Biochemistry - I

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Lecture –7
Enzymes –I

Welcome, we will start our discussion on Enzymes. We considered what proteins are. We have studied protein structures the constituents of proteins that are the amino acids and their properties.

What we are going to start today is our study on enzymes which will cover about three lectures and we will be studying about the Enzyme mechanisms of a few enzymes which will be basically Ribonucleus, Lysozyme and Serine proteins. As we go along you will see what they mean (Refer Slide Time 1:12 min).

Now you heard about enzymes and you know that you need them in the body for digestive purposes, you need them actually for any process that goes on in the body. So whether it is your respiratory process or it is your digestive process or what ever other process is going on all of them are enzymatic processes. They are specific enzymes that are involved which bring about specific reactions and you will see how specificity is extremely important in the way enzymes act.

So basically all Enzymes are protein catalysis. The protein catalysts are catalytic in nature but they are proteins. And what we have here is you know about catalysts they alter the rate of a chemical reaction without undergoing a permanent change in structure. So this is exactly what the protein is going to do also. We will see how it is exactly is going to change. It is going to change the rate of the chemical reaction but the protein itself is not going to undergo any change even though it is going to bring about drastic change in the reaction (Refer Slide Time 2:31 min).

Now this is the typical picture that all of you have seen, this typical diagram gives you the free energy of A + B where these are the reactants and your P + Q are the protons in these case. So what do you have? You have a ΔG of reaction which is the G of (P) + G of (Q) - G of (A) and G of (B). It is $G_{\mathbf{final}} - G_{\mathbf{initial}}$. And what is this? This is your transition state your activation states that this activation barrier has to be overcome to get you from the reactants to the products (Refer Slide Time 3:09 min).

Now if you look at this, this is a specific nomenclature that we have put here which is some times called as the daggers sign. So we have a $\Delta G^{\ddagger}$ that tells us this is the energy of activation for the reactants to go to this transition state.

Now, if we have a catalyst, how does this reaction profile change? it changes in such a manner that further reaction A + B is going to P + Q. now we have a catalyzed portion of the reaction or catalyzed reaction in which our activation energy has decreased.

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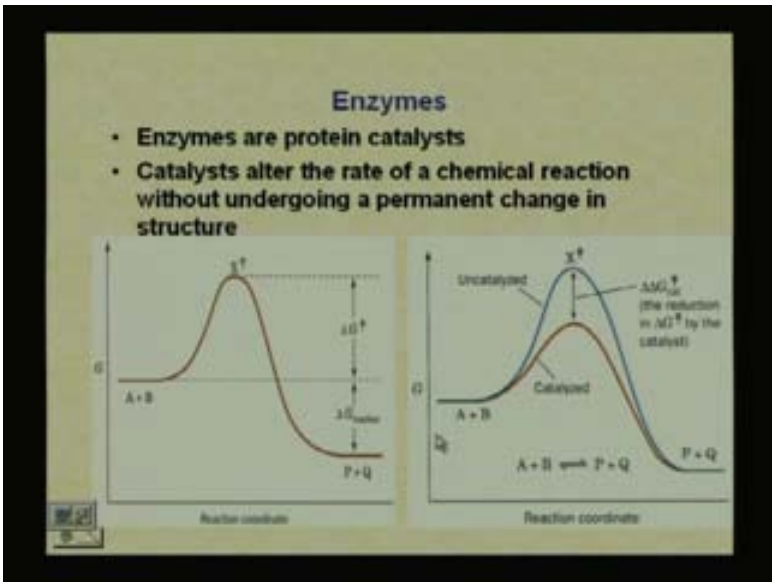

What does it do in decreasing? The reaction is catalyzed which means that the rate has been enhanced. But the ΔG itself has nothing to do with the rate. You have to remember the ΔG as a thermodynamic quantity but the activation that we are considering is a kinetic quantity. This distinction is extremely important when we are considering enzyme catalysis.

Now what do we have in an enzyme? It is basically a protein, a protein that has a specific structure to it and what it looks like is if we look at a specific protein say we have the protein that is shaped like this, we have particular cleft in our protein. Now if this is our enzyme it is going to react with a particular substrate. What is the substrate is going to do? The substrate is going to fit into this active site. Then what do have in the active site? in the active site we have which is say this region here is marked with particular residues or specific residues that are going to act on the substrate to give you the product but the enzyme will remain as it is, remember the enzyme is the catalyst (Refer Slide Time 5:30 min).

So we have the Active site that is lined with residues and sometimes contains a co-factor. The Active site residues have several important properties. So the residues that are in the active site of enzymes have specific charges so their charges could be partial, you could have a dipoles, you could have a helix dipoles because you have to remember that the specificity of an enzyme is the reactant that is coming to act on enzymes so that is going to have specific groups. The specific groups will interact with what ever residues lie in the active site. So we have charge, we have pKa.

If you remember when we studied amino acid properties I mention the pKa and Hydrophobicity are very important properties because the pKa is going to determine whether you have a protonated or deprotonated or a zwitterionic form amino acid. And if you remember I mentioned that the Hestidine amino acid residue has a pKa of 6 and it lines a lot of active sites of enzymes. The sole region being the pKa of Hestidine where we are talking about this pKa value this pKa of Hestidine close to the physiological pH of 7.4 so it can act not only as a base it can also acts as a acid.

