Biochemistry
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Lecture - 08
Enzyme II

We continue our discussion on enzymes and enzymes kinetics.

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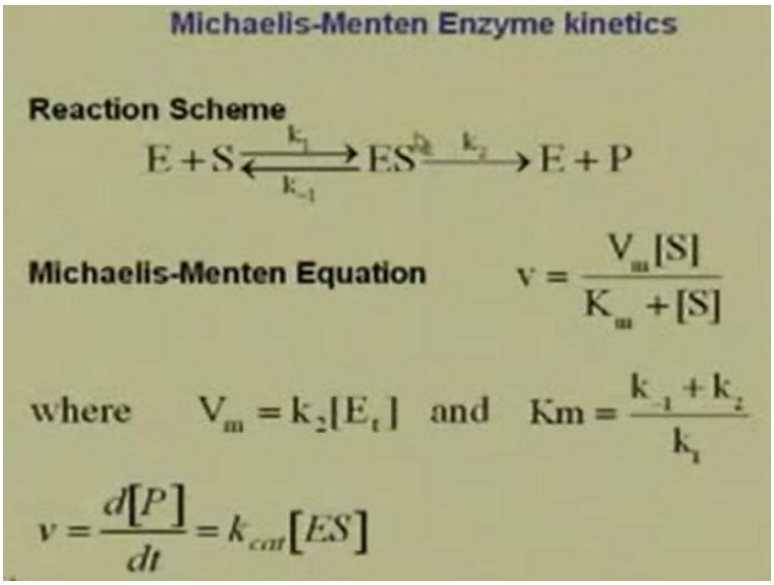

What we discuss last time was Michaelis-Menten enzyme kinetics. Now for this we have a particular reaction scheme where we have the enzyme react with the substrate to form what is called an enzyme substrate complex. Now in this enzyme substrate complex, we have equilibrium as to what is called pre-equilibrium step which then finally dissociates in to the product E + P.

Now you recognize that the enzyme is a biological catalyst. So what happens is the enzyme retains its structure so that it can further on bind another substrate to transform the substrate again to the product. We learnt that there were six types of enzymes and belonging to different classes depending on the type of reaction that they catalyzed. Now the overall Michaelis-Menten equation works out after a few assumptions and a bit of algebra works out to what is called the velocity of the reaction with a Vmax.

Now this Vmax which is also written as Vm or Vmax is the maximum velocity that the enzyme can attain considering the total enzyme concentration. This reaction can have a limiting value for the velocity of the reaction which is given by the k2 which is also we call the Kcat, catalytic kinetic rate constant and the total enzyme concentration because the velocity cannot increase beyond this value because the amount of enzyme is limited.

So the Vmax that we have is equal to k2[ET] and we have what is defined as a Michaelis constant that is a combination of the rate constants of these reactions. So we have k-1 + k2 divided by k1 and the velocity is actually the formation of the product which is Kcat [ES] where ES is the concentration of the enzyme substrate complex.

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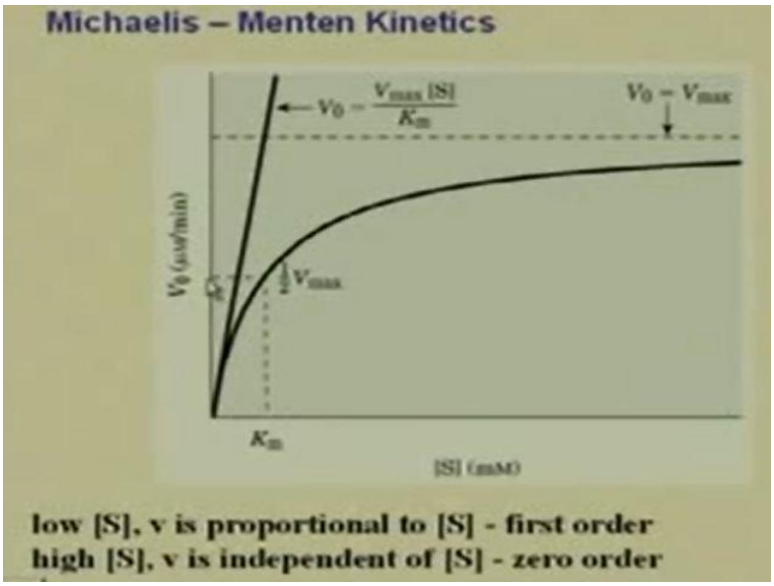

Now if we look at the overall expression that we have. From this expression, we can get what is called a rectangular hyperbola that is going to eventually plot the V0. Now why we called this the V0, I will tell you in a moment. So what we are doing is we are actually plotting a velocity versus a substrate concentration. Now what happens is as you increase the substrate concentration, because the enzyme has limited capability in taking in or forming an enzyme substrate complex.

This will reach a limiting value. And this limiting value is dependent on the total enzyme concentration that is available to you and this is Vmax, the maximum velocity available. Now if

we go back and look at the expression that we have here, we did this last time where if we substitute v as Vmax by 2 that is half the maximal velocity that is attainable, then what we can get is we can get the value for Km.

So Km is the substrate concentration for which 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 Km, what it means to have a high value for Km but for now we recognize that when we have a low substrate concentration. We have first order kinetics for this particular reaction where v is proportional to the substrate concentration.

