Experimental Biochemistry
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Lecture – 34
Enzyme Kinetics I

We begin our discussion on Enzyme Kinetics. We will cover this in 2 lectures and what we need to understand from this is what we mean by Enzymes; what we mean by Enzyme Catalysis; Enzyme Mechanisms and what are the specific features of Enzymes. Now, if we consider what enzymes actually are, they are the backbones the pillars of any biochemical process. Any biochemical process occurs through these enzyme reactions.

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So, when we consider enzymes in general; what we will study in this particular lecture is the enzymes as catalysts, the different types of enzymes and enzyme kinetics in general.

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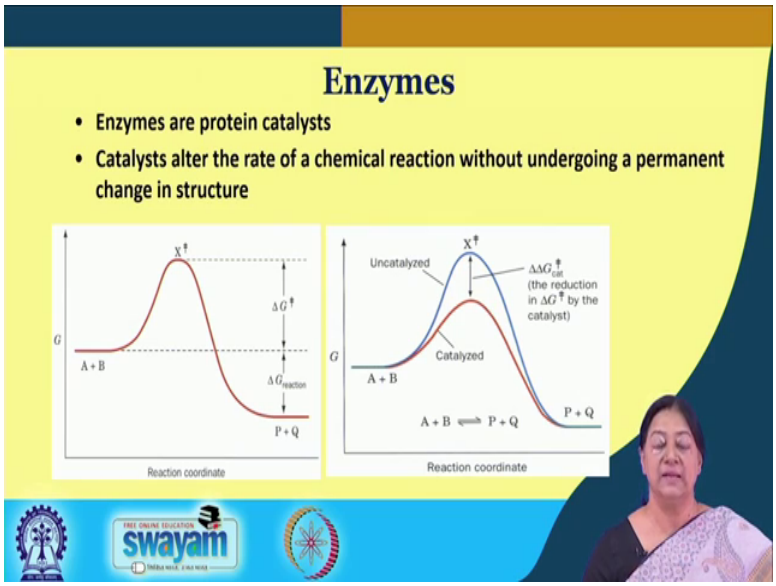

When we look at what enzymes are, these are protein catalysts which means that they alter the rate of a chemical reaction. In this case a biochemical reaction that will occur without a predominant change in the structure of the enzyme which will we will see as we go along.

So, if we have a general set where we are looking at a reactant going to a set of product and we have a certain activation barrier that has to be crossed. We know that when we have enzymes, if we have an uncatalyzed reaction this is what we have and if we have a catalyzed reaction, we know that we have a reduction in the activation energy. This is exactly what enzymes also do when they act as protein catalysts.

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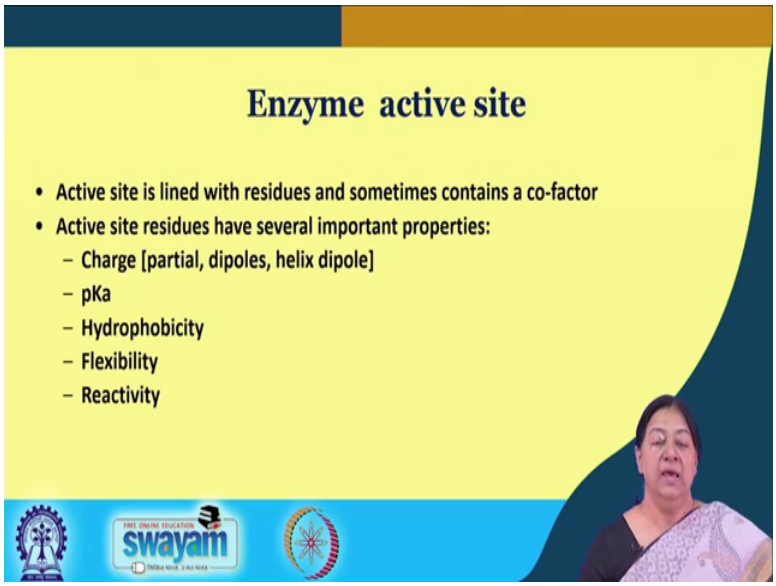

In the enzymes, we have what is called an active site something that we will see later on and we have amino acid residues in this active site. Because we have to remember that this enzyme is a protein and the important properties that this active site has is its Charge, its pKa, a specific Hydrophobicity, Flexibility and the definite Reactivity for the biochemical reaction to go on.

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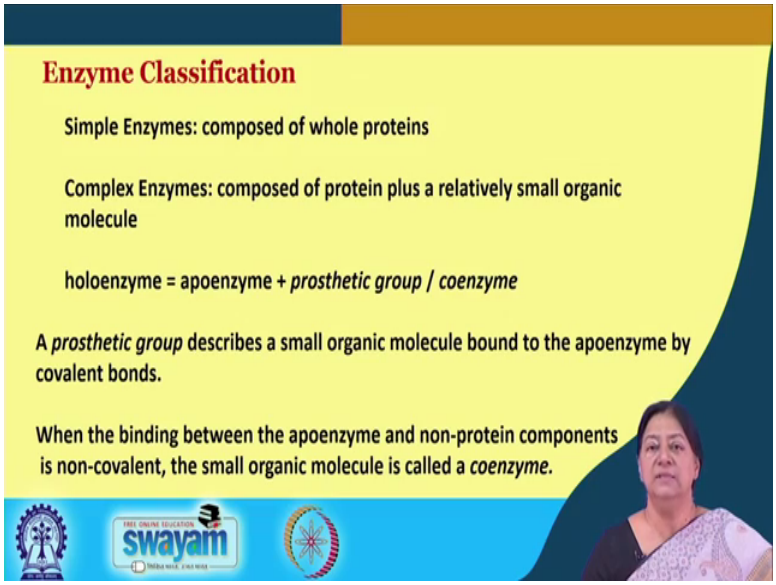

There can be simple enzymes; these simple enzymes are composed of whole proteins. The complex enzymes will be composed of the protein, but in addition to this, it can

have what is called a prosthetic group or co factor or it means might need even a metal for its activity or its enzyme catalytic activity to be complete.