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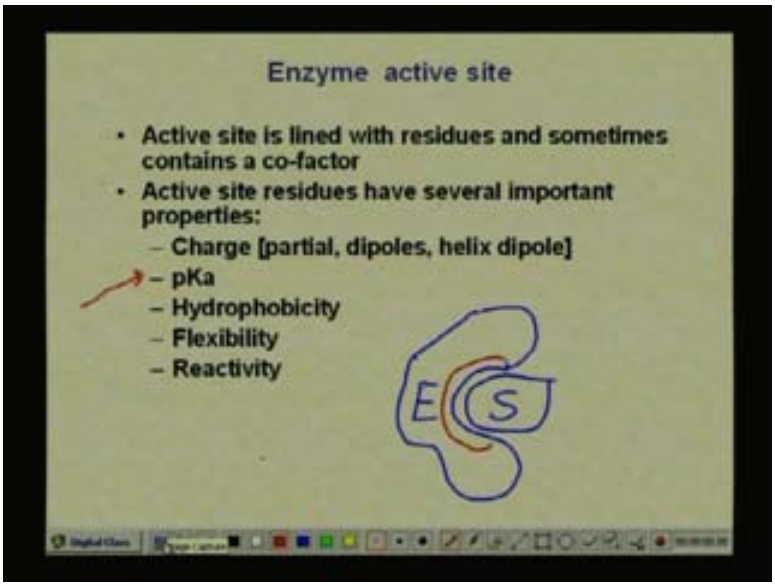

Another important property that we learnt about amino acid was the Hydrophobicity. So it is also going to be important in the active site residue lining. Then the Flexibility, because we should have residues move over to make way for the substrate to come in and definitely the reactivity.

So now we will go back to see what other properties do we have. So look at the enzyme active sites that we have here, these are three typical enzymes Chymotrypsin, Trypsin and Elastase where these are what we have in the active sites of these proteins. We will see what these proteins or what these enzymes do as we proceed in the course but for now what we have here is we have two Glycines under Serine and here we have two Glycines and then Aspartic acid. As we know the Aspartic acid is an acidic amino acid residue, it has a negative charge associated to it. So it is likely that the substrate that it is going to bind through is going to be the opposite charged one which is a positive one here. So what do we see here? We see here a Lysine.

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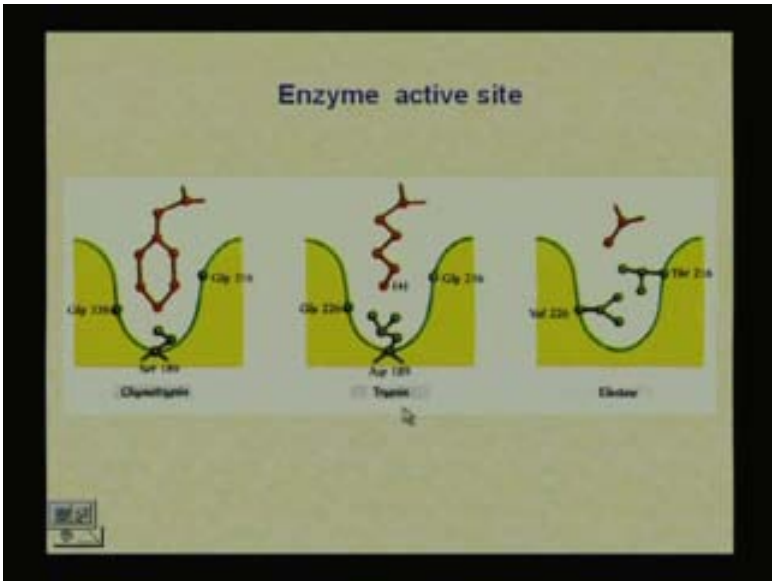

So if this is our enzyme active site such that it will accommodate a residue that is counter in charge to the active site residue and it will fix in the active site. So basically we should have a fit of the specific substrate that we are talking about in the enzyme active site. Again this is another protein called Elastase or another enzyme rather that we can call as Elastase. It has two different groups in its active site and it again acts on a different type of amino acid. So each of these active sites have their own properties in deciding which substrate it is going to act upon it (Refer Slide Time 9:51 min).

Now if we look at the enzyme classification in which we have Simple Enzymes and we have Complex Enzymes. What do we mean by them? For Simple Enzyme, we have an Enzyme that is composed of a whole protein and example of a simple enzyme would be Ribonucleus. Ribonucleus is composed of a whole protein so it is a simple enzyme.

If you have a Complex Enzyme, it is not the protein alone that can act on the substrate but it will be composed of a protein plus a relatively small Organic molecule. A Complex Enzyme is a holoenzyme which it is comprised of an apoenzyme again which is the protein part and a prosthetic group or a coenzyme. So for the Complex Enzymes we have a holoenzyme that is basically the whole enzyme, the holoenzyme that is comprised of an apoenzyme and a prosthetic group or a coenzyme (Refer Slide Time 10:55 min).

What is the prosthetic group? A prosthetic group is a small organic molecule bound to the apoenzyme which is a protein by covalent bonds. So if you have a holoenzyme that contains a prosthetic group then it is attached to the protein that is the apoenzyme by covalent bonds.

In the contrast, when the binding between the apoenzyme and non-protein component is non-covalent in nature then it is called a coenzyme. So that is the difference. So in this

simple very ordinary classification we would have two types in which one is the Simple Enzymes that comprises just of whole proteins for example Ribonucleus.

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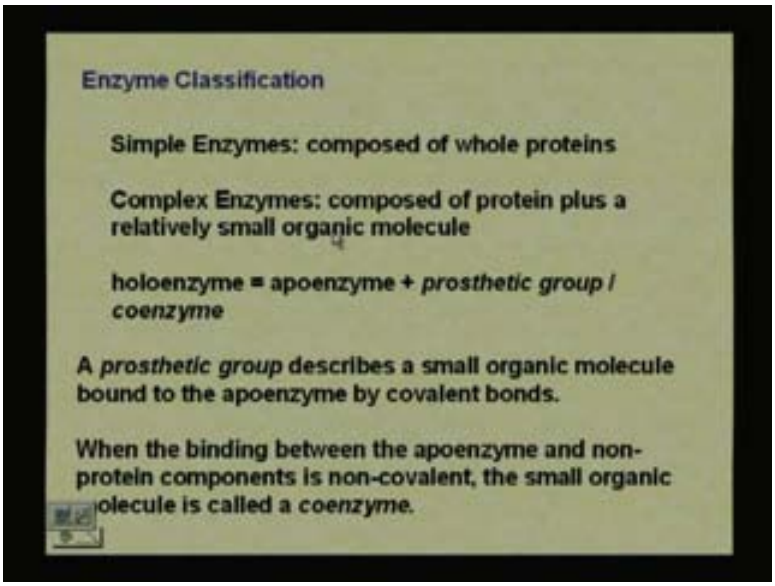

The other one is Complex Enzymes that have a protein part and along with a protein part they have a small organic molecule moiety either attached to it covalently or just associated to it. If it is attached covalently then it is called a prosthetic group or if it is just a non-covalent association then it is called a coenzyme (Refer Slide Time 12:21 min).

