Enzyme Science and Technology

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Module - II
Enzyme Structure
Lecture - 06
Primary Structure of Enzyme

Hello everyone. This is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And in the course Enzyme Science and Technology we are discussing about the different aspects of the Enzymes. And in this context in the previous module we have discussed about the Historical Development of the Field of Enzyme which is called as enzymology and then we have also discussed about the classification of the enzymes in the different groups.

So, what we have discussed, we have discussed that the enzymes are being been classified into the different group based on the detailed criteria which we have which the enzyme commission has adopted. And then subsequent to that we have also discussed about the enzyme nomenclature and we have taken an example of each class how we can be able to give the name of that enzymes.

And in the today's lecture or in this particular module we are going to discuss about the enzyme structures, you know that the enzyme is catalyzing a reactions right. It is converting the substrate into the product and it is very very crucial that the enzyme is recognizing the substrate and most of the substrates are having the three dimensional conformations.

So, enzyme also has to adopt the a suitable three dimensional conformation. So, that it can be able to recognize the substrate, it can be able to perform the reactions and that is how it can be able to convert the substrate into the product.

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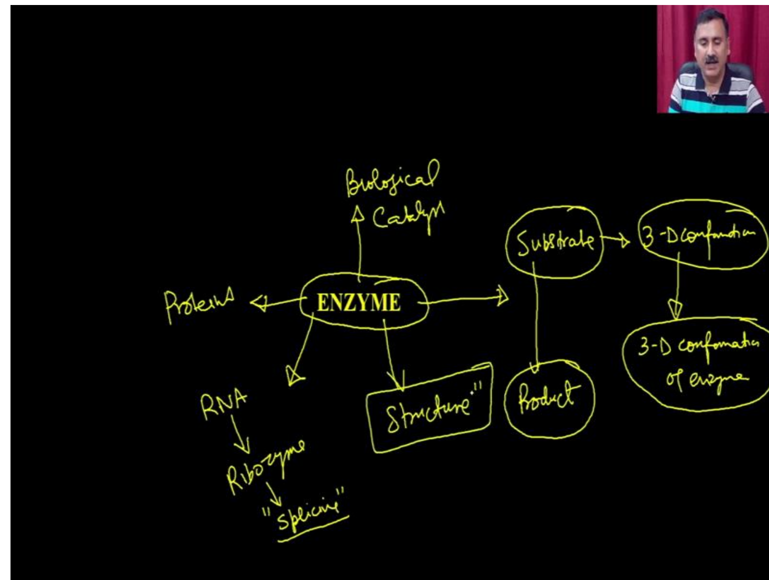

So, if we see the enzyme right, the enzyme are actually being central molecules and they are also been called as the biological catalyst. What they are doing? They are converting the substrate into the product, right. And in this process they are actually not changing the speed right; they are not being consumed right.

And enzymes in general are made up of the proteins, but there are exceptions where the enzymes are also being made up of the RNA molecules and these enzymes are called as the ribozymes. And ribozymes are involved in a very very crucial process that is called as the splicing, where they are actually removing the introns from the exons. So, that process anyway we are going to take up when we are going to discuss about the enzyme applications.

In today's lecture and enzyme is processing the substrate into generating the product because the substrate is having the required 3-D conformations, right. And these 3-D conformations are actually going to be identified by the 3-D conformations of the enzyme. If you recall in the previous module we have discussed how the enzymes are recognizing the substrate and how they are actually utilizing the different types of properties.

So, they are having a geometrical constraints, they are going to have the geochemical constraint and then they are also going to have the functional groups which are responsible for the substrate recognition and as well as the conversion of substrate into

the product. Now, as far as the enzyme structure is concerned, enzymes are actually having the detailed structure ok.

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And if you see the enzyme structure the enzyme structure is the ordered folding of the polypeptide chain that gives rise to the 3-D conformation known as the secondary structure of the protein such as the helices, beta sheets and loops. And the arrangement of the secondary structure give rise to the tertiary structure right, so this is the primary structure where you have the chain of the amino acids, these primary structures when they fold they are actually going to give you the secondary structures.

And within the secondary structure we have the alpha helices, we have beta sheets and we have the turns. And then when the secondary structures are actually connected via the unstructured loops to arrange themselves in a protein structure it allows the secondary structure to change their directions. And the tertiary structure define the function of a protein enzymatic activity or the nature of structural protein.