So, we have what is called holoenzyme, an apoenzyme or a prosthetic group plus the or the coenzyme. The prosthetic group is a small organic molecule that is bound to the apoenzyme by covalent bonds. When the binding between the apoenzyme and the non protein component is non covalent, this is called a coenzyme.

Now, what happens in enzyme reactions is it may so happen that the enzyme requires this prosthetic group or the coenzyme or even a metal for the reaction to be complete, for the reaction to go forward.

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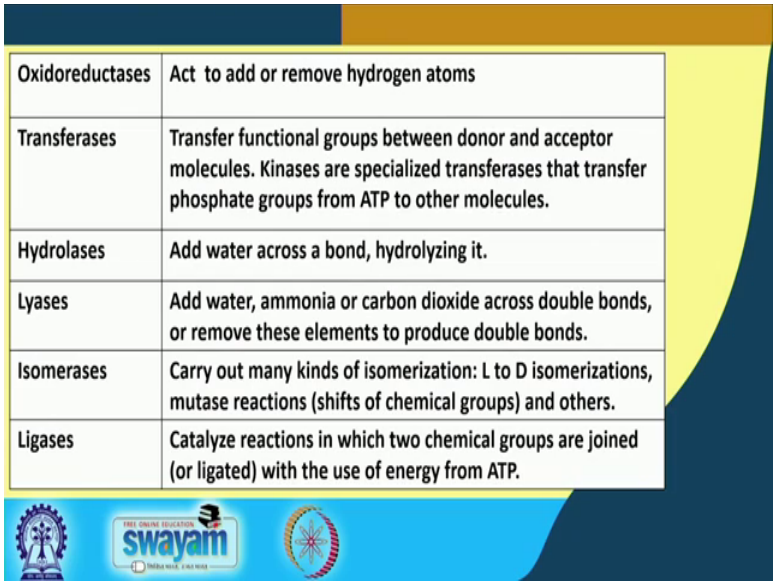

The different classes of enzymes are Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerizes and Ligases. So, these are the six different types of enzymes that catalyze specific reactions.

So, if we were to add water across a hydrogen bond resulting in hydrolysis, this would be a hydrolyzed. If we are transferring a specific functional group a chemical group between donor and acceptor molecules, we would have a Transference. And if the transferred moiety the chemical moiety that is being transferred happens to be a phosphate this is called a Kinase. If we have Ligase; that means, we are linking to different chemical groups together. If it is an Isomerase; it is an isomer isomerization

reaction. If it is an Oxidoreductase, it is the addition of the removal of hydrogen atoms or an oxidation reduction reaction. So, these are the specific definite types of enzyme classifications that are present.

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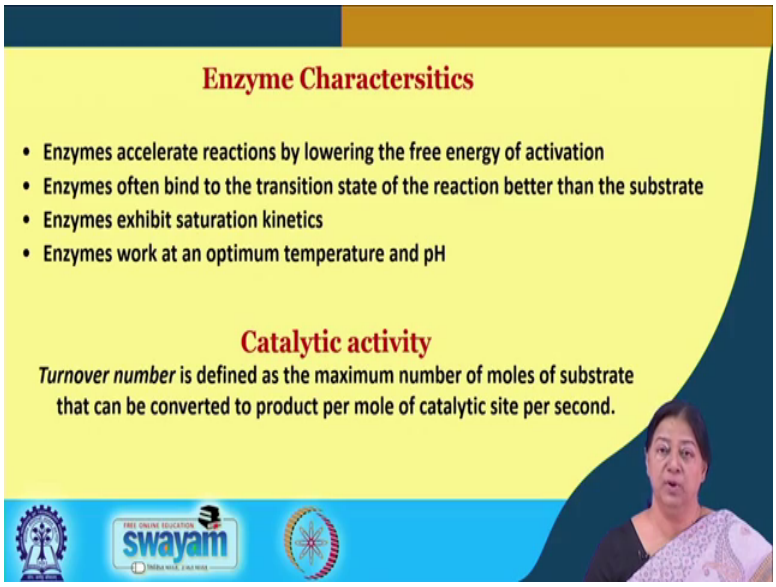

When we look at enzyme characteristics, they accelerate the reactions. As we saw by lowering the free energy of activation, they often bind to the transition state of the reaction better than the substrate and they exhibit what is called saturation kinetics. We will see what that means in a moment and they work at an optimum temperature and an optimum pH. We have to remember that these enzymes being proteins any extreme of pH we learnt will denature the protein, any extremes of temperature will also have an effect on the protein structure which in turn will affect the enzyme activity active site and the specific catalytic reaction or the enzymatic activity the enzymatic ability will be lost.

So, the catalytic activity is defined or is rather measured in terms of what is called a turnover number. What do we mean by this? It is the maximum number of moles of substrate that can be converted to product; product per mole of catalytic site per second.

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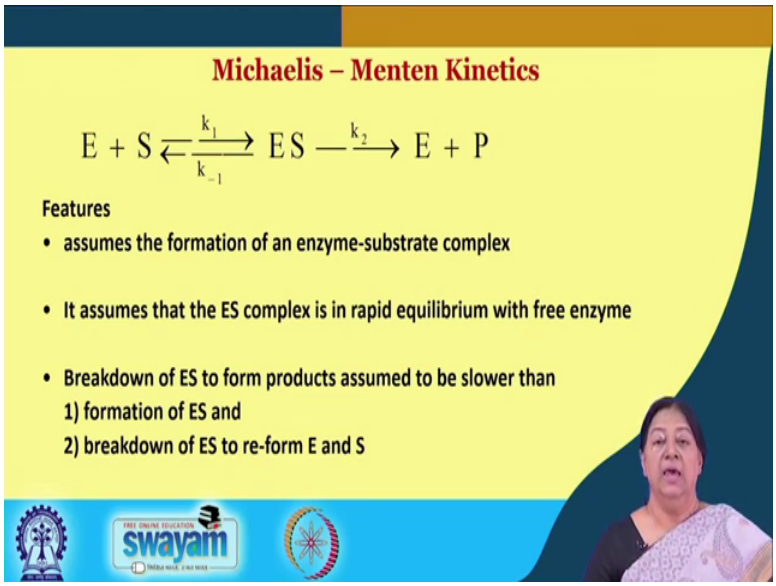

In the Michaelis - Menten Kinetics, we will not go into the complete details or work out this kinetics because this is something you have done previously. We have to remember or we have to just understand the methodologies or the features associated with Michaelis - Menten Kinetics.