There are other classifications of enzymes which you will have to know about. They are on the type of reaction that they work upon. You have to recognize that these enzymes are biological catalysts, they are proteins themselves and they act on specific substrates.

Now we have classification and we have six different groups of Enzymes based on what a substrate or what reaction they are going to catalyze. Now these are different groups of Enzymes that we have here. If you look at the names at the end they all of them are suffix by as ‘es’. So the first one an Oxidoreductases from the name itself suggest that it would be a redox type of reaction that it would catalyze. So Oxidoreductases would act on the chemical groupings either to add or remove hydrogen atoms.

So from the name itself you can actually figure out the function of the specific enzyme. So what is the Oxidoreductases doing is it involved in a redox reaction so it acts on groups to add or remove hydrogen atoms (Refer Slide Time 13:50 min).

The next one is Transferases. What is that going to do? It is going to transfer, since you are looking at specific reactions it is going to transfer a functional group between the donor and the acceptor so that would be a Transferases. We have a special name for groups that transfer phosphate they are called Kinases. Later on when we will be doing a

bioenergetics you can see how Kinases are extremely important in the transfer of phosphate from ATP to other molecules because this ATP is our currency of energy.

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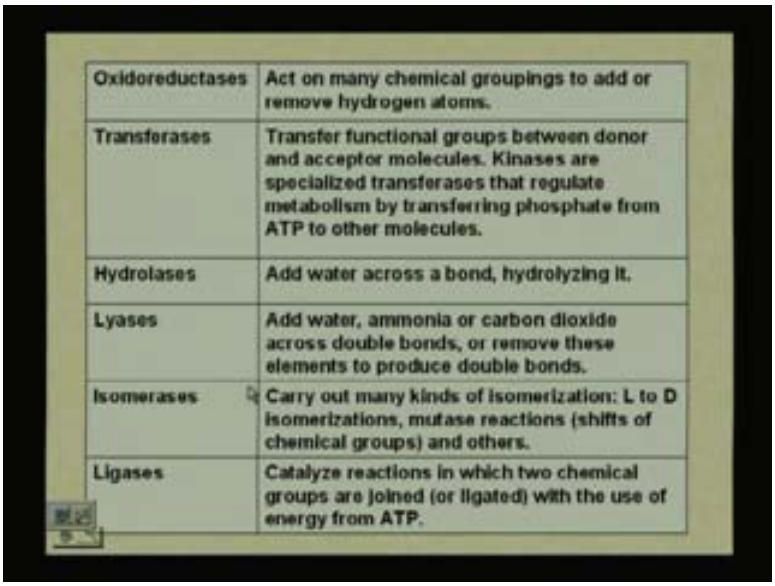

This something that probably you studied in your 12th standard where you know that ATP is our energy currency, it provides all the energy. so there is a specific name for the transfer of the high energy phosphate and the phosphate transfers are done by Kinases (Refer Slide Time 14:57 min).

So Transferases would transfer a functional group between a donor and acceptor molecule and the Kinases are the special groups of Transferases that transfer phosphate groups. Then we have Hydrolases. What does the name mean? The name mean is hydrolysis. So we have Hydrolases that would add water across a bond. in the event of doing that it hydrolysis the bond.

Then we have Lyases. What do Lyases do? They add water, ammonia or carbon dioxide across double bonds, or they remove these elements to produce double bonds. So the Lyases are the ones that are going to add the small molecular moieties across double bonds or remove them to form double bonds.

Next one is Isomerases. The name itself is sounding like isomer. So what is that going to do? It is going to bring about isomerization. An isomerization reaction is going to be catalyzed by an Isomerase. So it will carry out or catalyze the isomerization say from L to D form. So basically what it is doing? It is shifting the chemical groups. If you have an asymmetric carbon atom then you will know you can have a L form or a D form in which only the difference is in the position of the groups.

So this Isomerases can change the position of the groups about the asymmetric carbon atom giving rise to an either L isomer or a D isomer so we have the name Isomerases (Refer Slide Time 16:47 min).

The last one is Ligases. The ligation means linking together. So a Ligase would catalyze reactions in which two chemical groups are joined or ligated with the use of energy from ATP. So a Ligase would catalyze the reaction where they would be chemical groups that are joined together (Refer Slide Time 17:15 min).

So these are the six different classes of Enzymes that are used. We will see later on what is called the universal enzyme classification. Each enzyme has its own identity that sort of looks like an IP address, like a computer has an IP address your enzyme has an enzyme classification in which looks exactly like an IP address like 3.102.11.3 that is an enzyme classification that would belong. So what the first number actually is which group it belongs to here.

So if it were one dot something it would be Oxidoreductases. So that amounts to be enzyme classification. So what are the six types we have? We have a Oxidoreductases, a Transferases, a Hydrolases, a Lyases, a Isomerases and a Ligases. And what you need to know is which reactions are catalyzed by which enzymes and all you actually need to follow is what the name means. If you understand what an Ligases means then you know will that the chemical groups are joined by this enzyme. If you know what an Oxidoreductases means then you will know that it is involved in a redox reaction.

