Biochemistry
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Lecture No -07
Enzymes I

We start our discussion on enzymes we have considered what proteins are? We have studied protein structure the constituents of proteins that are the amino acids and their properties.

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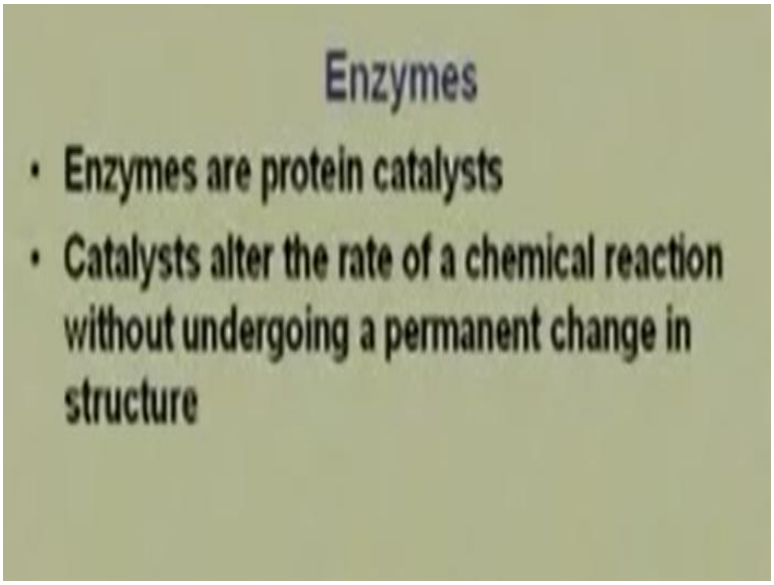

What we are going to start today is our study on enzymes which will cover about three lectures and we will be studying enzyme mechanism of a few enzymes which will be basically ribonuclease, lysozyme and serine proteases. As we go along you will see what they mean. Now you have heard about the enzymes.

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 your digestive process or whatever other process is going on all of them are enzymatic processes. There are specific enzymes that are involved that bring about specific reactions and you will see how specificity is extremely important in the way enzymes act.

Basically all enzymes are protein catalysts okay they are protein catalyst in that they are catalytic in nature but they are proteins. And what we have here? You have now known about catalyst what do they do? 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. It is going to change we will see how it exactly does that. 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 a drastic change in the reaction.

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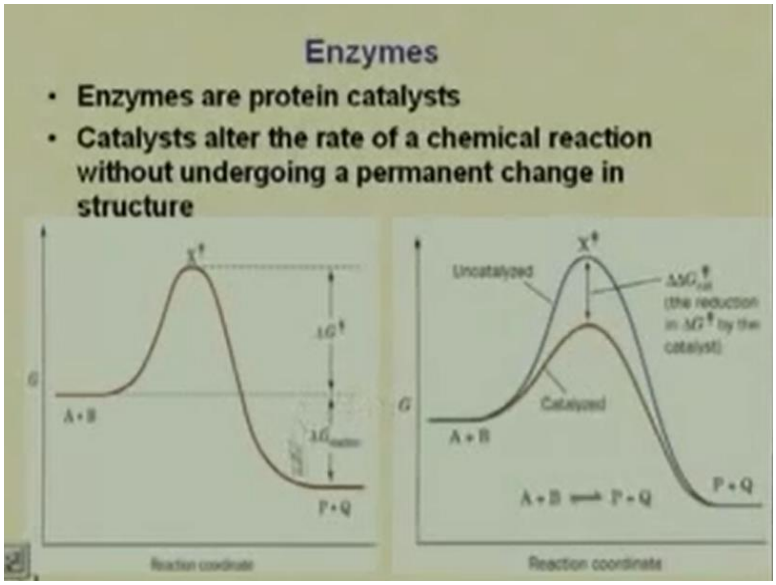

Now this is a typical picture that all of you have seen a typical diagram that gives you the free energy of A + B. What are these? These are the reactants and your P + Q in these case are the products so what do you have? You have a delta G of reaction which is what? It is the G of P + G of Q - G of A and the G of B right it is G final - G initial. What is this? This is your transition state, your activation state that has to be.

This activation barrier has to be overcome to get you from the reactants to the products. Now if you look at this there is a specific nomenclature that we have put here which sometimes is called as dagger sign. We have a delta G dagger that tells us that 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 for the reaction A + B going to P + Q. We have now a catalyzed portion of a reaction or a catalyzed reaction in which our activation energy has decreased. In decreasing what does it do? The reaction is catalyzed. It means that the rate has been enhanced okay, but the delta G itself has nothing to do with the rate. The delta G you have to remember is a thermodynamic quantity right but the activation that we considering is a kinetic quantity. This distinction is extremely important when we are considering enzyme catalysis.

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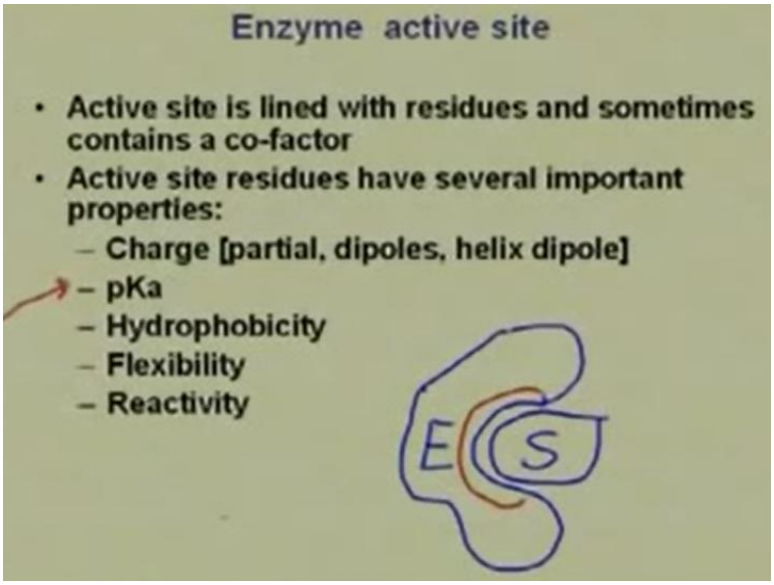

Now, what do we have in an enzyme. It is basically a protein. A protein that has a specific structure to it and what it looks like is if we look at the specific protein say we have the protein that is shaped like this. We have a particular cliff in our protein now if this is our enzyme it is going to react with the particular substrate now what is the substrate going to do?