The first idea that we have to have is that the enzyme has an active site. This active site binds a substrate. When it binds a substrate it forms what is called an enzyme substrate complex. This enzyme substrate complex then dissociates to regenerate the enzyme and give a product. Now this enzyme is again ready to take in another substrate. So, we have an enzyme substrate complex formed in what is called a pre equilibrium reaction. The rate constant for the forward reaction is k 1; the reverse is k minus 1. Once this E S complex is formed, it dissociates to form the E plus P.

So, the specific features here are we assume the formation of an enzyme substrate complex and it assumes that the E S complex is in rapid equilibrium with the free enzyme. The breakdown of the enzyme substrate complex now to form products is assumed to be slower than the formation of the E S as well as the breakdown of the E S to reform E and S.

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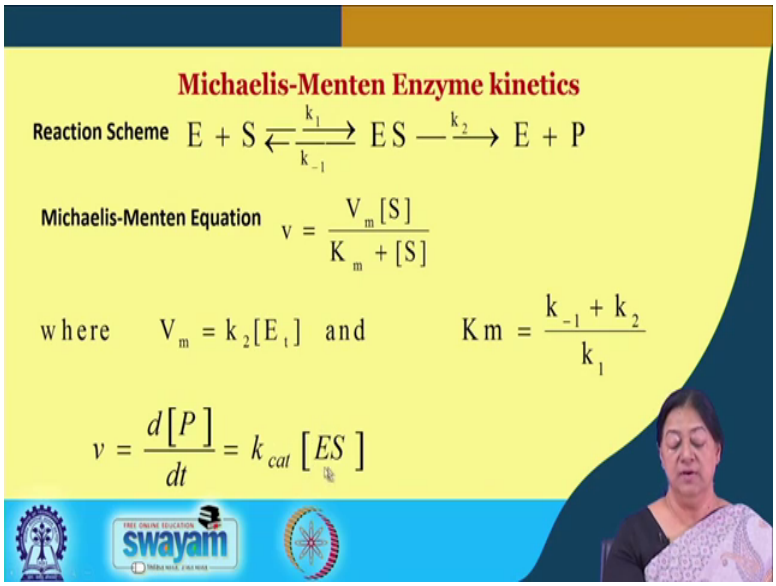

In the reaction scheme that we just learnt, we can apply what is called a steady state approximation to the E S complex. What do we mean by this? We look at the rate of formation and the rate of destruction of the enzyme substrate complex.

This means that where the forward reaction is the only way by which the enzyme substrate can be formed and the decay can occur through either k minus 1 or k 2. If we now work out some algebra based on enzyme kinetics, we will come up with what is called the Michaelis - Menten equation. The Michaelis - Menten equation tells us that this reaction has a certain velocity to it. What do we mean by this velocity? It is the formation of the products or the decrease of the reactants or in this case the substrate because we have a specific conversion reaction.

Any kinetics is associated with a reactant to product and how do we monitor this? We monitor this either by the depletion of the reactant or the formation of the product and we would have specific methodologies to look at this. If we have say a pH; a hydrogen or a proton formation or reduction, we can monitor this through pH. If we have an absorbance, if we have a color change, we can use spectroscopy which is by far the most widely used method to look at enzyme kinetics.

Now, what we have here is we have a terminology call we have a term called Vm. This is the maximum velocity possible and it considers the total enzyme concentration which means that that if all the enzyme want to can be converted into the product through the k

2 step, we would have the V max. This Km quantity is known as the Michaelis - Menten constant. This constant you can see is a measure of the k minus 1, the k 2 by the k 1. What is the k minus 1 and the k 2? These are where the enzyme substrate complex is disintegrated or it is destroyed. But the k 1 is where it is formed. The V as we said is a formation of the product with time and it is the k cat, the catalytic rate constant with the E S that is the enzyme substrate complex.

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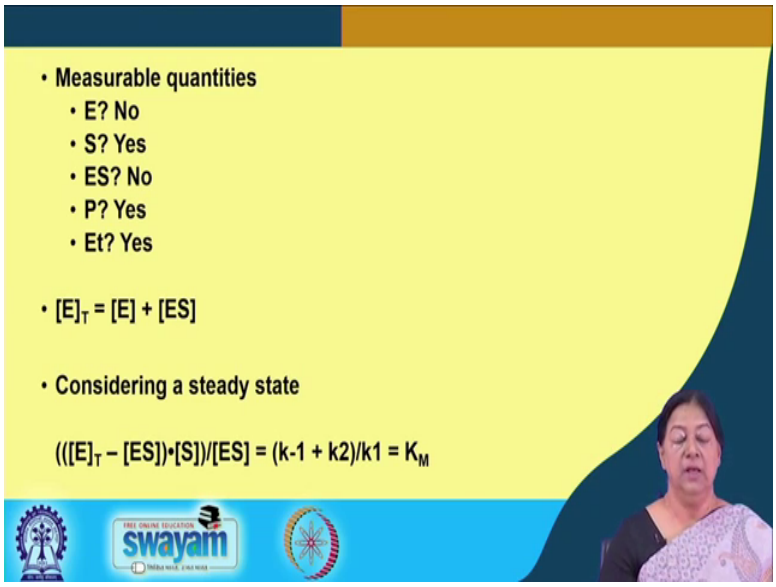

Now, what can we measure? Because this is extremely important when we are trying to understand what we can do. Since being an experimentalist you will know whether you can measure the enzyme as the reaction is going on. Whether you can measure the substrate? Since you added the substrate to the solution or to your experimental setup, you know how much substrate you have added.

You know how much product is being formed and what you do know is you know the total amount of enzyme that you have added; part of this enzyme is going to be in the free form; part of it is going to be in the enzyme substrate complex form. So, the total enzyme concentration is going to be the free enzyme plus the enzyme that has gone in to form the E S complex. So, this is where we have the total enzyme that is equal to the free enzyme plus the E S complex.