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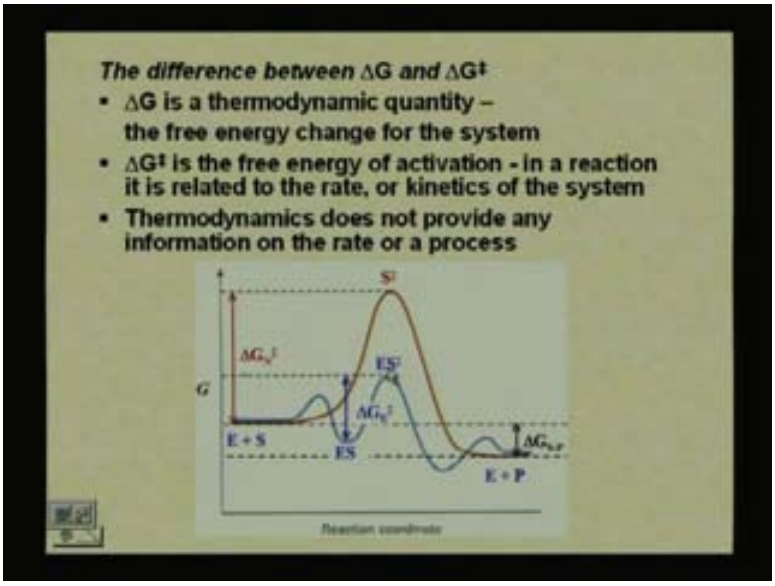

Now we come to this thing that I was talking about the ΔG and the $\Delta G^{\ddagger}$. What do we have here? We have not an A + B or P + Q anymore we now have an enzyme and a substrate. The enzyme and the substrate actually form an enzyme substrate complex.

We will go into the details of exactly how this is formed or what is actually going on here but we have an activated enzyme substrate complex here that is eventually will lead us to the product. So we have a ΔG associated with that. What is this ΔG? This is Thermodynamic quantity and it is the free energy change for the system. But the $\Delta G^{\ddagger}$ that we have here is the free energy of activation in a reaction, it is related to the rate, or the kinetics of the system.

You know that the Thermodynamics does not provide any information about the rate of the system. It is just going to give you the ΔG thermodynamic quantity that is going to tell you whether the reaction is spontaneous or not. (Refer Slide Time 20:07 min). So if we just consider two reactions here say we have a ΔG for an uncatalyzed reaction that corresponds to a 107 KJ and then we have $\Delta G^{\ddagger}$. If we put '‡' means it is an activation. When I say this is there for the catalyzed reaction is this going to be larger or smaller than that, smaller because now my reaction is catalyzed the energy for the activation has decreased.

So if I want to find out how the rates have changed which reaction or which equation do I used? Which expression do I used? I used Arrhenius expression which is $k = A\, e^{-\Delta G^{\ddagger}/RT}$ (Refer Slide Time 21:30 min).

So what can I do? I can find a k value. Now this k is always for rate, K is for equilibrium. It should never the other way round and you should never make that mistake. The K is always for equilibrium and small k is always for the rate. So we have a k for the uncatalyzed reaction, we have a k for catalyzed reaction.

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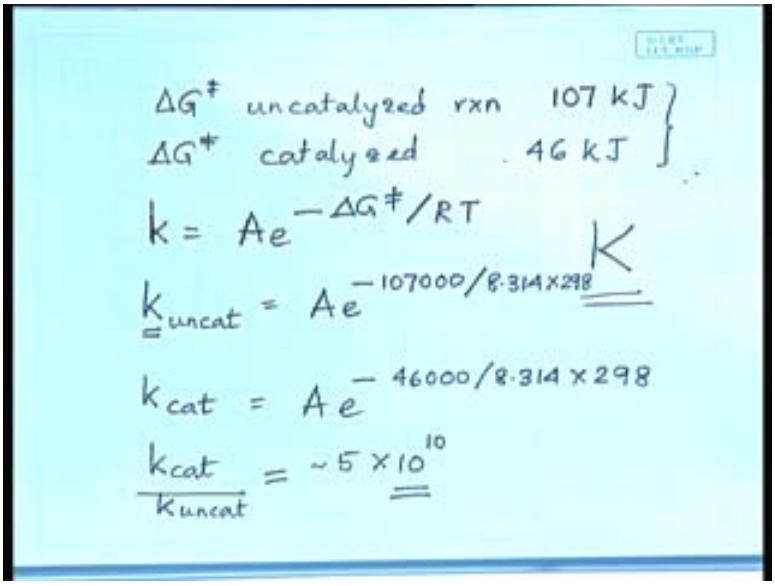

How do I determine it? All I have to do is plug in the values. Here A and if we consider the pre exponential factor to be the same so I have to be careful about units here which are kilojoules 8.314 and by default we usually use 298.

What about my catalyzed? A $e^{-46000/8.314x298}$ remember to use the right R also 298. So I can find out how the rate has increased for the catalyzed reaction as compared to the uncatalyzed reaction. It turns out that this is approximately 5×10^{10}. So I am saying that when I have reduced this energy of activation I have increased the rate 10^{10} folds basically (Refer Slide Time 23:26 min).

So I have a uncatalyzed reaction a catalyzed reaction and then I have associated to it. The change in the rate is due to reaction being catalyzed whether the catalyst happens to be an enzyme or any other catalyst it is the same thing.

So what do we have here? We have our enzyme plus substrate and we have our enzyme plus product. So what is our catalyst? Our catalyst is the enzyme. If you notice that what do we have in the beginning, in the very first I showed you that we had A + B going to P + Q, here I have E + S going to E + P. So E is the catalyst that is transforming the substrate to the product but itself it is remaining unchanged. So it is not like a normal reaction where we would have A + B going to P + Q here it is E + S going to E + P where E remains as it is in this reaction .(Refer Slide Time 25:00 min).

What do Enzymes do? Enzymes accelerate reaction by lowering the free energy of activation and they do this by binding the transition state of the reaction better than the substrate. Enzymes exhibit saturation kinetics.

Now how do I determine the Catalytic activity of an Enzyme? There must be a measure that tells us whether this Enzyme is good or bad. So the measure of that activity of an enzyme is called its turnover number.

