Biochemistry
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Lecture –8
Enzymes –II

Welcome, we continue our discussion on Enzyme and Enzyme kinetics. What we discussed last time was Michaelis -Menten Enzyme kinetics. Now we have a particular reaction scheme where we have the enzyme react with the substrate to form an enzyme substrate complex. In this enzyme substrate complex we have an equilibrium as to called a pre equilibrium step which the finally dissociates into the products E + P.

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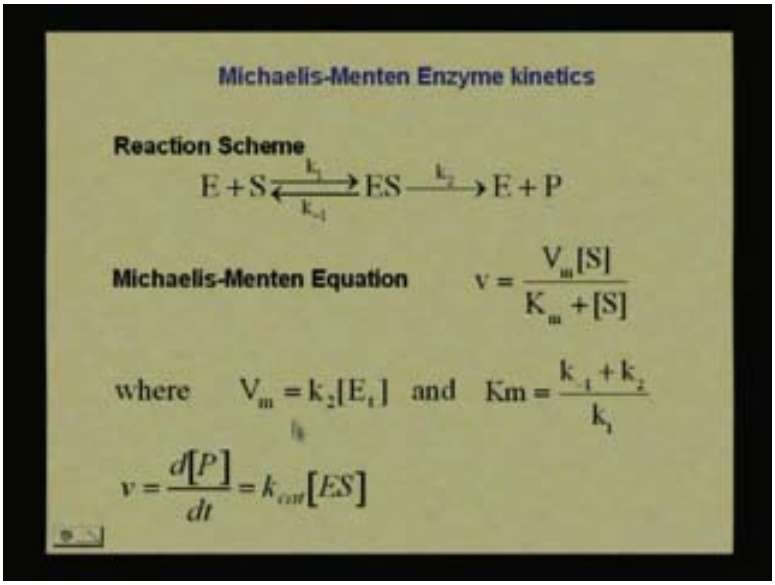

Now you recognize that the enzyme is a biological catalyst. So what happens is the enzymes retain its structure so that it can further on bind another substrate to transform the substrate again to the product. And we learn that there are six types of enzymes and belonging to different classes depending on the type of reaction that they catalyzed.

Now the over all Michaelis -Menten equation works out after a few assumptions and a bit of algebra works out to the velocity of the reaction with a V_{max}. This V_{max} which is also written as V_m or V_{max} is the maximum velocity that the enzyme can attain considering the total enzyme concentration.

So this reaction can have a limiting value for the velocity of the reaction which is given by the k_2 which is also called as k_{cat} the catalytic kinetic rate constant and the total enzyme concentration because the velocity cannot increase beyond this value because the amount of enzyme was limited. So the V_{max} that we have is equal to $k_2 E_T$ and we have

what is defined as Michaelis constant that is the combination of the rate constants of these reactions (Refer Slide Time 02:50 min).

So we have $(k_{-1} + k_2)/ k_1$ and the velocity is actually the formation of the product which is k_{cat} [ES] where [ES] is the concentration of the enzyme-substrate complex. Now if we look at the overall expression that we have from this expression we can get a rectangular hyperbola that is going to eventually plot the V_0. So what we are doing is we actually plotting a velocity versus a substrate concentration. Now what happens is as you increase the substrate concentration because the enzyme has the limited capability in forming an enzyme substrate complex this will reach a limiting value and this limiting value is dependant on the total enzyme concentration that is available to you and this is V_{max} the maximum velocity available.

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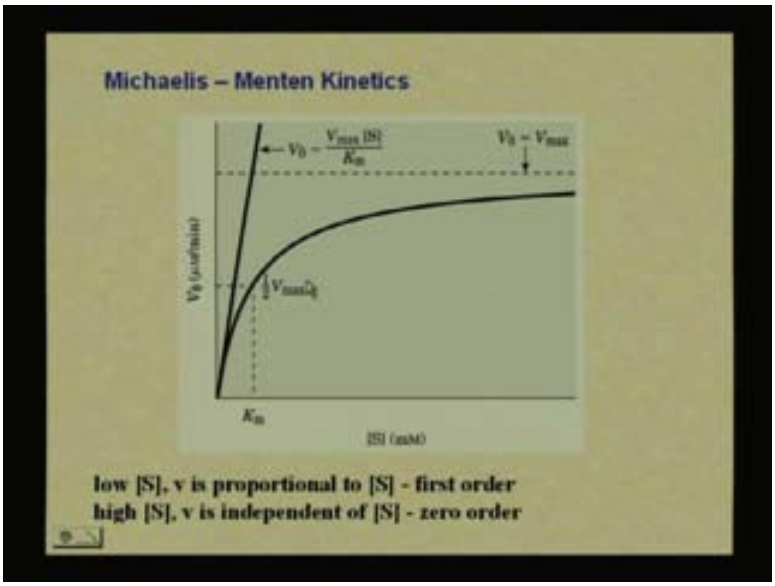

Now if we go back and look at the (Refer Slide Time 01:16 min) expression that we have here we did this last time where if we substitute V as $V_{max}/2$ which is half of the maximal velocity that is attainable then what we can get is we can get the value for K_m. So K_m is a substrate concentration for which the half of the maximal velocity is attained that is the definition for the Michaelis constant.

Now we are going to look at some features of the Michaelis constant as to what it means to have a low value for K_m, what it means to have a high value for K_m. But for now we recognize that when we have a low substrate concentration we have a first order kinetics for this particular reaction where V is proportional to the substrate concentration. Here we have a V_0 defined as V_{max} [S]/ K_M (Refer Slide Time 4:58 min).

Now you recognize this is actually the Michaelis Menten form where we actually have a K_m + S as the denominator but since S is extremely small usually less than 10% of the total substrate concentration. We consider this as the initial velocity and this is what is used later for actual enzyme kinetics.

When we consider the low substrate concentrations, the velocity is proportional to substrate concentration where we have a first order reaction and at higher [S] it becomes zero ordered independent of the amount of substrate that you have because there is limited capacity for the enzyme taken the substrate to form the enzyme substrate complex.

So what are the features of these? The features of kinetics by Michaelis- Menten are that the formation of an enzyme-substrate complex occurs. It assumes that this enzyme substrate complex is a rapid equilibrium with the free enzyme.

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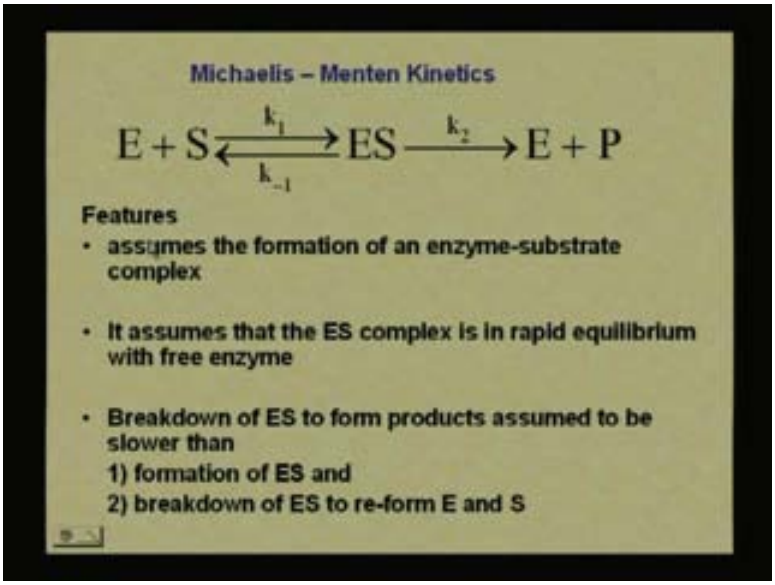

So here we have a rapid equilibrium that is the pre equilibrium step and the breakdown of the enzyme-substrate complex to form the products is slower than the formation of ES.
So if this reaction is slow what is this make? This makes it the rate limiting step of the reaction. So what we assume here is that the breakdown of ES to actually form the products is assumed to slower than the formation of ES and also slower than the breakdown of ES to reform E and S.

So here we are saying that both k_{-1} and k_2 are small because we are saying that the breakdown of ES to form the products is also slow and to go back to form the enzyme and the substrate back again is also a slow process.

So these are the features that have come into Michaelis-Menten kinetics to actually get into the form of the expression that we get (Refer Slide Time 7:15 min).

Now what do we have here is we have k_2 as a rate limiting step, we have k_{-1} also as a small step. So what do we have? Basically we have an association of the enzyme and the substrate to form the enzyme substrate complex.

So what we can actually define here is we can define an equilibrium considering the rate constants k_1 and k_{-1}, we can define an equilibrium. Now this equilibrium can be an association. What is this association? It is E + S to form ES that is an association reaction. What is that K? It is the enzyme-substrate complex divided by the concentrations of the enzyme and the substrate and at equilibrium this is our equilibrium constant.

