Basics of Biology
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Module – V: Molecules of the Life (Part-II)
Lecture 20
Proteins (Part-I)

Hello everyone, this is Doctor Vishal Trivedi from department of biosciences and bioengineering, IIT Guwahati. And what we were discussing? We were discussing about the bio molecules. And in this context in the previous module, we have discussed about the three biomolecules, we have initially discussed about the nucleic acids. And as we discussed that the nucleic acid is actually carrying the genetic information from the one generation to the next generations. In most of the organism, we have the DNA as the genetic material, but in some organism, we have the RNA as the genetic material as well.

In addition to that, in the previous module, we have also discussed about the carbohydrates, we have discussed about the structure and the function of the carbohydrates. And then, subsequent to that, we have also discussed about how the carbohydrates are going to be utilized in the different metabolic pathways, so, that it can be able to utilize for energy production. And then, in the last lecture, we have also discussed about the lipids, and what we have discussed? We have discussed about the structure of the lipids, we have discuss about the classification of the lipids, and then we have taken of the few of the classical examples, and then we have also discussed about the function of these lipid molecules.

In today's lecture, we are going to start discussing about the new biomolecule, and the new biomolecule what we are planning to discuss is the proteins. So, as you can see that the proteins are actually being considered as the building blocks, and these protein molecules are required for maintaining the whatever the losses we have. Suppose, there is a loss of protein then we require the protein to replenish or if there will be a damage, then also we require our protein to reconstruct the damaged portion. Apart from that, the proteins are also been responsible or also been functional as the enzyme.

So, and that these enzymes are required for running the different types of metabolic pathways. I am sure we have all already discussed a lot about the role of these enzymes into the carbohydrate metabolism, when we were discussing about the glycolysis and the Krebs cycle. So, subsequent to that, let us start discussing about the protein molecules.

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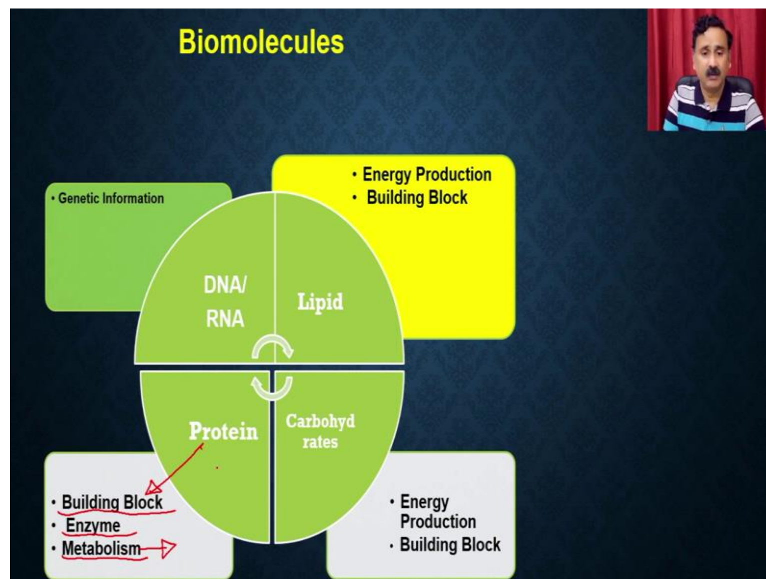

So, as we said, protein is a biomolecule and it is required for the as a building block. So, it is required at the building block, then it is required as an enzyme, and then it is also required for running the metabolic reactions. Proteins are even responsible for making the different types of structures in the body, such as like we could be able to stand and walk because of the fibre which are made up of the protein. So, all these we are going to discuss in this particular module. So, let us discussing about the protein molecules.