We have here a V0 defined as Vmax [S] by Km. Now you recognize this is actually the Michaelis-Menten form where we actually have a Km + S as the denominator but since S is extremely small usually less than 10 percent 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 high S it becomes zero order independent of the amount of substrate that you add because there is limited capacity for the enzyme to take in the substrate to form the enzyme substrate complex.

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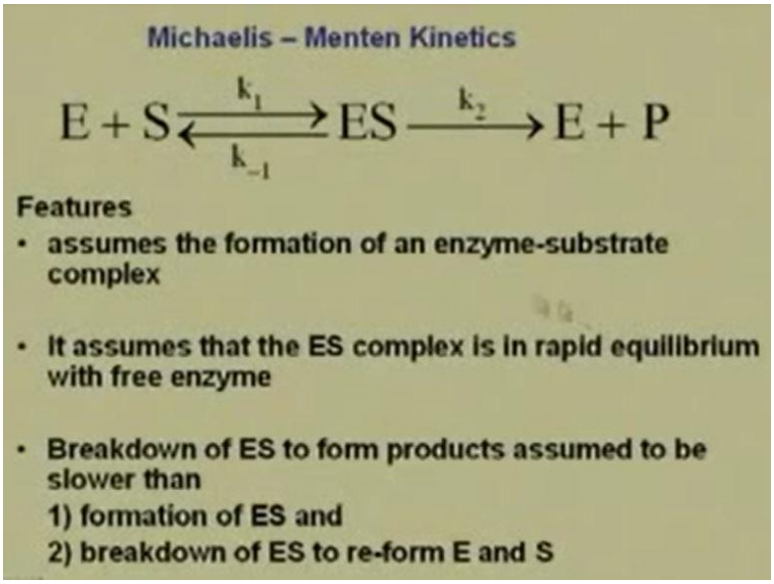

So what are the features of these? The features of kinetics considered by Michaelis-Menten are that the formation of an enzyme substrate complex occurs it assumes that this enzyme substrate complex is in rapid equilibrium with the free enzyme. So we have a rapid equilibrium here that is the pre-equilibrium 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 does this make this? This makes it rate limiting step of the reaction. What we assume here? That the breakdown of ES to actually form the products is assumed to be slower than the formation of ES and also slower than the breakdown of ES to reform E and S. What are we saying here? We are saying that both K-1 and k2 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.

These are the features that have come into Michaelis-Menten kinetics to actually get in to the form of it the expression that we get. Now what do we have here? We have k2 as a rate limiting step. We have k-1 as also a small step. So what do we have? We have basically an association of the enzyme and the substrate to form the enzyme substrate complex. So what we can actually define is? We can define equilibrium here, considering the rate constants k1 and k-1 we can define equilibrium.

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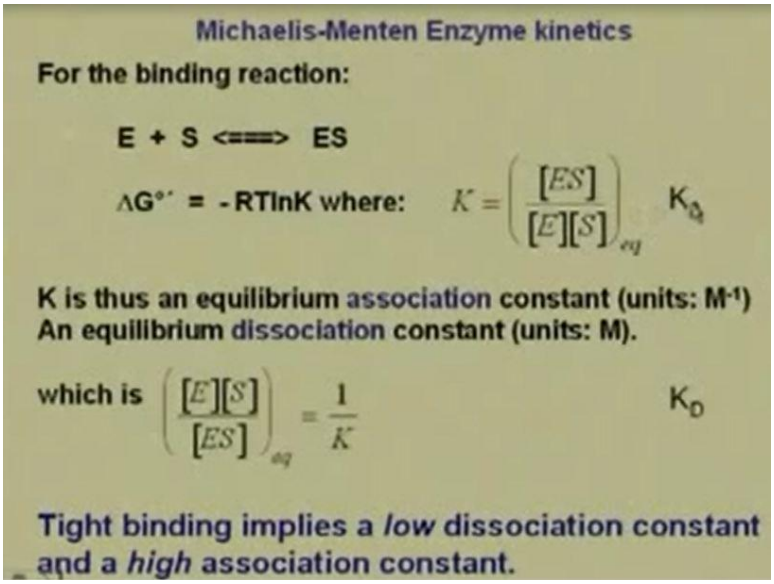

Now that equilibrium can be an association. What is this association? It is E + S to form ES that is an association reaction. What is that k? It is the enzyme substrate complex divided by the concentrations of the enzyme and the substrate and at equilibrium. What is this? This is our equilibrium constant. If this is our equilibrium constant we can find a Delta G not for this. So what we have is for this particular binding reaction, we have an E + S that takes us to the enzyme substrate complex and we have an association constant associated with it.

You recognize that the units of the association constant are mole inverse because the enzymes substrate complex is in Moles. The enzyme is in Moles and so is the substrate. Now if I go for the reverse of this reaction. I am considering a dissociation of the enzyme substrate complex back in to the enzyme and the substrate. So that is also equilibrium from the reversed side. So I have the expression which is just one by K where I have an enzyme concentration and a substrate concentration divided by an enzyme substrate concentration that makes the units of a dissociation constant molar units.

So what I have is initially. I have an enzyme and a substrate that forms the enzyme substrate complex. The formation you will have to remember you can just think of it. It is in an association so it is called an association constant because you are associating the enzyme and the substrate to form the enzyme substrate complex. Since this is an equilibrium, now what can I relate this to. I can also relate this to the rate constants so what will my K be it will be k1 divided by k-1.

