Enzyme Science and Technology
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Module - VII
Enzyme Substrate Interactions
Lecture - 31
Enzyme-Substrate Interactions (Part-I: Chromatographic Methods)

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course Enzyme Science and Technology. And so far what we have discussed, we have discussed about the historical aspects of the development of the field of enzymology, subsequent to that we have also discussed about how you can be able to isolate the gene of your interest.

So, that you can be able to clone that into a suitable vector. And then you can be able to use that for enzyme production. And while you are producing the enzyme, you have different options in terms of the chromatography to purify the enzymes. And once you have the purified enzyme, you can be able to use that or you can actually be able to test that enzyme to catalyzing the different types of reactions.

So, if you recall in the previous lecture, we have discussed about the importance of the enzyme in running the different types of metabolic pathways. So, we have discussed about the carbohydrate metabolism, we have discussed about the lipid metabolism and we have also discussed about the protein metabolisms and all these metabolic pathways, whether they are belonging to the catabolic pathway or the anabolic pathways are tightly controlled by the different types of enzymes.

So, in today's lecture, we are going to discuss about how the enzyme is interacting with the substrate and what are the different techniques you can be able to use to measure the interactions.

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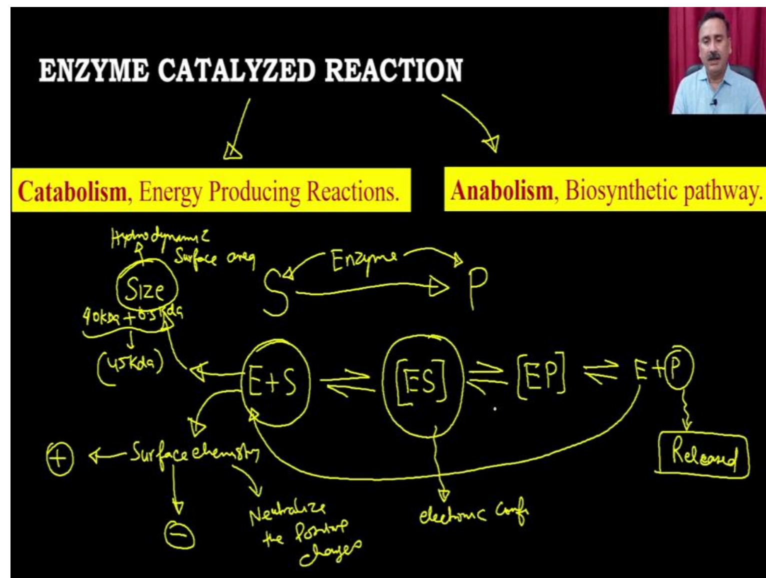

So, if you recall in the previous module, we have discussed about that the enzyme are catalyzing the different types of reactions. These reactions are either falling into the catabolic reactions or the anabolic reactions. But if an enzyme wants to run the catabolic reactions or the anabolic reaction, it has to process the substrate.

So, what exactly the in both of these pathways, what the enzyme is doing is it is converting a substrate into a product, right, with the help of the enzyme. And in this process, the enzyme is interacting with the substrate and enzyme is releasing the product. Now, this enzyme substrate interaction, when it occurs, it actually modifies or it actually changes many things in the enzyme substrates.

So, the first thing what happened is that when the enzyme is interacting with the substrate, if you write this reaction, you will going to say that it is actually going to form the enzyme substrate complex. Then this enzyme substrate complex is actually going to be get converted into the enzyme product complex.

You can have the single strain substrate or you can have multiple substrate, whatever the way, it ultimately the you are going to have the enzyme substrate complex. This enzyme substrate complex is going to be get converted into the enzyme product complex. And then it is actually going to be get converted into the enzyme plus product. And this product is actually going to be released from the enzyme and that is how it is actually going to be produced.

And this enzyme is actually going to return back to the original state and that is how it is actually going to be ready for processing the another molecule of enzyme. So, this means the enzyme when it interact with the substrate, it actually goes through the different types of you know modifications or transitions. So, let us see what are the different types of modifications it actually brought, right.

So, in the enzyme when it interact with the substrate, it actually changes the size, right. So, because if you can imagine that the enzyme is probably will be 40 kg right and the substrate could be of maybe like 0.5 kg. So, if it is like that, the ultimately it is going to be 45 kg when the substrate is reacting. So, it actually going to change the size Not only the size it actually going to change the hydrodynamic surface area, ok.

So, it is actually going to change the size in terms of increasing its diameter. So, it is actually going to change the hydrodynamic surface area. Apart from that when the enzyme are substrate are interacting with each other, it is actually going to change the surface chemistry. So, it is actually going to bring the either the positive charge or it is actually going to mass.

So, it actually can bring the positive charges into the enzyme or it actually can bring the negative charges in the enzyme or it may sometime actually going to neutralize the existing positive charges, ok. Apart from that, the enzyme substrate interaction is also going to because when the enzyme and substrate is going to interact with each other, it is actually going to form the enzyme substrate complex.