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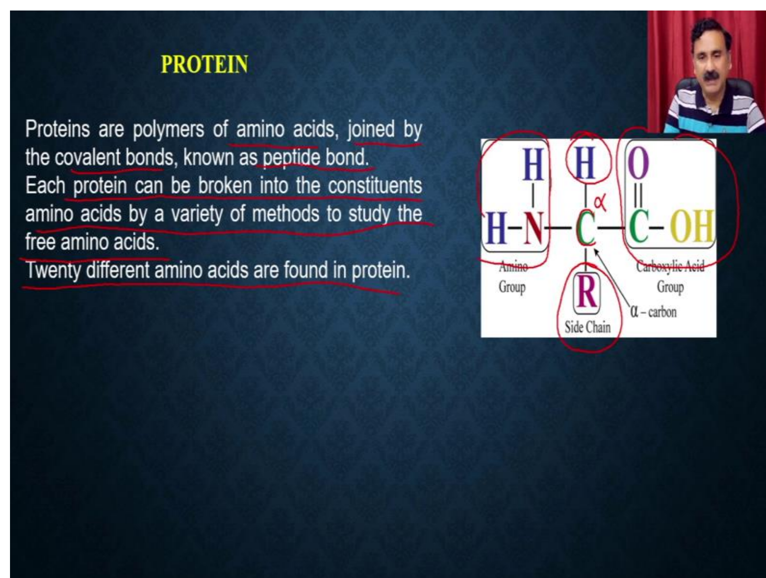

So, protein are the polymer of the amino acids, and they are joined by a covalent bond, which is known as the peptide bond, each protein can be broken into the constituent amino acid by a variety of methods to study the free amino acids. So, what you can see here is that this is a

generalized structure of amino acids, what you will see is, you have a carbon in the centre which is called as C alpha-carbon, and then this C alpha-carbon is attached to the 4 groups. You have amino group, you have a carboxyl group, and then you have the H, and on the fourth chord side we have a side chain. So, depending upon the side chain, we can have the different types of amino acids. So, there are 20 different types of amino acids which are present in the proteins, and these amino acids are being joined by a covalent bond known as the peptide bond.

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So, what is the structure of the amino acid? So, amino acids share the common structures, all the 20 amino acids are the alpha-amino acid with a common structure, each amino acid has a carboxyl group, and the amine group attached to the primary carbon, which is called as the alpha-carbon. They differ from each other in terms of the side chain, or the R group.

The side chain varies in a structure chemical nature and that influence the overall property of that particular amino acid. So, what you see is this is the generalized structure of the amino acid, where we have the C alpha-carbon, and that C alpha-carbon is attached to one side as the amino group, the other side is called as the carboxyl group.

And that is why the name suggests is the amino acid. So, amino acid means the compound which will have the amino group on one side, and the acid group on to the other side, and in between it is going to be connected to the H as well, as the functional group which means the R group. So, depending on the R group, the amino acids could be of different types. The side chains actually vary in terms of the structures, chemical nature, and that has influence on the overall property of the amino acid. Each carbon is attached to the four different groups there

is what we have discussed already. This is a C alpha-carbon, and it is connected to the four different groups, making it a chiral center to give the stereoisomers.

Because this C alpha-carbon is connected to the four different functional groups, like the functional group number one, which is the amino group. Functional number group number two, which is the acid group, and then it has the hydrogen which is the third group. And then the it has a side chain or the R group which is the four side. So, because of these four different groups attached to this particular carbon, this carbon is actually is a chiral center. So, it actually gives the stereoisomers. There are two common forms of the stereoisomers calls as the enantiomers found in the amino acid. These are the non-superimposable mirror image to each other.

So, this is what you see here is actually a enantiomer. So, what you see here is actually a mirror image. So, if you see all the groups what is present on this side, is present on to the this side in this particular amino acid. This is the example of the L-amino acid versus the D-amino acids. So, compared to these, all the amino acids only the glycine is the only amino acid which does not have the chiral center, because glycine the R chain is H. So, that is why the glycine does not show any kind of the chiral center.

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So, amino acids are classified by the R group. As different amino acids are classified based on the side chain or to the R group, these 20 amino acids are denoted by the first letter, or the three letter codes. So, these are three few amino acid, what you see here? So, we have the 20 different types of amino acid depending upon the side chain.

For example, we have the arginine, glutamine, phenylalanine, tyrosine, tryptophan and so on. So, we have the lysine glycine and all that. And all these amino acids are denoted by the two convention, one is the single letter conventions or third, and the second is the three letter conventions.

So, this is very easy to remember. So, if one how the people are, you can actually be able to remember the single letter or the three-letter convention is. For the single letter, you have to just go with the first alphabet of that particular amino acids. For example, in the case of alanine. So, alanine the first alphabet is A.

So, I can say that the alanine single letter code is A. Whereas, if I want to go with the three letter codes, I can just take the initial three alphabets. So, that the three-letter code is actually going to be Ala. So, the same way you can actually have the other amino acids also like for example, you can have the leucine. So, for the leucine, if I have to remember the single letter code it is going to be L, and if the three letter codes, then it is going to be Leu.