So you recognize that this we have a thermodynamic concept to this as well as a kinetic concept to it. 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 tight binding, a tight binding will mean that I do not have dissociation. If I have a tight binding, the dissociation is not easy right. So what does it amount to in the terms of complex form and the terms these constants.

If I have a tight binding I will not have dissociation so I will have a low dissociation constant but I will have a high association constant obviously because it is going to be one by if we consider KA, one by K is going to be our dissociation constant. If I have a high association constant it means that the enzyme and the substrate constant complex are bound tightly. So, what do we

have? We have an association and a dissociation if I have a high association constant what does it mean I have tight binding that means I am going have strong association. Strong association means, the enzyme and substrate are going to bind together very tightly.

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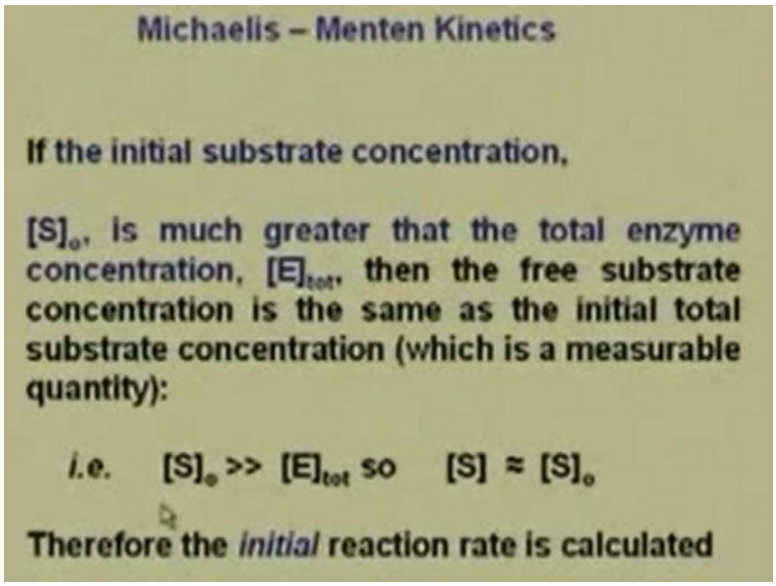

For the certain reactions in the Michaelis-Menten, there is another consideration that is made where we consider that the substrate concentration is very high. If the substrate concentration that is the initial concentration of the substrate is much greater than the total enzyme concentration, then there 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 off with this is very similar to considering a pseudo unimolecular reaction.

Where suppose you have your reaction and water what do you say? You said since the water is in excess you consider pseudo unimolecular reaction. 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 consider what is called an initial reaction rate which is the initial velocity remember I showed in the first curve.

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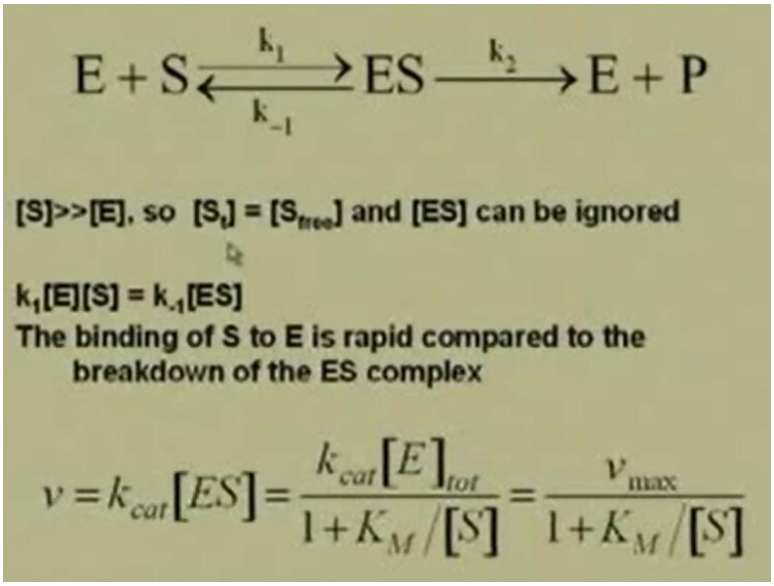

So what we have? We have the total enzyme concentration is equal to the free enzyme concentration and what are the other assumptions we make that the binding of the substrate to the enzyme is rapid so what do we have? We have a rapid formation of ES and the slow breakdown of the complex making k2 small and k-1 small. So what is k2? k2 is a rate limiting step. So what we have is we have this Kcat which is also refer to as k2 which we will see in moment why we call this Kcat and we have a specific expression related to RKcat and RKm.