So, these secondary structures they are being connected with the help of the unstructured loop and that is how they are actually going to be converted into the tertiary structures. And tertiary structures are actually going to be fold into the quaternary structure in those cases where the protein is having the more number of subunits.

So, as far as such the enzyme has the different types of secondary structure, different types of structural levels. It has the primary structures, it has secondary structure, it has tertiary structure and in some cases when the protein has a multiple polypeptide chains then it is also going to have the quaternary structure. So, let us start the discussing about the primary structures. So, primary structure is nothing but the sequence of the polypeptide chain.

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So, enzyme, so as I said you know enzyme could be the made up of the RNA molecules or enzyme could be made up of the proteins. Mostly the enzymes more than 99 percent enzymes molecules are made up of the protein molecules. And there are very small fraction of the enzymes which are made up of the RNA molecules and these enzymes are called as the ribozyme, right. So, we are not going to discuss about the ribozyme in this particular course.

So, enzyme is made up of the proteins and the proteins are actually the polymer of the amino acids which are joined by a covalent bond, known as the peptide bond. And the each protein can be broken into the constituent amino acid by a variety of method to study the free amino acids. There are twenty different types of amino acids which are present in the protein.

So, what you see here is, this is the general structure of a pro amino acids, where you have a central carbon and this is called as the alpha carbon and around this central carbon

you have the carboxyl group, right. And you have the amino group. So, its connected to the one side it is connected to the carboxyl group, on the side it is connected to the amino group and on one side it is connected to the hydrogen and then in the fourth side it is connected to a functional group that is called also the side chain.

And because of this the alpha carbon is a chiral centre and that is why the amino acids actually can be able to exhibit the isomerism; isomerization. So, it can actually be adopted the different types of transformation around the alpha carbons.

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So, amino acids are classified by the R group, but people have started discovering these amino acids they have given the name based on the food sources from which they have been isolated for the first time. For example the asparagine; asparagine was isolated from the asparagus ok. And say and the glutamate is isolated from the wheat gluten, whereas the tyrosine is isolated from the cheese and in the Greek the tyros means the cheese that is why the name of the amino acid is tyrosine.

Similarly the glycine, glycine is derived for its name due to its sweet taste because in the Greek's you have the glycos which is called as the sweet. So, these are the conventional name which have been given a, but later on people have started giving the name based on the systematic scientific way of doing it and that is how they have given these kind of names.

As I said you know remembering these single letter code or the triple letter code is very important, because when we are going to write the sequence of the amino acids or for a particular protein you cannot write the full name right and that is why these single letter or the triple letter codes are being used in that particular cases.

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Now, the amino acids are as I said you know amino acids are classified based on the R group. So, we have the 20 different amino acids and all these amino acids are varying in terms of the different types of properties. So, what you see here is I have given you a composite table, where I have given you the three letter codes or the single letter code. Then as far as the molecular weight is concerned, the molecular weight is also depending on the side chain as well as the other groups.

So, you can see that the molecular weight is 89 in the case of alanine, whereas molecular weight is 204 in the case of tryptophan. So, it actually varies a lot but on a generalized way, the amino acid molecular weight is considered to be 110 Dalton ok Dalton. So, if I say if there will be a question right that what will be the; how many amino acids are present in a 20 KDA protein? Ok.

So, if this is a generalized question what you can do is the number of amino acid if you want to calculate, then what I will do is I will just take the 20,000 KDA, 20,000 Dalton and I will divide that by 110. And then what you are going to get is you are going to get the number of amino acid.

So, that is a generalized term, if I say ok you should calculate the number of amino acids present in the actin protein, then and if I give you sequence of that amino acid or the sequence of that particular protein, then the or; if I say ok I give you a sequence of the I mean actin protein or you give me the molecular weight, then in that case the situation is going to be different. Then what you have to do is, you have to first count the number of amino acids right.

For example if I say number of glycine. So, suppose the number of glycine are 10 right for example. So, you can just go with the glycine table right, and then you say oh glycines molecular weight is 75. So, 75 into 10 is 750 Dalton. And that is how you are actually going to do for the calculations you are going to calculate the number of other amino acids like how many aspartates are present, how many arginines are present, how many lysine are present, how many tryptophana are present.