And this in this traditional state, the electronic configuration of the enzyme is also going to be changed, right. So, it is going to have the, because when the substrate is binding, it is actually going to have the unstable state. So, it is actually going to have the different types of the interactions, right.

And so, this is all about the, how the substrate is reacting and interacting with the enzyme, but the central question is that how and why the enzyme is processing the substrate and what are the different factors which are governing the interaction of the enzyme with the substrates.

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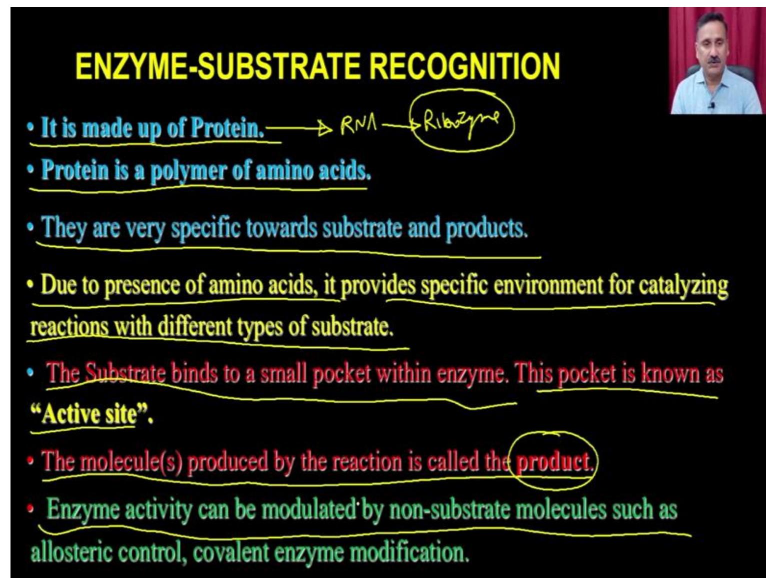

So, as we said, you know, in the past also that enzyme is made up of the protein. A minor fraction of the enzyme is also made up of the RNA which are called as the ribozymes. And we are not dealing with the any type of inter discussion about this class of enzymes. Protein is a polymer of amino acids and they are very specific towards the substrate and the product, right? As we discussed, right?

Just now that the enzyme is recognizing the substrate. Due to the presence of amino acid, it provide the specific environment for catalyzing the reactions with the different types of substrates. And the substrate binds to a small pocket within the enzyme. This pocket is known as the active site. The molecular, molecules produced by the reaction is called as the product, right, enzyme activity can be modulated by the non-substrate molecules such as the allosteric molecules as well as the covalent modifications.

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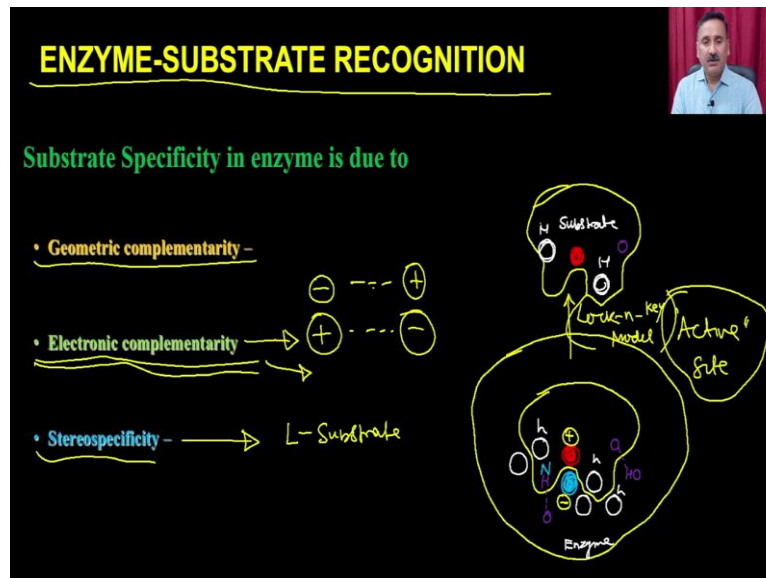

And how the enzyme substrate recognition works, right? Because before the enzyme is going to interact with the substrate, it has to recognize the substrate, right. So, you can imagine that if this is a substrate and this is the enzyme, it is actually going to react at a very small portion of the enzyme which is called as the active site.

So, active site is very, very active in terms of recognizing the substrates. And you can see that the substrate is nothing but the three-dimensional structure, right. So, it is actually going to have the some of the positive charges, could have the hydrogen bonding donors, it could have a hydrogen bonding acceptors and all that.

So, you can see that these are the hydrogen bonding donors and acceptors, right? And you can have the positive charges; you can have the Van der Waal interactions and so on. So, first thing what it actually going to decide whether the enzyme is going to recognize the substrate is the geometric complementarity, which means the 3D structure or the substrate is matching with the 3D structure of the pocket of the active site or not, right.