A substrate is going to fit in to what we call this active site. Now what do we have in the active site? We have in the active site which is say this region here that is marked with particular residues. Specific residues that are going to act on the substrate to give you the product but the enzyme will remain as it is because remember the enzyme is a catalyst so what do we have? We have the active site that is lined with residues and it sometimes contains a co-factor.

We will see what these mean in a moment, the active site residues have several important properties. Now this is what we have to be particularly careful about so the residues that are in

the active site of the enzymes have specific charges. So their charges could be partial you could have dipoles you could have a helix dipole because we have to remember that the specificity of an enzyme is the reactant that is coming to act on the enzyme so that is going to have specific groups.

The specific groups will interact with whatever residues lay in the active site. We have charge, we have pKa. Now if you remember when we studied amino acid properties I mentioned that the pKa and the hydrophobicity are very important properties why because the pKa is going to determine whether you have a protonated or deprotonated or (()) (6:46) form of your amino acid and if you remember I mentioned that the histidine, amino acid residue has a pKa of six.

It lay in a lot of active sites of enzymes the sole reason being that the pKa of the histidine is close to the physiological pH of 7.4 okay so it can act we will see how it does that later on in a later class. It acts not only as a base it can also act as an acid. Another important property that we learnt about amino acids was the hydrophobicity so that is also going to be important in the active site residue lining. The flexibility because we have to have residues move over to make way for the substrate to come in and definitely the reactivity.

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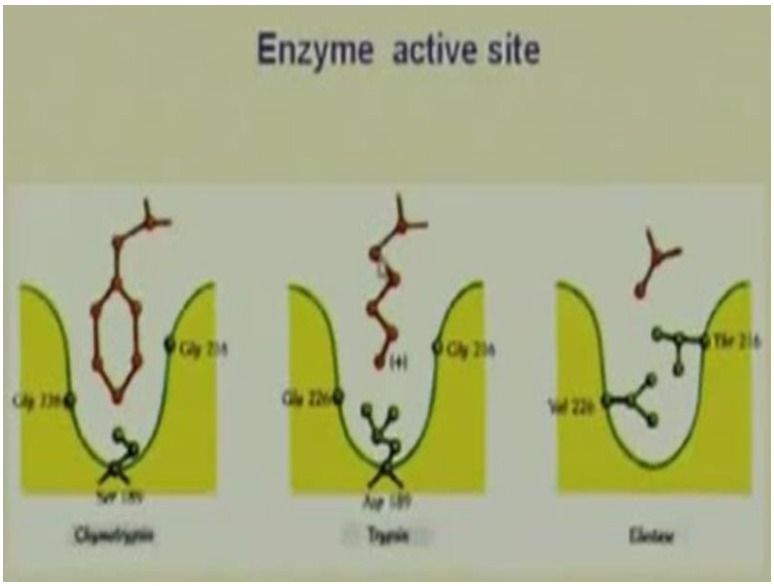

Look at the enzyme active sites that we have here. These are three typical enzymes chymotrypsin, trypsin and the elastase. Now, these are what we have in the active sites of these

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 and a serine. Here we have two glycines and as aspartic acid. The aspartic acid we know is an acidic amino acid residue.

It has a negative charge associated with it. So it is likely that the substrate that it is going to bind to is going to be of the opposite charge a positive one here. So what do we see here? We see a lysine here. If this is our enzyme active site, it is such that it will accommodate a residue that is countered in charge to the active site residue and it fits in the active site. So we have to have basically a fit of the specific substrate that we are talking about in the enzyme active site.

Again this is another protein called elastase another enzyme rather that we can call now call this elastase that has again two different groups in its active site and it again acts on a different type of amino acid. So each of this active sites have their now properties in deciding which substrate it is going to act upon.

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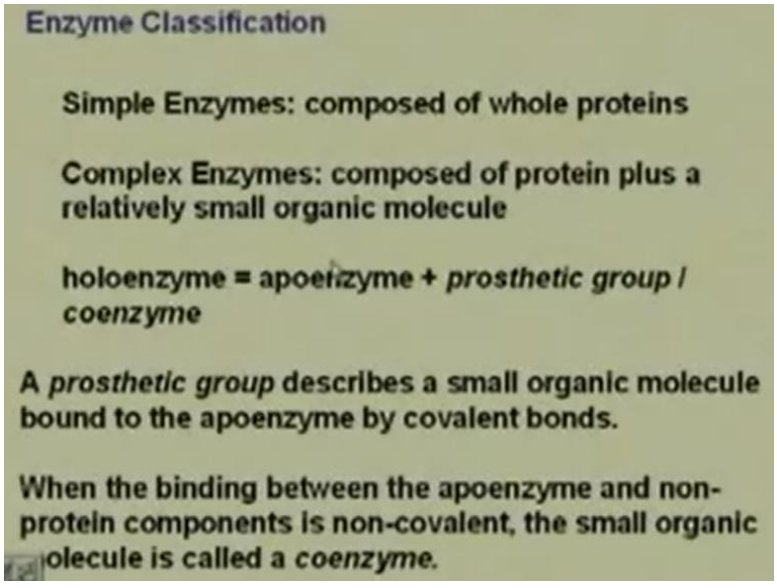

Now if we look at enzyme classification okay. We have simple enzymes and we have complex enzymes. What do we mean by them? For a simple enzyme we have an enzyme that is composed of a whole protein an example of a simple enzyme would be ribonuclease. Ribonuclease is composed of a whole protein it is a 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 + a relatively small organic molecule.