How would you define a catalytic efficiency? An efficient enzyme would be one that could transform more of the substrate product that would be an efficiency of an enzyme. So the turnover number is defined as the maximum number of moles of substrate that can be converted to product per mole of catalytic site per second. What do I mean by a catalytic site? Remember I showed you some diagrams where we have an enzyme active site, in that enzyme active site there are substrates that bind to the enzyme active catalytic site or the active site and they convert the substrate into product. As they do that the enzyme retains it structure and activity so that it can convert another substrate to product.

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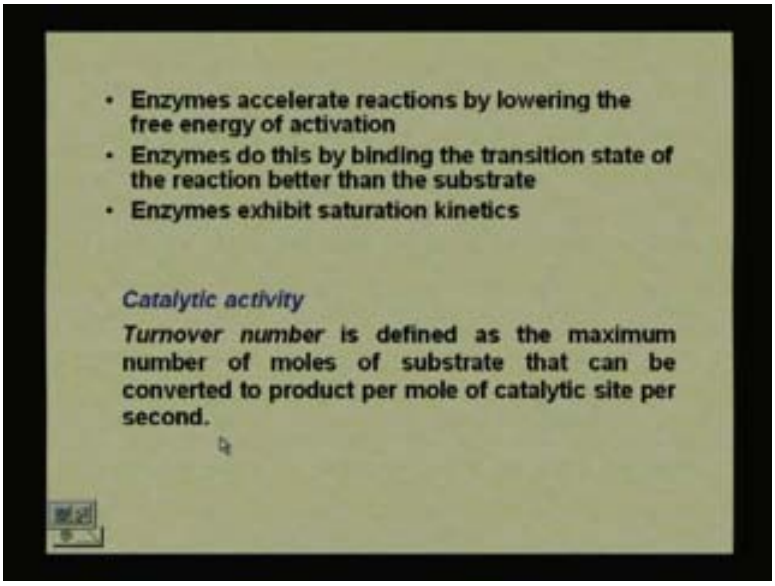

But there is a turnover number that is going to tell us the catalytic efficiency and that efficiency is measured by how many number of moles we can transfer of the substrate to the product, the conversion per mole of catalytic site per second because you have to remember it is a rate that we are considering (Refer Slide Time 27:21 min).

So you are looking at the maximum number of moles of substrate that can be converted to product per mole of catalytic site per second. Now, what happens to a protein when we heat it? It denatures and enzyme is a protein. So what do you have for your normal reactions when you increase the temperature? Certainly the rate increases.

For enzymes, you would expect that there would be an optimum temperature at which the enzyme is going to act because beyond that it is going to get destroyed. If it gets destroyed then the enzyme active site is destroyed as a result of that what is going to happen is the substrate cannot bind and obviously the product cannot formed (Refer Slide Time 28:15 min).

so we have an optimum temperature that if we do a percent activity versus temperature curve like this then we are going to have an optimum temperature for the highest activity of the enzyme. And you realize that at low temperatures the enzyme is not going to function, at high temperatures also the enzyme is not going to function because it has to have and optimum activity especially of course the activity is also going to considering the reactions that take place in our body then it is going to be an optimum of 37ºC (Refer Slide Time 29:00 min).

Then how about p^H? If we add too much acid to the protein then it is going to depend upon the amino acid composition of the protein. We have to remember that enzyme is a protein so the amino acid composition of the active site is extremely important in determining which pH is going to act that.

So if you look at here there are certain graphs here corresponding to the relative activity verses the p^H. Here the first one is Pepsin. if you remember you studied in school about Enzymes, if you had biology where Pepsin acts in our stomach where the acid is at low pH. The pH of the acid in your stomach is 2 so Pepsin acts at pH = 2. It means that there are certain groups in Pepsin that are active at a pH of 2.

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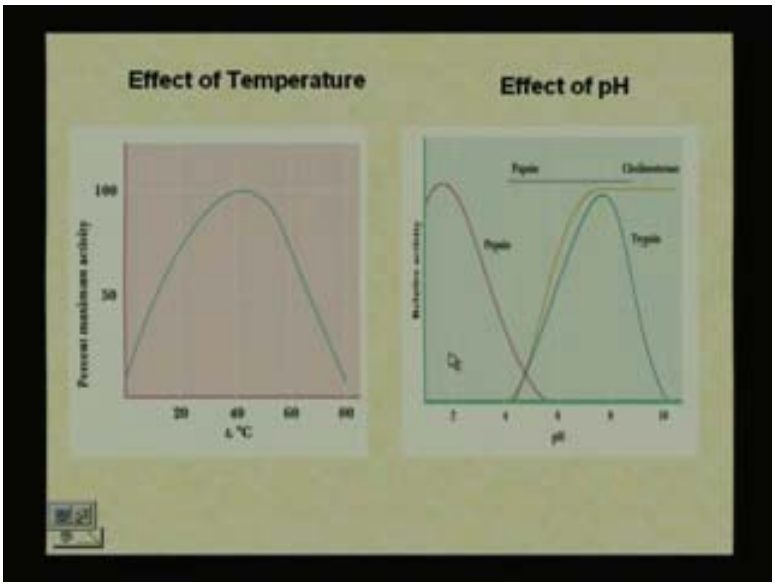

If we go to any other enzyme usually there are enzymes acted very low p^H or very high p^H the normal activity that you would see for example here you would see for Trypsin where you would found an optimum around 7 to 7.5 That would be the p^H optimum for a usual enzyme. Pepsin acts in the stomach where there is a high level of acidity.

So the effect of p^H on its optimum activity is at a pH of 2. In this case for example Trypsin the optimum p^H activity is around a pH of 7. That is the normal case that we would observe. (Refer Slide Time 31:16 min). Now, we will get into the Enzyme Kinetics. What is happening here is we have an Enzyme plus a Substrate that is going to form the Enzyme substrate complex. We have a pre equilibrium step in this Enzyme substrate complex. This step is our pre equilibrium step. This is a very simplistic way of writing an Enzyme Kinetic reaction but nevertheless we will consider the kinetics of this reaction for the definition of certain terminologies (Refer Slide Time 32:05 min).