And that is the basis of the lock and key model, right. Although some in some cases, this is not true, but in majority of the cases, the enzymes are actually having a pre defined 3 dimensional structure and that is going to be recognize the substrate. So, you can imagine that the there are could be many types of substrate molecules which may be matching with one portion of this 3 dimensional structures and that is how it is actually going to recognize.

So, a typical 3 dimensional structure will actually go and fit into this cavity. But that is not enough. It is actually should have the electronic complementarity also. This means it should have the different types of groups. So, for example, the positive is actually going to have the interaction with the negative and similarly the negative will have an interaction with the positive, right.

So, that is going to be also there true. That is also should match because there could be a possibility that you may have the similar kind of 3 dimensional structures, but it may not map the interaction. It may not map the electronic complementarity. So, for example, in this case, you have a hydrogen donor, right?

In this case, you have the hydrogen donor and this is actually the hydrogen acceptor. So, when this substrate will fit into this particular cavity, this hydrogen donor and this hydrogen acceptor are actually going to complementarity to each other and that is how they are actually going to form very strong bond.

Similarly, you have you know, the negative charges, you have the positive charges. So, if you have positive charge, it is actually going to meet with the negative charges, right. And the similarly, you can have the hydrophobic interactions, so, you can have the hydrophobic patches on to the substrate and that also will match it with the hydrophobic patches.

So, electronic complementarity is very important because it is actually going to give you the affinity parameters. It is actually going to distinguish even the closely related the molecules. For example, if we have if we talk about the hexokinase and glucokinase, hexokinase has a very relaxed specificity.

So, it actually can recognize the different types of substrate, whereas, the glucokinase is only specific for the glucose. So, it is only going to have the electronic complementarity only for the glucose, not for the other sugar molecule whereas, in the case of hexose kinase, it is going to have the electronic complementarity for the many type of sugar molecules.

And then we have the stereospecificity. So, if stereospecificity is also going to enzyme, is going to, you know, prefer a particular type of stereoisomer in the substrate. So, mostly the VR actually going to work with the L type of substrate rather than the D type

of substrate because the L type of molecule or isomeric molecules are mostly being present.

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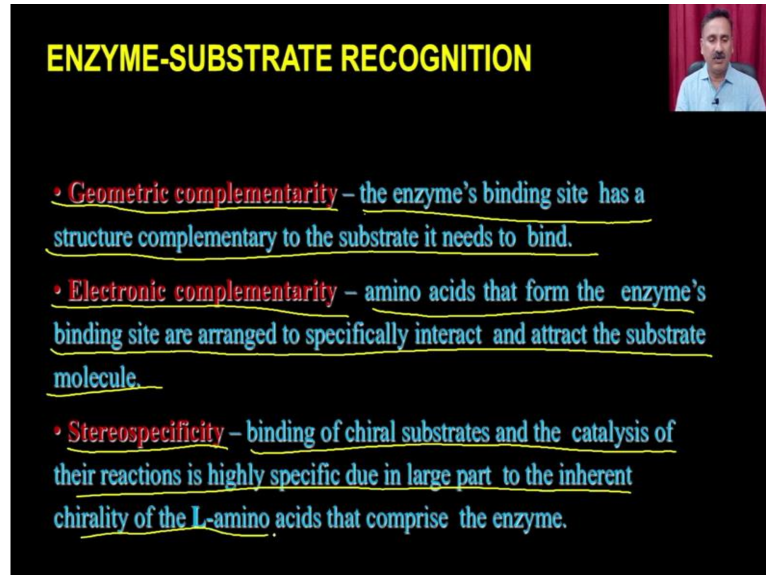

So, this is what I have given in the next slide that geometric complementarity, the enzyme, is actually binding site has a structural complementarity to the substrate. It needs to the bind. Then we have the electronic complementarity. So, amino acids that are formed the active site or the enzyme binding site are arranged to specifically interact and attract and substrate molecules.

And then we have the stereospecificity. So, the binding of the chiral substrate and the catalysis of their reaction is very highly specific due to in large part of the inherent chirality of the L amino acids that comprise the enzymes. Now, the big question comes that how the enzymes substrate are interacting and what are different modifications actually brought into the enzyme structures?

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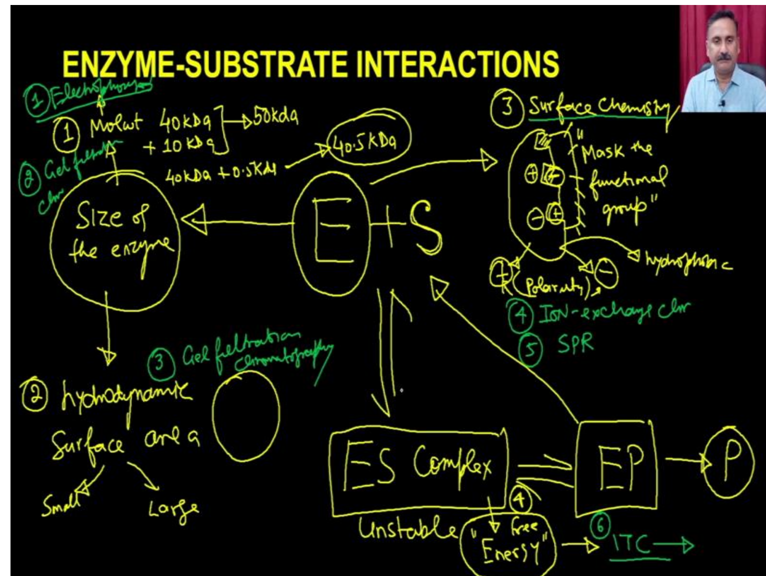

