Enzyme Science and Technology
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Module - IX
Enzyme Inhibitor Designing
Lecture - 39
Inhibitor Designing (Part-I: Traditional Approach)

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 how you can be able to do the enzyme assay in our previous module and how you can be able to study the enzyme kinetics.

And if you recall in the previous lecture, we have also discussed how you can be able to calculate the (Refer Time: 01:15) constant and other catalytic, vertically relevant parameters. So, in today's lecture, we are going to discuss about how you can be able to design the different types of inhibitors for the enzyme and what are the different approaches people are using.

Before we start this lecture, it is important to know that we are not going to extensively going to discuss about the, you know, the detailed procedure of the different approaches. What we are planning to do is we are just going to give you the brief idea. And so, that you can be able to get familiar with the different approaches and that is how you can be able to use them.

So, when we say about the inhibitors, right, inhibitor is a molecule which actually reduces the enzyme activity or it actually abolishes the enzyme activity. So, when you want to design the inhibitor, you are actually going to choose or you are actually going to set the different types of parameters to say that this particular molecule is going to be the inhibitor for this particular enzyme.

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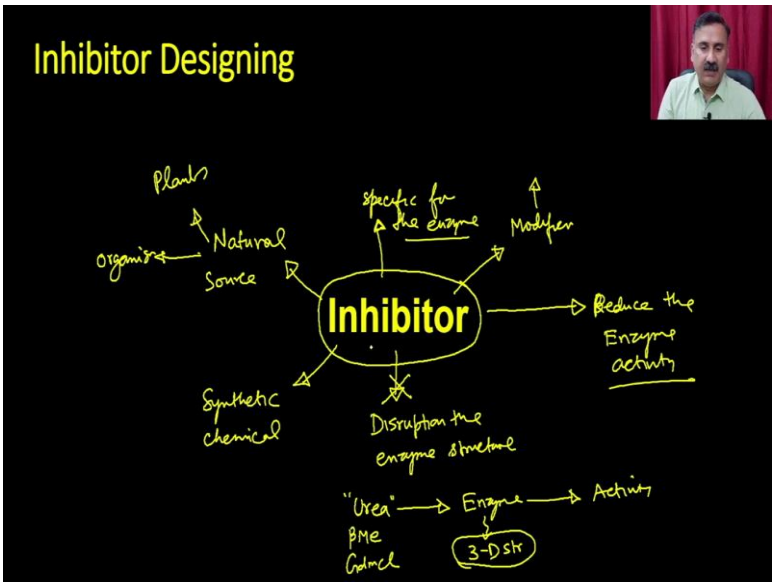

So, when we talk about the inhibitor, inhibitor is actually going to be a chemical molecule. It can be either from the natural sources or it can be from the synthetic sources. The first thing what is important about an inhibitor is that it is actually going to block or it is actually going to reduce the enzyme activity.

Now, when we talk about the inhibitor, that reduction in the enzyme activity should not be because the enzyme inhibitor is actually disrupting the enzymic structure. So, this is undesirable, ok. This means you cannot have an inhibitor which actually disrupt the enzyme structure.

For example, if you take the urea, for example, right. If I add a urea to an enzyme right, irrespective of whether the urea is going to be a inhibitor for the enzyme or not, it is actually going to destroy the activity, ok. It is actually going to destroy the enzyme activity because enzyme is no longer be able to maintain the 3D structure.

Ah Same is true for the other kinds of the denaturants. For example, you can have the beta mercaptoethanol or you can have the Gdmcl. So, all these kinds of you know the enzyme structure disruptors should not be considered as a inhibitor, ok. Now, inhibitor is actually going to reduce the enzyme activity. Inhibitor could be of the from the natural sources or they could be from the synthetic sources.

So, you can actually be able to synthesize the enzyme. So, they can be synthetic chemical molecule or they can be from the natural sources. When we talk about the natural sources, it could be either from the plants or it could be from the different types of lower organisms, right.

Some of the marine sources can actually be able to give you the molecule which are actually going to serve as an inhibitor. For example, many of the snakes, venom are also going to be considered as the, you know could be a source of inhibitor and all that or like scorpions poison and all that. Inhibiter is actually going to be, it should be very specific, right. So, it should be specific for the enzyme. So, it should not be a generic inhibitor like it should not be a amino acid modifier which is actually going to be an inhibitor.

So, it should not be a modifiers, right. So, for example, right if you cannot have a modifier like for example, if you have see some of the same is true like a beta mercaptoethanol. So, beta mercaptoethanol is actually going to, you know, change the disulfide linkages and other kinds of things.

Same is true for the other kinds of modifiers which are actually going to change the functional groups and all that. So, they are not going to be considered as a inhibitor. And when you want to design an inhibitor, you are actually going or you would like to start designing the inhibitor.

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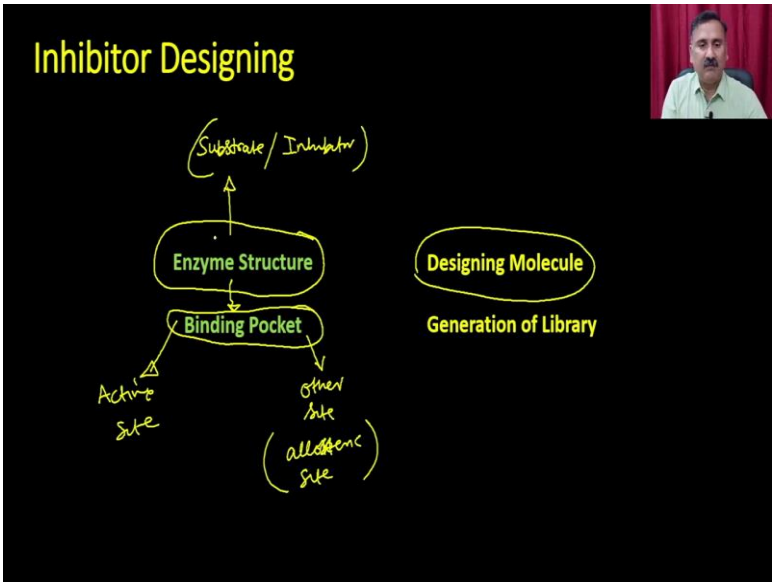

You have to consider the two parties, right. Or you are actually going to consider the two components. One, you are actually going to focus about the enzyme structure, right. And the other is you are actually going to design the molecule based on that, right. When you talk about the enzyme structures, you are actually going to see about the binding pocket. Now, binding pocket could be different, right.

So, you will see that when we were going to discuss about the different types of inhibitors, you will understand that the binding pocket could be the pocket where the substrate is binding. So, that pocket is called as the active site or the binding pocket could be the other site ok, or that is called as the allosteric site.

So, so it could be either the active site or the allosteric site. And that you will understand what you mean by the allosteric site and active site. So, before getting into the, what are the different types of enzyme structures you require and other things, we it is important to know that how the enzyme is actually recognizing the substrate or the inhibitor.

And what are the different types of models are available to explain the interaction of the enzyme with substrate and inhibitor. So, that it will help you to design the different better inhibitor.

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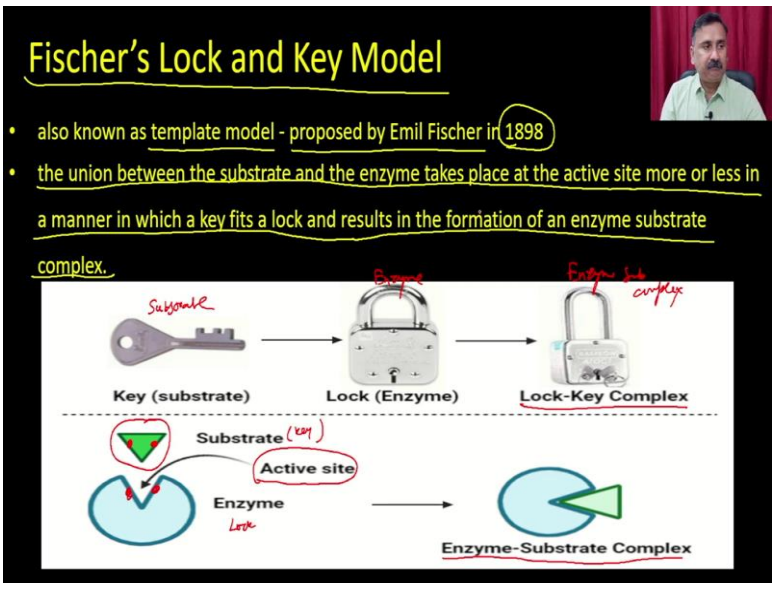

So, there are two models of explaining the how the enzyme is interacting with the substrate. One is the Fischer's Lock and Key model right, which is more very very

popular, right. So, Fischer's Lock and Key model is also known as the template model or and this template model or the Lock and Key model is proposed by the Emil Fischer in the year of 1898, right.