Now the first reaction we have here is a pre equilibrium which means it has a forward rate constant and a backward rate constant. If we consider the forward rate constant to be k_1 and the reverse rate constant to be k_{-1} and this to be k_2 then we have our enzyme plus substrate.

So basically if we just draw what it looks like? We have an Enzyme and we have Substrate then they will form an Enzyme substrate complex. Then what ever reaction is to occur or the active site occurs then we get back the enzyme but our product looks likes.

Because now the substrate has changed to the product but our enzyme remains the same i.e., what is unique about these catalyst.

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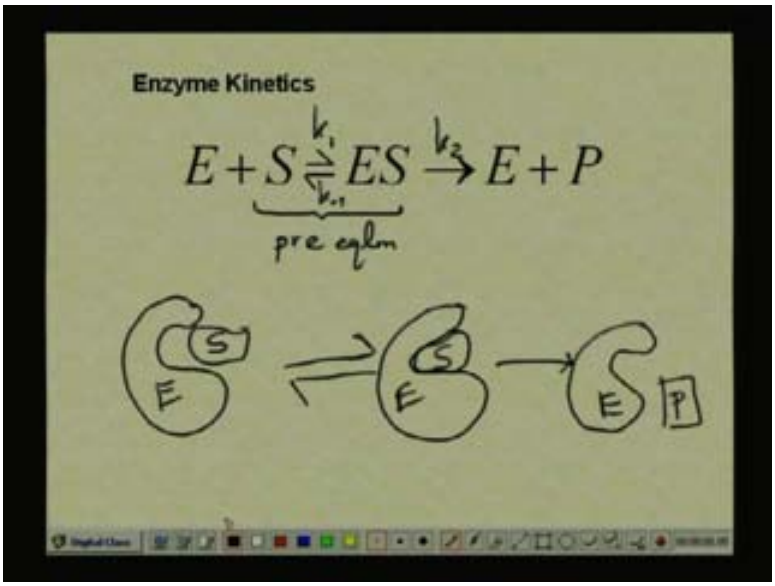

You have to understand that these are all chemical reactions that are going on in the active site but it reverts back to where it has to be after the reaction in the event forming the product. Now we will see how we can get to expression for Enzyme Kinetics. So we have E + S in a k_1 and a k_{-1} going to an ES complex forming E + P (Refer Slide Time 34:27 min).

Now I am going to define a velocity for the reaction. A velocity for the reaction is going to be v it is going to ν, this is going to be k_2 and the velocity of the reaction is going to be rate at which the products are formed. So it is going to be equal to k_2 [ES]. What is the rate at which products are formed? It is d[P]/dt. The d[P]/dt is the rate at which products are formed. So I have my d[P]/dt which is actually nothing but the velocity.

Now what do I want? I want an expression for ES, I have to consider d[ES]/dt to get an expression for ES (Refer Slide Time 35:37 min). So now if I have a steady state which you have studied what it telling is the rate of its destruction is equal to the rate of its formation. So the production and its destruction are at the same rate. So if I apply that to the expression that I have here, what can I write for d[ES]/dt? I can write k_1 enzyme concentration [E] substrate concentration [S] – k_2[ES] why minus is because it is being destroyed in the backward reaction so it is – k_2[ES]. So I have an expression that tells me that this d[ES]/dt is going to be k_1[ES] because it is formed by k_1 it is destroyed by k_{-1} and k_2. (Refer Slide Time 37:06 min).Now if I am to apply the steady states then I have to equate these two to zero.

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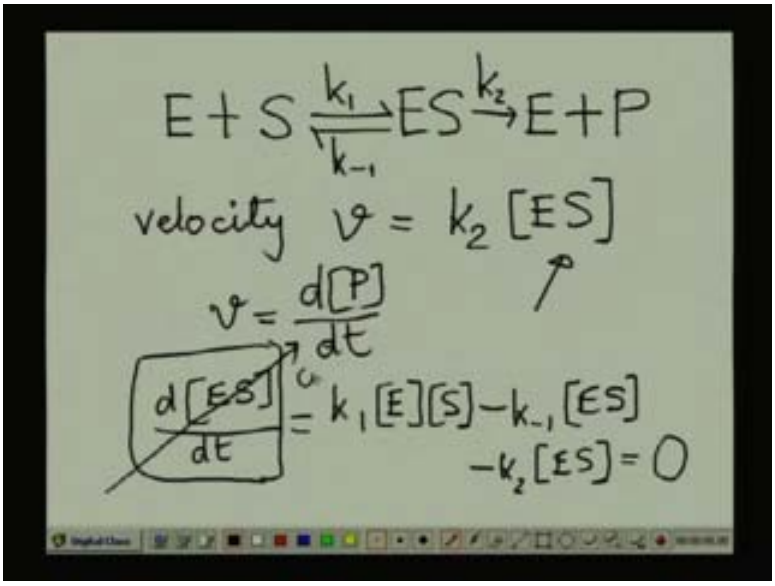

Why because its rate of formation is equal to the rate of destruction. So there is no change of the concentration of the Enzyme substrate complex with time. If there is no change with time then this quantity becomes zero because I am at a steady state.

Now if I am at a steady state therefore I will get a new expression for [ES]. Now what expression do I get for [ES]? It is going to I have d[ES]/dt was k_1[E][S] – k_{-1}[ES] – k_2[ES]. What did I do? I equated this two zero so I can get a value for [ES] which is going to be k_1[E][S] / (Refer Slide Time 38:58 min).

Now you have to remember that when you are doing this reaction there are certain quantities that you have measured, it is your experiment. What do you know? You know the amount of the substrate that you have added, you know the amount of total Enzyme that you have added but you do know at what time how much amount Enzyme substrate is actually being formed.