So, you can imagine that if an enzyme is there and it actually interacts with the substrate, it actually brings a lot of changes into the enzyme structure because when its enzyme is interacting with the enzyme structure, it is actually forming the enzyme substrate complex, right. And the enzyme substrate complex is an unstable state.

So, that is why it is actually going to get breakdowns and it is actually going to form the enzyme product because enzyme substrate complex is a very unstable entity. So, that is why the substrate is going to be get converted into products and this is also unstable. So, that is why ultimately the product is going to be released and the enzyme it is actually going to be reversed.

It will actually go back to the you know enzyme is going to be free, right that is why it is actually going to be interacting with the substrate molecule. Now, the big question comes that when the substrate is interacting with the enzyme, what are the different changes it actually broughts into the enzyme structure?

So, the first change what you see is that it is converting the enzyme into enzyme substrate complex. This means it is actually going to increase the size of the enzyme. So, size of the enzyme right size in terms of the molecular weight right which means it actually going to increase the molecular weight and it is more relevant when you when the enzyme is interacting with a proteinaceous substrate.

For example, if enzyme is 40 kDa and it is interacting with another proteinaceous substrate. For example, with 10 kDa then when it is actually going to form the enzyme substrate complex, but together it is going to be 50 kDa. Even for the small molecule also for example, if the enzyme is 40 kDa and it is interacting with a substrate of 0.5 kDa the change may not be very high, but it is still be going to the change the enzyme molecular weight.

Size in terms of also going to be change in the size of the hydrodynamic surface area. So, which means it is actually going to increase the size of the enzyme, ok. So, it is going to be a lower size, it can actually be able to change the hydrodynamic surface area. It can make it small or it can be able to make it large. So, it can actually make the enzyme little unfolded it can actually make the enzyme more compact.

So, the first modification is that it actually can increase the size of the protein. So, it is actually can increase the molecular weight it also can change the hydrodynamic surface area. The third thing is that enzyme is made up of. So, imagine that this is the enzyme which has the different types of active site right different types of groups right and it may have the positive groups, it may have negative groups and so on.

So, when the substrate is actually going to bind this site, right. For example, if this is a substrate which is actually going to bind and it has negative charges it has positive charges and so on. It is actually going to change the electronic configuration of this enzyme. So, it is actually going to mask the it is actually going to mask the functional groups right or functional charged groups or functional groups on the enzyme. So, it is actually going to change the mask of the functional group.

This means it is actually going to change the surface chemistry. So, the third modification is that it is actually going to change the surface chemistry of the enzyme and that could be in terms of the it may it bring the additional positive charge, it can actually be able to bring the additional negative charges, it can actually bring the it can bring the hydrophobic groups, right.

Because now what you see here is that this is an enzyme earlier it was having a positive group it has having a negative groups. Now, when the substrate is binding it is actually going to exhibit all the charge what is being present on the enzyme. So, if an enzyme is you know hydrophilic on one side and the hydrophobic on the other side you will can

imagine that earlier the surface of the enzyme is positively charged or negatively charged.

It means it is polar in group, but once the enzyme is binding this particular substrate its maybe getting converted into a hydrophobic surface. So, it is actually can have the positive and so, it actually can change the polarity of the molecule right either can bring the positive charges or it can bring the negative charges or it can actually bring the hydrophobic groups.

Apart from that when the enzyme is interacting with the substrate it also changes the energy level of the enzyme. So, it is actually going to bring lot of changes in the free energy of the molecule, right. So, it is actually going to change the free energy and all these properties. So, so, the first is its going to change the size. So, molecular weight it is going to change the hydrodynamic surface area.

So, it is going to have the increase in size or decrease in size. The third is its going to change the surface chemistry and the fourth it is actually going to play with the free energy of the molecule. So, it is actually going to have the modulation of the free energy as well. And all these properties can be studied with the help of the different types of different types of techniques.

For example, with if you want to know whether the molecular weight is going up or down you can be able to do like the electrophoresis right if and you can also if you are going to know whether the molecular weight is changing or not, you can actually be able to do the electrophoresis, you can also be able to do the gel filtration chromatography and so on.

Similarly, if you want to see whether the hydrodynamic surface area is changing or not then you what you can do is you can use the third technique and that is called as the gel filtration chromatography right. So, you can actually be able to use the chromatography techniques. Similarly, if you want to see the changes in the surface chemistry then you can actually be able to choose.