And then you just keep multiplying like that and if you add all those numbers then it is actually going to tell you that, what is the molecular weight of the actin proteins? So, this is just a generalized term, right. So, this is the molecular formula what is being given, for each and every amino acid, then it is the residue formula, what is given and then you have the residue weight, which is may like if you remove the water molecule, then this is going to be the molecular weight.

Then it is also giving you the PK values and if the amino acid has two functional groups then it is also going to give you the PK b as well and it is also going to give you the PI value. So, these PK values are actually going to calculate used to calculate the charge on that particular amino acids, whereas the PI is actually going to calculate the charge.

So, its going to let you to calculate the charge of that particular amino acids at that particular PH. So, in a particular PH so you know that at that particular PH the amino acid is going to be neutral ok.

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Now, the amino acids are classified by the R group. So, you can have the different types of R groups, you can have the non polar aliphatic R groups. So, the R group in this amino acids are non-polar and the hydrophobic examples includes are the alanine, valine, leucine, isoleucine, glycine, methionine and proline. Then we have the aromatic R groups which means, these are the groups where you going to have a benzene ring.

So, these groups are going to be contained the benzene ring, whereas in this case it is not going to contain the benzene ring, it is going to be a linear chains. So, the R group in these groups are hydrophobic side chains for example, the phenylalanine, tyrosine and the tryptophan, right. So, you might have to remember the structure of these amino acid, if you want to understand the functional role or the crucial role going to be played by these amino acids.

Then we have the polar uncharged groups which means these amino acids are not going to have the charge which means they are not going to be positive or negative charges, but they are polar in nature. So, the R group in these amino acids are uncharged and they are more polar than the hydrophobic amino acids. Example includes are serine, threonine, cysteine, asparagine and the glutamine.

Then we have the negatively charged R groups which means they are actually going to be called as the acidic amino acid, because they are actually going to impart the negative

charge into the protein. So, the R group in this amino acids are acidic with the net negative charge examples are the aspartate and the glutamate.

Then we have the positively charged R groups and these amino acids are called as the basic amino acids, they are actually going to give you the positive charges and the R group in these amino acids are basic with the net positive charges examples includes are the arginine and the lysine.

Now, when you take a protein and you want to calculate, you want to know what will be the amino acids are present, what you can do is you can just do a acid hydrolysis. And if you do the acid hydrolysis it is actually going to give you the mixture of amino acids, right. Now once you have the mixture of amino acid you can resolve these amino acids onto a thin layer chromatography.

So, thin layer chromatography is a technique, which actually is going to give you the spot for the each and every individual amino acids and depending on the intensity of that particular amino acid and depending on the number of spots what you are going to get from this particular protein. You can be able to calculate the number of amino acid as well as the different; the type of amino acids present in that particular mixture. So, that you are going to do when you are going to analyze the amino acids.

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So, how you are going to do the analysis of the amino acids. The thin layer chromatography technique is an analytical chromatography to separate and analyze the amino acids from the protein. In this method the silica or the alumina as a stationary factor, stationary phase is coated onto a glass or the aluminium foil as a thin layer.

And then a sample is allowed to run in the presence of the mobile phase. In comparison to other chromatography technique, the mobile phase runs from the bottom to top from the by the diffusion, whereas in most of the chromatography technique the mobile phase runs from the top to bottom by the gravity.

So, what you are going to do is, you are going to take a thin aluminium foil, right. And or the glass plates and then this aluminum foil you are actually going to make a thin film of the alumina or the silica. And then what you are going to do is you are going to apply your amino acid which you want to dissolve, right. And then you are going to keep this into a solvent system.

So, once the solvent is going to run in from the bottom to top, it is actually going to take up the your amino acid also along with that and then it is actually going to give you the spot as per the and its going to give you the mixture of the amino acid and that is how its actually going to resolve. Now, what you see here is in the TLC you are going to have the movement of the solvent from the bottom to top.

Because of the diffusion compared to that in a conventional chromatography, you are going to see always running of the mobile phase from the top to bottom. For example, if you run a column right, the column you run from the water from top to bottom, whereas in the case of thin layer chromatography, the solvent runs from the top to bottom because of the diffusion.