And what it says is that the union between the substrate and enzyme take place at the active site more or less in a manner in which key fits into a lock and results into a formation of enzyme substrate complex. So, this is what it is actually going to say, right. So, it says that you can imagine that you have a key, right.

So, every key is actually having its corresponding lock, right. So, you can actually not, you cannot be able to use the alternate key for this particular lock, right. So, only this particular key is actually going to fit into the lock and that is how it is actually going to give you the lock and key complex.

Why it is happening because the every key or the key, in this case the key is actually behaving as a substrate whereas, lock is actually precipitated by the enzyme and this is actually going to be called as enzyme substrate complex. So, every key which is substrate is actually going to have its well-defined three dimensional structure and this three dimensional structure is actually going to fit into a well-defined three dimensional active side, right.

So, this active side is or this is actually the lock and this is actually the key, right. And because they are actually complementary to each other, they are actually going to make a complex and that is how they are actually going to make the enzyme substrate complex.

Apart from this, if you recall when we were discussing about the how the enzyme is recognizing the substrate. So, it also requires the different, the compatibility of the different types of functional groups and it also requires the compatibility of the substrate in terms of the isomerise, isomorphism or so.

So, for example, you can have the some of the groups which are present on to the active site on to the substrate. And these groups should actually match with the groups on to the active site and that is how they will actually going to make a tight complex and that is how they are actually going to form the enzyme substrate complex. So, this is the model what is being what is being proposed by the Emil Fischer in the year of 1898.

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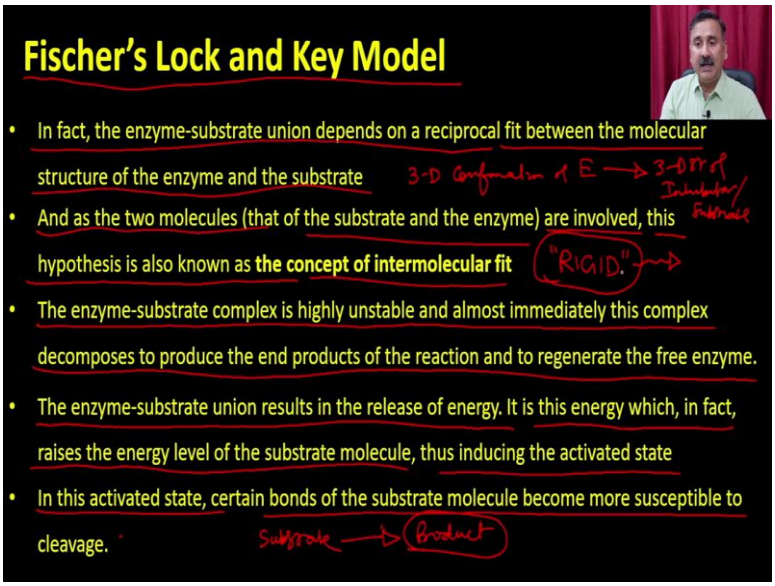

So, what it says also is in fact, the enzyme-substrate union depends on the reciprocal fit between the molecular structure of the enzyme and the substrate which means the three dimensional conformation of enzyme will match to the three dimensional structure of the inhibitor or substrate, ok.

So, as the two molecule that is the substrate and the enzymes are involved, this hypothesis is also known as the concept of intermolecular fit, right. The enzyme substrate complex is highly unstable and almost immediately this complex decomposes to produce the end product of the reaction and to regenerate the free enzyme which means as soon as the enzyme and substrate are making a complex.

The intermolecular rearrangement occurs and that is how the enzyme substrate is getting converted into the product and that product is getting going to be released from the enzyme. And then this free enzyme is actually going to bind the new set of substrates.

The set substrate enzyme substrate union results in the release of energy. Is this energy which in fact, raises the energy level of the substrate molecule and thus inducing the activated state, in this activated state the certain bonds of the substrate molecule becomes more susceptible to the cleavage and that is how the substrate is actually going to be get converted into the product.

Now, remember that as soon as the substrate is getting converted into the product, the product will not going to have the exactly the identical three dimensional structure as the substrate. And that is how the product is actually going to have the lower affinity to into the active site and that is how the product is actually going to be released from the enzyme and it will actually free the enzyme and that is free enzyme is actually going to start the binding the new substrate and that is how this cycle will continue.

What is the basic problem in this particular lock and key model is that its it actually expect that the active site or the binding site is actually going to be very rigid which means it does not have any kind of flexibility. And you know that rigidity is not actually been there. Because there are so much conformational changes there are so much you know movement of the side chains of the of the residues what are present in the active site and that is how it is actually catalyzing the different types of reactions.

I am sure you might have seen when we were talking about the how the enzyme is catalyzing the different types of reactions, you might have seen that there are. So, many active site residues serine, thionine and all that and their movement is actually driving the changes into the substrate and that is how the substrate is getting product and changes into the product and all that.

So, rigidity the concept of x is your assumption that the active site is a rigid body was one of the major drawback of this particular lock and key model. So, it was actually very very you know attractive it was you know explaining many of the phenomena and all that, but it was not been able to explain why the active site should be very rigid.

Because if it said it is rigid it will not going to facilitate the movement of the residues and all that. So, that is why to explain to make it more comfortable to explain this particular phenomena the another model is being produced and that is called as the Koshland's induced fit model, ok.

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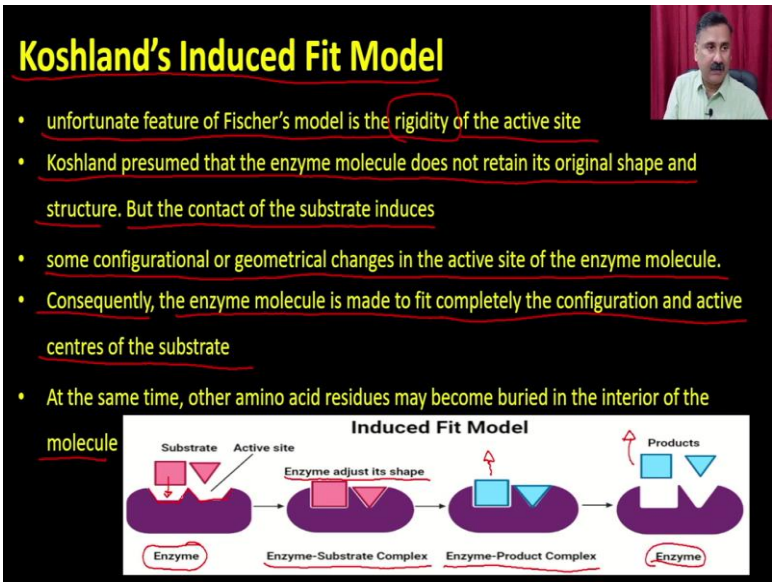

So, what is Koshland's induced fit model is that so, unfortunate feature of the Fischer model is the rigidity of the active site which means it says that active site is a three dimensional confirmation and it remain like that and it should not change, but that is not the case, right.

So, what Koshland Koshland presumed that the enzyme molecule does not retain its original shape and structure, but the contact of the substrate induces some conformational configurational or the geometrical changes in the active site of the enzyme molecule. Consequently, the enzyme molecule is made to fit completely the configuration and the active center of the substrate.

At the same time other amino acids are tube may become buried in the interior of the molecule. So, this is what it says that it says that the enzyme is not having the predefined three dimensional structures or the predefined three dimensional binding site. So, you can imagine that this is actually the active site which is very relaxed in terms of the binding site and you will see that there are two different types of substrate or the substrate is having the two different types of groups one is square another one is triangle, right.

So, when the substrate approaches towards the enzyme it actually induces the conformational changes into the enzyme, conformational as well as the configurational changes which means it is also asking the enzyme to change its site chains. And that is

how the enzyme adjusts its shape and that is how it is actually going to form the enzyme substrate complex. And once this happens the enzyme is actually going to catalyze the reactions site, right.

So, it is actually going to withdraw the group, so it is actually going to make the changes within the substrate and that is how the substrate is actually going to form the product and that is how it is actually going to form the enzyme product complexes. And once the enzyme product complex is formed these enzyme products complex are actually going to have the lower affinity and it is also going to induce the conformational changes and that is how these products are actually going to be released from the active site.