You can actually be able to use the ion exchange chromatography because ion exchange is going to be ion exchange chromatography or you can be able to use the SPR or the Surface Plasmon Resonance, right. And when you are expecting that there will be a

change in the free energy you can be able to do the many change many type of technique.

But you can use what we are doing discuss in this particular course is the isothermal calorimetry because you can be able to measure the energy of the complex. You can be able to measure the energy of this free enzyme and if there is a change you can be able to say that the enzyme is interacting with the substrate. So, let us discuss how we can be able to exploit some of these techniques and you can be able to use for studying the enzyme and substrate interactions.

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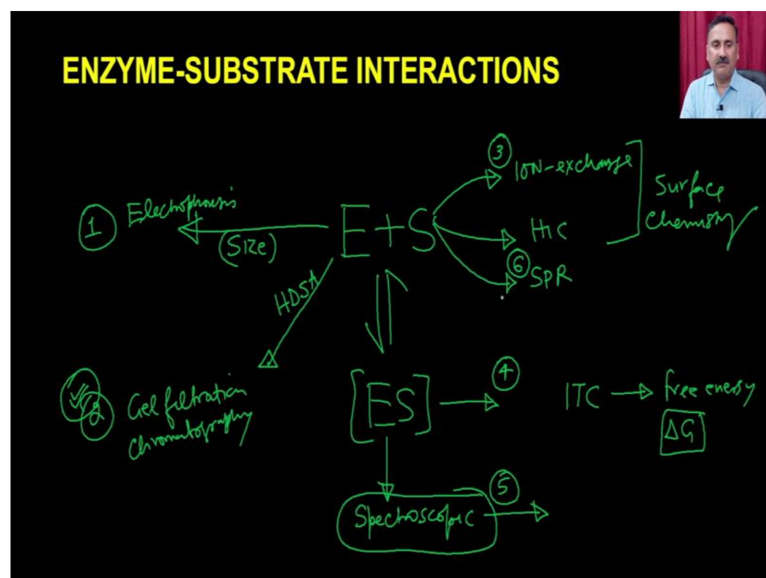

So, enzyme substrate interaction as I said you know enzyme is going to interact with the substrate and it is actually going to use and form the enzyme substrate complex. And in this process, it is going to have increased in size. So, if you have the increase in molecular weight, you can be able to use the electrophoresis.

So, electrophoresis is one technique that you can use to see whether there will be an increase in size or not right so, there will be an increase in size so, size is one parameter right. And the second is you are going to see whether the gel filtration chromatography is. So, gel filtration chromatography can be used right. So, gel filtration chromatography can be used for measuring the hydrodynamic surface area right. So, you can actually be able to see how much is the size of that ball, right.

And then third is you can actually be able to use the ion exchange chromatography in case you want to expecting or you can actually be able to use the HIC in case you are expecting that there will be and you can also use the SPR in case you are measuring change in the surface chemistry of the molecule and the surface is interacting.

And the fourth is you can also be able to measure with the help of the ITC and you can actually be able to measure the free energy of the molecule and you can be able to calculate what will be the change in delta G and that is how it is actually going to tell you whether the substrate is interacting with the enzyme or not.

So, let us start with the first technique and the first technique is how you can be able to use the chromatography technique. In this particular aspect you can also be able to use because you know the when the enzyme and substrate are interacting with the enzyme and forming the enzyme substrate complex it also going to bring the changes in terms of the spectroscopic parameters right.

So, it is actually going to bring the additional variations in terms of the pharmacophores. Because you know that as we discussed right and when the enzyme is interacting with the substrate it is actually going to change the surface chemistry and that may actually be able to change the electronic configuration of the molecules and that can be mapped even by the spectroscopy.

So, that is also going to be one of the technique what we are going to discuss. So, the first technique is this, second is this, third is ion exchange chromatography, fourth is ICT and the fifth is spectroscopy and the sixth is SPR. So, let us start with the chromatography.

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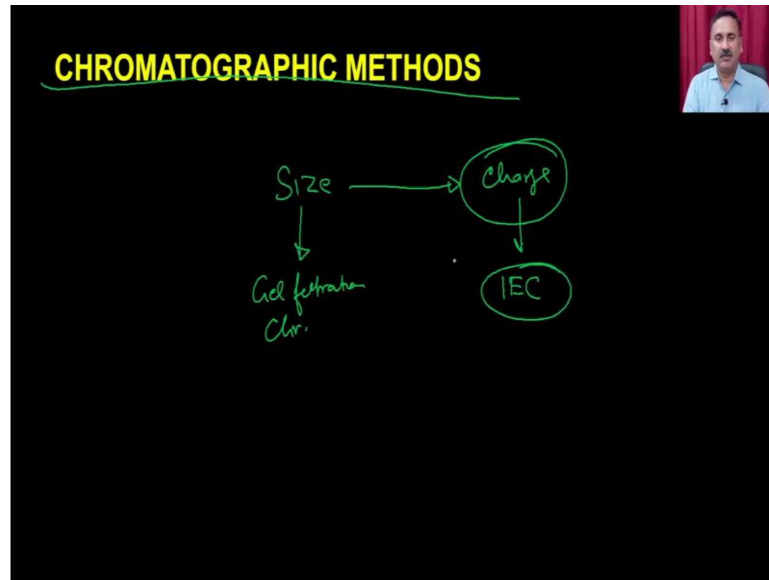

So, in the chromatography we can have the two chromatography technique because we want to measure the size and we can also be able to measure the charge chemistry or the surface chemistry right. So, we also can measure the charge. So, size we can measure with the help of the gel filtration chromatography and charge we can actually be able to do with the ion exchange chromatography.

