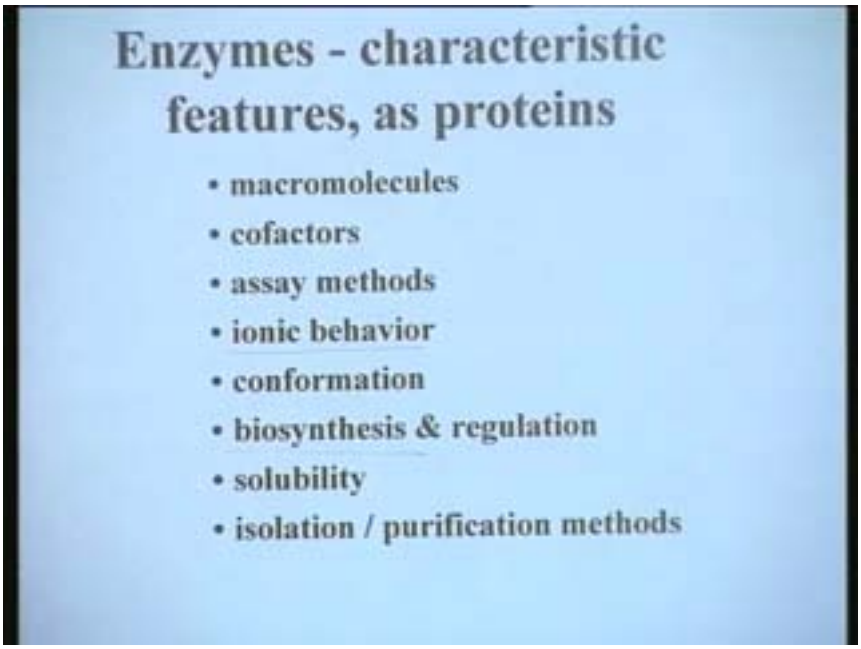

Quaternary structure has relevance in the case of oligomeric proteins that means the proteins that consist of more than one polypeptide chain. The arrangement of these polypeptide chains into a particular formation can result in certain interaction which can form catalytic sites.

The arrangement of different polypeptide chains in an oligomeric protein is termed as quaternary. In fact each chain will have its known tertiary structure and then these globular structures will assemble in a format. Example is lactate dehydrogenase which is a tetramer consisting of four polypeptide chains. Slight shift in these individual units can totally change the functional behavior of the enzyme. This has led to one of the diagnostic method particularly for the patients who are suffering from cardiac problems the lactate dehydrogenase from the heart muscles undergo the quaternary structural change. And the change in quaternary structure is related to the disease behavior. That is one of the diagnostic tools. So in fact even the conformational aspects are important for the applications.