As sample runs along with the mobile phase it get distributed into the solvent phase and the stationary phase, right. When it runs it is actually going to be you know going to get distributed. So, either it will go with the solvent fronts right, so you can see that all the solvent molecules are running, right. So, either; so if this is the molecule either it will go along with the solvent front or it will actually going to remain with the silica front, right.

And because of that it is actually going to be distributed because that kind of affinity and that kind of differential behaviour is going to be different for the different amino acid

molecules. The interaction of the sample with the stationary phase retard the movement of the molecule whereas, the mobile phase implies and the as the effective force. So, what will happen is that when you are going to apply the sample on to this, it is actually going to experience for example, this spot, right.

So, if you have a spot here, it is going to experience two different types of forces. It is going to have a driving force right, so driving force is actually going to be exerted by the solvent molecule. So, solvent molecule is going to try to push this molecule towards the top side because it is running, right.

But whereas, it is actually going to make the interaction with the these silica particles which are present on this TLC plate and because of that there will be a retardation forces. So, that retardation forces is going to be by the matrix molecule. And because of that it is going to have the two opposite forces. One which is going to be on towards the top side and the other one is going to be on to the bottom side. So, its going to be distributed, so and it is going to be immobilize onto the plate.

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Now, suppose the force caused by the mobile phase is F m and the retardation force by the stationary phase is F s. So, then the effective force on the molecule will be F m minus F s. So, this is what I am going to; I was trying to explain you, right. So, if this is the spot on this spot you are going to have the upward forces, which is called as F m or the

mobile force by the mobile phase, whereas it is actually going to have the retardation forces, which is going to be caused by the stationary phase which is called as the F s.

So, this molecule is actually going to run effectively by a force which is going to be F m minus F s. Now, the F m minus F 1, so where this molecule is going to be stop, right. It is going to stop the place where the F m is going to be equivalent to F s, right. And that F m minus F s is going to be different for the different molecule and that is why they are actually going to be present at the different places.

For example, for this molecule the F m is too big for the F s, which means it is still having a effective charge at this point and that is why the this molecule runs for the longer period of time. But at this point when it got immobilized, again for this one also the F m is equivalent to F s. So, the molecule is immobilized on the silica gel where the F m is going to be equivalent to the F s and the position is controlled by the multiple factors.

Nature or the functional group what is present onto the molecule or the analyte. So, if the molecule is going to interact with or suppose it has a functional group and it is going to interact with the silica particle, then it is actually going to increase the F s. If it is going to increase the F s, then the F m minus F s is going to be small, right.

And if this is the phase, then it is actually going to be immobilized towards the starting points. Then it is also going to be depend on the nature on the composition of the mobile phase. So, depending on the mobile phase also it is actually going to be different, right. If the mobile if the molecule is very soluble into the mobile phase, then the F m; the value of F m forces are actually going to go up, right. And that is why it is actually going to run very far away from the spotting place, right.

Then it also depends on to the thickness of the stationary phase because that also is going to have the effect on to the retardation forces and then it also has a functional group. So, functional group what is present onto the stationary phase. So, apart from the functional group, what is present onto the analyte molecule?

The functional group if the functional groups are also present on to the silica particles like for example, if you take the silica particle or if you take the functional silica particle,

they may have the higher affinity for this particular molecule. And as a result the F s will actually go up and if the F s will go up, it is going the F m minus F s is going to be small.

And that is why it is actually going to immobilize very soon and it is going to be closer to the spotting points. So, you can imagine that if I want to see the differences like, what I can do is or if I want to know where the molecule is going to immobilize, what I can do is once it got immobilized I can take a distance from this molecule to the origin. So, this is the origin point. So, for example, at this point, I have started putting the spot of the mixture and then the solvent started running, right.

So, solvent when the solvent reached to the end of the plate or it reaches to at least the 75 percent, then what I can do is I can just stop this, I can develop this spot and then I will calculate the distances what is run by the solvent and I can also calculate the distances run by the individual spot. So, for example, this is the spot number 1, this is the spot number 2, this is the spot number 3. And the distance of the spot number 1 is d 1, the distance of the spot number 2 is d 2 and the distance of the spot number 3 is d 3.