And then the enzyme is actually going to be ready to start the new cycle.

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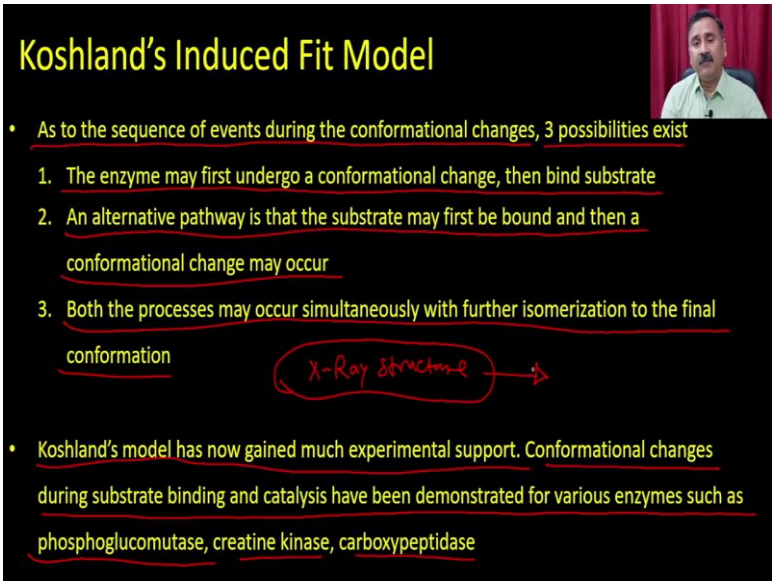

So, as to the consequence of event during the conformational changes there are three possibilities exist. The enzyme may first undergo a conformational changes and then bind the substrate. An alternate pathway is that the substrate may first be bound and then a conformational changes may occur. Both the process may occur simultaneously with the further isomerization to the final conformation. So, what it says is that there are two three possibilities.

One that the enzyme is actually you know substrate will go and you know induce the conformational changes and that is how it is actually going to bind the substrate more

you know with more affinity. The other is that the substrate will first go and bind and then there will be a conformational changes and that actually results into the binding of the substrate with more affinity.

Either of these processes are actually going to say that the substrate enzyme is active site is not rigid it is fluidic, it is actually going to have the capacity to adopt the conformation of the substrate and accordingly it is actually going to change the conformation.

So, Koshland models has now gained much experimental support. The conformational changes during the substrate binding and catalytic has been demonstrated for various enzyme such as phosphoglucometanase, keratinase kinase, carboxy peptides and so on.

There so many examples where people have actually it is you know determine the X-ray structures and what they found is that the even if they are solving the X-ray structure it is not it is actually giving a indication that there are conformational changes being induced by the substrate, ok.

So, Koshland model is also very very popular and it is explaining many of the phenomena, but it is does not mean that the lock and key model is also not relevant. So, both of these models are explaining, in some cases the enzyme has the predefined three dimensional conformations and that is how it is binding the substrate. In some cases, the substrate is binding and inducing the conformational changes.

So, the best model which actually can explain the enzyme substrate interaction could be a mixture of both that you are actually requiring a definite three dimensional structures to bind the substrate, but the sometimes the substrate is actually going to induce to achieve the best conformation or sometime you are also already having the best conformation exist, ok. So, this is all about the tool models what are being used or what are been proposed to explain the enzyme interaction with the substrate.

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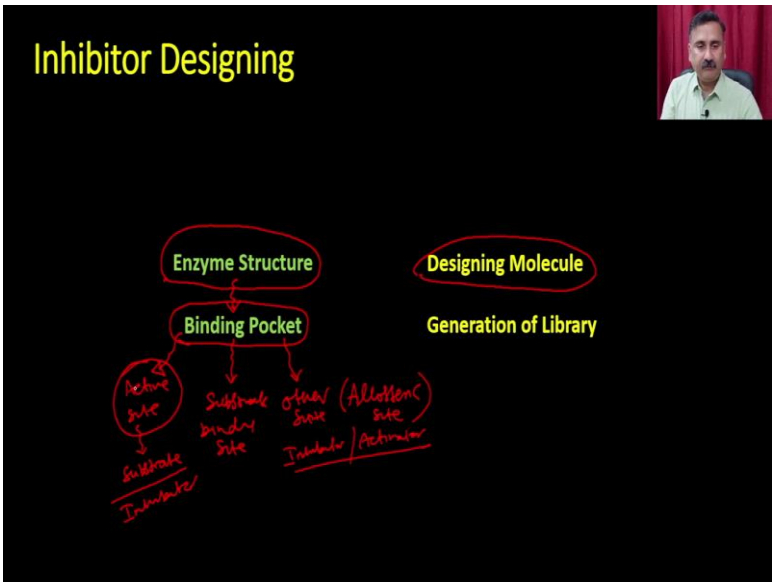

Now, when we want to use this, you are actually going to have the two counterpart one is the enzyme substrate the other one is called as the designing molecule. So, this means when you talk about the enzyme substrate you are going to talk about the binding pocket, ok.

And as I said in the previous lecture also in the previous slides that the binding pocket could be of two types, it could be the active site the site where you are actually going to have the binding of the substrate and sometime it is also going to bind the inhibitor or you are actually going to have the other site where you are actually going to have the binding of inhibitor or activator, right.

So, these other site is also called as the allosteric site and this allosteric site the purpose of this allosteric site is to you know to modulate the enzyme activity. So, when we talk about the active site will active site has a pre-defined space within the binding pocket and the binding pocket could be bigger it could actually have the active site, it could also have the substrate binding site and it could actually be able to have a combination of both.

So, substrate binding as the binding pocket could also have the substrate binding site. And in many of the cases the enzyme may have the substrates separate binding site, it may have separate active site and the combination of both. Sometimes the active site also bind also within the substrate binding site, sometime it may not be, ok. So, when we talk

about when you say about binding site pocket you have three components active site, substrate binding site and the allosteric site. So, let us first discuss about the active site.

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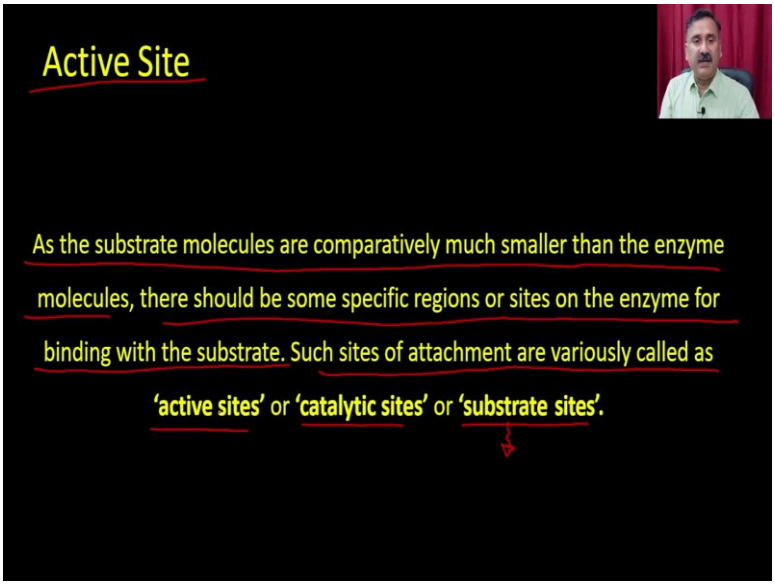

So, active site as the substrate molecules are comparatively much smaller than the enzyme molecule there should be some specific region or site on the enzyme for the binding with the substrate. Such site of the attachment are variously called as active site, the catalytic site or the substrate binding site. And as I said you know substrate binding site could be different, could be within the active site, ok.