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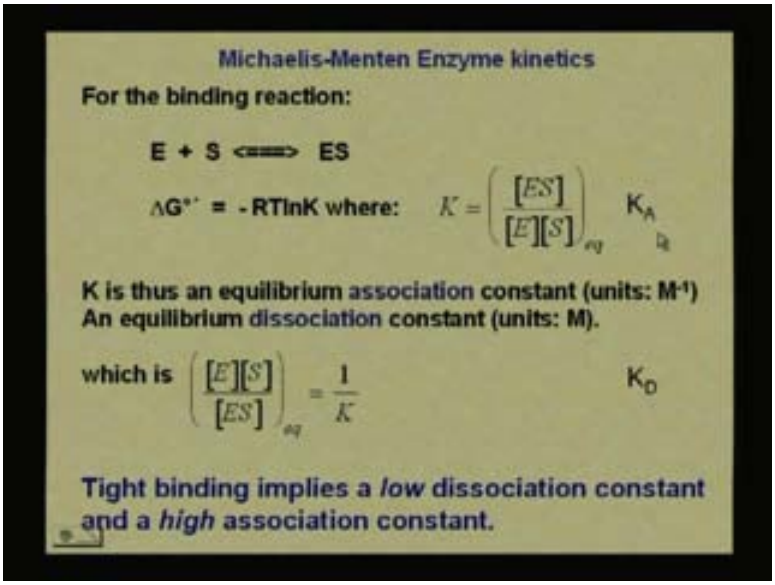

If this is our equilibrium constant then we can find a ΔG^o for this. So what we have is for this particular winding reaction we have an E + S that take us to the enzyme-substrate complex and we have an association constant associated to it. You recognize that the units of the association constant are $mole^{-1}$ because the enzyme-substrate complex is in moles, the enzyme is moles and so it is the substrate (Refer Slide Time 8:40 min).

Now if I go for the reverse of this reaction I am considering a dissociation of the enzyme-substrate complex back into the enzyme and the substrate. So that is also equilibrium from the reverse side. So I have the expression which is just 1/K where I have an enzyme concentration, a substrate concentration divided by an enzyme substrate concentration that makes the units of dissociation constant as molar units (Refer Slide Time 8:40 min).
So what I have is initially I have an enzyme and I have a substrate that forms the enzyme-substrate complex. The formation is an association. So it called an association constant because you are associating the enzyme and the substrate to form the enzyme-substrate complex. Since this is equilibrium now I can also relate this to the rate constants. So what

will my K be? It will be k_1 / k_{-1}. So you recognize that we have a thermodynamic concept to this as well as a kinetic concept to it (Refer Slide Time 10:03 min).

So we have an association constant and the reverse of this is going to be dissociation constant and the units will change accordingly. Now if I have a tight binding a tight binding would mean that I do not have dissociation. If I have a tight binding the dissociation is not easy. So what does it amount to in terms of these constants? If I have a tight binding I will not have dissociation. So I will have a low dissociation constant but obviously I will have a high association constant because if we consider the K_A then 1/ K is going to be a dissociation constant (Refer Slide Time 10:53 min).

So if I have a high association constant it means that the enzyme and substrate complex are bound tightly. So what do we have? We have an association and dissociation. If I have a high association constant means I have tight binding. That means I am going to have strong association. Strong association means the enzyme and substrates are going to bind together very tightly. Now there is another consideration that is made for the certain reactions in Michaelis-Menten where we consider that the substrate concentration is very high.

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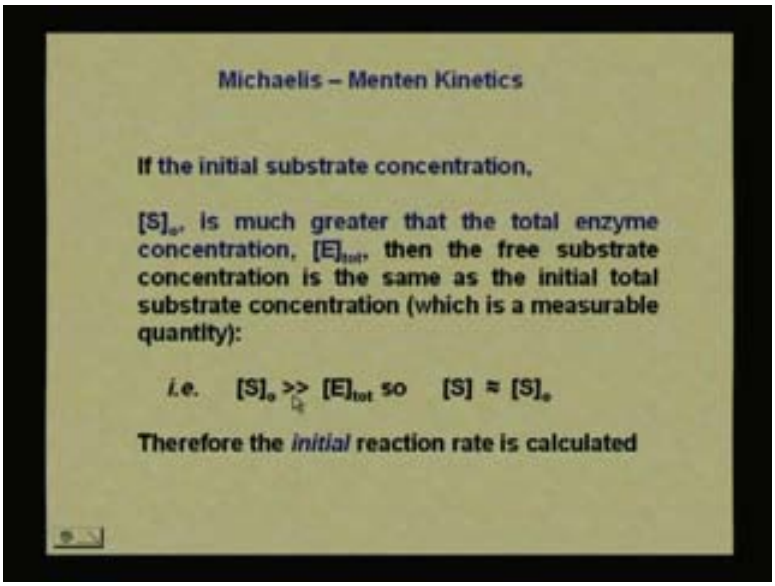

If the substrate concentration that is the initial concentration of a substrate is much greater than the total enzyme concentration then there are certain assumptions that we can make where we can consider the free substrate concentration to be approximately equal to the initial concentration that we started of with. This is very similar to considering a Pseudo Uni molecular reactions where suppose you have your reaction in water then what do you say? You will say since water is excess so you consider this as Pseudo Uni molecular reaction (Refer Slide Time 12:23 min).

So here you have the enzyme and the substrate and you consider that the substrate is in very high concentration compare to the enzyme. And what we have is we have consider initial reaction rate which is the initial velocity remember I showed you in the first curve.

So we have the total enzyme concentration is equal to the free enzyme concentration and what are the other assumptions that we made is the binding of the substrate to the enzyme is rapid. So what do we have? We have a rapid formation of ES and a slow breakdown of the complex making k_2 small and k_{-1}. So what is k_2? k_2 is a rate limiting step.

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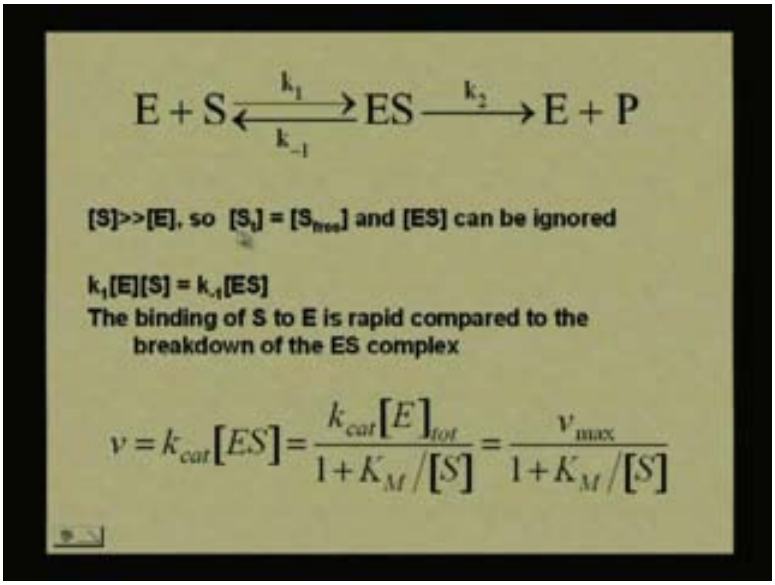

So we have this k_{cat} which is also referred to as k_2 and we have a specific expressions related to our k_{cat} and our K_m.

Now what is this k_{cat}? This k_{cat} is a direct measure of the conversion of substrate to product. It tells you a turnover number where the number of substrate molecules actually are forming product per enzyme molecule per second is called the turnover number. Like how many substrate molecules can be formed into product or can be turned over into product because nothing is happening to the enzyme molecule it is a catalyst. So what is it doing? It is transforming the substrate to the product. So it has to be a measure of its efficiency. The measure of its efficiency is going to come by this K_m value.

Now we also know that the over all rate of a reaction is limited by the slowest step of the reaction which is the rate determining step or the rate limiting step.

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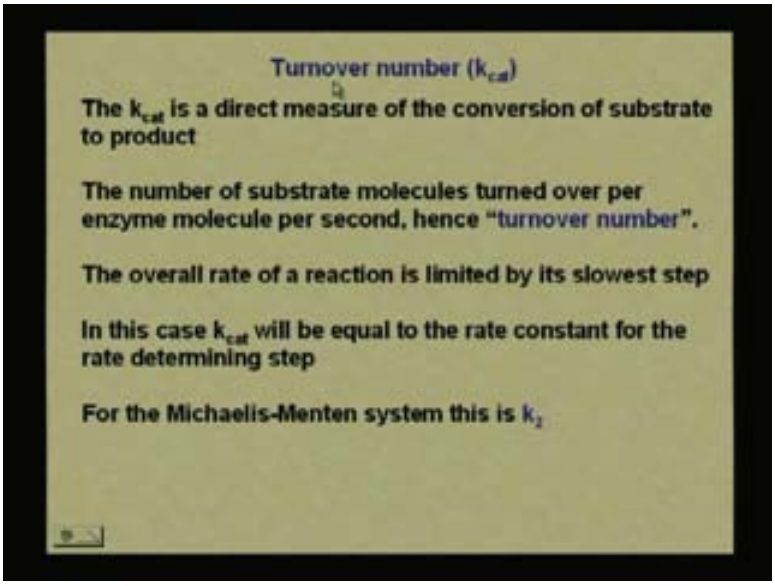

So what will be the k_{cat}? The k_{cat} will be equal to the rate constant for the rate determining step. And the rate determining step is k_2 because k_2 is the one that is forming the products.