So, what I can do is I can just calculate the R f values and R f value what is the formula is that the distance of the analyte right, distance of the analyte spot which is like d in this case. So, d x divided by the distance of the solvent. So, distance of solvent like the in this case ds ok. And this is going to be fractional. So, the maximum R f what is possible is 1 and it going to be the fraction of 1 right, because this is the ratio, right.

And this R f value is going to be dependent on to the; on to the solvent system right, depending on the solvent what you have taken, depending on the matrix material and depending on the conditions in which it is run. So, if you are going to maintain all the three constant, right. If you maintain the same solvent system, if you maintain the same matrix and if you run it under the identical conditions the R f value is not going to be changed.

Even if you run it for example, if I run it for 50 centimeter, it is going to be distributed accordingly. So, that for example, if I have R f value of 0.5 ok. So, if I run it for 50 centimeter, the spot is going to be formed as 20 centimeters; 25 centimeter. If I run it on 100 centimeter right, then the spot is going to be formed at 50 centimeter. Because it is going to maintain the ratio of R f is equal to 0.5.

So, if I R f is 0.5, which means the ratio of dx to ds is going to be 0.5. So, that does not depends on the how much length you are going to run the TLC plates. It is going to be always be immobilized at the 50 percent distance, right. If the R f value is 0.5, so this R f value is constant and that is why the R f value can be used to characterize the different types of amino acids.

Now, the question comes, how you can be able to determine the R f values? You can actually be able to run the TLC plate that you can be able to run the thin layer chromatography and that is how you can be able to calculate.

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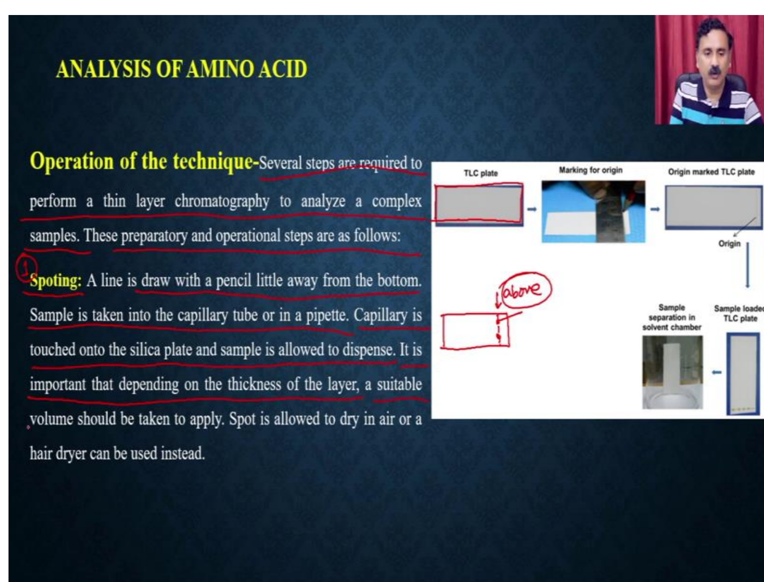

So, how you can actually be able to run the thin layer chromatography? Several steps are required to perform a thin layer chromatography to analyze the complex samples these preparatory and operational steps are as follows. So, in the step number 1 you are actually going to make the spoting.

But before that you are actually first going to take a thin layer plate. So, what you see here is this is the thin TLC plate, right. So, what you are going to do is what you take the TLC plate; you cut the TLC plate as per the number of sample what you are going to place, right. On the width wise and its height is also going to be as per the chamber of your TLC plate right.

So, you are going to run it in a chamber, right. And then what you are going to do is you are going to take a scale and you are going to put a line and this line should be above to the solvent front, right. Because it is going to dip right, ultimately you are going to dip this, so then you put a line and then on this line you are actually going to put the spots. So, the line is drawn with a pencil little away from the bottom, the sample is taken into the capillary tube or in a pipette.

So, what you can do is, just take the sample into a capillary tube, the capillary is touched on to the silica plate and sample is allowed to dispense. So, what will happen is when you touch the silica, when you touch the capillary to the that particular silica its actually going to suck the sample automatically by the action of diffusion, right. So, it is important that the depending on the thickness of the layer a suitable volume should be applied, spot is allowed to dry in air or a hair dryer can be used instead.