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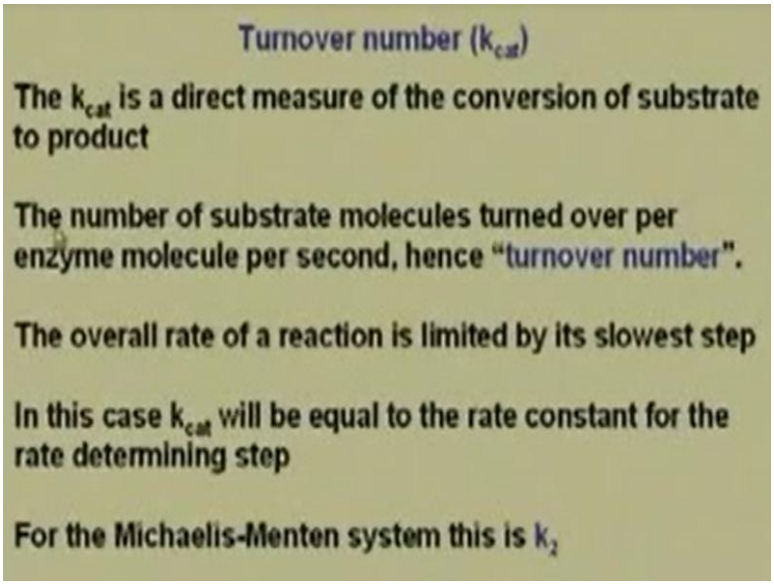

Now what is this Kcat? This Kcat is actually a direct measure of the conversion of the substrate to the product. It tells you what is called a turnover number which I mentioned the first day when we started enzymes. Where the number of substrate molecules that are actually forming the

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.

What is it doing? It is transforming the substrate to the product. So there has to be measure of its efficiency. The measure of its efficiency is going to come by this Kcat value. Now we also know that the overall rate of a reaction is limited by what, the slowest type of there action which is the rate determining step or the rate limiting step. What will be the Kcat? Kcat will be equal to the rate constant for the rate determining step.

Which is the rate determining step? It is k2. Why is it k2 because that is the one that is forming the products. Since k2 is the 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 become the slow step of the reaction. This becomes the rate limiting step or the rate determining step of the reaction and for the Michaelis-Menten system the Kcat is actually the k2 value. What can I do with these values?

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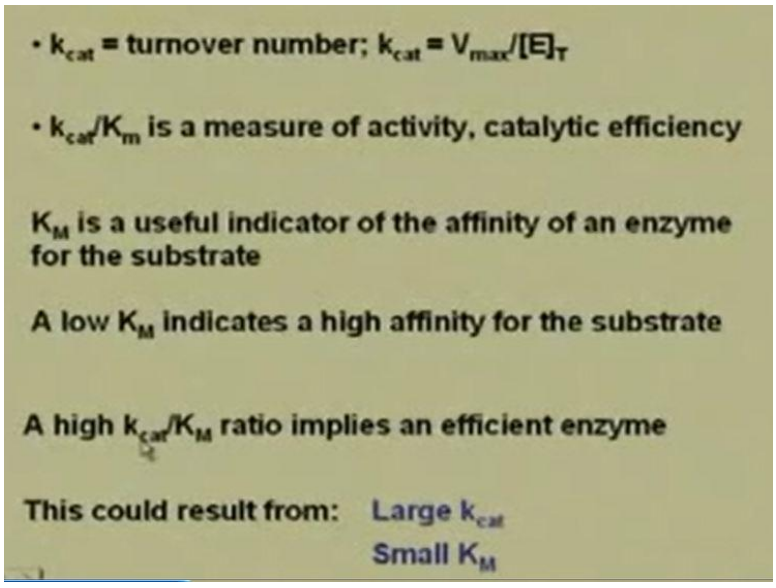

So Kcat is our turnover number so what is our Kcat. We know that Kcat into the total enzyme concentration is the maximum velocity attainable right? Because what did we do initially. We wrote k2 total enzyme concentration that is going to give us Vmax. What is our Kcat? It is Vmax

by the total enzyme concentration. Now the Kcat by Km is a measure of activity or rather the catalytic efficiency.

Km, we understand is a useful indicator of the affinity of an enzyme for the substrate. Why is that so? It is because it is relating the rate constants of the formation of the reactions. What is the Km combination of? It is a combination of k1, k-1 and k2 which are all and what is Kcat? Kcat is actually k2 so together they can actual give us some idea about the activity of the catalytic efficiency.

If we have a high value for Kcat or k2 then what do we have? We have a high measure of the catalytic efficiency so the enzyme is more efficient. Now suppose we have a low Km value. We have a high affinity for the substrate so what we want is for an efficient enzyme we would want a high Kcat by Km ratio and what could this be a combination of? This could come from either a small Km value making this ratio large or we could have a large Kcat value again making this ratio large.

What is ratio a measure of? It is a measure of how efficient your enzyme is. 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 kinetic constants that we have.

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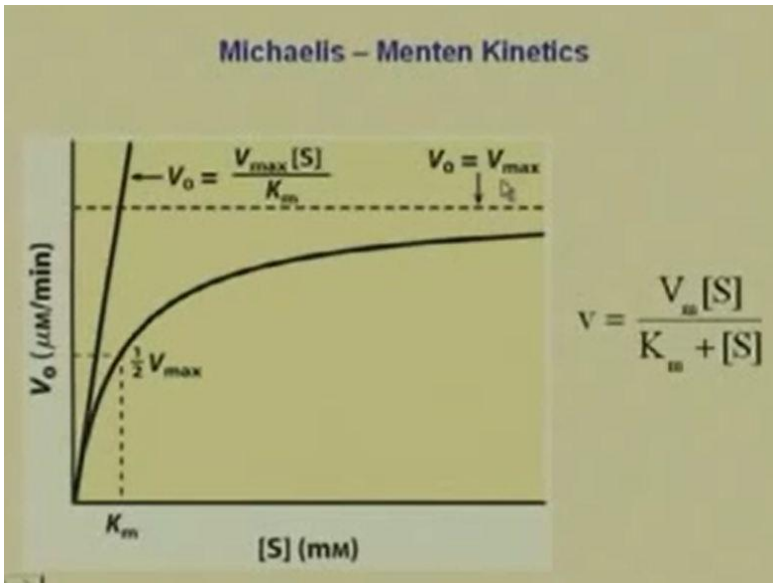

So this is our Michaelis Menten kinetics. We have Vmax, we have Km, we have a velocity and we have substrate concentration. Now what happens is if I know the total enzyme concentration that I start off with there are certain aspects or there are certain measurable quantities that I have.