So what you know is you know these quantities, you know what S is and you also know what the total enzyme concentration is. Now at any time T the total enzyme concentration is going to be the what ever free Enzyme is left plus what ever has formed as the Enzyme substrate complex because the enzyme cannot go anywhere else, it either is free or it has formed an Enzyme substrate complex. So that is what our E_T amounts to (Refer Slide Time 40:07 min).

So if I change the expression, instead of writing the Enzyme concentration I can write E_T – [ES]. I can write this for the free enzyme where do I have this quantity in my expression here.

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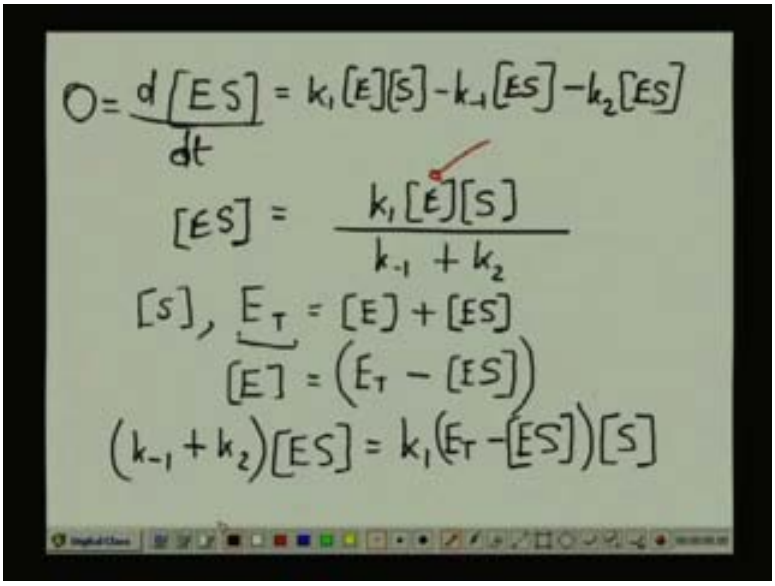

So this is where we are going to substitute this. So if we do that what do we get is (k_{-1}+ k_2) [ES] = k_1 (E_T – [ES]) [S] which is what I have. So here I have another expression for [ES] which I can take over on to the left hand side. So if I do that I will amount on the right hand side to k_1 [E_T] [S]. if take this [ES] part over on to the left hand side then what I will end up with on the right hand side is a k_1 [E_T] [S] and what I will have on the left hand side is a (k_{-1} + k_2 + k_1 [S]) / [ES] and this going to be equal to k_1 [E_T][S] (Refer Slide Time 43:07 min).

Now here I am going to use some more information. The velocity of the reaction where it was k_2 [ES] and here I am going to use another term. If this is k_2 [ES] then I can define V_{max}. The V_{max} is the maximum velocity that the enzyme can attain and that is the measure of total Enzyme concentration because of all the Enzyme want to react then it would attain the maximum velocity possible. This is something you should get very clear (Refer Slide Time 44:07 min).

If you considering the maximal velocity it is highest reaction rate that can be attained because all of you enzyme is going to be saturated with the substrate. So it is going to be k_2 E_T. So basically if we work out this whole reaction by substituting ν as k_2[ES] and putting V_{max} as k_2 [E_T] if we multiply both sides by k_2 because we are going to put ν instead of k_2[ES].

so I am going have on the left hand side (k_{-1}+ k_1 [S]) [ES] + ν because we are looking at this and this together with the S and the k_2 and the [ES] form the ν. and what do we have on the right hand side is we still have k_1 E_T [S]. Now if I multiply both sides by k_2, I can put a ν_{max} form here. Eventually what we are going to get is we are going to define another quantity that is going to be a combination of the rate constants. it is the Michaelis constant that is equal to the term (k_{-1}+ k_2) / k_1. This is another quantity where we are going to use.

And the final expression after you do all the algebra is going to work out to $v = v_{max}[S] / (K_M+[S])$. This is your final expression that you are going to get after you do all the algebra.

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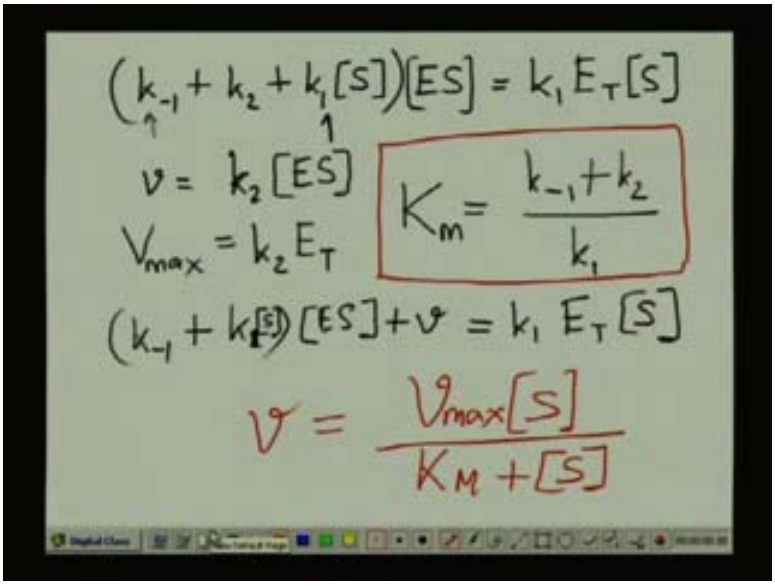

Now if I want to have a physical interpretation of what K_M value actually is then what you can get is the expression is $v = v_{max}[S] / (K_M+[S])$. Now if I make this velocity $v_{max}/2$ then if I just say that the velocity is half attained the v_{max} is half attained then I have $v_{max}/2 = v_{max}[S] / (K_M+[S])$ and if you work out the algebra you will get this specific case as $K_M = [S]$ (Refer Slide Time 48:38 min).