So since k_2 is the step that is forming the products. So this becomes our rate limiting step because we know that the breakdown of the complex is slow and this becomes the slow step of reaction. So this becomes the rate limiting step or the rate determining step of the reaction. And for the Michaelis-Menten system the k_{cat} is actually the k_2 (Refer Slide Time 15:10 min).

Now what can we do with these values? So k_{cat} is a turnover number. So what is our k_{cat}? We know that k_{cat} into the total enzyme concentration is the maximum velocity attainable. Because what did we do initially is we wrote k_2 the total enzyme concentration that is going to give us V_{max}. So our k_{cat} is V_{max} by the total enzyme concentration.

Now the k_{cat} / K_m are a measure of activity or rather the catalytic efficiency. We understand that K_m is a useful indicator of the affinity of an enzyme for the substrate. Why is that so? Because it is relating the rate constants of the formation of the reactions.

So K_m is a combination of k_1, k_{-1} and k_2 which are all. And k_{cat} is actually k_2. So together they can actually give some idea about the activity of the catalytic efficiency.

If we have a high value for k_{cat} or k_2 then we have a high measure of catalytic efficiency. So the enzyme is more efficient. Now suppose we have a low K_m value then we have a high affinity for the substrate.

So what we want is for an efficient enzyme we would want a high k_{cat} / K_m ratio. And what this could be a combination of? This could come from a either a small k_M value making this ratio large or we could have a large k_{cat} value again making this ratio large and this ratio is a measure of how efficient your enzyme is.

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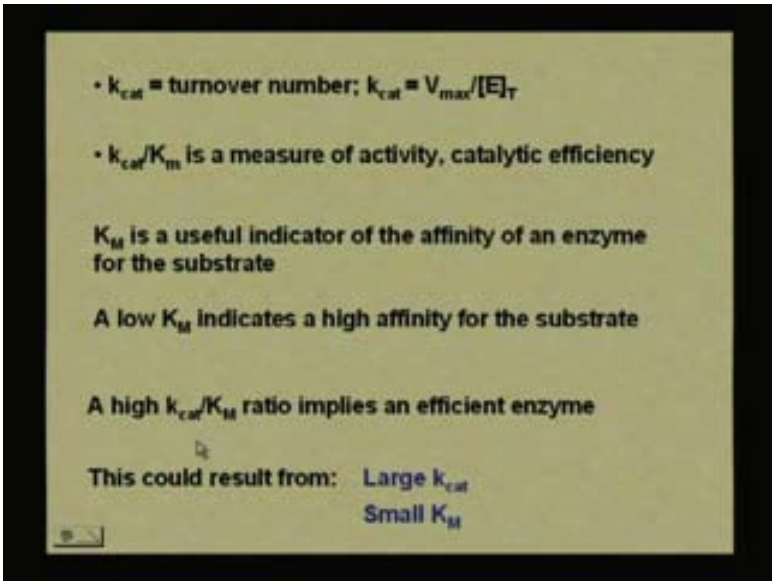

So from Michaelis-Menten Enzyme kinetics there are certain aspects of enzymes that we can consider about the efficiency of an enzyme in terms of the kinetics constants that we have. So this is our Michaelis-Menten Kinetics. We have a V_{max}, we have a K_m, we have a velocity and we have a substrate concentration.

Now what happens is I know the total enzyme concentration that I start of with, there are certain aspects or certain measurable quantities that I have. What are these measurable quantities? The measurable quantities are total enzyme concentration, total substrate concentration and actually my initial velocity. why because if I consider my curve here this is the velocity of the reaction, this is the substrate concentration and this I do not know where V_{max} is where it is actually going to sought of go (Refer Slide Time 18:40 min).

So this is some quantity that I actually cannot determine. But what I can determine is I can determine what the V_0 values are at low concentrations. So what do I have? I can find out what an initial velocity is. We go back to the slides here (Refer Slide Time 18:59 min).

We look at what the initial velocity is. So what do I have? I have an initial velocity that is eventually going to get me to my V_{max} if I have enough substrate concentration. But still I do not get a good value for my V_{max}. So how do I calculate my V_{max}?

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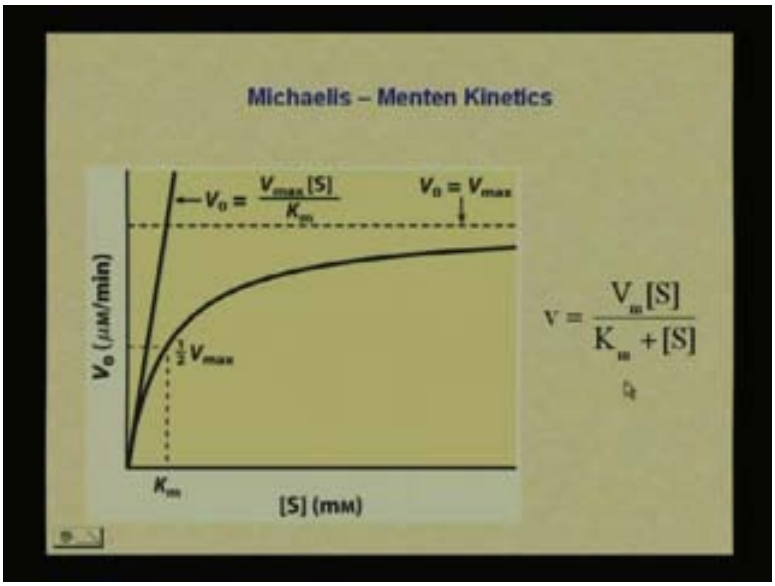

If I look at the expression I have a velocity that is equal to the $(V_{max} + [S]) / (K_m + [S])$.

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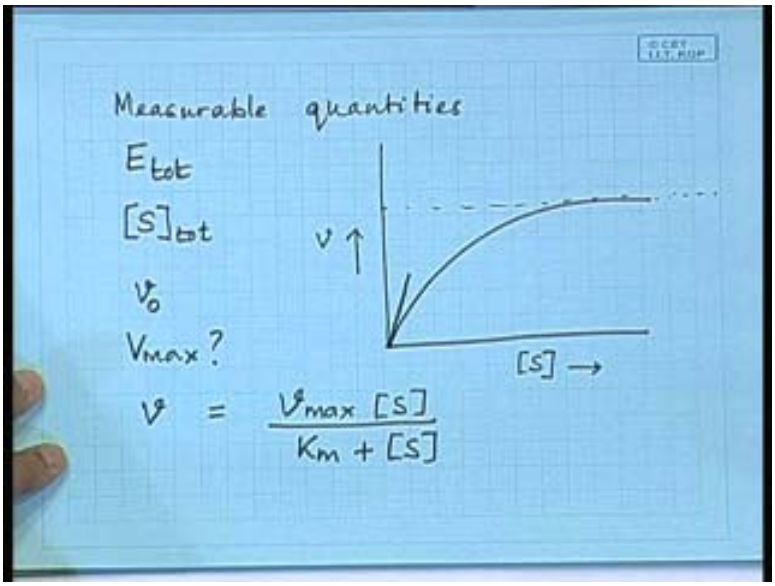

Now if I take the reverse of this what can I do? I can take 1/ V that is going to give me km $(K_m + [S]) / (V_{max} + [S])$.

If I just separate out these two quantities then I get this. I consider 1/ V from the Michaelis, I just inverted the Michaelis-Menten expression and I work out an expression where I have the form of y is equal to m x + c. (Refer Slide Time 20:51 min)

So what can I do? if I plot 1/ V versus 1/ [S] this is some thing I know because I know what substrate concentration I start out with and I can find out from my curve. This is initial value for V_0.