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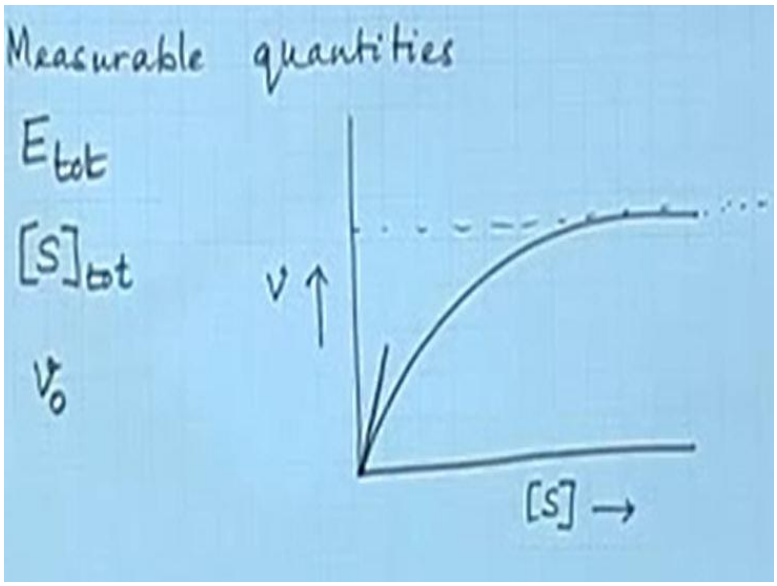

What are these measureable quantities? The measurable quantities are total enzyme concentration, total substrate concentration and actually my initial velocity. Why? Because if I consider my curve here, what is this? This is the velocity of the reaction. This is the substrate concentration; this I do not know where Vmax is actually going to sort of go right. So this is some quantity that I actually cannot determine this way but what can I determine? I can determine at low concentrations what the V0 value is. What do I have? I can find out what an initial velocity is.

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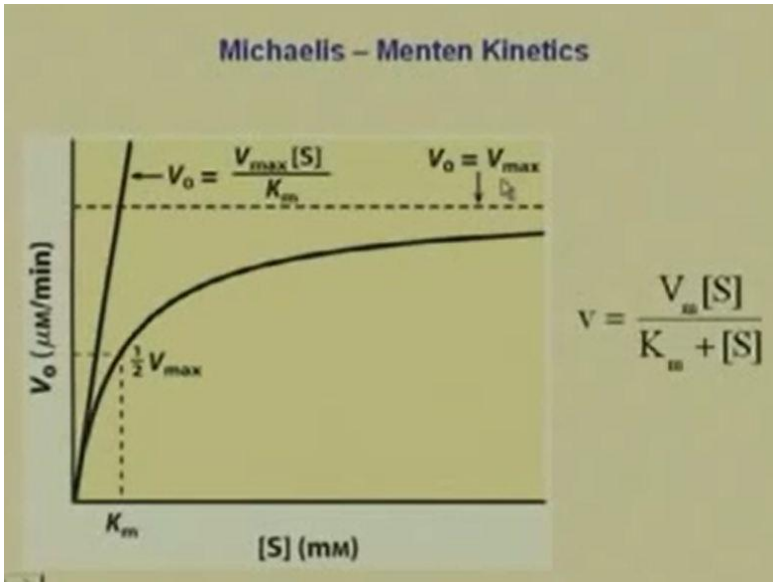

We will go back to the slides here. We can look at what the initial velocity is. So what do I have? I have an initial velocity that is eventually going to get me to my Vmax if I have enough substrate concentration but I still do not get a good value for my Vmax. So how do I calculate Vmax? If I look at the expression, I have a velocity that is equal to my Vmax substrate concentration divided by what Km + the substrate concentration. Now If I take reverse of this.

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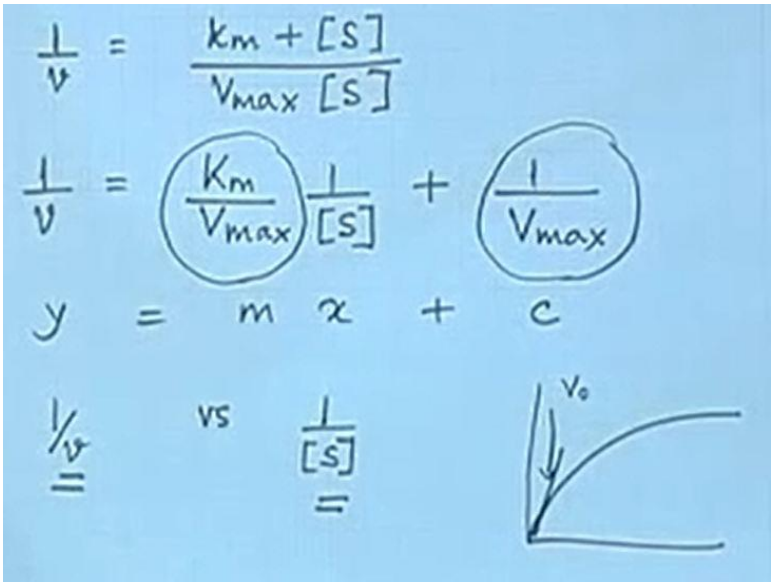

What can I do? I can take one by V that is going to give me what Km + [S] divided by Vmax[S] right. Now if I just separate these two quantities. What am I going to get here? That is what I get. I consider one by v from the Michaelis I have just inverted the Michaelis-Menten expression and

I work out an expression where I have the form of y is equal to m x + c right so what can I do if I plot one by v versus one by [S].