A complex enzyme is what is called a holoenzyme it is comprised of an apoenzyme which is the protein part and a prosthetic group or a coenzyme. So for the complex enzyme we have a holoenzyme that is the whole enzyme basically the holoenzyme that is comprised of an apoenzyme and a prosthetic group or a coenzyme. What is the prosthetic group? A prosthetic group is a small organic molecule that is bound to the apoenzyme which is a protein by covalent bonds that is what a prosthetic group is.

So if have a holoenzyme that contains a prosthetic group then it is an attached to the protein that is the apoenzyme by covalent bonds. In contrast, when the binding between the apoenzyme and the non-protein component is non-covalent in nature it is then called a coenzyme. So that is the difference, we have in this simple very ordinary classification we would have two types - one is the simple enzymes that comprised just of whole proteins for example ribonuclease.

Complex enzymes that have what do they have? They have a protein part and along with the protein part they have a small organic molecule moiety either attached to it covalently or just associated with it. If it is attached covalently it is called a prosthetic group if it just a non-covalent association, it is called a coenzyme. There are other classifications of enzymes which you will have to know about which we will be doing in the next slide.

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 based on what substrate or what reaction they are going to catalyst we have classifications and we have six different groups of enzymes these are:

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Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms.
Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules.
Hydrolases	Add water across a bond, hydrolyzing it.
Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds.
Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others.
Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP.

These groups I will go in to detail about this, the first one now all these enzymes are called if you look at the names at the end they are suffixed by "ases". An oxidoreductases, from the name itself suggests that it would be a redox type of reaction that it would catalyze. An oxidoreductases would act on the chemical grouping either to add or remove hydrogen atom so from the name itself you can actually figure out the function of the specific enzyme.

What is the oxidoreductases doing? It is involved in a redox reaction it acts on groups to add or remove hydrogen atoms. A transferases what is that going to do? It is going to transfer what transfer what 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 bioenergetics, you will see how kinases are extremely important in the transfer of phosphate from ATP to other molecules. Because ATP is our currency of energy okay this is sometime that you studied in your 12 standards where you know that ATP is our energy currency. It provides all the energy so there is a specific name for the transfer of this high energy phosphate and the phosphate transfers are done by kinases.

So transferases would transfer a functional group between a donor and acceptor molecule and the kinases are the special groups of transferases that transfers phosphate group. Hydrolases what is

the name mean? Hydrolysis okay so we have hydrolases that would do what they add water across the bond. In the event of doing what happens it hydrolysis the bond. We have lyases what do lyases do? They add water, ammonia or carbon-di-oxide across double bonds or they remove these elements to produce double bonds.

What do they do? The lyases they are the ones that are going to add the small molecular moieties across double bonds or remove them to form double bonds. Isomerases what does the name sound like? An isomer so what is it going to do? It is going to bring about an isomerization. An isomerization reaction is going to be catalyzed by an isomerase so it will carry out or catalyst the isomerization say from an L to a D form so basically what is it doing it is shifting the chemical groups.

If you have an asymmetric carbon atom you know you can have an L-form or a D-form. What is the difference? The difference in the position of the groups so this isomerize can change the position of the group about the asymmetric carbon atom giving arise to either an L isomer or a D isomer so we have the name isomerases. Ligases what are ligation mean? Linking together right 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 a reaction were there would be chemical groups that are join together. So these are the six different classes of enzymes that are used we will see in later on and what is called 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 which looks exactly like an IP like a 3.102.11.3 that is an enzyme classification that would belong. The first number actually is which group it belongs to here. If it were one dot something it would be an oxidoreductase. So that amounts to the enzyme classification so what are the six types we have? We have an Oxidoreductases, a transferases, a hydrolases, a lyases and an isomerases and a ligases.

What you need to know is which reactions are catalyzed by which enzymes and all you need to actually follow is what the name means if you understand what a ligases means you know that the chemical groups are joined by this enzymes. If you know what an oxidoreductases means what is it means? It is involved in a redox reaction.

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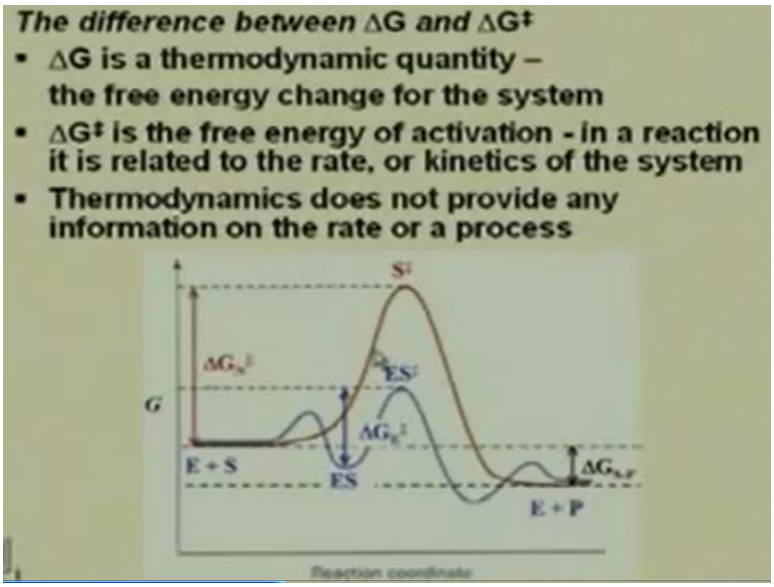

Now we come to this thing that I was talking about, about the Delta G and the Delta G daggered. What do we have here? We have now not an A + B or a P + Q any more. We now have an enzyme and a substrate. What is the enzyme doing? The enzyme and the substrate actually form what is called an enzyme substrate complex. We will go in to 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 going to lead us to our product.