So, if we have a measure to understand how much free enzyme is present or how much E S complex is formed, then we since we know any one of these if we know any one of

these, then we know the other one. Because we know the total amount of enzyme that we have started off with. So, we consider what is called a steady state as I just mentioned and we can get what is called the Michaelis - Menten constant.

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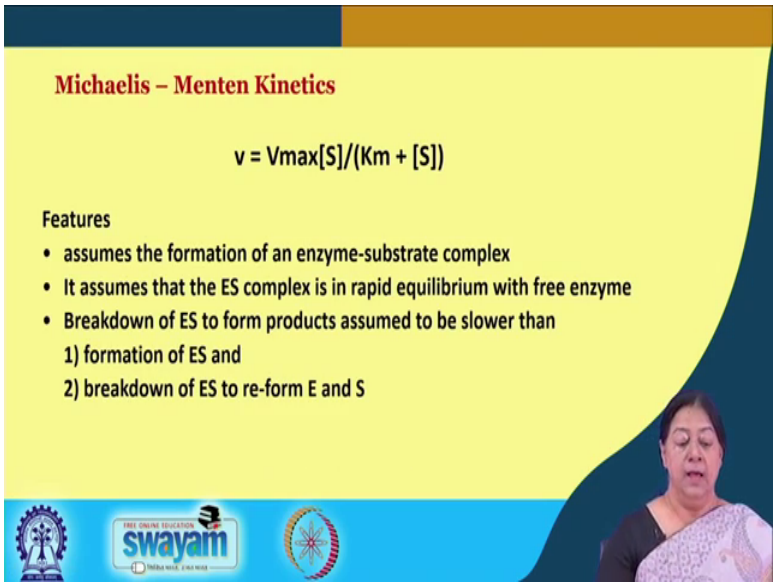

So, the features assume that we have this as we mentioned before the formation of the enzyme substrate complex, the breakdown and a specific michel Michaelis - Menten Kinetics given by an expression.

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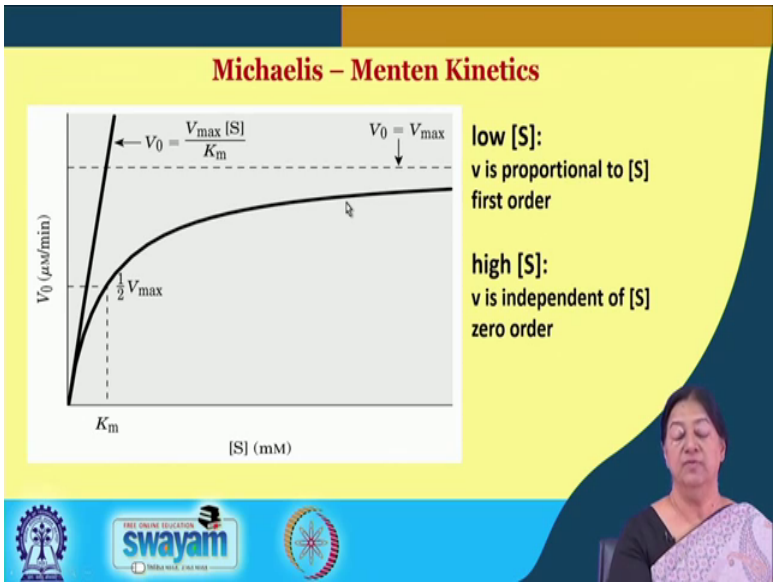

So, we have what is called here. So, this is the substrate concentration and this is the velocity of the reaction, the initial velocity of the reaction. So, as we increase the substrate concentration, there are certain features that we have to look at in this graph. There is an initial velocity that is taken by this tangential part from the hyperbolic curve that we get here. There is a plateau or a saturation kinetics attained; why is this? We have to remember that the enzyme is a protein, it has an active site to it. So, there is a limit to the number of substrate molecules, this active site can hold.

So, however much we increase the substrate concentration, it will reach a maximum when all the active sites of all the enzyme molecules present in the solution have been filled up. But what do we see in terms of kinetics in the initial part? We see that low substrate concentrations, we have the V proportional to the S following first order kinetics.

But as we have a higher substrate concentration V is independent of S, there is no change in the velocity. We can reach a V max and it is zero order. And what do we have here? If this is the V max value, half of the V max based on the Michaelis - Menten equation, the expression relates to the Km. So, the substrate concentration at which half the maximum velocity is attained is equal to the Michaelis - Menten constant.

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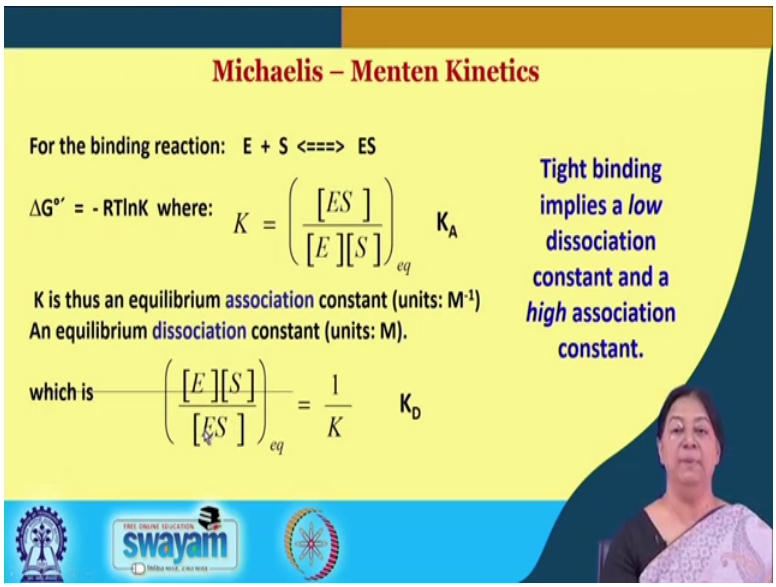

So, we have the kinetics and we have certain terminologies here that we have to look at. So, we have what is called if we look at this reaction the pre equilibrium reaction, the E

plus S forming the E S complex. This is an equilibrium constant associated with the equilibrium concentration of the enzyme substrate com complex the E and the S. This is what is called an association constant. So, it is an equilibrium association constant and we can see that the units here are mole inverse. If we take the reverse of this, we will have what is called a dissociation constant which is E S by the E S complex and the units here are mole. This will be something that you will also study when you look at protein binding, where we will have association and specific binding constants associated with your reaction.