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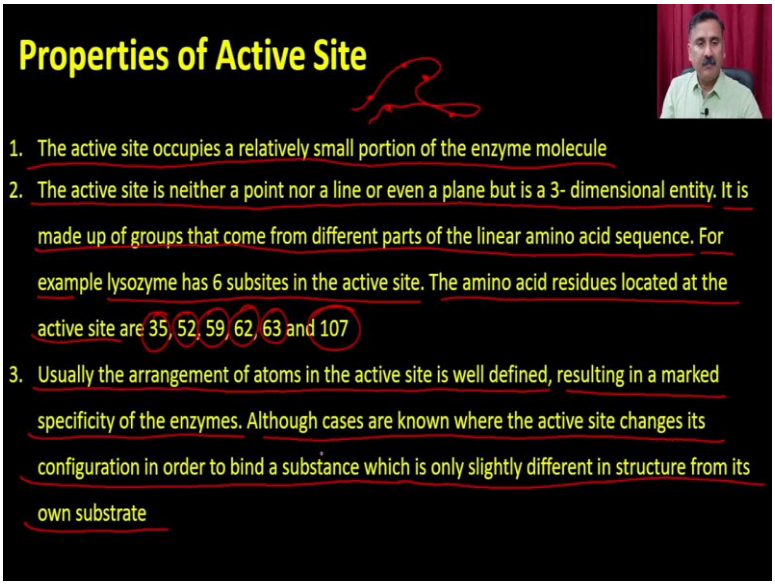

So, what are different properties of the active site? The active site occupies a relatively small portion of the enzyme molecule. So, it is very small in comparison to the total structure of the enzyme. The active site is neither a point nor a line or even a plane, but it is a three dimensional identity, it is made up of the group that come from the different parts of the linear amino acid sequences.

For example, the lysozyme has three subsite in the active site. The amino acid residues located at the active sites are the 35, 52, 59, 62, 63 and 107. So, you can actually have the active site which may actually coming from the different types of amino acids and these may be coming from a different distant location within the amino acid sequence.

Usually, the arrangement of the atoms in the active site is well defined resulting into a marked specificity of the enzyme although cases are known where the active site changes its configuration in order to bind a substance which is only slightly different in the structure from its own substrate.

So, this is the third point what you see that here actually it says that the active site is not a rigid space, it actually can have the fluidity and it actually can change the configurations and as well as the conformations to bind a particular substance.

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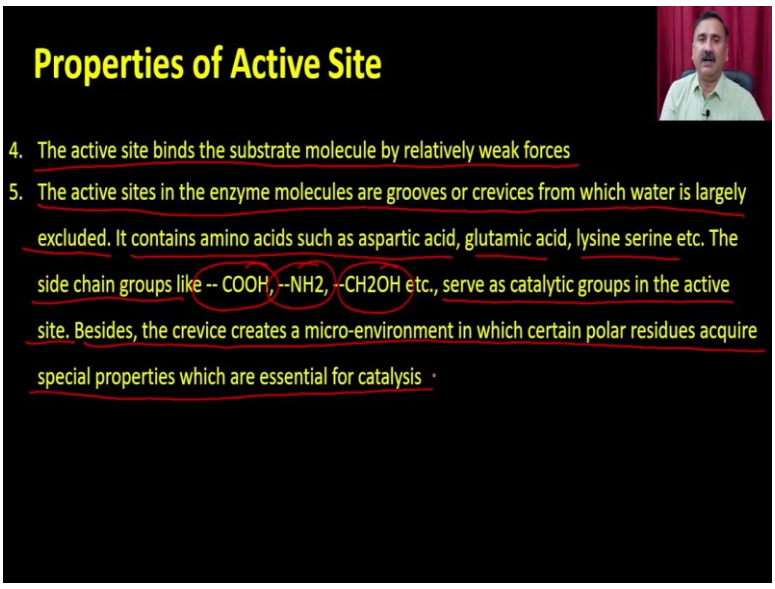

The active site bind a substrate molecule by relatively weak forces and the active site in the enzyme molecules are groups or services from which the water is largely been

excluded. It contains the amino acids such as aspartic acid, glutamic acids, lysine, serine etcetera. The side chain groups like the acid groups, amine groups, alcohol group etcetera serve as the catalytic group in the active site.

So, many of these groups are actually facilitating the either the extraction of the protons or extraction of the electrons or the exchange of the electrons from the substrate to the product because you know that the substrate is actually getting converted into the product simply by breakage of some bonds and deformation of few bonds, right.

So, that is the purpose of having the these functional groups. So, they are actually very active in terms of the you know either acting as a electrophile or the nucleophile and that is how they are actually facilitating the breakage as well as the formation of bonds. Besides the service created a micro environment between the certain polar residue acquire the special property which are essential for the catalytic side. Now, the first question comes how we can be able to identify the active site on a enzyme.

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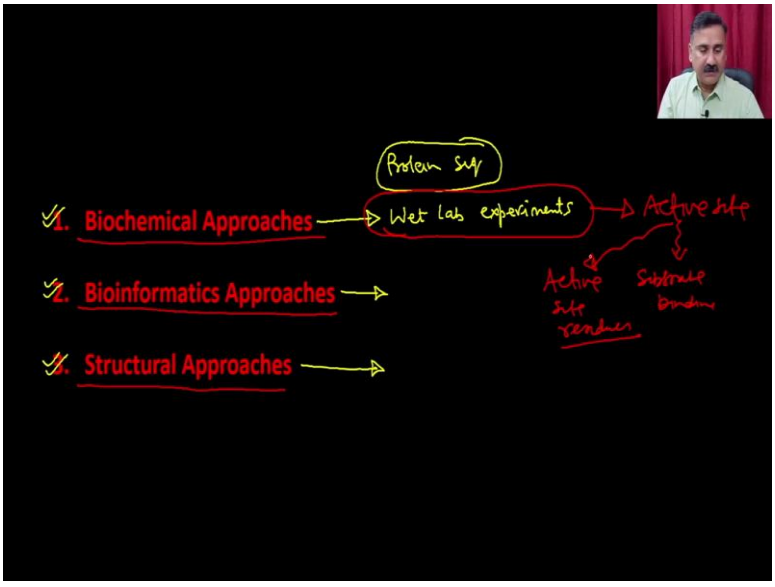

So, there are three approaches when can use. So, approach number one is the biochemical approach. Approach number two is the bioinformatics approach and the third is the structural approach. So, approach, biochemical approach means you are actually going to do the wet lab experiments right, to know where the active site is, right. And you know that active site means the site which actually going to have the substrate

binding and the place which is actually going to have the residues, right. So, active site residues which are actually going to facilitate the catalysis.

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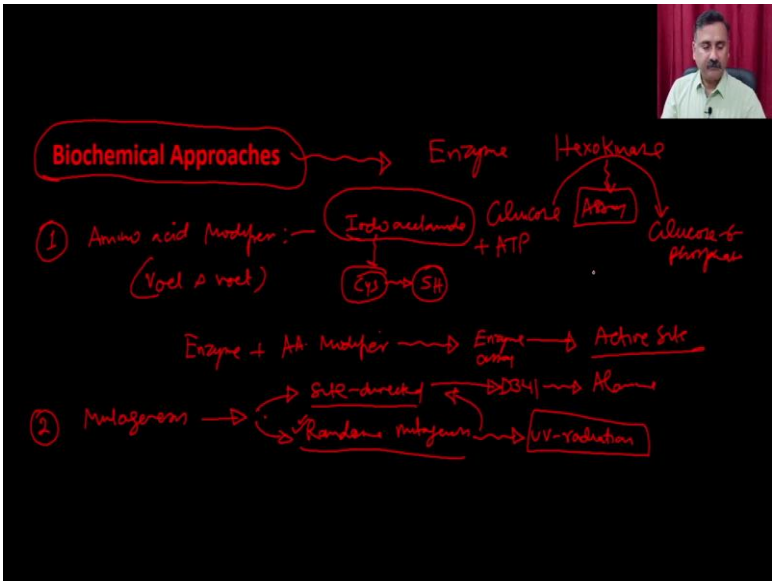

Now, how we can actually be have the different types of biochemical approach, right. So, biochemical approach it depends on the enzyme, right. So, imagine that we have an enzyme, for example, we have the hexokinase, ok. Now, hexokinase what is the activity? Hexokinase is actually going to have the glucose plus ATP and it is actually going to form the glucose 6 phosphate, right.

Which means it is actually taking one of the phosphate from the ATP and transferring that onto the glucose and forming the glucose 6 phosphate, ok. Now, this activity can be measured by one of the assay, right. So, you can actually have a very strong assay to measure the activity of this particular enzyme. Now, the first thing what you can do is you can actually be able to use the first approach where you can actually be able to use the amino acid modifiers, ok.

So, until the you know or before the pre genomic errors until the people were not doing very you know the cloning and other things were not very common, right. So, you can actually be able to use the amino acid modifiers. And there are a list of amino acid modifiers, what you can actually be able to go through from white and white and other kind of biochemistry group.

And what they do is they are actually either modifying the you know the groups or for example, iodoacetamide, ok. So, iodoacetamide. So, iodoacetamide is an amino acid modifier right, and it is actually going to modify the cysteine residue, right. So, if you treat the hexokinase, right. So, what you do is you take the enzyme ok, and treat with amino acid modifier.