So we have a delta G associated with that what is this delta G? This is the free energy change for the system. It is a thermodynamic quantity. It is the free energy change for the system but the delta G dagger that we have here what is that? That is the free energy of activation in a reaction it is related to the rate or the kinetic of the system. Thermodynamics you know does not provide any information about the rate of the system. It is just going to give you the delta G thermodynamic quantity that is going to tell you whether the reaction is spontaneous are not.

So if we just consider two reactions here say. We have a delta G.

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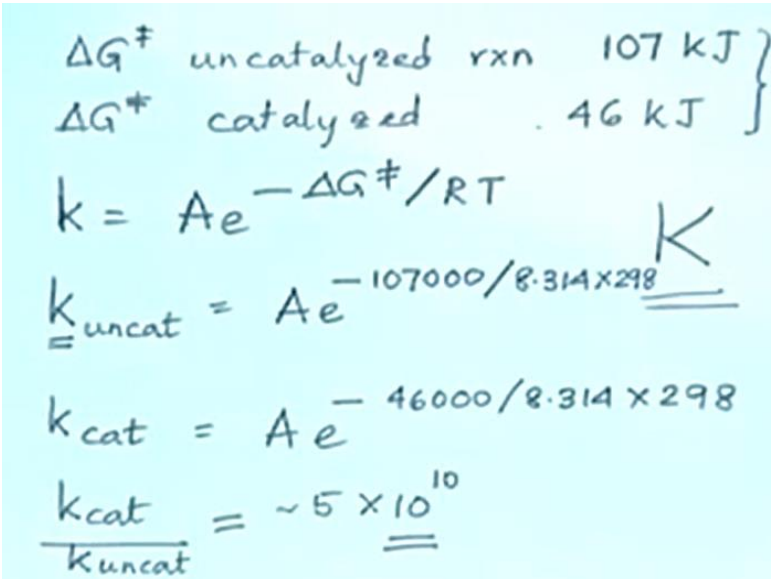

For an uncatalyzed reaction that corresponds to a 107 kJ if I put this, what this is mean? It means it is the activation. When I say this is there for the catalyzed reaction is this going to be larger or smaller than that. Smaller. Why? Because my reaction is now catalyzed my energy for the activation has decreased.

So if I want to find out how the rates have changed which reaction or which equation do I use? Which expression do I use? I use Arrhenius expression. What is Arrhenius expression? Ae to power - delta G so this is our Ae now by RT so what can I do? I can find that k value now this is small k small k is for rate always. Capital K is for equilibrium. It is never the way round and you should never make that mistake. K capital - K is for equilibrium and small k is always for the rate.

So we have a small k for the uncatalyzed reaction. I have a small k for the catalyzed reaction. How do I determine it? All I have to do is plug in the values here. If we consider the pre-exponential factor to the same so I have to be careful about units here kJ Ae to power -107000 by 8.314 and by default we usually use in to 298 right. What about my catalyzed? Ae - 46000 by 8.314 remember to use the right R also in to 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 into 10 to the power 10.

So what I am saying that when I reduce this energy of activation I have reduced this energy of activation and what have I done I have increased the rate 10 fold basically. So I have an uncatalyzed reaction and a catalyzed reaction and then I have associated with it. The change in the rate due to the 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 + substrate and we have our enzyme + product.

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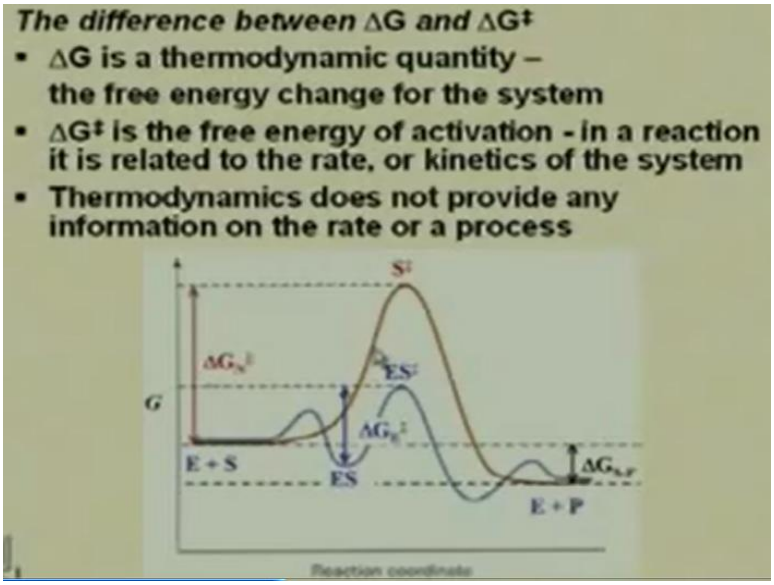

What is our catalyst? A catalyst is the enzyme. If you notice, what do we have in the beginning? In the first slide that I showed you what was the first slide? We had A + B going to P + Q what do I have here. I have E + S going to E + P. So what is my E? My E is my catalyst that is transforming the substrate to the product but itself is remaining unchanged. So it is not like a normal reaction while we would have A + B going to P + Q it is E + S going to E + P. E remains as it is in this reaction.