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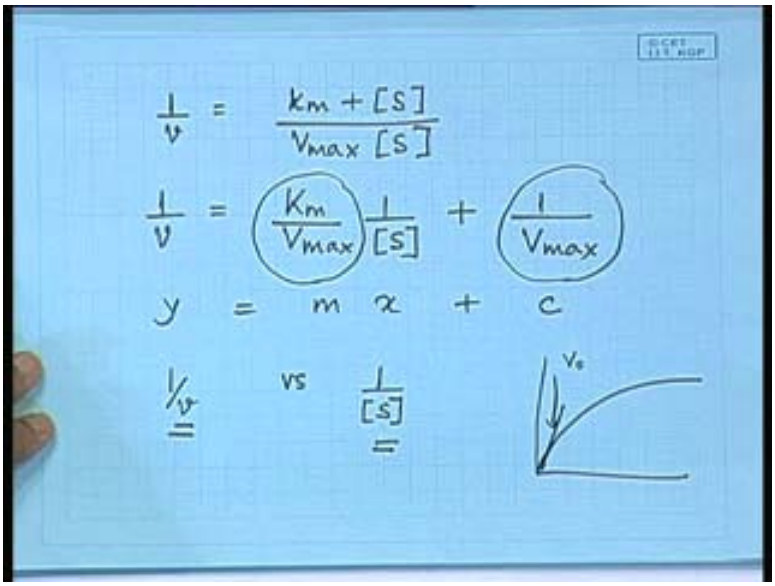

So this is the velocity. I know the substrate concentration. So what do I get? From the slope I get K_m /V_{max} and what do I get from the intercept is 1/ V_{max}. This is exactly what you can do to get a double reciprocal plot also known as the Lineweaver-Burke Plot that is extremely important in enzyme kinetics. There is no enzyme kinetic reaction or plot that you can do without doing a Lineweaver-Burke Plot. (Refer Slide Time 22:05 min)

So we have a 1/ V = (1 / V_{max}) + (K_M / V_{max}) 1/ [S]. What am I plotting is 1/ V on the Y-axis, 1/ [S] on the X-axis and what do I get is the intercept one by 1/ V_{max}, what is my slope is K_M / V_{max}.

So if we plot 1/V verses 1/ [S] then my slope is K_M / V_{max} and my intercept is 1 / V_{max}. So what can I determine from this? I can determine the maximum velocity attainable which I cannot do from a rectangular hyperbola normal Michaelis-Menten curve (Refer Slide Time 23:05 min). So I can find out what V_{max} is. If I know V_{max} and I know the total enzyme concentration then I can also determine k_{cat}.

I can find out what k_{cat} is because now I know what V_{max} is. And what are the quantities I know? I am doing the reactions so I obviously know what enzyme concentration that I

started of with so I know what k_{cat} is. And from here what else do I know is I know what K_M is.

So I can find k_{cat}/K_M. And what will that tell me? That will tell me how efficient my reaction or my enzyme-substrate complex formation for the whole enzyme kinetic reaction is.

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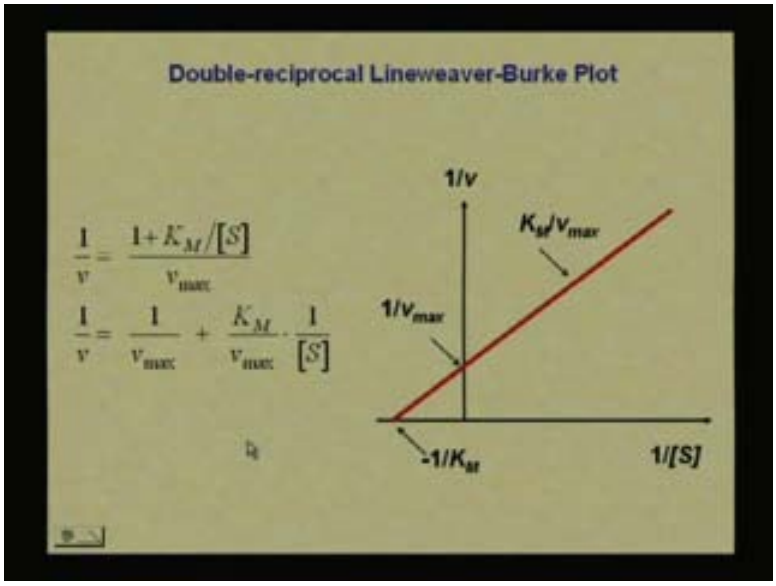

So now there is another plot that is also sometimes considered. so this is where we have Lineweaver-Burke Plot, there are other linear plots the other one that is commonly used Eadie-Hofstee plot that plots V versus V/[S].

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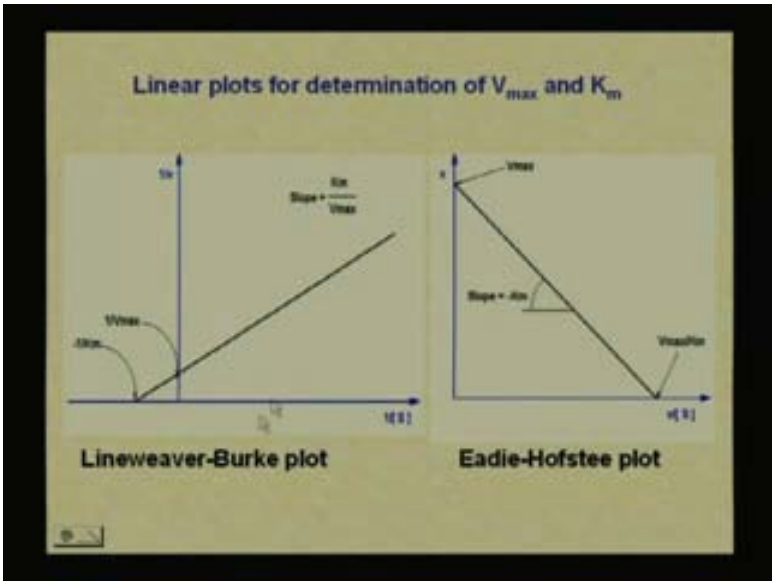

So if we plot V versus V/[S] again what you get here is you get the slope $-K_M$ and the intercept as V_{max}.

But the most important one and the one probably that you need to know is the Lineweaver-Burke Plot that is used for the determination of V_{max} and K_M which otherwise you could not determine. What happens if we have more than one substrate? That is also possible. So now we have a Bisubstrate Reactions.

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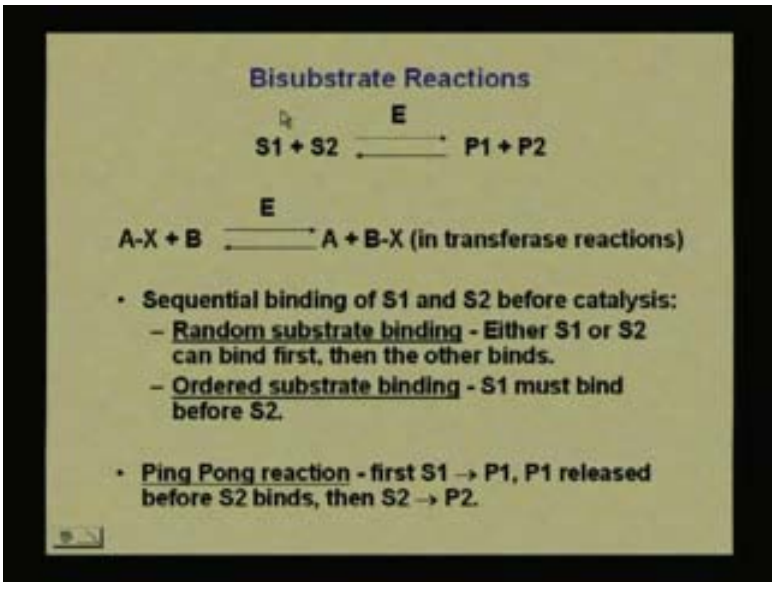

Now there are certain terminologies that are used here but we are not going into kinetics of this reaction. Since we have done enzyme kinetics there are certain terms that you need to know. For example what we have here is we have S1 + S2 so these are the two substrates that actually form two products P1 and P2.

Now, you recognize that we can have sequential binding which means that the enzyme can bind S1 then bind S2. So how can this sequential binding be? It can be of two types. It can be random where the enzyme can bind S1 or it can bind S2 first or we could have ordered binding. The ordered binding means that there is a definite order in which the substrates have to bind for the reaction to go on. You have to recognize that this is the catalytic reaction where S1 and S2 are going to P1 and P2 (Refer Slide Time 25:48 min).

So the enzyme has to form the enzyme-substrate complex. Now you could have the formation of a ternary complex where we have three components in the complex. What are these three components? It is the enzyme S1 and S2. Now how S1 and S2 are going to bind to E? Again we can have a sequential binding where S1 and S2 are going to bind randomly in any order it will not matter or we could have ordered binding where S1 has to bind before S2 for the products to form (Refer Slide Time 26:34 min).

So basically you first mixing up E and S1 then S2 forms into that complex where you have a complex with E S1 S2 which is then going to form the products. So this gives us sequential binding.