This is not true for all amino acid because you can also have the amino acids which is also be starting with an alphabet. For example, we have the arginine. So, if we have the arginine, then it is, for these kinds of amino acid, you cannot take the A, because A is already been allotted for the alanine. So, what we can do is we can just go with the sound, what is coming from this amino acid. So, what amino sound is coming? Arginine, so, when you speak the arginine, the sound comes as R. So, you can actually give the single letter code to R.

So, that is why these are the exceptions these are the things what you have to remember. For example, arginine you have to remember that the single letter code for arginine is R, and the triple letter code is Arg. Similarly, we have already been allotted the L to the leucine. So, we can actually cannot allot the L for the lysine. For the lysine, the single letter code is K, and thus, the triple letter code is Lys. The same is true for the other amino acid.

For example, the glycine you can easily take the single letter code as G, and the triple letter code as Gly. So, for the few amino acids, you might have to remember the single letter code, or the triple letter code, but all other amino acid, you are just going to follow the convention, that the first letter or that particular amino acid you are going to take as the single letter code.

The name of the amino acids was trivial or the classical in few cases derived from the food source from which they have been isolated the first. But this is a scientific way of giving the name of or the single letter or the triple letter code for these amino acids. But when the

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. And the glutamate for is isolated from the wheat gluten, whereas the tyrosine is isolated from the cheese.

And in the Greek the tyrosine is. No, in the Greek the tyros mean the cheese, that is why the name of the amino acid is tyrosine. Similarly, the glycine. Glycine is derived for its name due to his sweet taste, because in the Greek you have the glycos which is called as the sweet. So, these are the conventional name which have been given, 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 kinds of names.

As I said, 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. 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, 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 type 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 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.

So, if I say if there will be a question that what will be the, how many amino acids are present in a 20 kg protein? So, if it 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 kPa, 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 acids.

So, that is the generalized term. If I say okay, you should calculate the number of amino acids present in the actin protein. Then, and if I give you a sequence of that amino acid or the sequence of that particular protein, then the or if I say okay, I give you a sequence of the actin protein, 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. For example, If I say number of glycine. So, suppose the number of glycine's are 10 for example. So, you can just go with that glycine table. And then you say, glycine's 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 aspartic are present, how many arginine are present, how many lysine are present, how many tryptophan are present, and then you just keep multiplying that dat.

And if you add all those numbers, then it is going to tell you that what is the molecular weight of the actin proteins. So, this is just a generalized term. 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 may like you if you remove the water molecule, then this is going to be the molecular weight. Then it is also giving you the pKa values. And if the amino acid has two functional group, then it is also going to give you the pKb as well. And it is also going to give you the pI value.

So, these pKa values are actually going to calculate, used to calculate the charge on those particular amino acids, whereas the pI is actually going to calculate the charge. So, it is going to let you to calculate the charge of that particular amino acids at that particular pH. So, on a particular pH. So, you know that at that particular pH, the amino acid is going to be neutral.

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PROTEIN

Amino acids are classified by R groups:

NONPOLAR, Aliphatic R Group: The R group in this amino acids are non-polar and hydrophobic. Examples include are alanine, valine, leucine, isoleucine and glycine, methionine, proline.

AROMATIC R Groups: The R group in this amino acids are hydrophobic side chain. Examples include are Phenylalanine, tyrosine and tryptophan.

POLAR, Uncharged R Groups: The R group in this amino acids are uncharged and they are more polar than hydrophoc amino acids. Examples include are serine, threonine, cysteine, asparagines and glutamine.

Negatively Charged R Groups: The R group in this amino acids are acidic with net negatie charge. Examples include are aspartate and glutamate.

Positively Charged, R Groups: The R group in this amino acids are basic with net positive charge. Examples include are Arginine and Lysine.

Now, the amino acids are classified by the R groups. So, you can have the different types of R groups. You can have the non-polar aliphatic R groups. So, the R group in these 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 chain. So, the R group in this groups are hydrophobic side chains. For example, the Phenylalanine, tyrosine and tryptophan.

So, you might have to remember the structure of these amino acids, 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 there 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 proteins. So, the R group in these 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 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 acid hydrolysis, and if you do the acid hydrolysis is actually going to give you the mixture of amino acids. Now, once you have the mixture of amino acid, you can resolve these amino acids on to 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 acid. 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 analyse the amino acids.