Now, what amino acid modifier is going to do is it is actually going to modify all the functional groups. So, you remember that cysteine has the this group, right. So, what it will going to do is it is actually going to modify the SH groups, right. So, as soon as the SH group is being modified the cysteine will not going to participate into the catalysis, right.

And that is how it is actually going and then what you are going to do is you are actually going to do the enzyme assay. And it will actually going to tell you whether the cysteine is involved into the activity of will be present in the active side or not, right. The second approach is the mutagenesis.

Now, mutagenesis approach will work only when you are working with an enzyme for which the sequence of the amino acids are known, for which the genome is known and for which the gene is known, ok. So, then in that case what you can do is you can do the two types of mutagenesis, you can do the site directed mutagenesis or you can actually be able to do the random mutagenesis, ok.

Normally people if they do not know anything about the active site, they what they do is they are actually going to do the random mutagenesis followed by the site directed mutagenesis. So, what is site directed mutagenesis means? Site directed mutagenesis means I am going to modify the 341, ok. So, 341 amino acid I am going to modify. Suppose this is aspartate. So, D349, I am going to modify to alanine, ok. So, that is called site directed mutagenesis.

Random mutagenesis means you are actually going to do the random mutations. Random mutations either by the UV radiations and all other kinds of sources. And that is very very crude, ok. So, random mutations is same as amino acid modifiers and site directed mutagenesis when you are actually going to know pin pointedly that there is a aspartate, there is a aspartate 341 which may be involved. So, you will actually modify and then you will going to check the enzyme assay whether it is working or not, right.

So, these are the two approaches biochemical approaches one can use, right. And you can actually be able to answer the questions where the active site is and you can be able to know these are the three residues which are actually been involved into the catalysis and that is how you can be able to know the active site.

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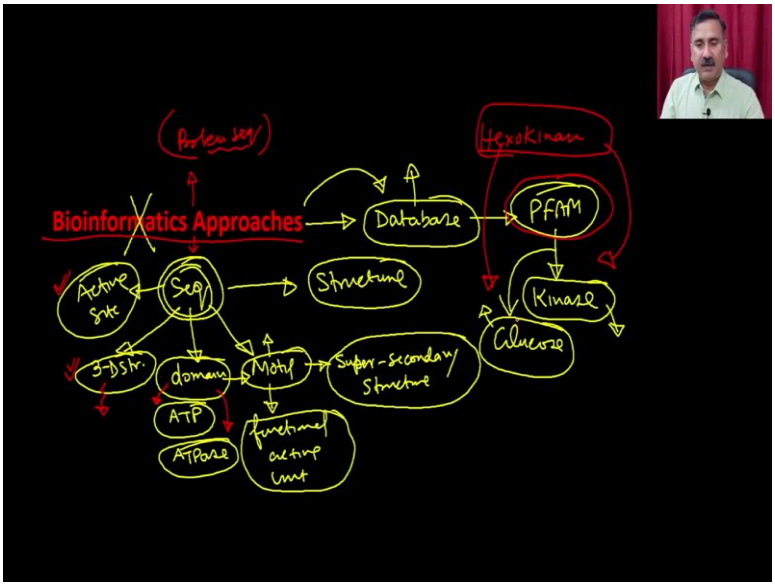

Then the second is bioinformatics approaches. So, bioinformatics approaches relies on to the amino acids or the protein sequence, right. So, it relies on the protein sequence, right. So, when you do the protein sequence analysis you have the two options. One you can actually take the sequence and it is actually can be able to you know compare it with the different types of other sequences and that is how on based on that it is actually going to tell you the location of the active site.

Because in your sequence the active site position is not known, but if you take this particular sequence and you will identify the homologous sequences and if you compare them for the homologous sequences, you know where the active site is, right. So, based on that you can be able to do the active site analysis.

Taking these into things you can actually be able to do a three dimensional structures. You can actually be able to you know make that three dimensional structure and that is how the three dimensional structures will allow you to visualize the 3D structures or visualize the active site. You can actually be able to take this sequence and you can actually be able to identify the domains.

So, you can actually be able to say whether there is a ATP binding domain present in that particular sequence or not, whether this particular domain has the ATPase activity or not, because this particular sequence is actually forming the domain which is actually the ITPs domain and other proteins. And then you can also be able to say motifs you can actually be able to identify in this particular sequence the different types of motifs. You can actually be able to calculate the super secondary structures and so on.

So, taking these into account you can be able to calculate the different types of properties of this particular sequence. You can identify the active site; you can actually be able to visualize the active site in a three dimensional structures. You can actually be able to identify the different types of domains, motifs and all that. Apart from that you can actually be able to take this sequence and put it into the database.

For example, you can actually go through the Pfam database and it is actually going to tell you whether this particular protein is a kinase, whether it is actually having the you know the binding sequences for binding the glucose or not. So, for example, when we are talking about the hexokinase, right. So, hexokinase if it is a hexokinase and if you blast that into the Pfam database, it will say that it has a kinase domain, it will say that it has a glucose binding site and all that and you can actually be able to do that.

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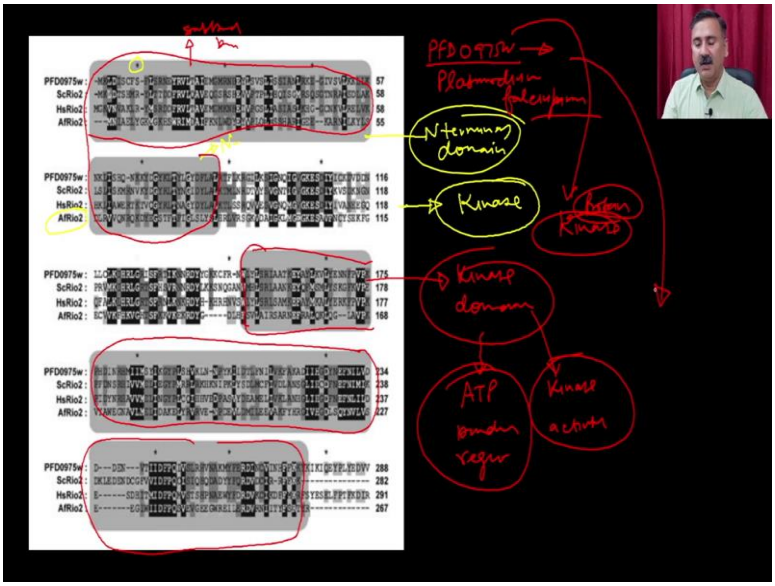

Let us take a clear example. So, in this particular thing this is actually a hypothetical protein which is called as the PFD0975W, ok. So, this is a hypothetical protein from the

Plasmodium calciferol, right. And so, when you talk about the you know the hypothetical protein you do not know anything about this particular protein, but when we actually try to identify the homologous sequences from the database what we could found is that this is actually the N terminal domain.

The first what you see here right, this is the N terminal domain what is present and it is actually going to be responsible for the substrate binding. Whereas, this is this sequence what you see right, this sequence ok, because it is matching exactly with the another homologous protein is actually the kinase domain, ok.

And based on this we have said that the PFD0957W is a protein kinase, ok. And it is actually going to have the substrates ok, because the homology is less in this particular region it is difficult to identify the substrate simply by looking at this. But at least we will know that it has a ATP binding cassette, right.

So, it has the ATP binding region and it actually has all the crucial amino acids which are responsible for the kinase activity. So, taking this into account we can actually be able to say that it is actually a protein kinase and it will actually go into further catalyzation is required it is because simply by bioinformatics you cannot be able to do.

So, you actually have to do the lot of bioinformatics biochemical experiments mean you have to clone this protein, you have to over-express this protein and you have to test for what are the substrate it is actually going to phosphorylate.

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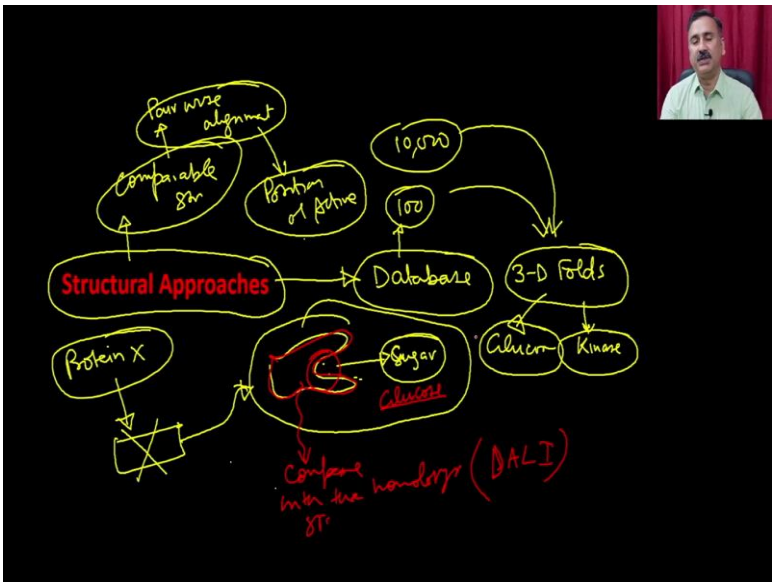

And then the third approach is the structural approach. So, a structural approach and the bioinformatics approach are actually going to have the different is going to have the same flow actually. Because when you have the proteins you can actually be look at into the databases and its actually going to tell you and taking into the information from the database you can be able to have the three dimensional structure of this particular protein.