Another mechanism is called the Ping Pong mechanism where E first binds S1 so P1 is formed then E binds S2 and P2 is formed. But S1 forms P1 so P1 is released before S2 binds. It is much like the previous kinetics that we consider. So there are two specific types of reactions that we have here which are Sequential binding and Ping Pong reactions for Bisubstrate types.

So this is what we mean by a random order. We have the enzyme. What can the enzyme do? It can form ES_1 or ES_2 a random order and then what happens is it forms the ternary complex which means that you have ES_1 and ES_2 together.

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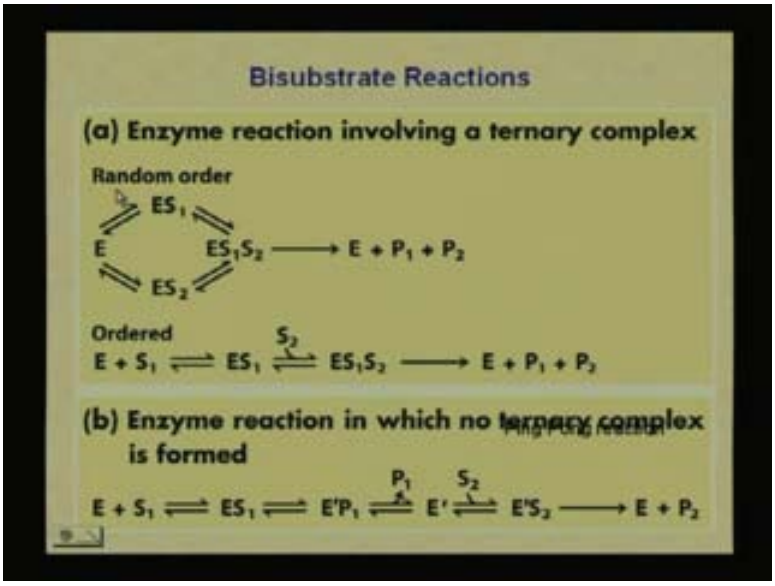

So these three components in the complex make it a ternary complex. So now you can see from this reaction scheme that E could either form ES_2 or it could form ES_1, it could form either of them.

Then if this forms S_1 obviously S_2 has to bind now and if this forms S_2 first then S_1 has to bind now. So we form the ES_1S_2 ternary complex. Then we have product formation where $E + P_1 + P_2$ (Refer Slide Time 28:26 min). Now in the enzyme reaction where we do not have a ternary complex form which is actually called the Ping Pong reaction we have $E + S_1$ forming ES_1 and what is important about the Ping Pong mechanism is that P_1 has to be released first. So we have $E + S_1$ form ES_1 the first product is released before the next substrate binds.

So what happens is the binding of S_1 forms an E'. so this E' is not E there is some change that has come across in the enzyme structure which after release of P_1 then it binds S_2 to form and $E'S_2$ complex which on losing P_2 will form E back. You have to remember you start of with E and you have to end with E (Refer Slide Time 29:40 min).

In the Ping Pong mechanism if we look at this part up to EP_1 here or other $E' + P_1$ if we consider the normal Michaelis-Menten kinetics that will be considering all along we had E + S go to ES but what did is we form after that E + P not an E' (Refer Slide Time 30:00 min).

So the enzyme comes back after because you have to remember this is a Bisubstrate reaction. So there are two substrates involved two products being formed. You can either have a formation of a ternary complex where you have ES_1S_2 formed in a random order or a sequential order where you have the products formed or you could have a Ping Pong reaction where you have an enzyme and a substrate form an enzyme-substrate complex

ES_1 that releases P_1 before it binds S_2 then it releases P_2 and then enzyme gets back to its original form where it can now bind another S_1 and the reaction can go on (Refer Slide Time 30:50 min). So we are talking about the interactions of enzymes and now we have to consider active site of the enzyme.

What is there in the active site that is so important that we have a Bisubstrate reaction or we have single substrate reaction there are specific interaction sites of the enzymes that have to bind to the substrate.

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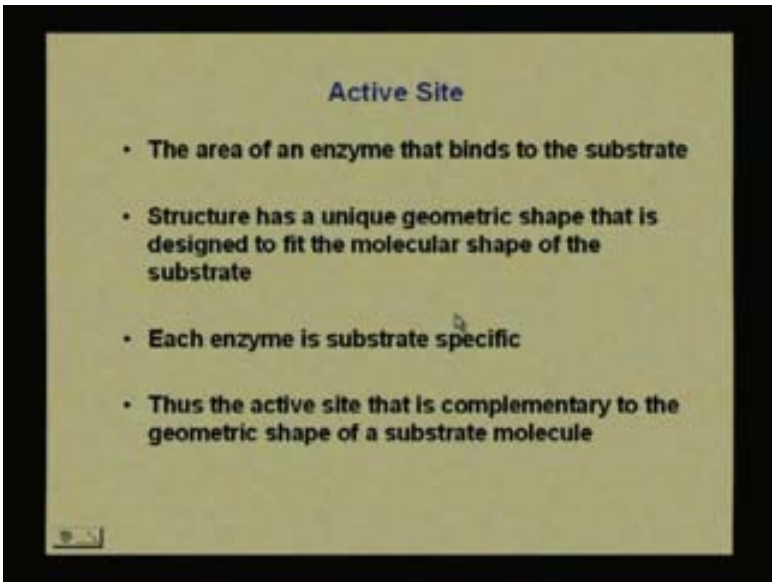

The structure has a unique geometric shape that is designed to fit the molecular shape of the substrate. That is obviously essential. Then each enzyme is substrate specific it is extremely specific. The way enzymes work is just miraculous it is extremely a substrate specific. So the active site is complementary to the geometric shape and there are other interactions or other features that we are considering or we will consider where we have the active site of the enzyme.

Suppose we are looking at the hydrolysis of Sucrose this is just a schematic where we have the enzyme. This is the active site of the enzyme.

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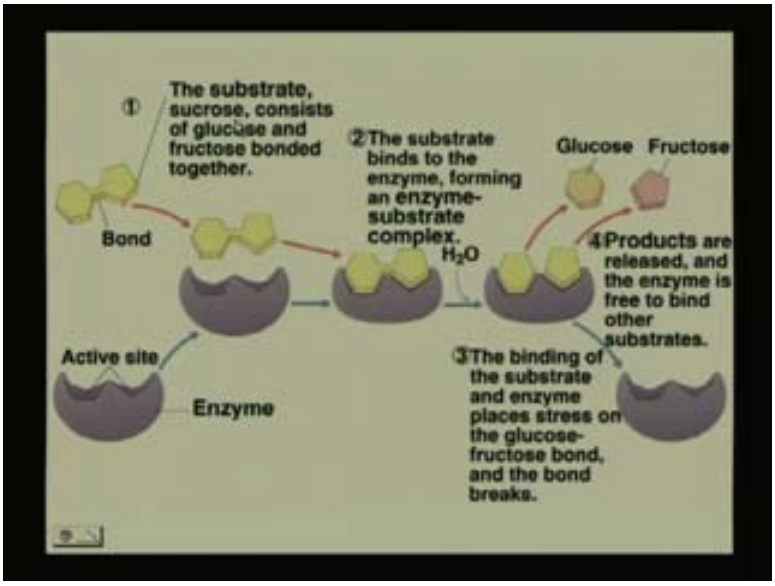

Now you see this is shaped in such a way that it is going to bind the six membered ring. This is geometrically shaped in such a way that it is going to bind the five membered ring. So what happens is we have this particular bond that is going to be cleaned. So the products are going to be Glucose and Fructose. Here this is Sucrose.

So we have a Sucrose which is a combination of Glucose and Fructose but it is connected so we have to cleave this. So this enzyme is going to cleave that (Refer Slide Time 32:43 min).

So we have the active site that exactly fits. You cannot fit it the other way you will not be able to fit it. It has to fix in exactly this orientation exactly this geometry. So the substrate binds to the enzyme. So what do we found is we found the enzyme-substrate complex. The substrate binds to form the enzyme-substrate complex.

Then what happens is we have a hydrolysis that occurs and we have the breaking of this bond that is an enzymatic mechanism which we do not have to consider right now. Then what happens is the products are released. On release of the products we have the enzyme exactly in the form that we started of with. So it can bind them with the Sucrose and go through the same thing over again. So it will keep on doing that obviously that is what is happening in our bodies all the time. (Refer Slide Time 33:50 min)

So we have a particular enzyme that is going to fit at the active site. But what we have to notice here is at the geometry is extremely important in the way the Sucrose is going to bind to this particular enzyme.

Now what we need to know is see this is a picture of an active site of an actual enzyme and this is the substrate. You can see how snuggly it fits in here. That is a showing you how the substrate can actually fit into the active site of the enzyme.