Now, when we have tight binding, it means that we are looking at a low dissociation. A tight binding means that the E S complex concentration is high. If this is high K D is low. So, if we want tight binding, we want to look for a low K D and a high association constant. What does this mean? Now, when we have an enzyme substrate complex form, it has to be a very delicate balance.

We do not want the substrate to bind too tightly because then it will the equilibrium will be shifted only to one direction. We have to have an equilibrium, where this because the enzyme has to be free and by the substrate, it has to be a relatively loose non covalent binding. In most of the cases, there are covalent reactions that also occur, but we will see that the enzyme goes back to its initial stages to be ready to take up another substrate.

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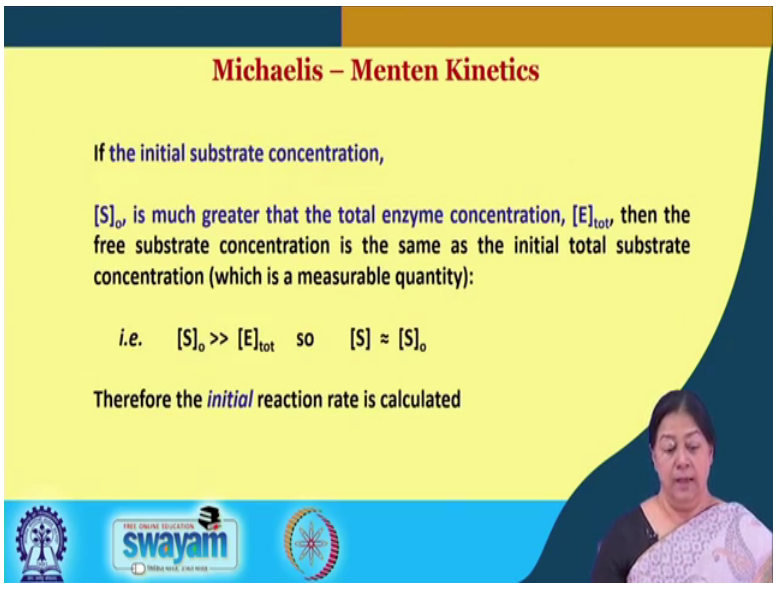

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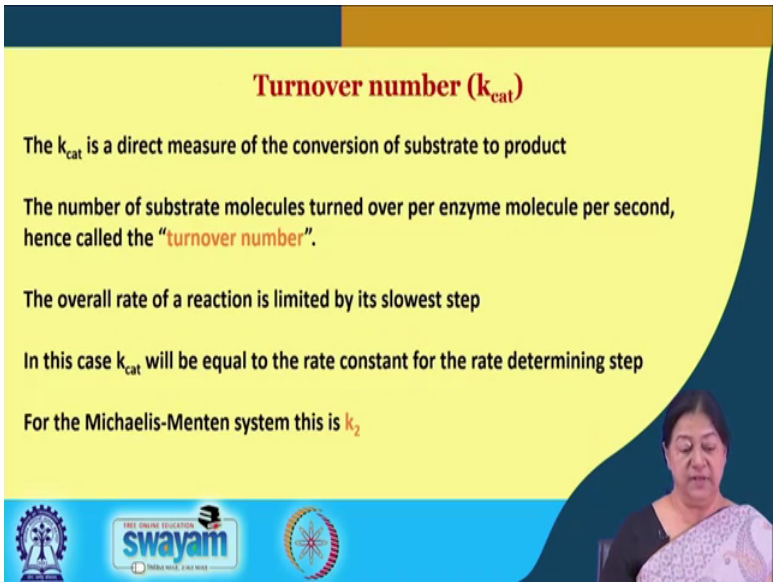

So, if we have a specific turnover number or let us look at this; so we have if the for the initial substrate concentration, we look at an S 0 that is much greater than the total enzyme concentration. What does this mean? It means that we can make certain approximations.

The certain approximations will allow us to calculate the initial rate of the reaction which will help us in determining what the Michaelis constant is. Now, the k cat is a value that gives us a direct measure of the conversion of the substrate to the product. It is the number of substrate molecules turned over per enzyme molecule per second and the overall rate of reaction just like any kinetic reaction is limited by its slowest step, the rate determining step. In this case, the k cat will be equal to the rate constant and for the Michaelis - Menten system this is k 2, where we have k 2 E S going to E plus P.

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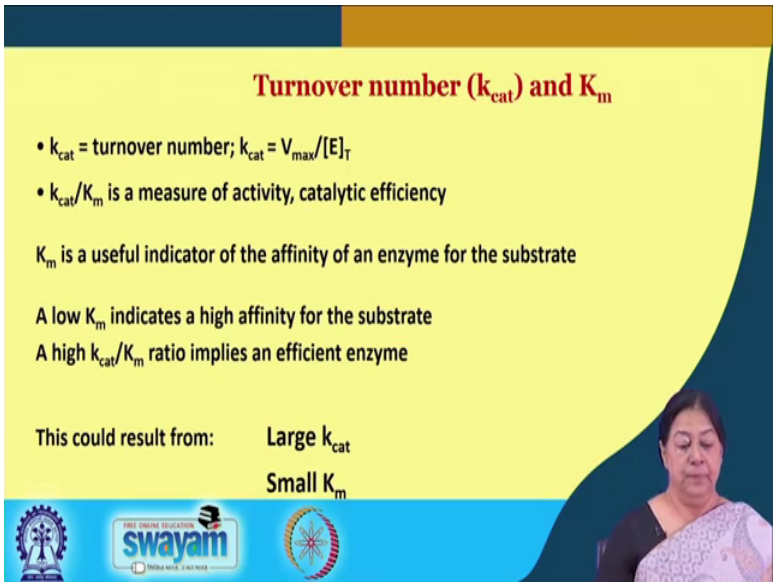

So, we have the turnover number which is defined as the V max by the E T, the total concentration of the enzyme present and the Km value that we have the Michaelis - Menten constant is a useful indicator of the affinity of an enzyme for the substrate. If we have a low Km, we have high affinity and then, if we have a high k cat by Km ratio, this implies it is an efficient enzyme. It will work better in terms of having either a Large k cat or a Small Km.