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ANALYSIS OF AMINO ACID

The thin layer chromatography technique is an analytical chromatography to separate and analyze free amino acids from proteins. In this method, the silica or alumina as a stationery phase is coated on to a glass or aluminium foil as thin layer and then a sample is allowed to run in the presence of a mobile phase (solvent). In comparison to other chromatography techniques, the mobile phase runs from bottom to top by diffusion (in most of the chromatography techniques, mobile phase runs from top to bottom by gravity or pump). As sample runs along with the mobile phase, it get distributed into the solvent phase and stationery phase. The interaction of sample with the stationery phase retard the movement of the molecule where as mobile phase implies an effective force onto the sample.

Distance travelled By the solvent
Distance travelled by The various dyes
Solvent
Matrix

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 analyse the amino acids from the protein. In this method, the silica or the alumina, as a stationery factor, a 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 chromatographic 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, and on or the glass plates. And on this aluminium foil, you are actually going to make a thin film of the alumina or the silicone, and then what you are going to do is? You are going to apply your amino acid which you want to dissolve, 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 it is going to give you the mixture of amino acid and that is how it is actually going to dissolve.

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. If you run a column, 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 gets distributed into the solvent phase and the stationary phase. When it runs, it is actually going to be going to get distributed. So, either it will go with the solvent front.

So, you can see that all the southern molecules are running. So, either so, if this is the molecule either it will go along with the solvent front, or it will actually be going to remain with the silica front. 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 retards 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 onto this, it is actually going to experience.

For example, this spot. So, if you have a spot here, it is going to experience two different types of forces, it is going to have driven force, 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. But whereas it is actually going to make the interaction with these silica particles which are present on this TLC plate, and because of that, they will be retardation forces.

So, that retardation forces are going to be by the metrics molecule, and because of that, it is going to have the two opposite forces. One which is going to be on toward the top side, and the other one is going to be on to the bottom side. So, it is going to be distributed, so and it is going to be immobilized onto the plate.

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Now, suppose the force caused by the mobile phase is Fm, and retardation force by the stationary phase is Fs. So, then the effective force on the molecule will be Fm minus Fs. So, this is what I am going to, I was trying to explain you. So, if this is the spot, on this is spot you are going to have the upward forces which is called as Fm, 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 Fs. So, this molecule is actually going to run effectively by a force, which is going to be Fm minus Fs.

Now, the Fm minus F1. So, where this molecule is going to be stopped, it is going to stop the place where the Fm is going to be equivalent to Fs, and that Fm minus Fs 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 Fm is too big for the FS, 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 Fm is equivalent to Fs.

So, the molecule is immobilized on the silica gel, where the Fm is going to be equivalent to the Fs, 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 Fs. If it is going to increase the Fs, then the Fm minus Fs is going to be small. And if this is the case, then it is actually going to be immobilized towards the spotting 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, if the mobile, if the molecule is very soluble into the mobile phase, then the Fm the value of Fm forces are actually going to go up. And that is why it is actually going to run very far away from the spotting place. Then it also depends on to the thickness of the stationary phase, because that also is going to have the effect onto the retardation forces, and then it also has functional group. So, functional group what is present on to the stationary phase.

So, apart from the functional group, what is present on to the analyte molecule, the functional group, if the functional groups are also present on to the, the silica particles like for example, if you take the silica particle or if you take the functionalized silica particle, they may have the higher affinity for this particular molecule. And as a result, the Fs will actually go up and if the Fs will go up, it is going, the Fm minus Fs 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 sporting points.

So, you can imagine that if I want to see the differences, I 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.

So, solvent runs, the solvent reached to the end of the plates 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 a spot number 2, this is the spot number 3, and the distance of the spot number 1 is d1 and the distance of the spot number 2 is d2, and the distance of the spot number 3 is d3.

So, what I can do is, I can just calculate the Rf values, and Rf value what is the formula is that the distance of the analyte, the distance of the analyte spot, which is like d in this case, so, dx divided by the distance of the solvent, so, distance of solvent like the in this case, ds. And this is going to be fractional. So, the maximum Rf what is possible is 1 and it going to be

the fraction of 1, because this is the ratio, and this Rf value is going to be dependent on to the solvent system, depending on the solvent what you have taken, depending on the metrics material, and depending on the conditions in which it is run.

So, if you are going to maintain all the three constants, if you maintain the same solvent system, if you maintain those same metrics, and if you run it under the identical conditions, the Rf value is not going to be changed. Even if you run it for, for example, if I run it for 50 centimetre it is going to be distributed accordingly. So, that for example, if I have Rf value of 0.5.