And then what you can do is what you can actually compare this three dimensional structure with the homologous sequence homologous structures, right. So, you can actually be able to use a server which is called as DALI server and that is actually going to tell you that ok, there is a cavity here, this cavity is actually going to bind the glucose molecule.

And using that you can actually be able to define the active site. If you want the structural things you can actually be able to calculate the 3D folds and all that, right. So, these are the three different approaches to identify the binding pocket, you can actually be able to identify the active site.

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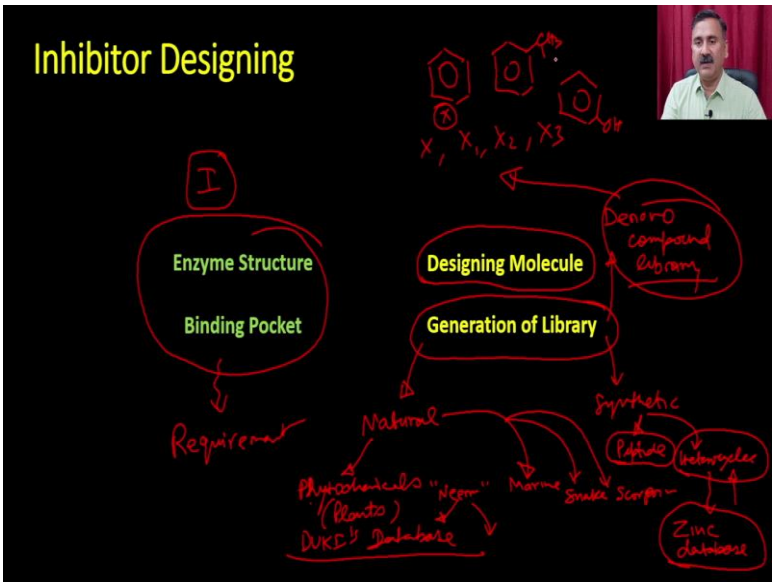

So, once the active site is known then half part is over right, you are going to know what are the requirements of this particular area ok, which means component one is clear, ok. Now when you want to design the molecules, you have to design or you have to generate a library, ok.

This library where you are actually going to have the different types of design molecules right, this library could be a natural library or it could be a synthetic library which means it is actually can be a library which manmade right, or it could be actually a natural library.

Natural library there are different sources of natural library. For example, you can have the different types of phytochemicals, what you can actually be able to do, right. So, you can actually be able to take the phytochemicals from the different types of plants and you can actually be able to use that, ok.

So, you can actually be able to use the Duke's database and that actually is going to tell you that what are different phytochemicals are present. For example, if I want to know what are the different phytochemicals are present in the neem, I can do is I can take this neem plant and I can just blast it into the Duke's database.

And it is actually going to tell me that these are the reported phytochemicals what are present in the neem, ok. The other sources are natural sources are like the different types

of organisms. So, you can have the marine organisms, you can have the you know the snake or you can actually have the scorpions or you can have the other kinds of natural sources, right. And then you can have the synthetic library. So, you can have the you know the peptide based library.

So, you can have the different types of molecules, you can have the peptide based inhibitors, you can have the heterocyclic compounds. And there are a lot of libraries what are present where you can actually have the peptide library and heterocyclic library. For example, you can actually be able to use the Zinc database. And the Zinc database is actually going to be very extensive or comprehensive database to give you the information about the different types of heterocyclic library.

And apart from that you can actually be able to use and calculate make your own Denovo compound library, right. This means you can actually be able to have [FL] different types of library like for example, X, X 1, x 2 like that, ok. For example, you have a benzene for example right, this is X, ok. Now what I do is I will modify I will modify and put CH 3 that is X 1, ok. I will put I will modify like this I will put OH here that will be X 2.

So, if I put different types of groups ok, I am designing the different types of molecules and that is how testing these will actually going to guide you which one is better. So, for example, if CH 3 is better OH is reducing then I will what I will do is I will not put the OH here, I will put oh somewhere else or I can just make the modification somewhere here and all that. So, these are you know that will actually going to iteratively if you do that it is actually going to allow you to design a very good molecule and that is how you can be able to design a true library.

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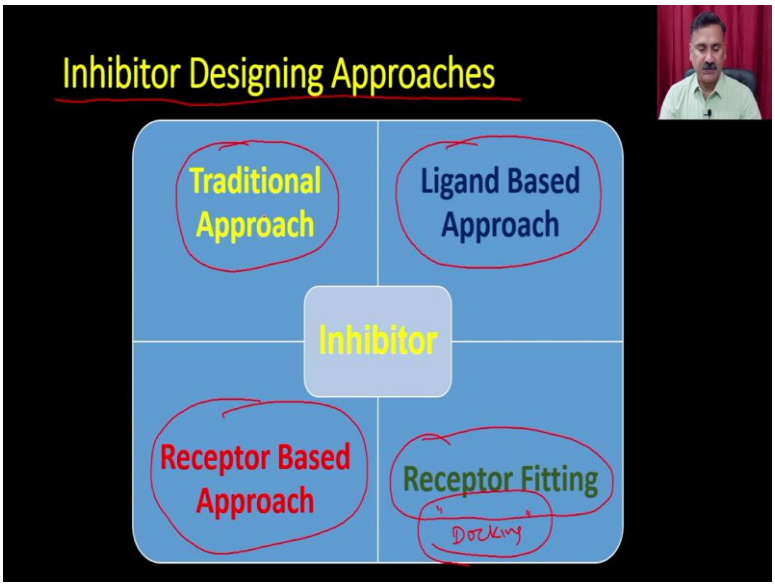

Now, when we talk about the inhibitor designing approach. So, you can have the four options one is you can have the traditional approach ok, you can have the ligand based approach, you can have the receptor based approach and you can also have the receptor fitting or I will say you can actually have the computational approach where you are going to use the docking and related software's.

So, what is the difference between the traditional approach and the modern approaches or the targeted approaches. So, all these three are targeted approaches where a traditional approach is where you are actually going to have the no information about the enzyme or the structures.

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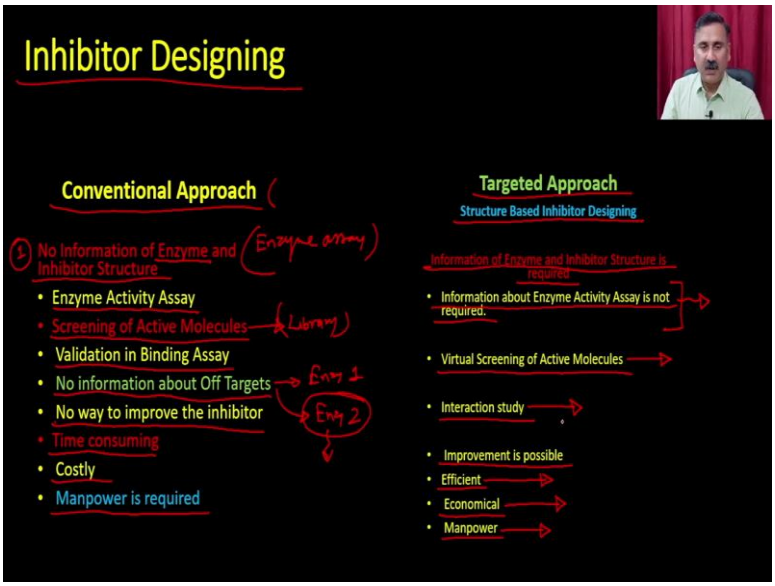

So, what are the difference between the inhibitor designing when you go with the conventional approach or I will say the traditional approach or the targeted approach. When the targeted approach means structure based inhibitor designing, ok. So, one of the major advantage of the conventional approach is that it does not require the enzyme right, it does not require the structure of the enzyme or the inhibitor.