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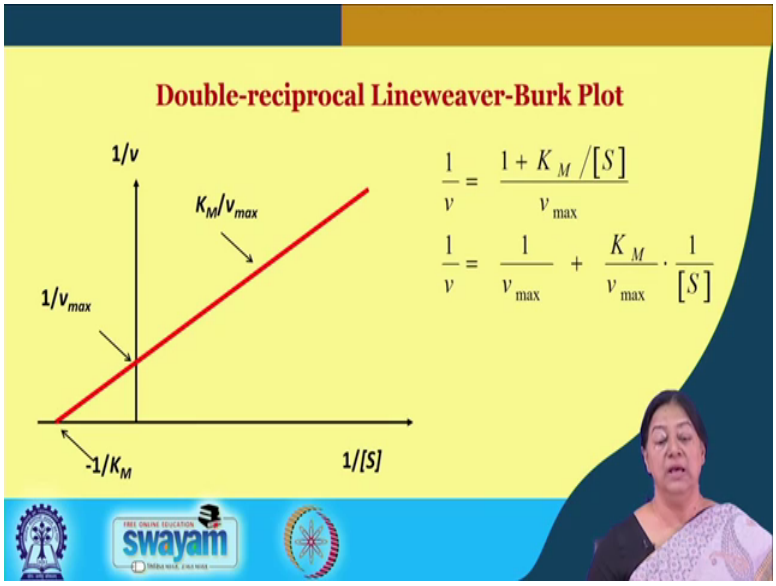

Now, how do we measure all this? How do we calculate all this? This is given by what is called a Lineweaver-Burk Plot where if we look at the Michaelis - Menten kinetics, we look at a graph where we have a hyperbolic curve. It is very difficult in that case to determine the actual value of the V max because we do not know where the V max will or at what velocity it will be attained.

So, in this case we have if we rearrange the equation and we plot what is called a double reciprocal plot, where we have 1 by V versus 1 by substrate concentration. This can be a straight line as you can see from the expression here and what we have here is the y intercept here is 1 by V max and the intercept on the x axis is minus 1 by K m. So, we straightaway get the values of V max and Km, the Michaelis constant as well as the maximum velocity attained from this double reciprocal Lineweaver-Burk Plot.

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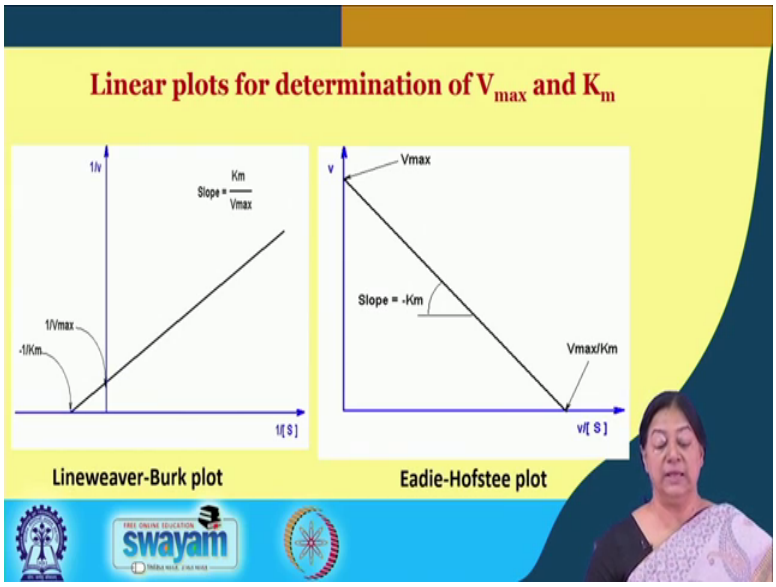

There are other plots also that can give you the V max and the Km. Another common plot is the Eadie-Hofstee plot, but the Lineweaver-Burk plot is something that is used more often for enzyme kinetics and this will be demonstrated to you in the experimental part of this lecture.

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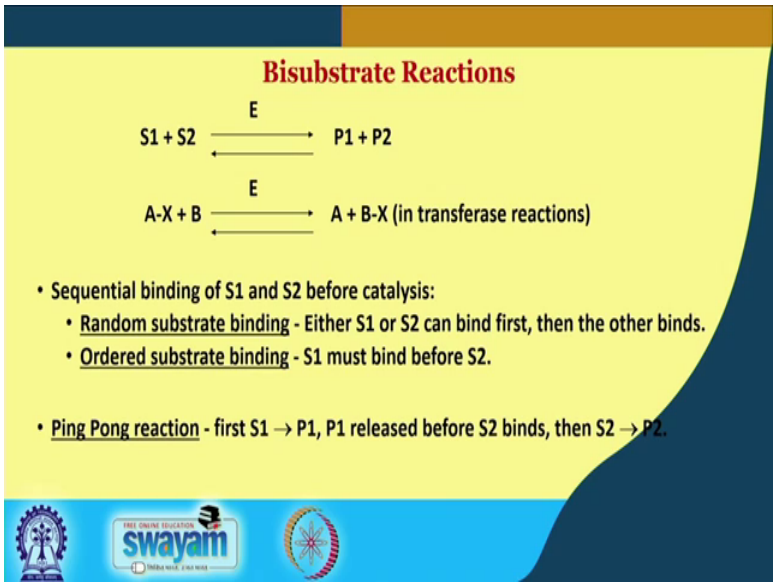

There are other ways in other ways in which we have certain reactions, the some are called bi substrate reactions; what do we mean by that? We can have two substrates. If we have two substrates and how does this work? It means we have S 1 and S 2 and we can have two products.