So, if I run it for 50 centimetres, The spot is going to be formed as 20 centimetres, by 25 centimetres, if I run it on 100 centimetres, then the spot is going to be formed at 50 centimetres, because it is going to maintain the ratio of Rf is equal to 0.5. So, if Rf is 0.5, which means the ratio of dx to ds is going to be 0.5. So, that does not depend on the how much length you are going to run the TLC plates, it is going to be always be immobilized add the 50 percent distance, if the Rf value is 0.5.

So, this Rf value is constant, and that is why Rf value can be used to characterize the different types of amino acids. Now, the question comes how you can be able to determine the Rf values, you can actually be able to run the TLC plate, that you can be able to run that thin layer chromatography and that is how you can be able to calculate. So, how you can actually be able to run the thin layer chromatography?

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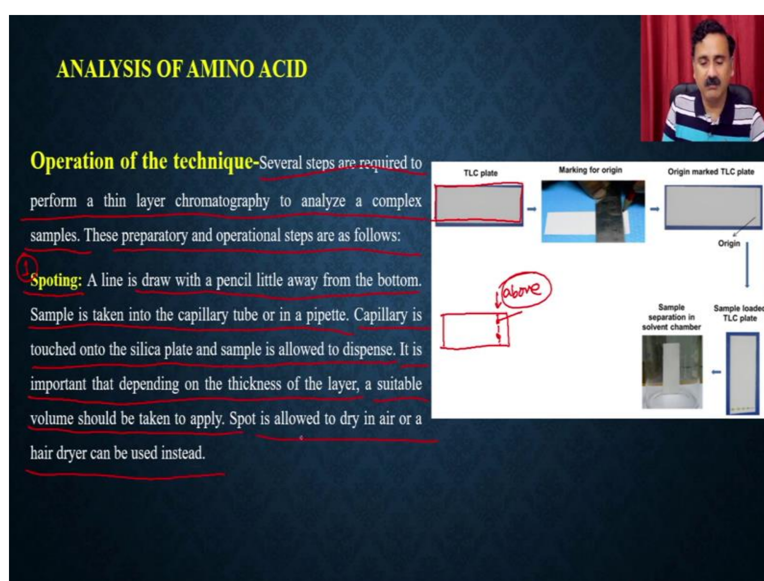

Several steps are required to perform a thin layer chromatography, to analyse the complex sample, these preoperative and operational steps are as follows. So, the step number 1, you are actually going to make the spotting, but before that, you are actually first going to take a thin layer plate. So, what you see here is this is the thin TLC plates.

So, what you are going to do , is was to take the TLC plate, you cut the TLC plate as per the number of sample what you are going to place on the width wise, and its height is also going to be as per the chamber of your TLC plate. So, you are going to run it in a chamber. 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. Because it is going to dip, 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 onto 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, it is actually going to suck the sample automatically by the action of diffusion. 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 hairdryer can be used instead.

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ANALYSIS OF AMINO ACID

Running of the TLC: Once the spot is dried, it is placed in the TLC chamber in such a way that spot should not be below the solvent level. Solvent front is allowed to move until the end of the plate.

Analysis of the chromatography plate- The plate is taken out from the chamber and air dried. If the compound is colored, it forms spot and for these substances there is no additional staining required. There are two methods of developing a chromatogram-

Staining procedure- In the staining procedure, TLC plate is sprayed with the staining reagent to stain the functional group present in the compound. Forex. Ninhydrin is used to stain amino acids.

TLC plate

Marking for origin

Origin marked TLC plate

Origin

Sample separation in solvent chamber

Sample loaded TLC plate

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 depending on the type of the solvent.

So, when you have to cover this with some, for the lids, either you can just put a cap, and why this 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 moment.

After the so, then you are going to place the TLC into this. 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. The analysis of the chromatography plate, the plate is taken out from the chamber and air dried, if the compound is coloured, its formed the spot and these substances, there is a no additional staining required, there are two methods of developing a chromatography.

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 coloured, 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 like the ds. 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 Rf value by dx by the ds.

So, for this the position of the spot is very important. So, if it is coloured 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. But if it is not, then you have to go with the staining procedures. In the staining procedure, the TLC plate is sprayed with the 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 to you can actually go with the non-staining as well.