So, you can actually be able to if you have the enzyme assay you can be able to identify a inhibitor ok, because it is actually going to does not require that. So, you what you require? You require enzyme activity assay; you require the screening of the active molecules.

So, you require a library of molecule ok, this means you require the molecules in hand, ok. Then you require the validations in the binding assays and one of the major drawback is there will be no information about the off targets, right. So, if it is enzyme 1 for which you are screening it could be possible that it may be affecting the enzyme 2 and this enzyme 2 could be useful or could be good for the for the for the host.

Since you do not know the enzyme at the inhibitor structure. So, you do not know how the enzyme inhibitor is actually inhibiting the enzyme, what are the different crucial residues are important and all that. So, that is why you are not going to have you would not have any ability to improve the inhibitors. It is time consuming because it is based on

the enzyme assay, then screening and real wet lab experiments. So, it is actually very time consuming.

Apart from that it also requires lot of biochemicals and reagents and all that. So, it is also costly and it also requires a trained manpower to perform the enzyme assays and to calculate the to analyze that data and to know whether the enzyme inhibitor is inhibiting enzyme or not.

Compared to this in a targeted approach you require the information of the enzyme and the inhibitor structure is required because here you are actually going to see whether the inhibitor is binding into the active site or not and all those kind of information. So, the inhibition information about the enzyme and the inhibitor structure is essential if you want to make the targeted approach.

You do not require the information about the enzyme activity assay because you are not going to perform the activity assay in the first step, right. First you are actually going to analyze the enzyme and the inhibitor structures and see whether they are compatible with each other or not, you are going to perform the virtual screening of the active molecule. So, it is actually very very fast and it does not require the you to perform the activity assay.

Interaction studies also you computational you can be able to perform the interaction studies, since you know the interaction studies you are based on the interaction studies, you can be able to change the groups onto the inhibitor and that is how they will say always a possibility that you can be able to improve the inhibitors. It is efficient because it is a computational driven.

So, it is actually going to be very very fast, it is economical because you are not going to consume any kind of costly you know biochemicals and other kinds of reagents. And it also require the trained manpower compared to this, ok.

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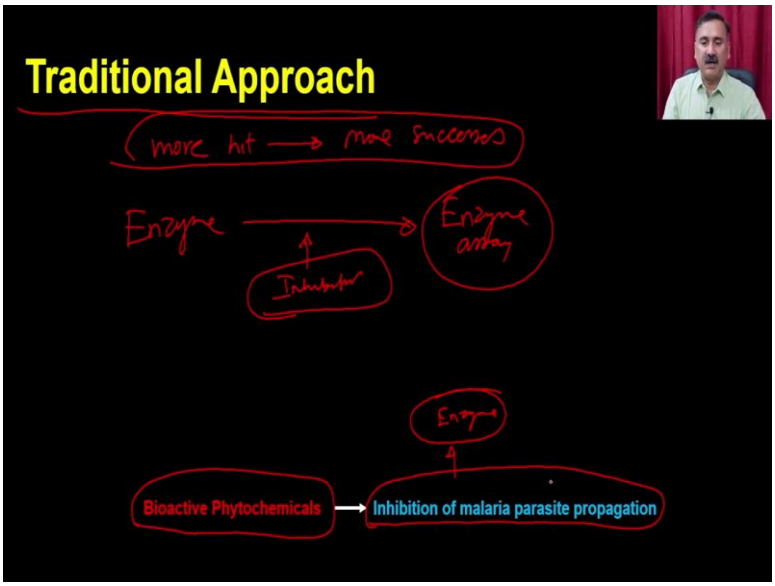

So, let us talk about first the traditional approach. So, in a traditional approach if I say traditional approach is called as more hit more success. So, that is the main motto of the traditional approach. In a traditional approach what you require? You require an enzyme and you require an enzyme assay ok, and that is how you can be able to screen the different types of inhibitor, right. And using this data you can be able to say whether this inhibitor is inhibiting the enzyme assay or not.

So, for example, we have taken an case study where we are actually going to screen some of the bioactive phytochemicals and we say whether this biochemi these phytochemicals are inhibiting the growth of the malaria parasite or not, ok. So, this inhibition is because of the inhibition of some enzyme.

So, and, but in a conventional traditional approach you are not being very bothered about the enzyme. So, here instead of assay we are actually going to see whether it is these bio phytochemicals are disrupting the malaria parasite propagation or not.

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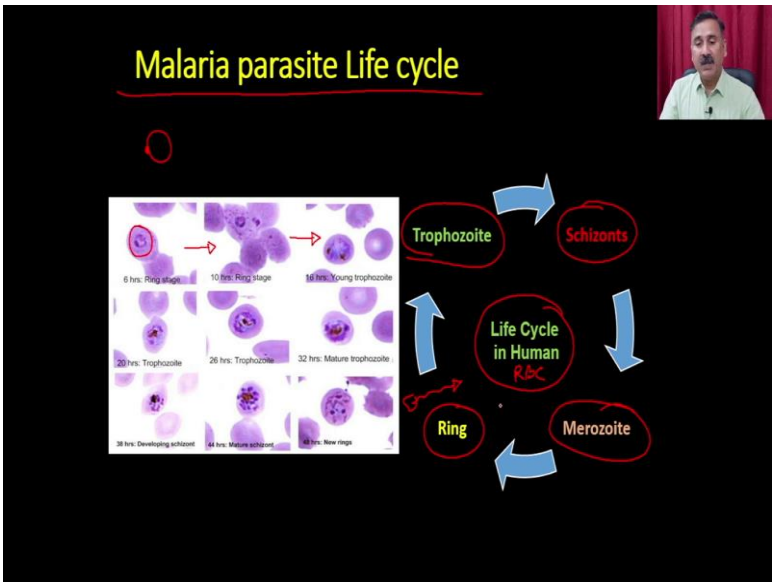

So, when before getting into this you have to understand about the malaria parasite life cycle. So, malaria parasite life cycle within the human host is within the two different organs one is the liver stages the other one is the RBC stages. So, the life cycle in the human is within the RBC is having the four stages. One is the ring stage, the other one is the trophozoite then the schizonts and the merozoite.

After the merozoites it is actually going to infect the new RBC's and that is how the parasite is actually going to increase the number. And these are the different stages what you these are the ring stage you see this it is its like this, right. And that is why this is called as ring stage. When the ring goes for the 10 hours it goes into and develop into the mature ring and then the mature ring gets developed into the trophozoite and so on.

So, these are the different stages what you can actually be able to identify microscopically and that is how you can have a little to say if I take the ring stage and if I treat this with the phytochemicals right, whether the ring will get converted into tropho and schizonts, ok.

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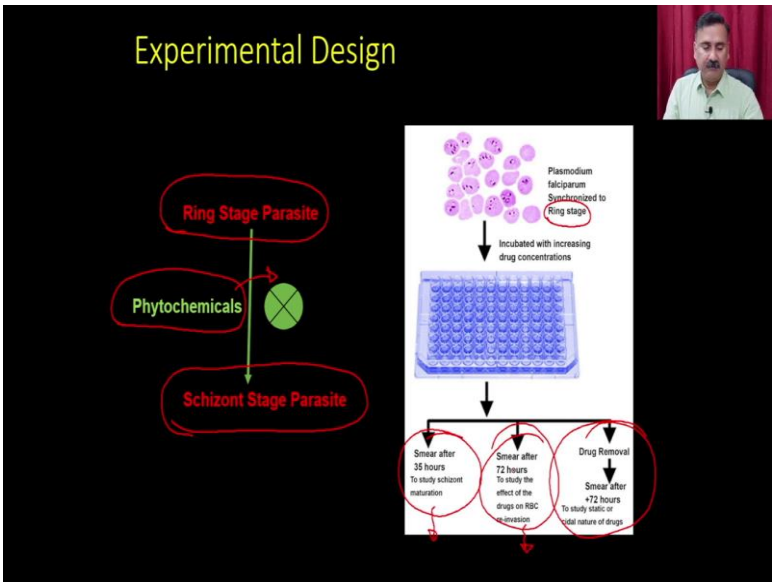

So, the experimental method experimental procedure is like this you are actually going to take the ring stage parasite, you are treating it with the phytochemicals and expecting that the phytochemicals are actually going to interfere with some of the enzyme what are present in the ring stage parasite. And that is how it is actually going to not align the ring stage parasite to form the schizont stage parasite.