Suppose we have a reaction like this where we have A X plus B and X is transferred to B from A in a transferase reaction. They can be sequential binding of S 1 and S 2, random substrate or ordered substrate. This will depend upon the active site. It may so happen that if it is a random substrate binding, it would not matter whether S 1 bound first or S 2 bound first. But in an ordered substrate binding, it would mean that S 1 would have to bind first before S 2 can bind. This may result in some variation in the enzyme structure which we will see later on.

In the Ping Pong reaction, S 1 has to bind first; P 1 has to be released, then the because the enzyme has a different structure then. Then, enzyme the different enzyme let us call it enzyme E prime will then bind S 2 form P 2 and then go back to its original formation.

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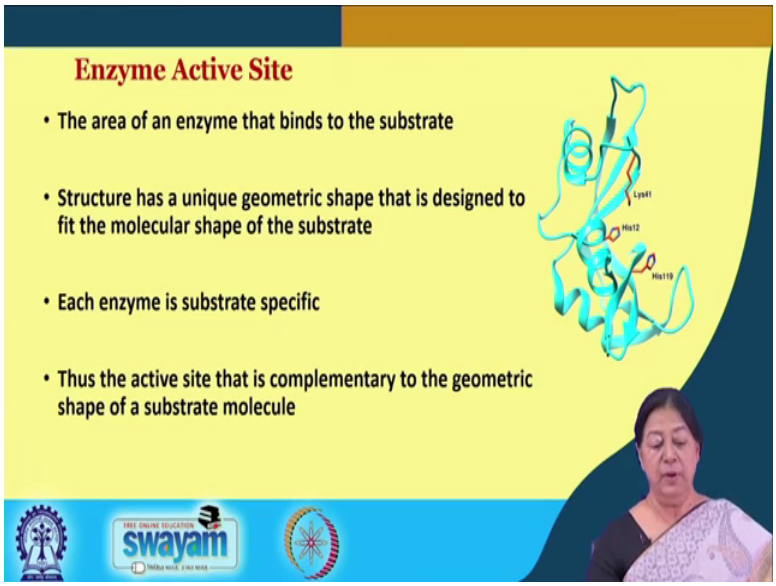

If we look at what an enzyme active site is, I have been talking about the enzyme active site, this is the area of the enzyme that binds the substrate. The structure has a unique geometric shape that is designed to fit the molecular shape of the substrate.

So, there is a shape complementarity, in addition to a chemical complementarity for the addition. Each substrate is extremely substrate specific; each enzyme is very substrate specific. So, the active site is complementary to geometrical shape of the molecule. If we look at this geometrical shape, we will look at the mechanism of the specificity, this is (Refer Time: 19:35) and we will see how the shape has been defined or realized or how it is actually shaped to bind the substrate.

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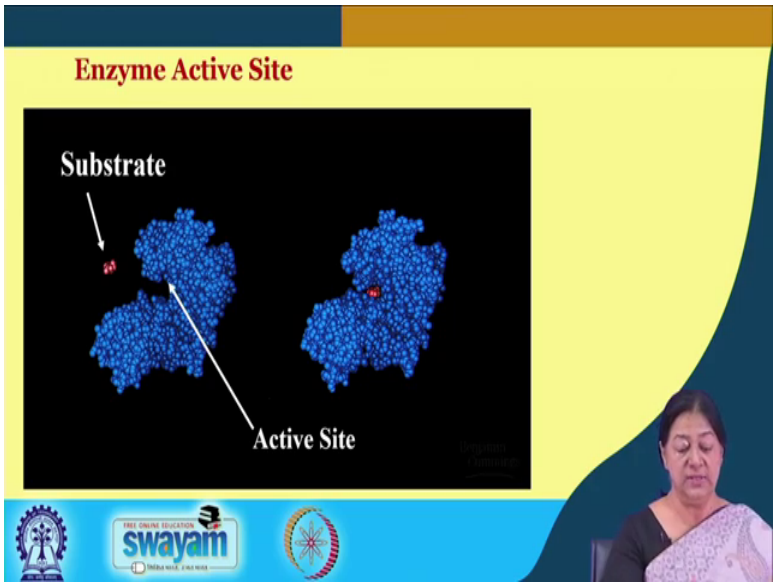

So, let us look at this. If this is our enzyme molecule; this is our active site and this is up substrate; this is the enzyme substrate complex, where we have the binding of this small molecule to the active site of the enzyme for a particular reaction to occur.

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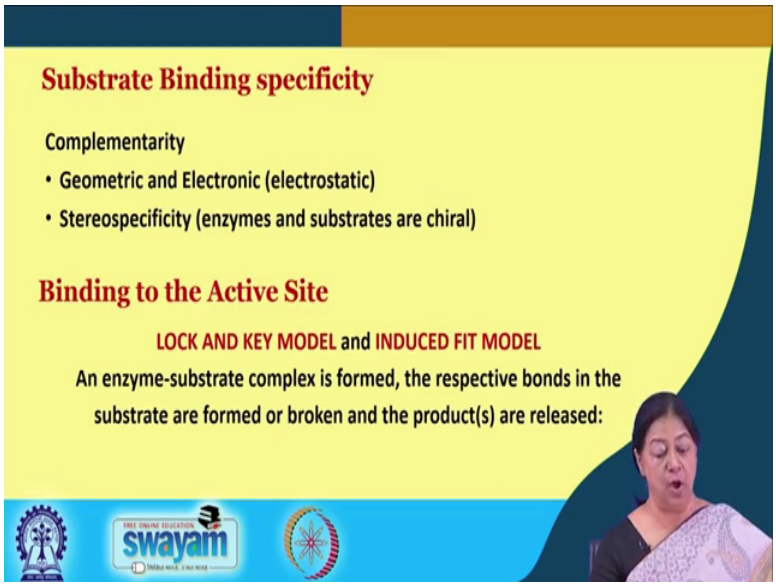

So, we have Substrate Binding Specificity, Geometric Electronics, Stereospecificity and the Binding to the Active Site is defined by two different methodologies either a Lock and Key model like fit a key to a specific lock or an Induced Fit model. What happens is in both cases an enzyme substrate complex is formed, then there are specific bonds that

are formed or broken and the products are released. Let us look at these two different types.