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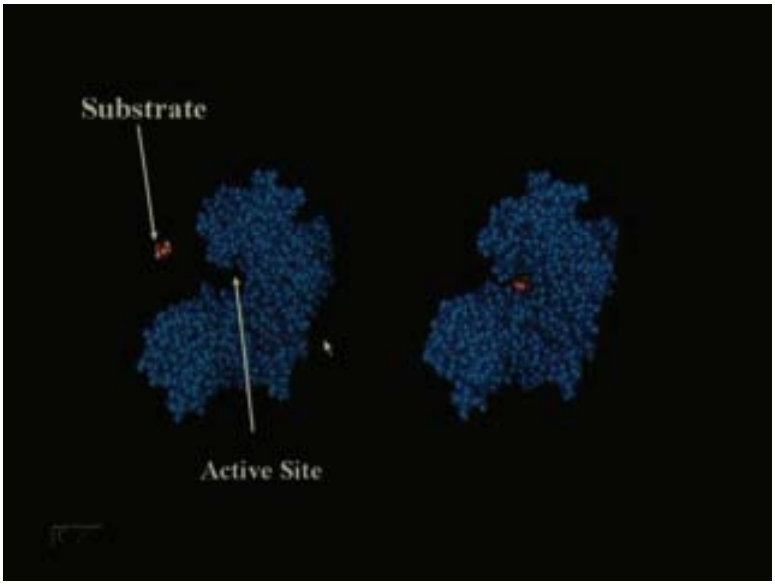

What are the amino residues that actually can form the active sites of enzymes? now you recognize that what are the interactions that are going to place they are going to be non covalent type of interactions because we do not have a covalent linkages that is formed why because the enzyme has to lose the substrate or rather it has lose the product after the transformation. So once we form the enzyme-substrate complex there are certain Amino Acid residues that can form these interactions.

Now, what are these? These are the charged residues Glutamine and Aspartic acid. What can it act as? It can act as a proton donor. So this is a specific type of reaction that it can act on. As a proton donor where it is going to form this, but you realize that this is going to take place then the pKa is low (Refer slide Time 35:26 min).

So in the reaction where this is going to take place is to be at low pH. Suppose where these reactions such as this takes place in our bodies is in the stomach where we have an enzyme called Pepsin.

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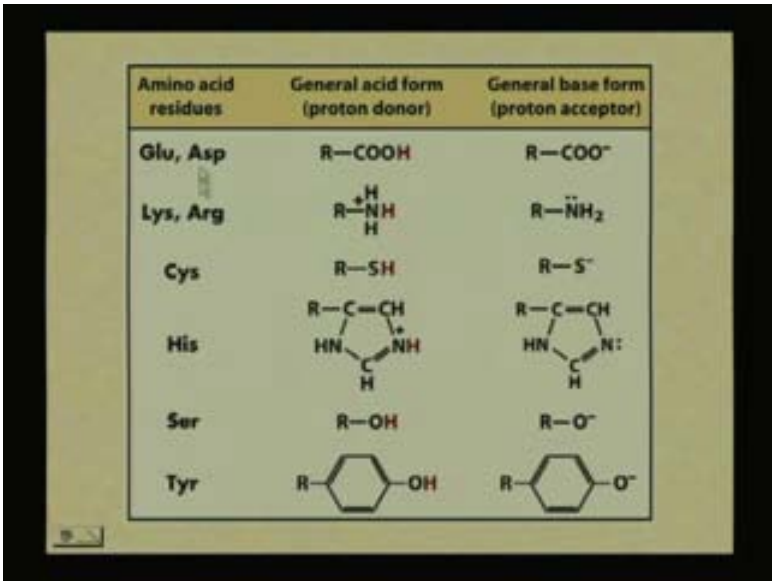

This enzyme works at acidic pH which means that it is probably lined the active site of the enzyme is probably lined by these amino acid residues.

We can have Lysine or Arginine. What is that going to do? That is also going to act as a proton donor or if this is in this form it can act as a proton acceptor. So we can have two forms of each of these. For Cysteine we can have R-SH or we can have $R\text{-}S^-$ which is going to act as a proton acceptor. And the most important one you have to remember is Histidine because this pKa is around 6 (Refer Slide Time 36:27 min).

So what do we have here? At some point Histidine can act as a proton acceptor and also it can act as a proton donor and this equilibrium is at a pH of six or close to six. Next one is the Serine. It has R-OH. What is this R? Basically this is the rest of the amino acid or the rest of the polypeptide chain. Then we have RO^-. So what is it doing here is again proton donor and this species can act as a proton acceptor. (Refer Slide Time 37:03 min). And then we have Tyrosine. If this is the proton acceptor then this can be the proton donor.

So we will see how in certain enzymatic mechanism works actually. For example we will do one specific example where we have Histidine, in one of the steps it can acts as a proton donor, in another step of the reaction it can acts as a proton acceptor. It is just because you have to remember that it has get back to its original form (Refer Slide Time 37:38 min).

This is what I showed you last time. So we have a specific Serine that is going to act on an enzyme called Chymotrypsin. We will look at the specific mechanism of Chymotrypsin and we will see how -OH of Serine actually takes parts with this reaction.

We have Aspartic acid here and a lysine here. What happening here is we have a particular electrostatic interaction that is an ionic interaction that is pulling this particular substrate into the enzyme active site.

Again, we have an Alanine that is coming in an interaction where we are going to have another hydrophobic residue.

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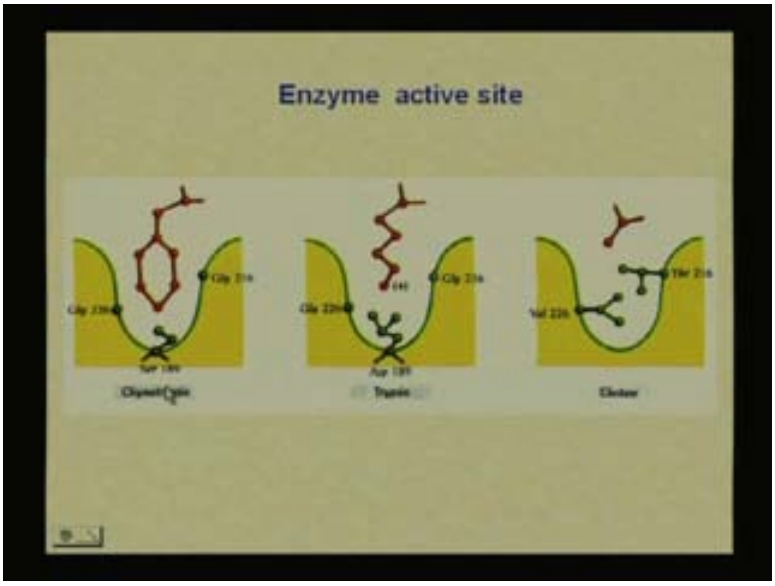

So what do we have? We have a complementarity in terms of geometric complementarity and electronic complementarity.

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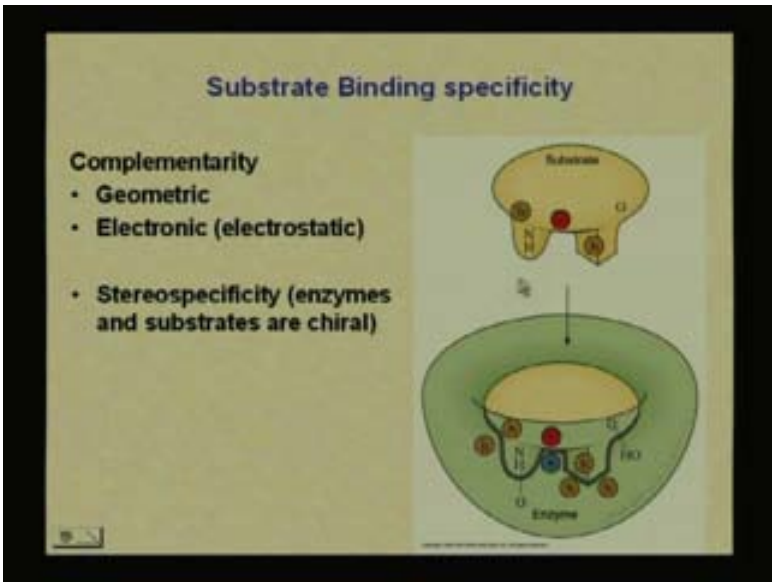

And considering the stereospecificity of the enzymes and the substrates that are usually chiral which because they are proteins and the proteins are composed of L Amino acids. And substrates are also you will see how specific they are later on.

So we have a substrate that looks like this and we have a particular enzyme that looks like this. So this H means it is hydrophobic in nature, this NH means that it has an amine group to it, a minus sign means it has a negative charge to it. Then we are going to have another hydrophobic group and oxygen here. Now if this particular substrate wants to look for a specific enzyme active site where it is going to bind then what would you expect for a hydrophobic interaction? Some other hydrophobic residue closed by because you have to remember that the substrate complex has to form for forming of the product (Refer Slide Time 39:29 min).