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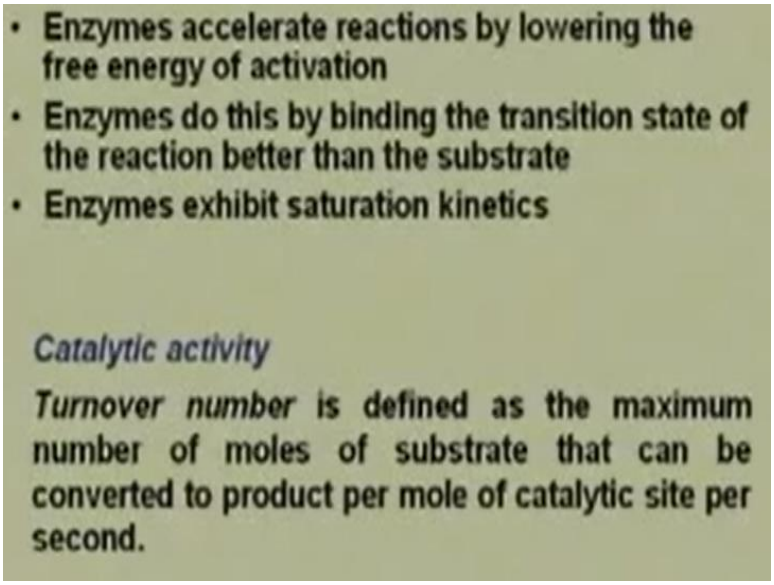

What do enzymes do? Enzymes accelerate reaction by lowering the free energy of activation. How do they do this? They do this by binding the transition state of the reaction better than the substrate. Enzymes exhibit saturation kinetic. We will see what this means in a moment. Now how do I determine the catalytic activity of an enzyme? There must be a measure that tells me whether this enzyme is good or bad.

What is the measure of that activity? The measure of the activity of an enzyme is called it is turnover number okay. The turnover number is defined so how would you define a catalytic efficiency? An efficient enzyme would be one that could transform more of the substrate to the product right 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 the catalytic site? Remember I showed you some diagrams where we had an enzyme active site. So what are doing? 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 in to product as they do that the enzyme retains its structure and activity so that it can convert another substrate to product. 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 conversation per mole of catalytic site per second because you have to remember it is a rate that we are considering. You are looking at the maximum number of moles or substrate that can be converted into product per mole of catalytic site per second.

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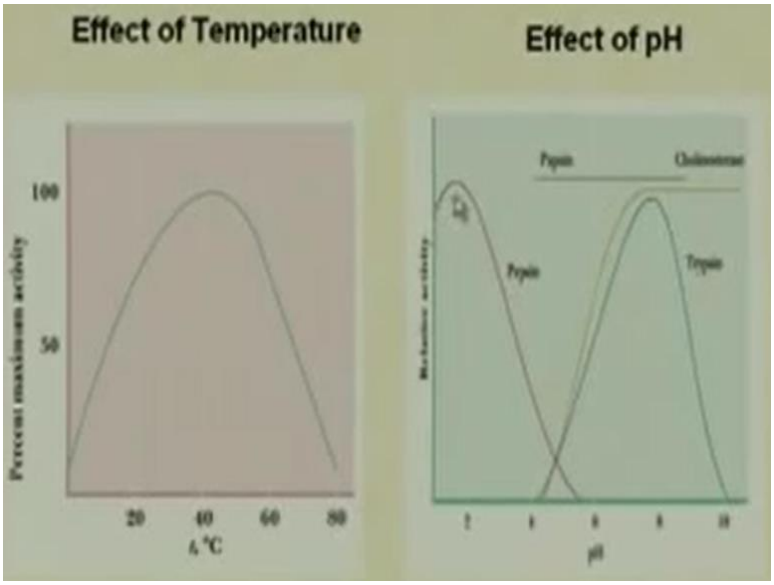

What happens to a protein when we heat it? It denatures. An enzyme is a protein so what do you have for normal reactions when you increase the temperature the rate increases right for enzymes what would you except you would except that there would be an optimum temperature at which the enzyme is going 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?

The substrate cannot bind and obviously the product cannot be formed. So we have an optimum temperature that if we do a percent activity versus temperature curved like this. Then we are going to have an optimum temperature for the highest active 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 an optimum activity especially of course the activity is also going to if considering the reactions that take place in our body then it is going to be an optimum of 37 degrees centigrade. How about pH? If we add too much acid to the protein it is going to depend

on what? It is going depend upon the amino acid composition of the protein. You have to remember the enzyme is a protein so the amino acid composition of the active site is extremely important in determining which pH is going to act at okay so if we look in the slide here there are certain graphs here corresponding to the relative activity versus the pH.

This first one here is pepsin. If you remember you have studied in school about enzymes if you had biology where pepsin acts in our stomach where the acid is at low pH. So the pH of the acid in your stomach is two. Pepsin acts as pH2. What does that mean? It means that there are certain groups in pepsin that are active at a pH of two. If we go to any other enzyme that usually there are very few enzymes that act at very low pH or very high pH.

The normal activity that you would see is what you see for trypsin here where you would find an optimum around 7 - 7, 7.4 or 7.5. That would be the pH optimum for a usual enzyme pepsin acts in the stomach where there is a high level of acidity. So the effect of pH on its optimum activity is at a pH of two. In this case for example trypsin, the optimum pH activity is around a pH of 7 okay that is the normal case that we would observe.