So what does this mean? It means the K_M or the Michaelis constant is the substrate concentration when half the maximum velocity is attained. So all you have to do is just do the algebra. Now we look at our Enzyme Kinetics we work through the whole set of expressions. What do we have from the expressions? We get Michaelis- Menten kinetics and this is the expression that we got there and $v = v_{max}[S] / K_M +[S]$.

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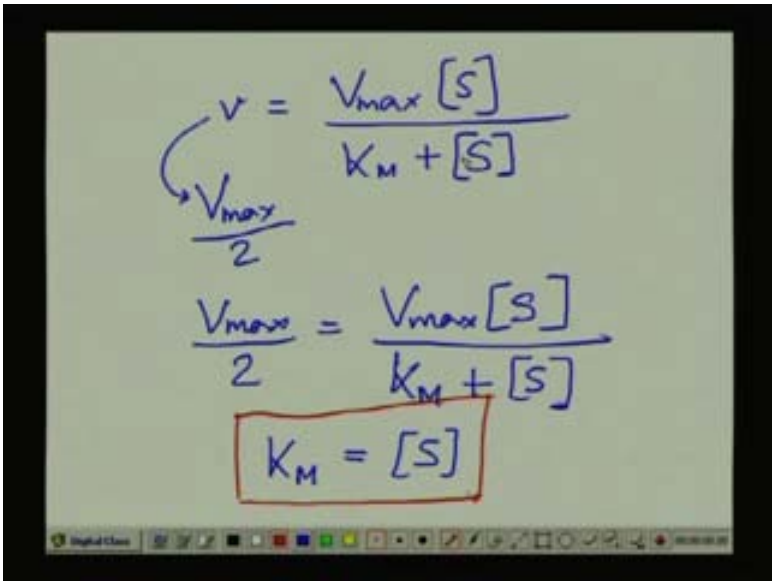

What are the features of Enzymes Kinetics or Michaelis -Menten kinetics? It assumes the formation of an enzyme -substrate complex. There is pre equilibrium where the enzyme - substrate complex is in equilibrium with the free enzyme.

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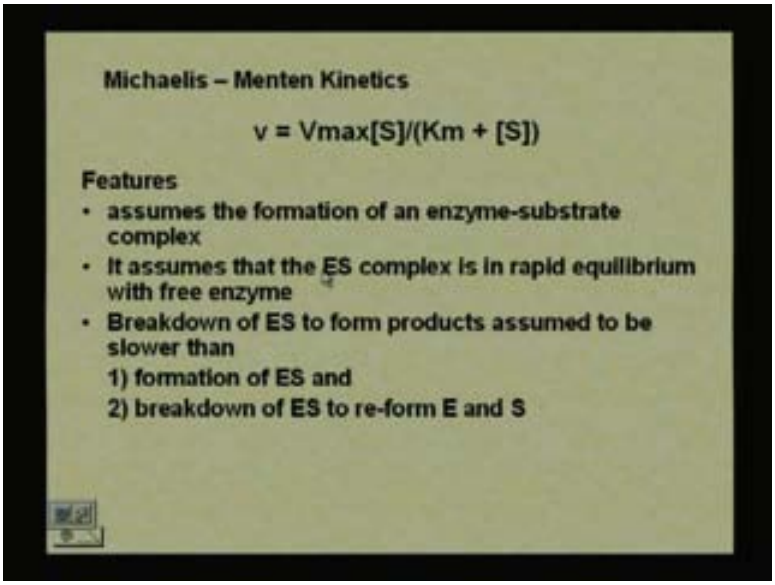

Then the breakdown of the enzyme is slower than the formation of ES and the breakdown of ES to re-form E and S. So basically what we are looking at is we are looking at a picture like this which exactly what happens. We have the velocity and we have the substrate concentration, as you increase the substrate concentration.

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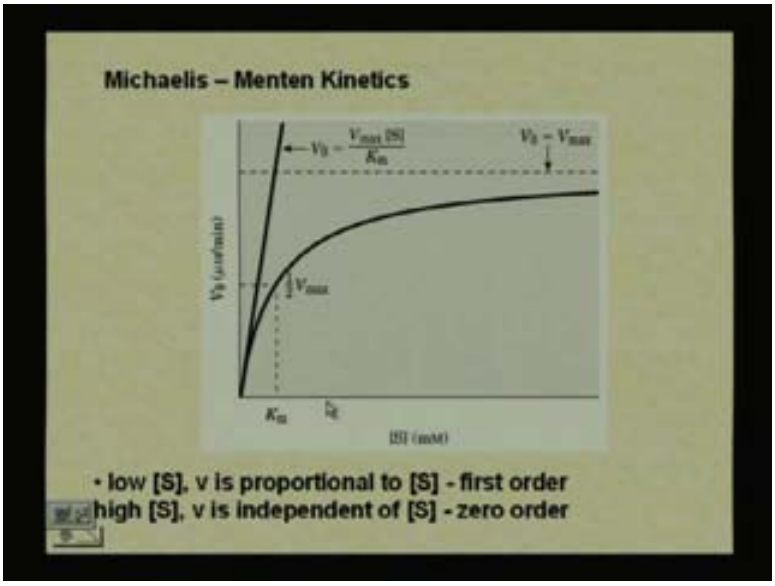

You are going to have saturation. Why? You are talking about an Enzyme which is a protein so it has a minimum number of active sites, you had a limited amount. So if even though you increase the substrate concentration the enzyme has a definite capacity to accept the substrate. So at a time you are going to attain saturation and at high substrate concentration the velocity is going to be independent of the substrate concentration where you have zero ordered kinetics. Initially you have first ordered kinetics at low substrate concentrations where ν is proportional to [S]. And what is K_M? This is ν_{max}, this is half of ν_{max}, this is K_M. it is a substrate concentration at which the maximum velocity is reached (Refer Slide Time 51:20 min).

So what we did today was we understand what enzyme kinetics is what enzymes actually do and their specific classifications based on the types of reactions that they catalyze. Next class we will consider more of this Enzyme kinetics and how we can inhibit the kinetics of enzymes.
Thank you!