So, let us start with the charge and how the charge can be used and exploit with the help of the ion exchange chromatography to measure the or to map the enzyme substrate interactions.

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So, for explaining this I have taken an example of the enzyme which is interacting with DNA. So, you can imagine that in this case the enzyme is having the positive charges and the DNA is having the negative charges and that is how they are interacting with each other.

So, in this particular so, DNA enzyme interaction so, in this approach the anion exchange matrix is incubated with the DNA and allow it to bind the DNA tightly. Now, the pure protein is passed through the DNA bound beads followed by the washing with the buffer to remove the unbound protein. Now, the DNA is eluted from the matrix either by adding the high salt or with the denaturating conditions.

Now, the fractions are tested for the presence of DNA and protein and the eluted protein is analyzed in SDS page and DNA in the agarose. So, this technique what we are talking about is more of a qualitative technique so, that you can be able to say whether this particular enzyme is interacting with the DNA or not.

You will not be able to measure or you will not be able to calculate the affinity parameter which means I am saying that you will not be able to measure the dissociation constant so, that constant Kd you will not be able to calculate. So, what you are going to do is you are going to take the anion exchange matrix into a column and then you are going to pass through the DNA.

So, when you pass through the DNA the bead is actually going to have the DNA molecule which is bound right. Because the bead is positively charged so, it is actually going to bind the DNA molecule. So, these are the DNA molecule right. And when you are adding the enzyme what the enzyme is going to do is it is actually going to have no affinity for the beads because enzyme is also positively charged right.

And you the and the bead is also positively charged. So, enzyme will not bind that beads, but enzyme will bind the DNA which is negatively charged. So, as a result the enzyme is going to bound to the DNA. And then what you are going to do is you are going to elute with the high salt. So, in the high salt presence the DNA is going to be eluted from the beads and that is how you are going to have the DNA protein complex which is going to be eluted.

And when you are going to analyze this onto SDS page it is actually going to an agarose it is actually going to give you the pattern of the protein and it is also going to give you the pattern of DNA.

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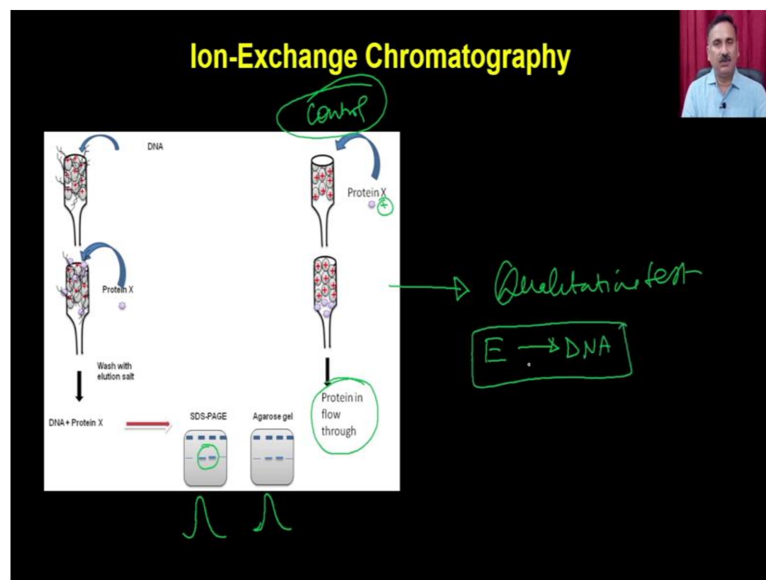

So, when you have that what you are going to see is that the pattern of elution of the protein is like this right. So, it is eluting and then you see this is actually eluting more in the center right. Similarly, the DNA is also going to elute in the same pattern right. So, it is going to be elute in the same pattern and since their pattern is matching that will say that the protein is binding to the DNA.

Now, as a control you are also going to run the protein onto the these positively beads. Since the protein is already positively charged, it will not going to bind the column. So, this is positively charged protein which you are adding into the anion exchange column it will not going to bind and it will come out.

So, that will be a control experiment to ensure that the protein itself is not binding to the matrix and that is how you are actually going to get a qualitative test whether this particular enzyme is interacting with the DNA or not ok. So, once you are done with this you can be able to use the more sophisticated techniques to measure the other kinds of kinetic parameters and you can be able to measure the other like affinity parameters.