This is something I know because I know what substrate concentration I start out with and I can find out from my curve this initial value for V0. The velocity I know. The substrate concentration I know. So what do I get? From the slope I get Km by Vmax and what do I get from the intercept one by Vmax.

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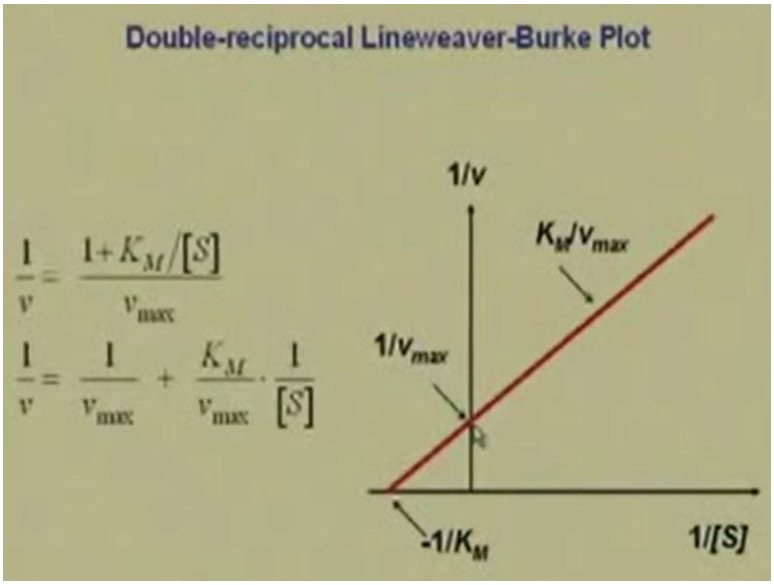

This is exactly what you can do to get what is called a double reciprocal plot also known as the Lineweaver–Burke plot that is extremely important in enzyme kinetics. There is no enzyme kinetic reaction or plot that you can do without doing a Lineweaver-Burke plot. What do we have? We have a one by V, a one by Vmax + a Km by Vmax, one by [S]. What am I plotting? One by V on the y-axis, one by [S] on the x-axis and what do I get is the intercept one by Vmax.

What is my slope? Km by Vmax so that is what we get so if we plot one by V, one by [S] what do I have here my slope is Km by Vmax and my intercept is one by Vmax. So what can I determine from this? I can determine the maximum velocity attainable which I cannot do from the rectangular hyberbola normal Michaelis Menten curve. I can find out what Vmax is. If I know Vmax and I know the total enzyme concentration what can I also determine Kcat. I can find out what Kcat is why?

Because I now know what Vmax is and what are the quantities I know. I am doing the reactions so I obviously know enzyme concentration I started off with so I know what Kcat is and from here what else do I know? I know what Km is. What can I find? I can find Kcat by Km and what will that tell me that will tell me how efficient my reaction or my enzyme substrate complex formation or the whole enzyme kinetic reaction is. Now there is another plot that is also sometimes considered.

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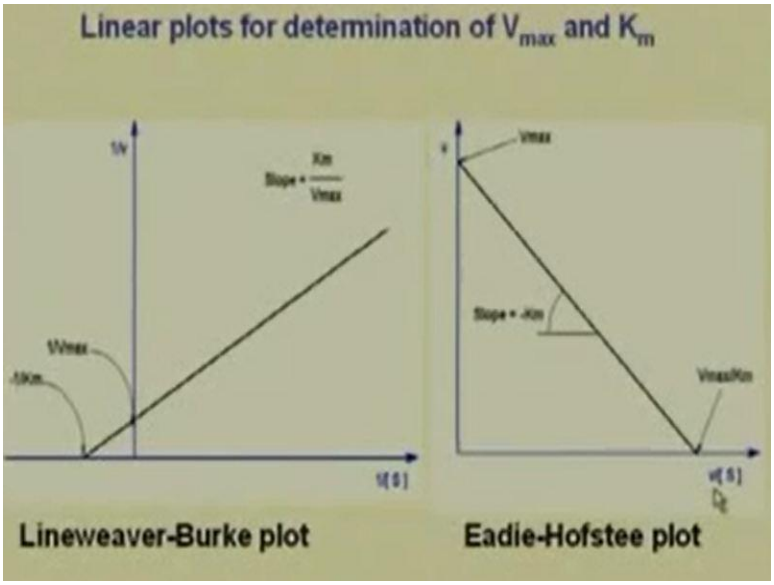

This is where we have our Lineweaver–Burke plot rather linear plots the other one that is commonly used is an Eadie-Hofstee plot that plots V Verses V by [S]. So if we plot V versus V by [S] again what you get here? You get the slope as minus Km and the intercept as Vmax. But the most important one and the one probably that you need to know is the Lineweaver –Burke plot that is used for the determination what? The determination of Vmax and Km which otherwise you could not determine.