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Then we have the running of the TLC, so once the spot is dried it is placed in the TLC chamber, in such a way that the spot should not be below to the solvent level. Solvent level front is allowed to move until the end of the plate. So, what you can going to do is just keep it into a TLC chamber. So, you can actually be able to develop a TLC chamber into a beaker or into a thin small chamber it is depending on the type of the solvent, right.

So, then you have to cover this, with a some you know with the lid right rather you can just put a cap right. And why it is important? Because so that the solvent what you have put is actually going to form the vapour and that vapour should be condensed otherwise it is not going to give you a upward movement. After the so then you are going to place the TLC into this right, so you are going to plate the TLC plate and make sure that this line should be above to the solvent front.

So, that it is not going to get dissolved into this solvent before running, right. The analysis of the chromatography plate, the plate is taken out from the chamber and air dried. If the compound is colored, it forms the spot and these substances there is a no additional staining required. There are two methods of developing a chromatogram. So, what you can do is, later on you can just take out this plate and then you air dry.

So, if the analytes like the amino acids are colored, then actually they are going to give you a spot. And then you can directly take the all the sort of measurements. So, you can actually know that this is the like the d s, right. So, this is the solvent, what you have run. And then you can just take the calculation of this and it is actually going to be dx and then you can actually be able to calculate the R f value by dx by the ds.

So, for this the position of the spot is very important, right. So, if it is colored compound there is no need to have the any kind of additional staining procedures or any kind of procedures you can actually be able to do this, right. But if it is not, then you have to go with the staining procedures.

In the staining procedure, the TLC plate is sprayed with a staining reagent to stain the functional group what is present into the compound. For example, the ninhydrin is used to stain the amino acids. So, if it is not then you can actually be able to use the staining procedures. If you want you can actually go with the non-staining procedure as well

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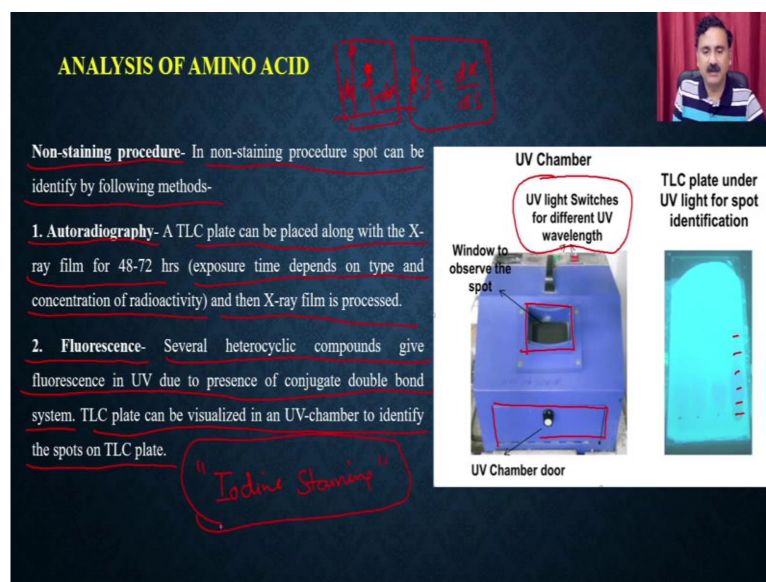

So, you can use the non-staining procedure, in the non-staining procedure spot can be identified by the following method. You can use the auto radiography, a TLC plate can be placed along with the X-ray film for 48 to 72 hours, exposure time depends on the time and the concentration of the radioactivity and then the X-ray film is processed. So, you can actually have the radioactive amino acids and that is actually going to be exposed to the X-ray film and then it is actually going to give you the spot on to the X-ray film.

And then you can do all the calculations from this particular spot, right. For example, you can do the F f calculations you can actually do the dx and ds and that is why you are actually going to get the R f value. And that R f value you can use for identification of that particular unknown spots. The second is you can do the fluorescence. So, several heterocyclic compound gives the fluorescence in UV due to the presence of conjugate double bond system. TLC plate can be visualized in the UV chamber to identify the spot.

So, what you see here is, this is the typical UV chamber where you have the UV bulbs and this is the chamber right, this is the; this is the lid of that particular chamber. So, what you can do is, just open from here and place the plates under this and then you can just turn on the UV lights. So, you can have the two different types of UV lights, which you can use as per the wavelength.