So here we will find another hydrophobic group closed by the hydrophobic group. For the NH we would expect some sought of hydrogen bonding that might hold it together, I would expect a positive charge at the active site for the negative charge that I have here. So that I have an electrostatic interaction, again a hydrophobic interaction for the other parts of the substrate and so on and so forth.

So now if you look at the active site we can see that once the substrate gets into the active site we will have a hydrophobic interaction here, we have a favorable hydrogen bonding here, we have an electro static interaction here, we have an another hydrophobic set of hydrophobic interactions here and again a hydrogen bonding here.

So all this is going to loosely associate the substrate with the enzyme into forming the enzyme-substrate complex that is eventually going to find out and then from this complex it will undergo certain reactions to form the product. And the enzyme will retain that is the H, the O, the plus, the two H's here and an OH. It will have to come exactly back to the same structure if it has to bind another of the substrate molecules (Refer Slide Time 40:45 min).

Now we have to consider the models in which this binding can actually take place. There are two models of binding to the active site. We are talking about binding of the substrate to the active site. The two models are called as Lock and Key model and then Induced Fit model.

You understand from the name itself the Lock and Key model where the Lock is the enzyme and the Key is the substrate. So you have a specific key that is going to fit into the lock so a specific substrate is going to fit into the lock that is our enzyme.

So in each case we have an enzyme-substrate complex is formed and the respective bonds in the substrate are formed or broken and the products are released in both cases because of each of these are talking about how we form the ES complex that is essentially what we are founding now (Refer Slide Time 41:41 min) .

So how we form it? We can form it in Lock and Key mechanism or Induced Fit mechanism. What are the features of this site or this model? In the Lock and Key model the enzyme binds a substrate in a region called the active site which we have seen. Only certain substrates can fit the active site. And what helps in the active site help the substrate bind? It is the R groups so either the hydrophobicity or the.

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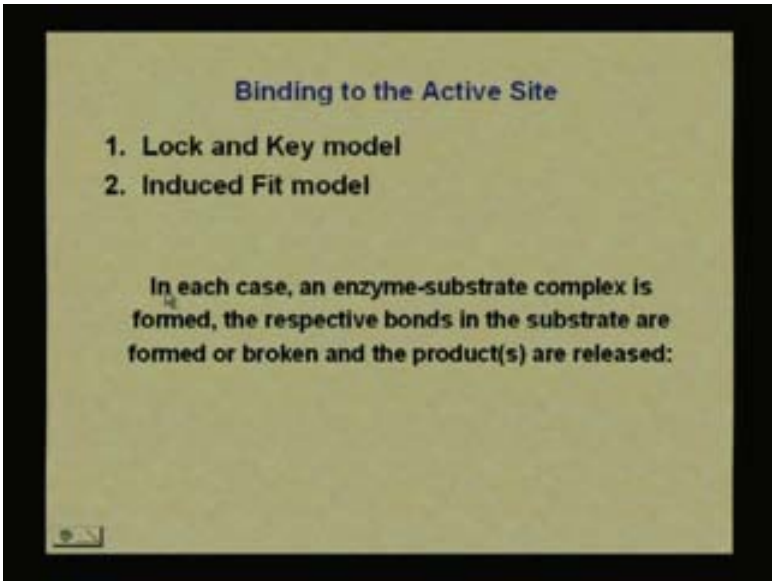

Positively charged or the negatively charged or the hydrogen bonding what ever you are talking about it is all the amino acid site chain that are actually eventually taking part in the reaction.

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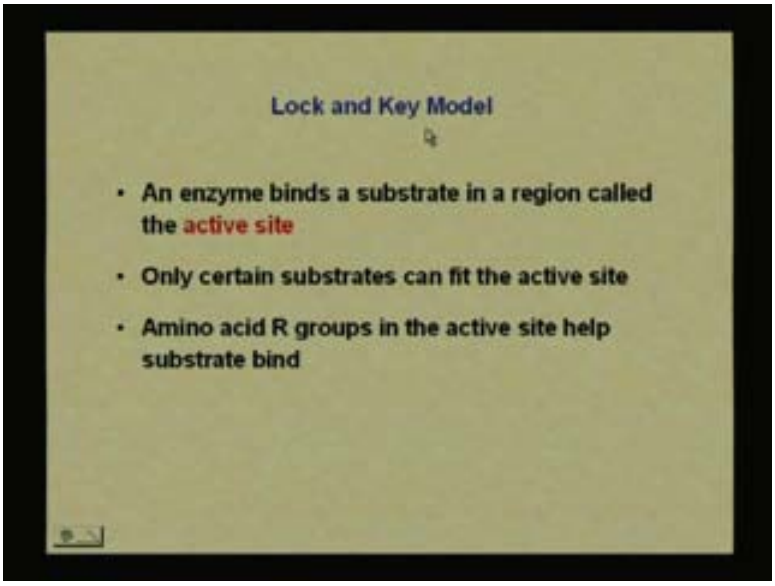

So we can have a Lock and Key model, we can have Induced Fit model to form the enzyme substrate complex. What is this says? It says that the Enzyme structure is actually is not as rigid as a lock it is slightly flexible. So what it does is the enzyme and subsequently its active site can adjust its shape to bind the substrate. So that is the slight modification that can occur due to the flexibility.

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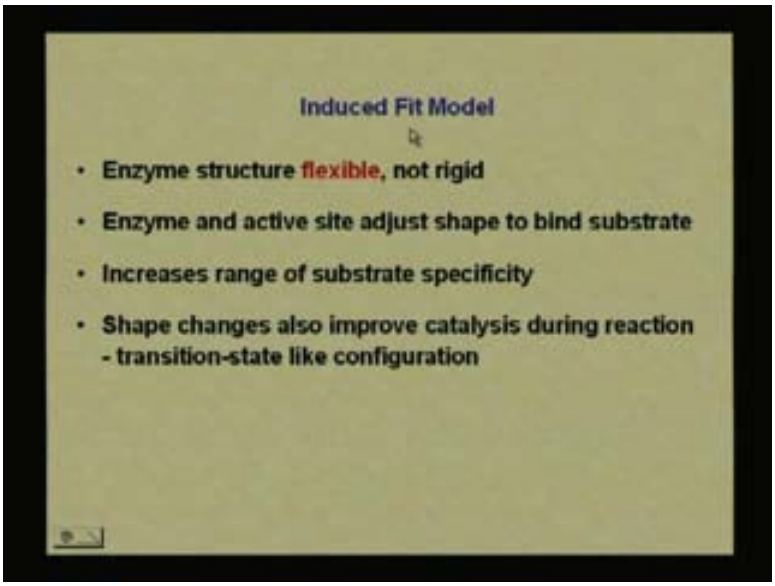

Now why I will say that this is flexible? I can say that the enzyme structure is flexible because we have all those torsional angles, we have all the bending can occur, a slight

change in the polypeptide chain can occur. And if this happens to occur near the active site just to accommodate the substrate then we called it an Induced Fit model.

The Enzyme structure will remain as it is but as soon as the substrate approaches it, it will modify itself so that it can bind the substrate. So the shape changes to improve catalysis during the reaction and it usually assumes a transition state like configuration (Refer Slide Time 43:41 min).

So this is what we mean by a Lock and Key model. This is our active site where we have our enzyme and this is our substrate. So what is happening is we have this as our key that fits exactly into the cleft of the active site. So it fits exactly in this and then you have your product formation what ever. Say this could analogous to that Sucrose where we have the Glucose and Fructose.

So in this what are the features of this? We have our enzyme, we have our substrate. the enzyme-substrate complex is formed that considers this enzyme to actually be slightly rigid in nature where the substrate fits exactly in that pocket or the cleft as it is called and we have product formation.

If we consider the Induced Fit model we have the active site that is actually these comprise the active site. So the enzyme remains like this if the substrate is happened. Now, as the substrate approaches the enzyme then what happens is there is an accommodation of the enzyme. So there is a slight change or flexibility associated with this that is going to result in an induced fits.

So now we should see that the shape of this is slightly changed to accommodate the substrate. So basically this is formed so that it can be cleaved in this case it is being cleaved (Refer Slide Time 45:29 min). So now we have the enzyme, the transition state conformation is formed and we have enzyme form the products.