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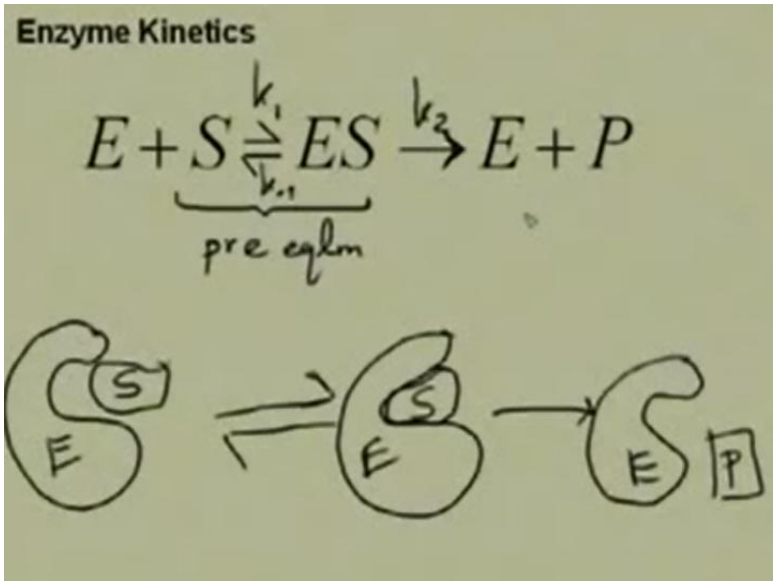

Now we get the kinetics what is happening here? We have an enzyme + a substrate that is going to form the enzyme substrate complex. Now in this enzyme substrate complex we have a pre-equilibrium step. This step is our pre-equilibrium step. This is a very simplistic way of writing an

enzyme kinetic reaction but nevertheless; we consider the kinetics of this reaction now for the definition of certain terminologies.

Now the first reaction that we have here is a pre-equilibrium which means it has a forward rate constant and a backward rate constant right if we consider the forward rate constant to be small k1 and the reverse rate constant to be small k-1 and this to be k2 then we have our enzyme + substrate. If we just draw what it looks like, we have say an enzyme and we have our substrate there are forming an enzyme substrate complex.

Then whatever reaction is to occur at the active site occurs then what do we get? We get back the enzyme but say our product looks like this right. Because as substrate has changed now to the product but our enzyme remains the same that is what is unique about these catalysts. You have to understand that these are all chemical reaction 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. So now we are going to see how we can get two expressions for enzyme kinetics.

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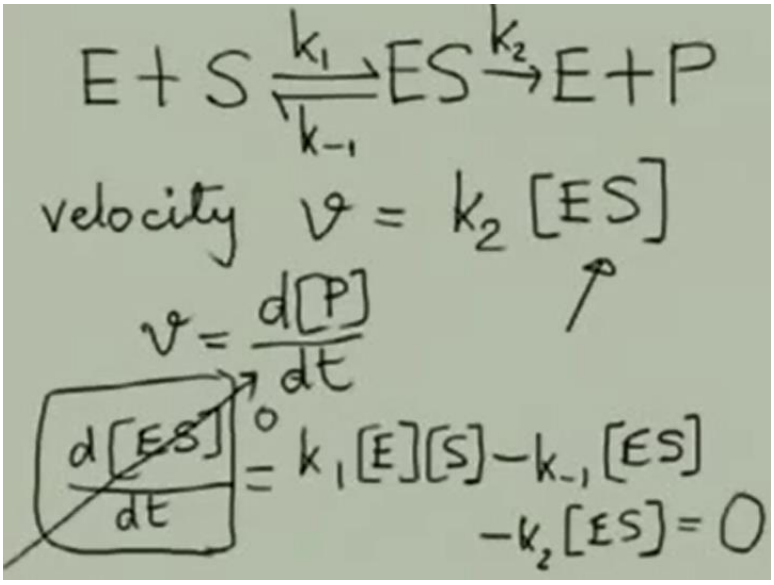

What do we have? You do this along with me. We have E + S going to in a k1 and k-1 going to an ES complex forming E + P. Now I am going to define a velocity for the reaction. A velocity for the reaction what is this velocity? It is going to be V; this is going to be k2 the velocity of the reaction is going to be the rate at which the products are formed okay so it is going to be equal to

k2 ES. What is the rate at which products are formed? It is d[p] by dt right 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. To get an expression for ES I have to consider d[ES] by dt right. So if I have now a steady state. You have studied steady kinetics. What is that? That is telling you that the rate of its destruction is equal to the rate of its formation right so the production and its destruction is at the same rate so if I apply that to the expression that I have here what can I write for ds dt.

I can write k1 enzyme concentration substrate concentration - why - because it is being destroyed in the backward reaction and - k2[ES] right. So what do I have? I have an expression that tells me that this ds/dt is going to be k1[E][S] because it formed by k1 is destroyed by k-1 and k2 now if I am to apply the steady state here. What do I equate this to?

I equate this to zero 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 is what. This quantity becomes zero because I am at a steady state right. So now if I am at the steady state now what I will get is?