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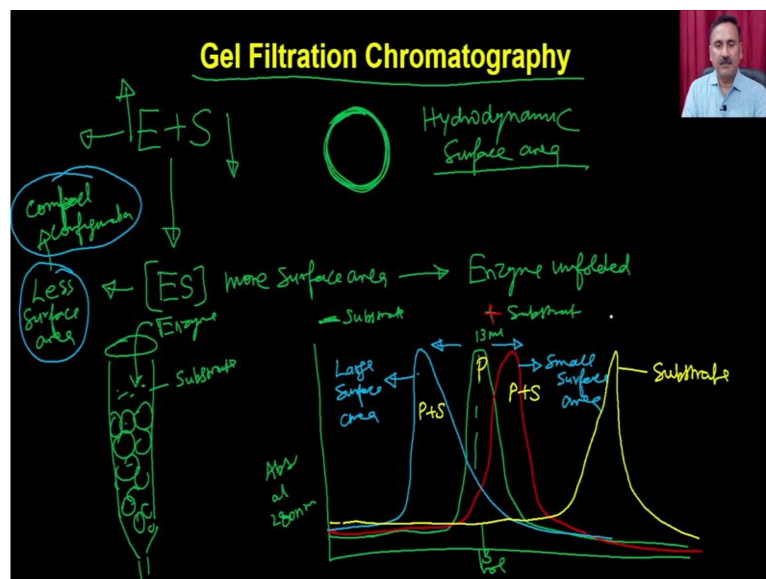

Now, let us move on to the gel filtration chromatography and I hope that you still remember that the what is the principle of gel filtration chromatography right. So, the gel filtration chromatography is basically separating the molecule based on the hydrodynamic surface area right.

Which means it is going to measure how much is the water is associated with the surface and this actually can be a very very robust tool to measure when the enzyme is interacting with the substrate it actually can bring the two different types of changes. It actually can increase the surface area or it actually can decrease the surface area.

So, it actually can increase the surface area or actually can decrease the surface area, which means when the enzyme is interacting with the substrate it can actually make the enzyme substrate complex right and that may have more surface area. This means the enzyme is going to be get unfolded then only it actually can have the larger surface area or in some cases it actually going to have the less surface area.

And when it is going to have less surface area the enzyme is going to be get more folded or I will say it is going to acquire a compact configuration. Now, how we can actually be able to study this. So, what we are going to do is we are going to prepare a column right. So, we are going to prepare a gel filtration column right with the beads right and you are going to analyze your sample right.

So, first you are what you are going to do is you are going to analyze the enzyme and while you are analyzing this buffer what you are adding and what you are using for equilibration can actually have the substrate right.

So, you can actually be able to do this experiment in two ways. One without substrate and the other is plus substrate. So, first you can just analyze the enzyme without substrate and when you do the when you see the chromatogram it is a chromatogram would be look like this So, this is the volume this is the absorbance at 280 nanometer right and initially you are going to just do without substrates ok.

So, when you do the without substrate its actually going to give you a peak like this ok imagine that if this peak is around 30 ok. So, it is this is a peak at 13 ml ok. Now, when you changed the substrate so, if it is in the presence of substrate. So, when you added the substrate, it actually can go in both the direction ok.

So, it actually can go either like this and can show you this way or it actually can go in the reverse direction and it can actually be able to show you a peak in this direction ok. So, ok now the red curve what you see this is the; this is the normal this is the enzyme right this is the enzyme without substrate and this is the; this is the substrate when it is making it more towards this right.

So, this is small surface area. So, this is actually going to be small surface area and this is going to be large surface area. So, basically when the enzyme will actually will make the you know get unfolded it will move in this direction ok and when the enzyme is going to

be more compact it is actually going to be in this direction. So, many substrates are you know behaving the enzyme with a different way ok.

So, it is very unpredictable it is very difficult to say whether the substrate will shift the enzyme in right direction or whether it will shift the enzyme in the left in direction, but majority of the substrate when they form the enzyme substrate complex, they actually make the surface area more less. So, it actually make the structure more compact rather than more unfolded, but there are examples where it actually also unforce the enzyme and it becomes more and more it becomes larger surface area.

Apart from this you also suppose to run the substrate alone because that also should tell you that whether the substrate is interacting or whether the where the substrate is eluting. So, if it is a substrate is eluting in a very very distant place next we had nothing to do with the enzyme then you are actually be very confirm about this particular experiments.

So, if you if I recall again that you are actually going to have the first you are going to run the protein. So, this is the protein this is the protein plus substrate this is also protein plus substrate it can go in either direction if the structure is going to be compact it will go in this direction if the structure will be less compact or I will say will acquire the larger surface area then it will actually go in this direction. And by this you can be able to calculate the surface area.

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Because the distribution coefficient has the relationship of the between the elution volume and the void volume so, the molecular size of a protein is related to the shape of the molecule and the relationship between the molecular weight and the radius of gyration is that R g is M to the power a where, a is a constant and depends on the shape of the molecule.