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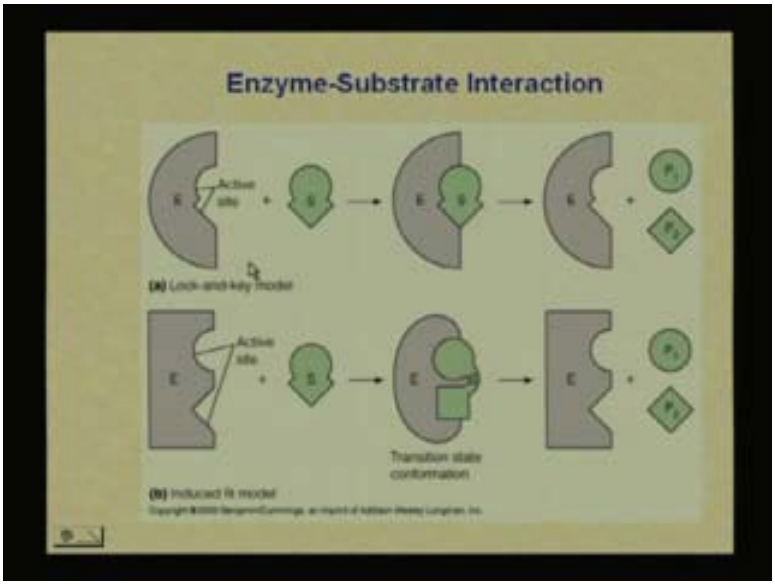

So we have these two models. So what we learnt here is we have the summary the summary here is Michaelis Menten kinetics that we consider today. What did we have? We had the V_{max}. What is this V_{max}? It is the k_2 or the k_{cat} versus the total enzyme concentration.

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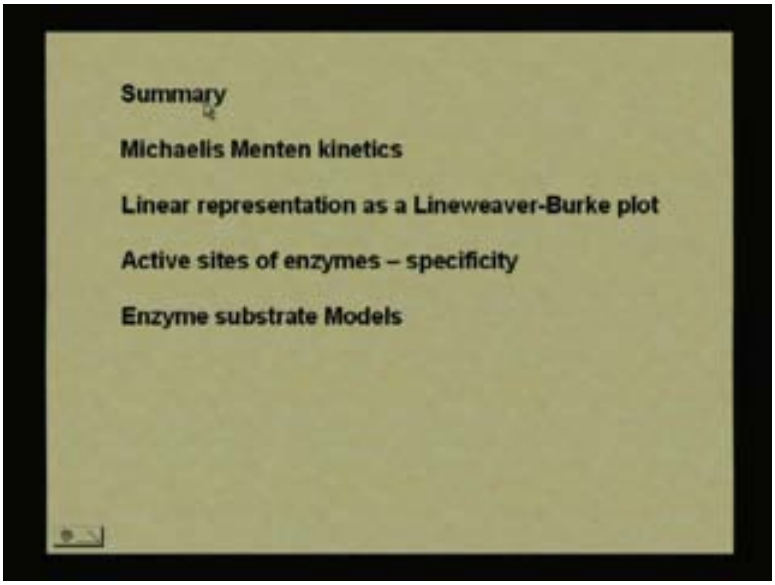

We were looked at a linear representation as a Lineweaver-Burke plot. What is this Lineweaver-Burke plot? It tells us how we can calculate V_{max}, from V_{max} we can get k_2 that means we can get k_{cat} and we can also get K_m. So k_{cat} and K_m will give us an idea about the catalytic efficiency of our enzyme.

And we consider the active sites of the enzyme. What are the active sites telling us? It tells us the specificity of the particular enzyme. How the specific amino acids side chains can form or can act rather as proton donors and proton acceptors in the active sites to bring out or bring about the particular complex formation or the particular reaction.

Also, we have the different enzyme substrate models that tell us how the substrate going to bind to the enzyme active site. And the two models that we considered here are a Lock and Key model and an Induced Fit model. What are the differences between these two models? In the Lock and Key model we have a relatively rigid structure for the enzyme where the enzyme and the substrate complex is going to form and in the Induced Fit model we have a slight change due to the flexibility to accommodate the substrate to the enzyme in forming the product (Refer Slide Time 47:35 min).

Now what we are going to do is we are going to work out a specific problem where we are going to find out the V_{max} of a particular reaction or particular enzyme substrate complex. So we have our enzyme concentration, we have the enzyme plus the substrate that is giving us the enzyme substrate complex that eventually gives us the E + P.

Now if I have a set of substrate concentrations then if I want to calculate what I need is I know that when I do this for a particular substrate concentration this is going to be my V_{max}. Now if I want to plot the Lineweaver-Burke plot I need 1/V, I need 1/[S]. As I go beyond here my reaction is no longer first order it gradually becomes zero order. So what I have to do is I have to conduct this particular experiment for a number of substrate concentrations. This is for one substrate concentration where I will calculate the initial velocity.

Say for substrate concentration one milliMolar then the velocity is 43 nanomoles per minute. So I have a particular velocity. What is this velocity? It is initial velocity associated with a substrate concentration of one milliMolar. Now I repeat the reaction with two milliMolar substrate. Then the velocity increases to 68 nanomoles per minute (Refer Slide Time 50:10 min).

Now, I go to five millimolars where I have 105 nanomoles per minute, then ten millimolars where I have 128 nanomoles per minute, twenty millimolars where I have a 144 nanomoles per minute. Now if I want to determine K_m and V_{max} from the data that I have here then what I need to do is I have to calculate 1/V and 1/[S]. Once I calculate 1/V and 1/[S] what I may go to get? I can plot the data on Lineweaver-Burke plot and that gives me some thing that looks like this. So what I have plotted is 1/V verses 1/[S] and what I have done is I have conducted the reaction at a variety of substrate concentrations and calculate the initial velocity for each of these substrate concentrations. Then what I do is I have plotted 1/V verses 1/[S] and here this is $1/V_{max}$ so I can calculate what the maximal velocity is and I can calculate what the K_m value is.

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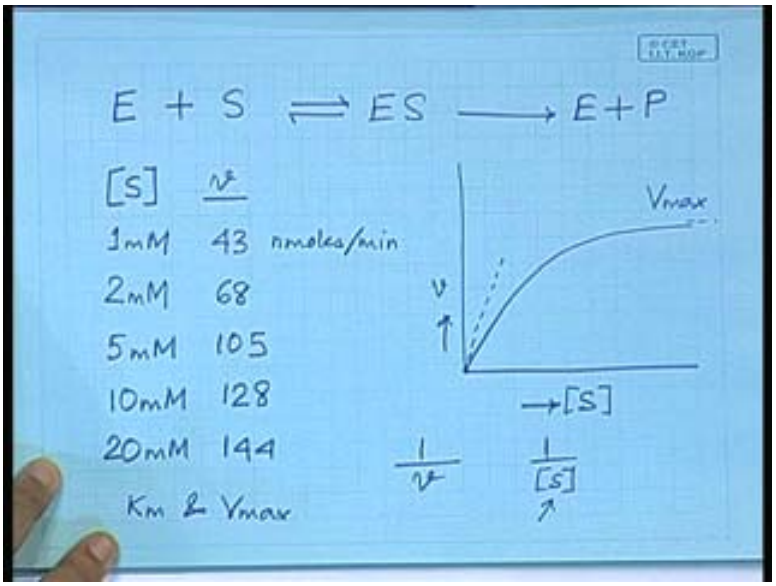

If I knew what the total enzyme concentration is then what else I could is calculate k_{cat}.

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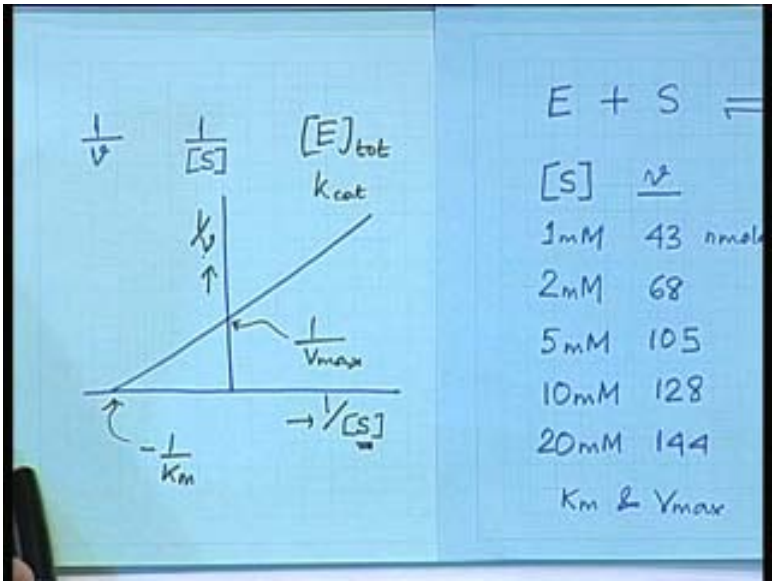

So this completes our discussion on Enzyme kinetics for reactions where we do not have any inhibition.

In the next class we will see how enzyme inhibition can occur and also what are effects on the different plots that we have and what are the different types of inhibition that can occur. Thank you.