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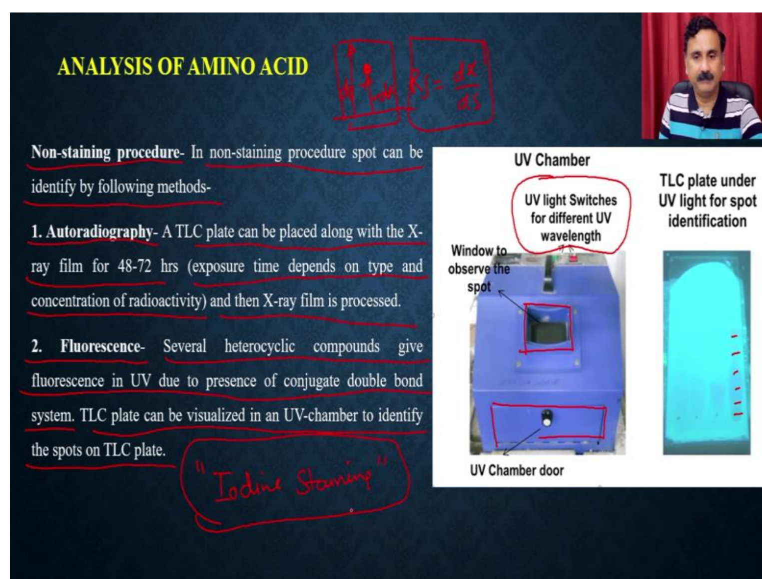

So, you can use the non-staining procedure. In the non-staining procedure, spot can be identified by the following methods, you can use the autoradiography, a TLC plate can be placed along with the X-ray film for 48 to 72 hours. Exposure time depends on the time and the concentration of radioactivity and then the X-ray film based 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 onto the X-ray film. And then you can do all the calculations from this particular spot. For example, you can do the Rf calculation, you can actually do the dx and ds, and that is why you are actually going to get the Rf values, and that Rf value can use for identification of that particular unknown spots.

The second is you can do the fluorescence. So, several heterocyclic compounds give 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, 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 light. 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 eyes. 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 the all the spots are visible, 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. So, you can actually be able to incubate that TLC plate into the iodine chamber, and this iodine chamber is actually going to stain the spots as well.

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PROTEIN

Proteins are polymers of amino acids, joined by the covalent bonds, known as peptide bond. A peptide bond is formed between carboxyl group of first and amino group of second amino acid with release of water. it is a dehydration synthesis or condensation reaction. The peptide bond has partial double bond character due to resonance and C-N bond is not free to rotate. But the bond between N-Cα and C-Cα can be able to rotate through dihedral angles designated by φ (phi) and ψ (psi). These angles can be able to rotate from -180 to +180 with few restriction. The Indian scientist G.N. ramchandran has determined the possible φ (phi) and ψ (psi) for a particular amino acid by synthesizing tripeptide with the amino of interest in the the middle. Based on these calculations, he has constructed Ramchandran plot to define the region of allowed rotation for amino acids present in a protein structure and proposed to use this to validate the 3-D structure of a protein model.

So, the proteins are, as I said, we discussed, 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 have 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 reaction.

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, 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 this 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. Ramachandran 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 tripeptide. So, for example, if he wants to calculate the phi and psi angle for A, he has, he has made a tripeptide with C and B, so you can keep changing these tripeptides. And that is how you can be able to calculate under different conditions what will be the different psi, phi angle is possible.

And, that is how you can be able to make a map between the psi versus phi. So, you can actually be able to make a angle, the map between the psi and phi, and then you can calculate that under different combinations how much the psi and phi angle are going to vary for the A molecule. And that is how you can say that, 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 Ramachandran plot, and that Ramachandran plot is used to define the region of the allowed rotation for the amino acid present in a protein structure.

And he bought, he proposed is that if you can use this particular type of plot to say, whether a salt 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 region. So, that is what you see here is that you are actually going to see the different regions, and it is going to be what you see here is the plot between, the 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, with this brief discussion about the Ramachandran plot and as well as the brief discussion about the amino acid, I would like to conclude my lecture here. In our subsequent lecture, we are going to discuss about the some more property, structural properties of the proteins. So, what we have discussed so far, we have discussed about the biochemical properties of the amino acids, and we have also discussed in detail about how you can be able to analyse the amino acid using that thin layer chromatography.

So, with this I would like to conclude my lecture here. Thank you.