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One is the lock and key model. In this site, where we have the active site of the protein, the enzyme binds a substrate in a region called this active site and we learnt that there are only certain substrates that will be able to fit the active site which means that if we have a circular substrate, it is not going to fit in the active site here. The substrate is shaped like this. It fits into the active site of the enzyme perfectly and the amino acid groups that are present here will fit in the substrate in a manner that allows for chemical complementarity as well and then, once we have the product formation. Then, we have the enzyme back to it is original form which can bind the next molecule of the substrate.

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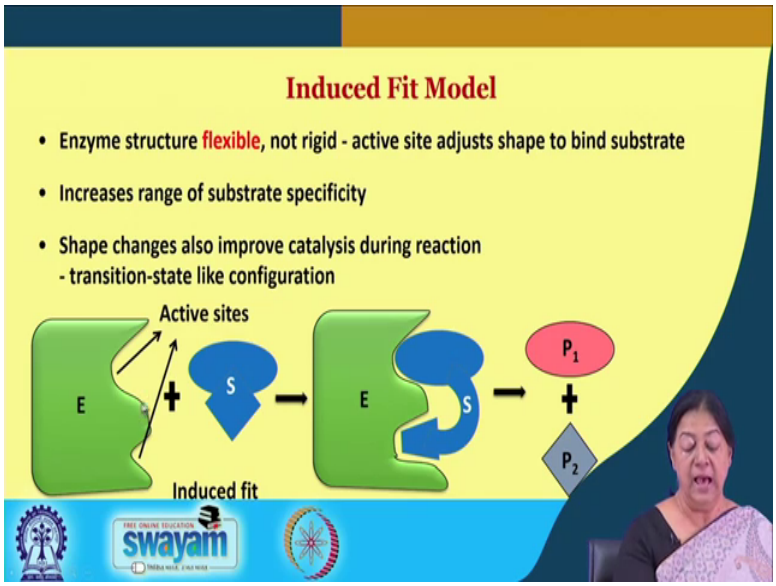

If we look at the induced fit model, in this case the enzyme structure is flexible. It is not rigid like in the previous case and the active site will adjust it is adjust its shape in such a manner so that it can bind the substrate in a manner which will allow for the catalytic reaction to occur. This increases the range of substrate specificity and there are shape changes that occur that improve the catalysis during the reaction and there are specific transition state like configurations that occur.

So, if we have a specific active sites that are shaped like this, we have the substrate that can come in and what you can see is the enzyme has modified itself or adjusted itself to accommodate for the substrate that is also being a chemical component, a chemical component. It can adjust itself, it will have changes in bond formation and then, we have the product formation. So, we have what is called a lock and key method; we have what is called an induced fit method. So, these are the two models that are usually used for enzyme reactions.

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We have given here two references; one referring to the Biochemistry book by Voet D Voet and another very good book about Enzyme Kinetics which gives you various reactions that can occur. Because what we just looked at is we just looked at the simplistic method, where we have the enzyme and the substrate bind together to form the enzyme substrate complex.

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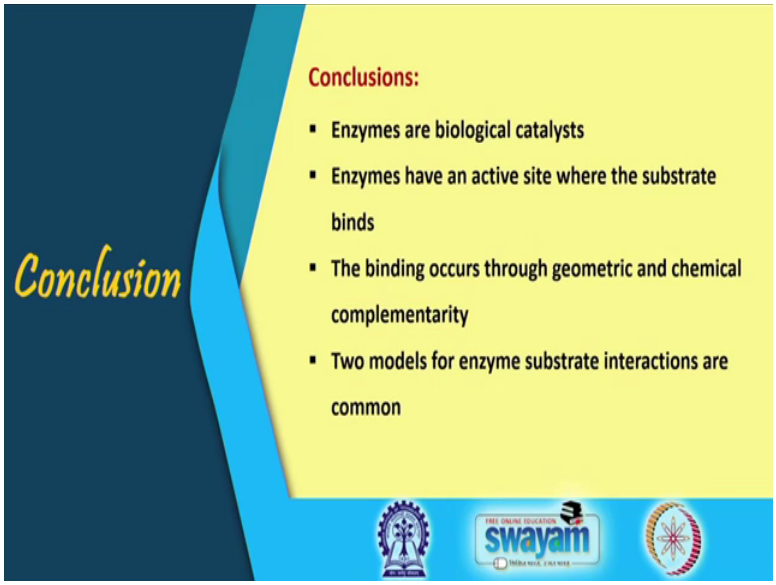

Now, we understood that the enzymes are biological catalysts. What does this mean? This means that there is an active site. There is a transition state, there is an activation

energy. Now, when this activation energy is reduced like in any kinetic reaction, we have a catalyst present. In this case our catalyst is an enzyme. The reaction is a biochemical reaction and the enzyme is a protein. When the enzyme is a protein, the protein can act by itself, if it is a simple enzyme. If it is a complex enzyme in addition to the apoenzyme or an in addition to the enzyme itself, it will have or it will require a prosthetic group or a coenzyme or even a metal ion for its catalytic reaction to occur..

The active site is lined by amino acid residues that have specific characteristics. We learnt of the different types of amino acid residues that can some of these will line the active site and we will see that there are large number of histidines actually that are present and this is because the pKa value of the histidines as I mentioned earlier in one of the classes is in such a range that it matches with your physiological pH. We have the binding that occurs to the active site through complementarity. What kind of complementarity? Either geometric or chemical complementarity.

So, if we have a hydrophobic active site, if we have an acidic active site, if we have a basic active site or if we have that can one can that can form aromatic by stacking or different types of interactions or hydrogen bonding. We want to have a non covalent interaction between the enzyme active site and the substrate and what is going to happen? The enzyme substrate complex is going to form and this is then going to release the product in a specific reaction.

Now, when the product is released, there are two models that are followed; the lock and key model and the induced fit model that will tell us whether we have our enzyme reaction occur in a manner that can be monitored and we will see a specific enzyme reaction, when the experiment is done in the experimental part and then, we will look at other activities of enzymes.

Thank you.