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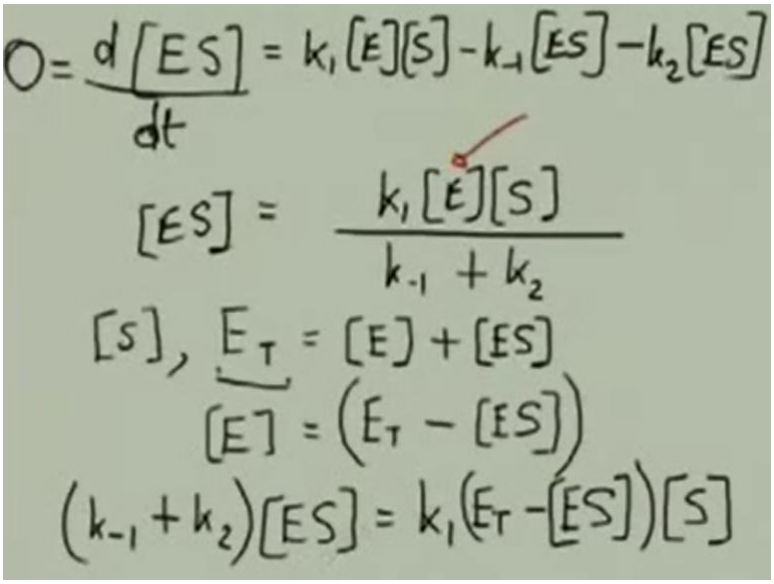

I will therefore get a new expression for [ES] now what expression do I get for ES? It is going to be What did I have? I have d[ES] by dt was k1[E][S] - k-1[ES] - k2[ES]. What did I do? I equated this to zero so I can get a value for ES. What is that? That is going to be k1[E][S] divided by k-1 + k2 fine. Now what do I know? We 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 substrate you have added.

You know the amount of total enzyme that you have added but you do not know at what time how much enzyme substrate is actually being formed right. So what you know is these are the quantities you know. You know what S is and you also know what the total enzyme concentration is right. Now any time T the total enzyme concentration is going to be what? Whatever free enzyme is left + whatever has formed the enzyme substrate complex because the enzyme cannot go anywhere else.

It either is free or it has formed an enzyme substrate complex right so that is what our ET amounts to. So now if I change the expression instead of the enzyme concentration I write ET - ES. I can write this for the free enzyme where do I have this quantity in my expression? Here so this is where we are going to now substitute this so do that. So if we do that what do we get?

We get k-1 + k2[ES] is equal to k1ET then - [ES][S] that is what I have right so what can I now do? I have another expression for ES here which I can take over on to the left-hand side right so if I do that I will amount on the right-hand side to k1 ET[S]. If I take this ES part over on to the left-hand side what will I land up with on the right-hand side a k1ET [S] and what will I have on the left hand side let us just do it.

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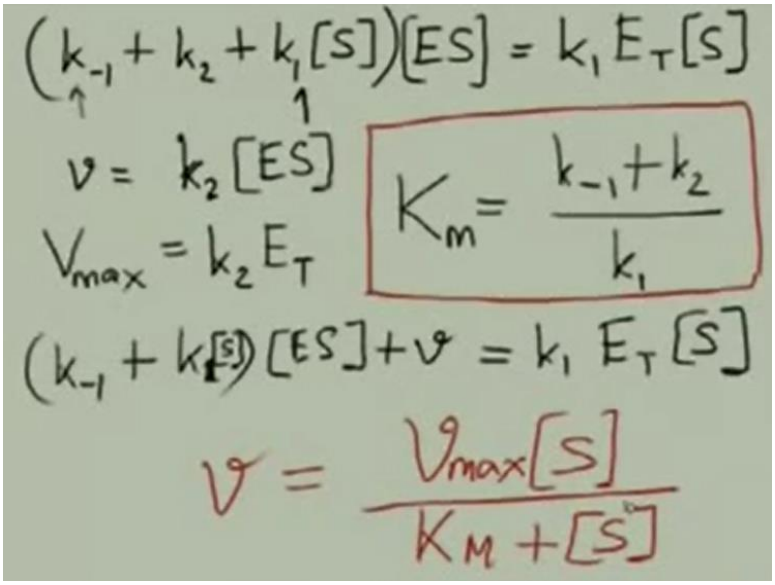

I will have on the left-hand side a k-1 + a k2 + a k1[S] all multiplied by [ES] and what is this going to be equal to k1ET[S]. Now we are going to use some more information here. The velocity of the reaction what was the velocity of the reaction? It was k2 [ES]. I am going to use another term here now if this k2[ES] I can define a Vmax. The Vmax is maximum velocity the enzyme can attain and what is that a measure of? That is the measure of the total enzyme concentration because if all the enzyme were to react then it would attain the maximum velocity possible.

Now this is something you should get very clear. If you are considering the maximal velocity it is the highest reaction rate that can be attained because all of your enzyme is going to be saturated with the substrate. So it is going to be k2ET. Basically, if we work out this whole reaction by substituting v as k2[ES] and putting Vmax as k2ET if we multiply both sides by k2 because instead of I am not working it out completely but it is just algebra instead of k2[ES] here we are going to put v.

So I am going to have on the left hand side a k-1 + a k1[S] + a v actually because we are looking at this and this together with the S and the k2 and the ES form the v and what do we have on the right hand side we still have k1ET[S]. If I multiply both sides by k2, I can put a Vmax 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 constant it is the Michaelis constant that is equal to you can write this down k-1 + k2 divided by k1.

This is another quantity that we are going to use and the final expression after you do all the algebra is going to work out to v equal to Vmax [S] divided by Km + [S] okay that is your final expression that you are going to get after you do all the algebra. If I want to have a physical interpretation of Km actually is the Km value if I want to have a physical interpretation of what it actually means, then what you can get is. What is the expression?