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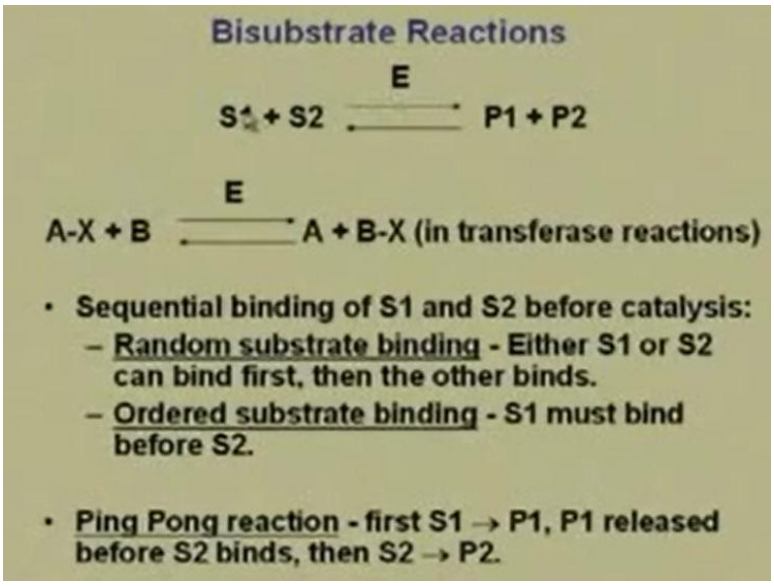

Now what happens if we have more than one substrate? That also possible okay so we have now what is called a Bisubstrate reaction. Now there are certain just terminologies that are used here but we are not going in to the kinetics of this reaction but since we have done enzyme kinetics there are certain 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.

What is that mean? It means that the enzymes 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. What is that mean? Ordered binding means 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 a catalytic reaction where S1 and S2 are going to P1 and P2. So enzyme has to form the enzyme substrate complex.

Now you could have the formation of what is called a ternary complex. What do you mean by a ternary complex? Where we have three components in the complex what are these three components then. It is the enzyme, S1 and S2. Now how are S1 and S2 going to bind to E again we can have what is called sequential binding where S1 and S2 are going bind randomly in any order it will not matter or we could have ordered binding where the S1 has to bind before S2 for the products to form.

It is basically mixing up E and S1 first 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, P1 is formed then E binds S2 and P2 is formed. But S1 forms P1 so P1 is released before S2 binds okay much like the previous kinetics that we considered. There are two types of specific types of reactions that we have here sequential binding and ping pong reactions for bisubstrate types.

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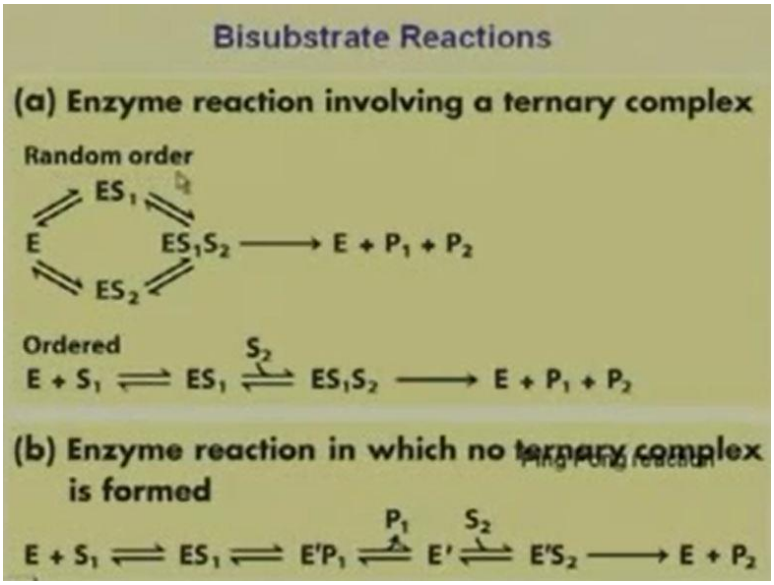

This is what we mean by a random order we have the enzyme what can the enzyme do it can form ES1 or ES2 a random order and then what happens it forms the ternary complex. What is a ternary complex mean? It means that you have E, S1 and S2 together so three components in the complex make it the ternary complex. Now you see from this reaction scheme that E could either form ES2 or it could form ES1 right it could form either of them then if this forms S1 obviously S2 has to bind now and if this forms S2 first then S1 has to bind now so we form the ES1S2 ternary complex.

Then we have product formation where E + P1 + P2. Now in the enzyme reaction where we do not have a ternary complex formed which is actually called the ping pong reaction. We have E + S1 forming ES1 and what is important about the ping pong mechanism is that P1 has to be

released first. So we have E + S1 form ES1 the first product is released before the next substrate binds. What happens is the binding S1 forms an E prime.

You notice there is an E prime here. So this E prime is not E. There is some change that has come across in the enzyme structure which after release of P1 then it binds S2 to form an E prime S2 complex which on losing P2 will form E back. You have to remember you start off with E you have to end with E. In the ping pong mechanism, so if we look at this part up to EP1 or rather E prime + P1.