So, a is 1 which is for rod a is 0.5 for the coil and a is 0.33 for the spherical molecules. So, when you are trying to do this experiment, you have to do parallel experiment to determine the molecular size right. So, this is just the qualitative test right you can also be able to very precisely be able to calculate the molecular sizes.

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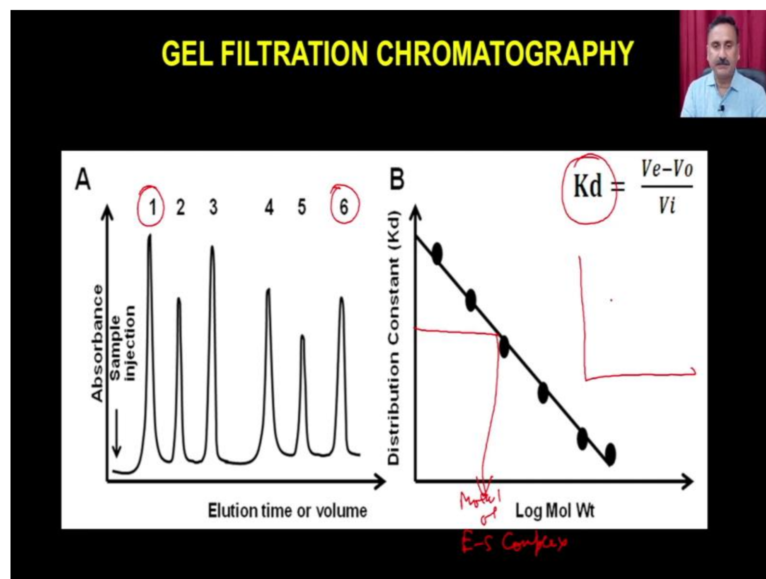

So, you can actually be what you are going to do is you are actually going to run the different proteins of the different sizes. So, you can actually have the larger protein and the smaller protein and all the 6 protein you can run. And then you are going to have the Kd versus log molecular weight as the calibration curve.

So, you can actually be able to run the calibration curve and that calibration curve you can be able to use to calculate the molecular weight of the enzyme substrate complex right. So, in this case the enzyme if you when you do the substrate interactions its enzyme is actually going to move either in the left hand direction or the right hand directions.

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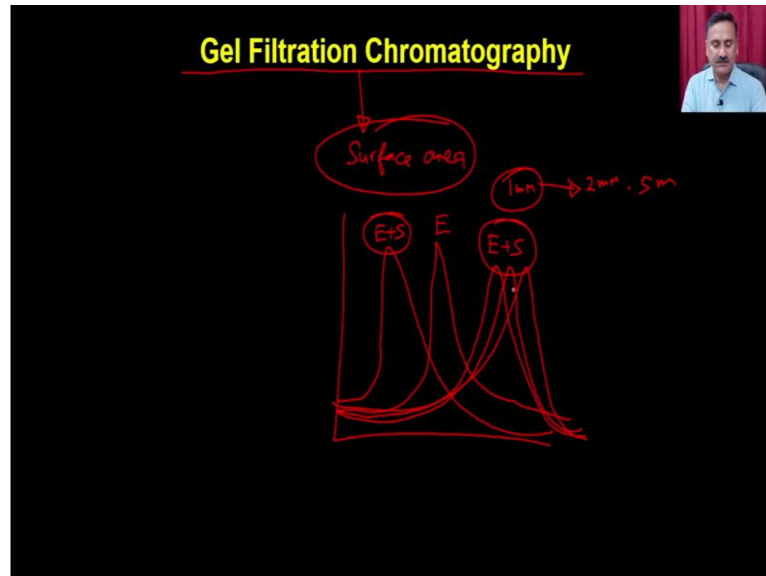

So, this is all about the gel filtration chromatography how you can be able to use the gel filtration chromatography for calculating the for monitoring the enzyme substrate interactions. And what we have said is that the enzyme is since the gel filtration is working on the surface area of the molecule you can be able to have the two possibilities when you have the enzyme. Enzyme is actually going to run like this when you have the sub.

So, this is the enzyme and when you have the substrate it actually can move in this direction or the substrate can actually be able to move in this direction. So, this is the enzyme plus substrate complexes. So, either of these conditions you can be able to have the modification you can have the mapping whether the this is actually been done with the substrate.

Because if this peak is true and suppose the you have taken the substrate as 1 millimolar right what you can do is you can increase the substrate concentration from 1 millimolar to 2 millimolar and then 5 millimolar and so on. So, when you do that, it is actually going to keep shifting in this direction. Ultimately it is actually going to get saturated at one point and that is how it is actually going to give you the clear idea that the enzyme is this shifting is because of the enzyme substrate interactions.

So, this is all about the how you can be able to use the chromatography to measure the enzyme substrate interactions. In our subsequent lecture we are going to discuss more

about the how you can be able to use the other techniques like the spectroscopy techniques or the ITC or SPR and as well as the electrophoresis to measure or to monitor the enzyme substrate interactions.

Thank you.