So, these are the things what you have, you have to first prepare the ring stage parasite, you are actually going to incubate these parasites with a different concentration of the inhibitors and then you are actually going to make the smear at 35 hours, 72 hours and after removal of the drug as well to see whether the drug is killing or the parasite or not. And then you are actually going to ask what will be the number of parasite present right, and what are the different types of parasite present, right.

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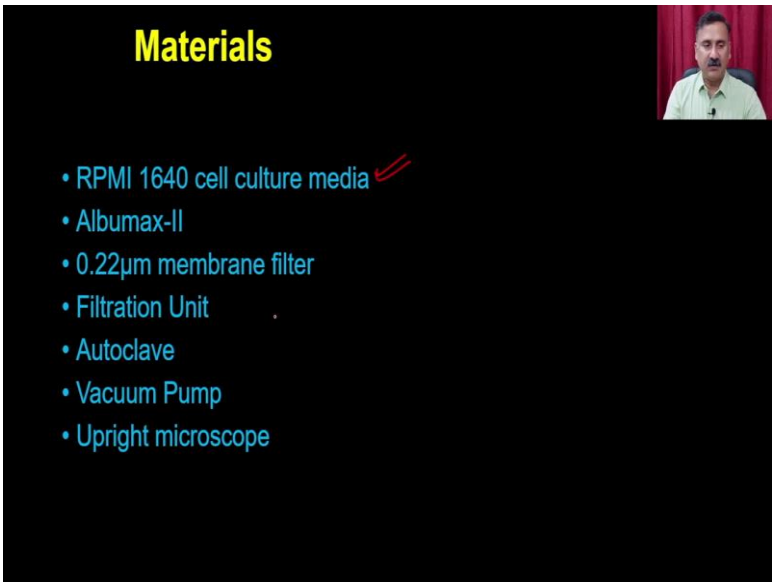

So, what you require? You require the these are the different types of reagents what you require. So, you require the cell culture media and the other kinds of things.

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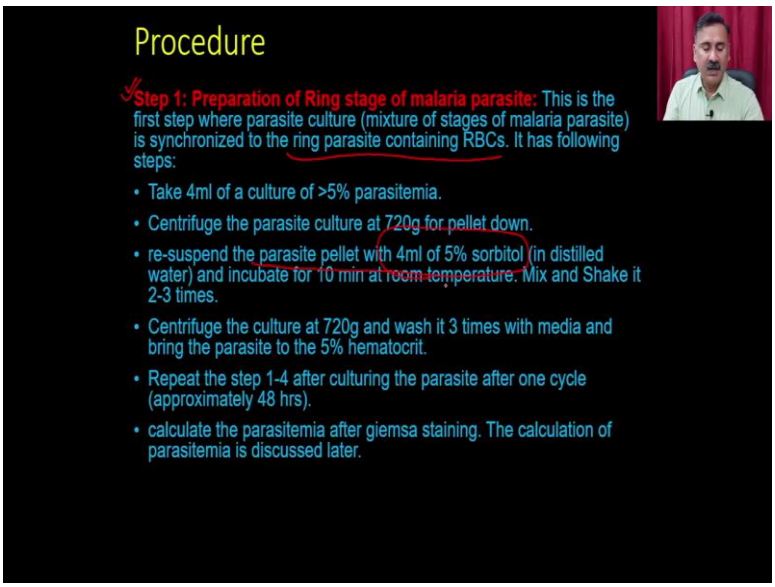

Within the procedure what you have step 1 you are actually going to prepare the ring stage of the malaria parasite. So, this is the detail protocol what you have to follow to prepare the ring stage parasite and I am not going to go through with the detail of this. So, what you are going to do is you are going to take the parasite and treat it with the

sorbitol and that actually is going to destroy or kill the trophozoite and the schizont stage containing cells and it is actually going to only give you the ring parasites.

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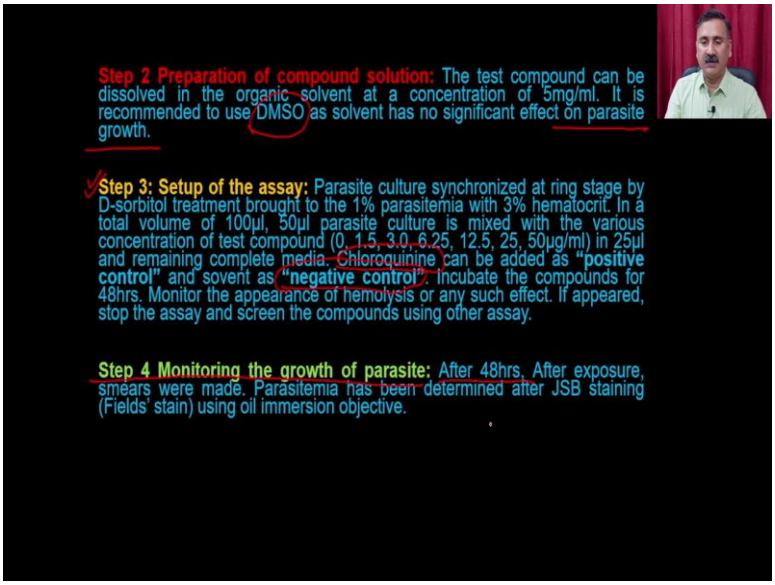

In the step 2 you are actually going to prepare the inhibitor compounds. So, most of these inhibitors are the soluble in organic solvents sometime they are soluble in water also. So, you are going to dissolve that according to the solvent and then you are going to ensure that you are going to take the solvent consultation to such an extent that it should not affect the parasite growth on its own.

Then in the step 3 you are actually going to set up the assay. So, you are going to take the ring sampleized parasites, you are going to incubate it with the inhibitor and as a negative control you are going to add the only the solvents, right. And as a positive control also you are going to take some of the known inhibitors.

And then in the step 4 you are going to monitor the growth of the parasite. So, after 48 hours you are going to take the smear, you are going to stain it and then you are going to determine the parasitaemia.

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And this is what exactly what you are going to see. What you are going to see is these are the morphologically healthy parasites, right. So, they are actually going to show you all different sets of stages trophozoite, schizont and all that whereas, in the case of the inhibitor treated parasites what you are going to see is you are going to see a dead parasite. So, these are the dead they do not have any kind of nuclei.

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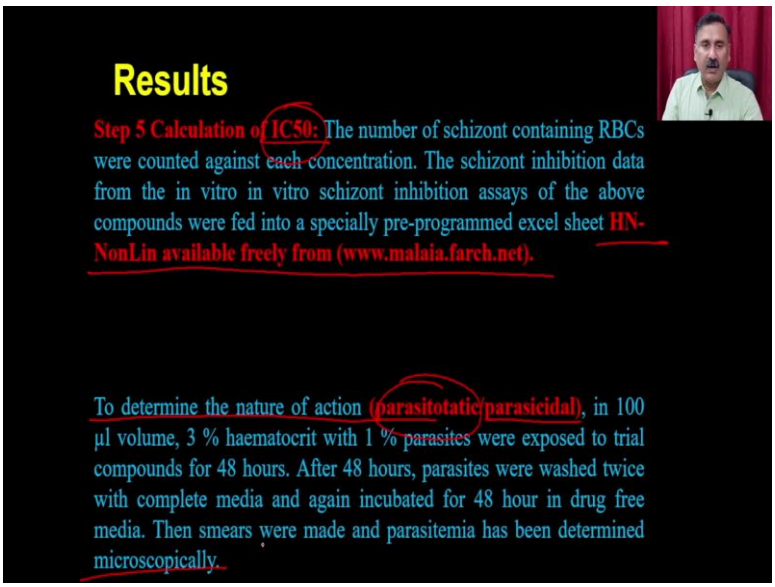

So, taking this into data, taking these data into account what you can do is you can actually be able to calculate the inhibitory concentrations, we can actually be able to

calculate the IC50 and you can be able to use this particular online tool to calculate the IC50. So, to determine the nature of the action which means whether the inhibitor is killing the parasite or whether the inhibitor is only stopping the growth you can actually be able to do the reactions without the inhibitor for 48 hours.

And that is how you can be able to know that also. So, this is all about the traditional approach what we have discussed. So, what we have discussed in this particular lecture? We have discussed about how you can be able to identify the active site and how are what are the different approaches you can use to identify the active site and what are different approaches you can use.

So, in this particular lecture we have discussed about the traditional approaches and we have taken the case study from the you know screening the inhibitor as the anti-malarial drug. So, in our subsequent lecture we are going to discuss about the other targeted approach like the ligand base approach, receptor base approach or the docking. So, till then I would like to conclude my lecture here. In our subsequent lecture we are going to discuss some more approaches to design the inhibitors.

Thank you.