And then from this size because the UV light is dangerous for the eye, so that is why you can actually be able to observe not directly, but through this particular spotting observing window. And what you see here is that this all the spots are visible, right. So, under the UV and then you can actually be able to use the camera or some other acquisition system and that is how you can actually be able to capture the image.

Apart from that you can also use the iodine. So, you can also use the iodine staining right, so you can actually be able to incubate the TLC plate into the iodine chamber and this iodine chamber is actually going to stain the spots as well.

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So, the proteins are as I as we discussed that proteins are the polymer of the amino acid, they are joined by the covalent bond known as the peptide bond. A peptide bond is formed between the carboxyl group of the first and the amino group of the second amino acid with the release of the water molecule. So, this is you will see that this is the amino acid number 1 and this is the amino acid number 2.

So, when they will go by with the condensation reactions. So, what will happen is that the OH of this acid and the H from this is actually going to combine. And that is why there will be a bond which is going to be formed between the two amino acids. And that is how there will be a loss of water and this is a dehydration reaction. So, it is a dehydration synthesis or the condensation reactions.

The peptide bond has partial double bond character due to the resonance and the C-N bond is not free to rotate. But the bond between the N to alpha right, N to the alpha and the C to C alpha can be able to rotate through a dihedral angle designated by the phi and psi. So, what you can; so this peptide bond is rigid it is not allowed to rotate, but the bond the, but the bond between the N to alpha and the C to C alpha can be able to rotate through the dihedral angle, which are designated as the phi and psi.

And these angle can be able to rotate from the minus 180 to plus 180 with the few restrictions. To exploit this particular type of phenomena, the Indian scientist G. N. Ramchandran has determined the possible phi and psi angles for a particular amino acid by synthesizing the tripeptide with the amino acid of interest in the middle. So, what he has done is, he has actually synthesized a tripeptide.

So, for example, if it he wants to calculate the phi and psi angle for A, he has; he has made a tripeptide with C and D. So, you can keep changing these tripeptide and that is how you can be able to calculate under different conditions what will be the different psi phi angles are possible and that is how you can be able to make a map between the psi versus phi, right.

So, you can actually be able to make a angle between the map between the psi and phi and then you can calculate that under different combinations how much these psi and phi angle are going to vary for the a molecule. And that is why you can say that ok, A will go from this psi angle to this phi angle.

And based on that he has actually developed a map which is called as the Ramchandran plot and that Ramchandran plot is used to define the region of the allowed rotation for the amino acid present in a protein structure. And he what he proposed is that he you can use this particular type of plot to say, whether a whether a solved protein structure is correct or wrong.

Because if it is incorrect, then the psi and phi angle are not going to be present in that particular defined regions, right. So, that is what you see here is that you are actually going to see the different regions and its going to be what you see here is the plot between the psi and phi and that shows the location of the different types of structures what is present in the protein structures and so on.

And that is how it is actually going to give you the distribution of that particular amino acid in the protein structures and how much its phi and psi angle are going to vary. So, for what we have discussed, we have discussed about that the enzymes are made up of the either the protein molecules or the RNA molecule.

Majorly enzymes are made up of the protein molecules, whereas there is a small fraction of the enzymes which are being made up of the RNA molecules and these enzymes are called as the ribozymes. So, in this case in this particular course we are not discussing about the ribozymes. And then the proteins are made up of the 20 different types of amino acids, these amino acids share a common structural feature.

So, that they have the central C alpha carbon, the C alpha carbon is attached to the amino group on one side, carboxyl group on the other side and then it is group attached to the different types of functional groups. And based on these functional groups the amino acid could be of different types.

So, we have 20 different amino acids, what is present in the protein structures. And we have discussed, how the different side chains are actually going to be responsible for providing the different types of properties into the amino acids and you can be able to generate the amino acids from the protein. So, that you can be able to study the different types of amino acids, what are present in the proteins and how you can be able to exploit the thin layer chromatography to study the amino acids which are present in the enzyme structures.

This is what we have discussed in our subsequent lecture we are actually going to discuss about, how you can be able to determine the primary structure ok. So, determination of the primary structure is a very very important aspect to understand how you can be able to know the primary structures. So, with this I would like to conclude my lecture here in our subsequent lecture, we are going to discuss about the determination of the primary structure.

Thank you.