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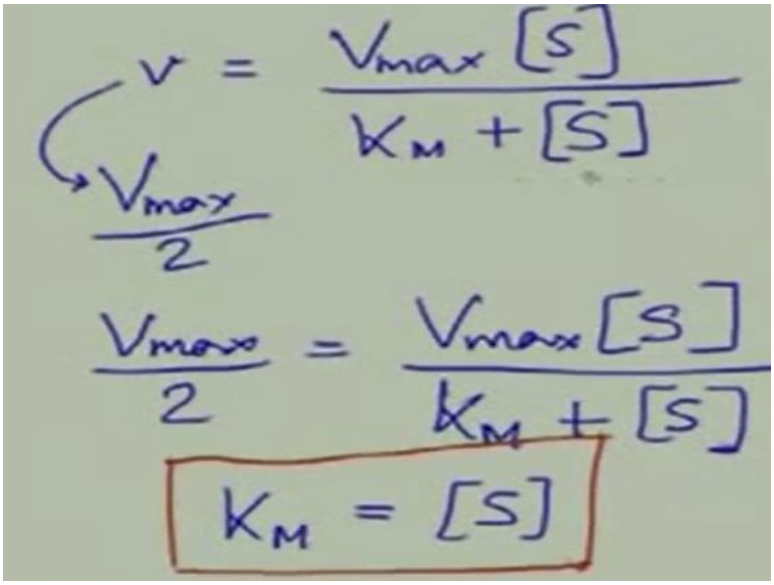

The expression is v equal to Vmax [S] divided by Km + [S] now if I make this velocity Vmax by 2. If I just say that the velocity is half attained the Vmax is half attained, then I have Vmax by 2 equal to Vmax[S] by Km + [S] and if you work out the algebra. You will get in this specific case Km equal to [S]. So what does this mean? It means that the Km or the Michaelis constant is the substrate concentration when half the maximum velocity is attained. All you have to do is just do the algebra.

Now let us go back. If we look at our enzyme kinetics we work through the whole set of expression. Now what do we have from the expressions

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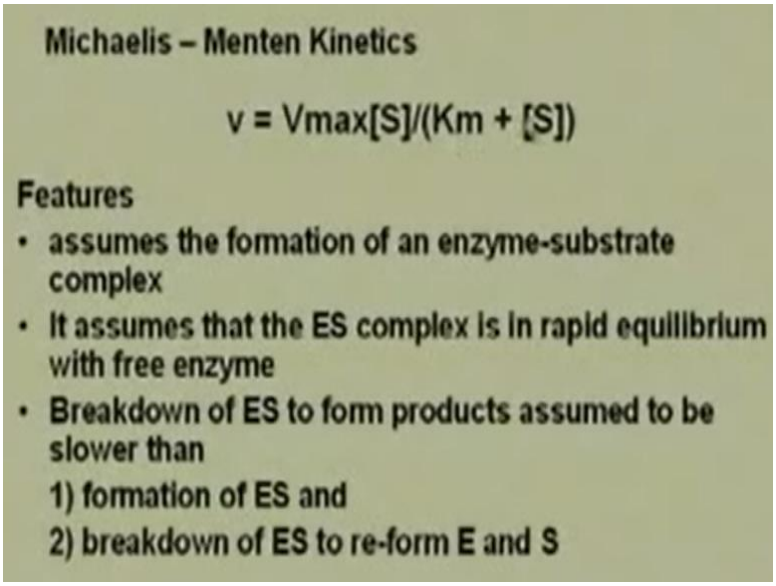

We get the Michaelis Menten kinetics and this is the expression that we got there. That v is equal to Vmax[S] divided by Km + [S]. What are the features of enzyme kinetics or Michaelis Menten kinetics as this is called? It assumes the formation of an enzyme substrate complex. There is a pre-equilibrium where the enzyme substrate complex is equilibrium with the free enzyme then the breakdown of the enzyme is slower than the formation of ES and the breakdown of ES to re-form E and S.

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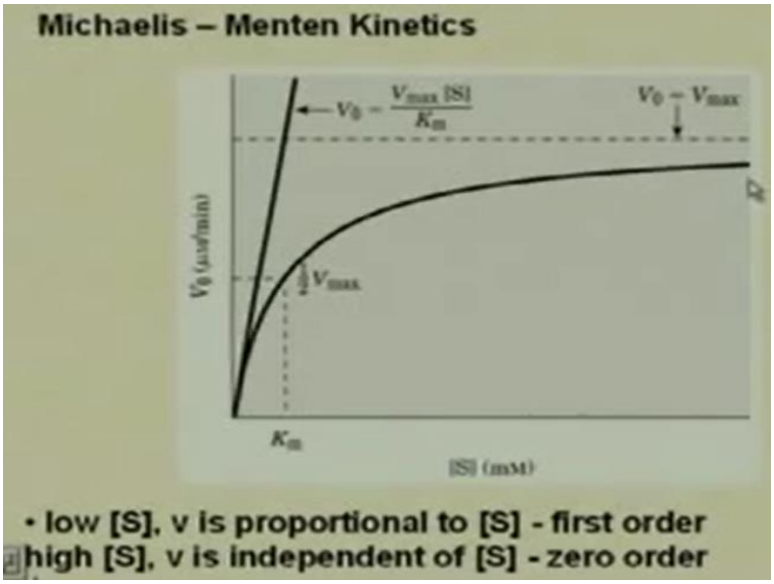

Basically what we are looking at is? We are looking at a picture like this. That is exactly what happens what we have is we have the velocity and we have the substrate concentration. As you increase the substrate concentration, you are going to have saturation. Why? You are talking

about an enzyme it is a protein it has a minimum number of active sites. You have a limited amount. Even though you increase the substrate concentration the enzyme has a define capacity to accept with the substrate.

At a time, you are going to attain saturation and that high substrate concentration it is going to be independent of the velocity is going to be independent of substrate concentration where you have zero order kinetics. Initially you have first order kinetics at low substrate concentration where v is proportional to [S]. And what is Km? This is Vmax, this half of Vmax, this Km. It is a substrate concentration at which half the velocity maximum is reached.

What we do today was understand what enzyme kinetics is? What enzymes actually do and their specific classifications based on the types of reactions that they catalyst. Next day, we will consider more of this enzyme kinetics and how we can inhibit the kinetics of enzymes. Thank you