If we consider the normal Michaelis-Menten kinetics that we have been considering all along we had E + S go to ES but what did we form after that E + P not an E prime okay. So the enzyme comes back after because you have to remember this is a bisubstrate reaction. There are two substrates involved two products being formed. You can either have a formation of a ternary complex where you have ES1S2 formed in a random order or a sequential order where you have the products formed.

You could have a ping pong reaction where you have an enzyme and a substrate form an enzyme substrate complex ES1 that releases P1 before it binds S2 then it releases P2 and the enzyme gets back to its original form where want can it do it can now bind another S1 and the reaction can go on.

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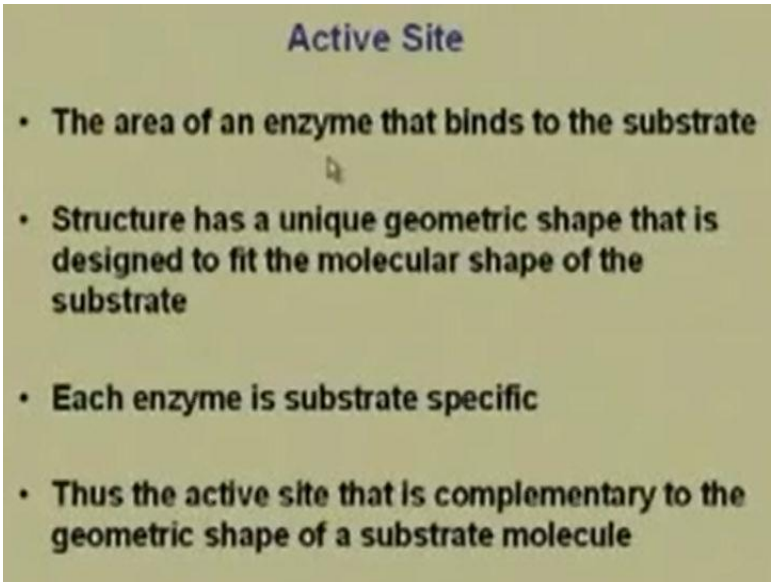

We are talking about the interactions of the enzymes and we now have to consider is the active site of the enzymes. What is there in the active site that is so important that we have a bisubstrate reaction or we have a single substrate reaction? There are specific interact sites of the enzymes that have to bind to the substrate. Now the structure has a unique geometry shape that is designed to fit the molecular shape of the substrate okay that is obviously essential. We will see how that works out.

Then each enzyme is substrate specific it is extremely specific, the way enzyme work is just miraculous. It is extremely end 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 will be considered where we have the active site of the enzyme.

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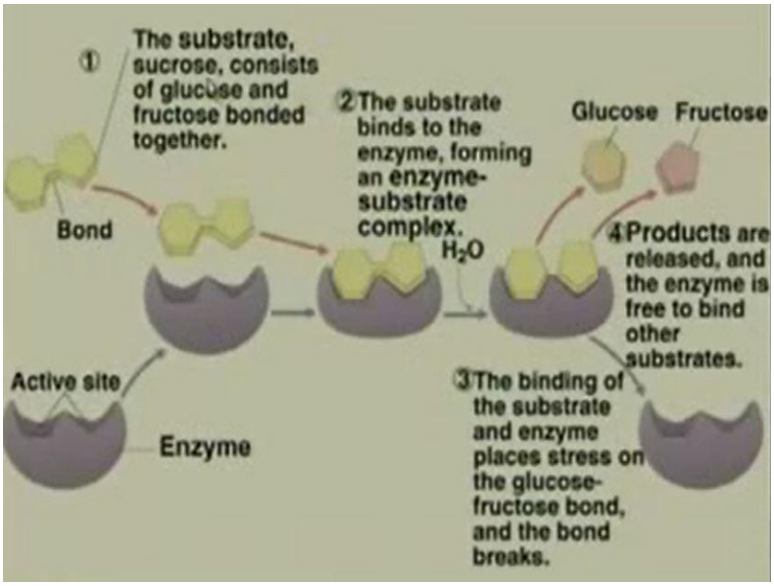

Now suppose we are looking at the hydrolysis of sucrose. This is just a schematic where we have the enzyme now what is there? This is the active site of the enzyme. You see that this is shaped in such a way that it is going to bind the six-membered ring. This is geometrically shaped in such a way that it is going to bind the five-membered ring. What happens is we have this particular bond that is going to be cleaved. So, our enzyme the products are going to be glucose and fructose.

What is this? Sucrose, so we have the sucrose that is a combination of glucose and fructose but it is connected so we have cleaved this. This enzyme is going to cleave that. 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 fit in exactly this orientation exactly this geometry. So, the substrate binds to the enzyme so what do we form the enzyme substrate complex.

The substrate binds to form the enzyme substrate complex. Then what happens we have an hydrolysis that occurs and we have the breaking of this bond that is an enzymatic mechanism which do not have to consider right now. Then what happens is the products are released. On release of the products what do we have? We have the enzyme exactly in the form that we started off with. So what can it now do?

It can go bind another sucrose and goes through the same thing over again fine. So it will keep on doing that obviously that is what it is happen in our bodies all the time. So we have a particular enzyme that is going to fit at the active site. But what we have to notice here is that the geometry is extremely important in the way the sucrose is going to bind to this particular enzyme.

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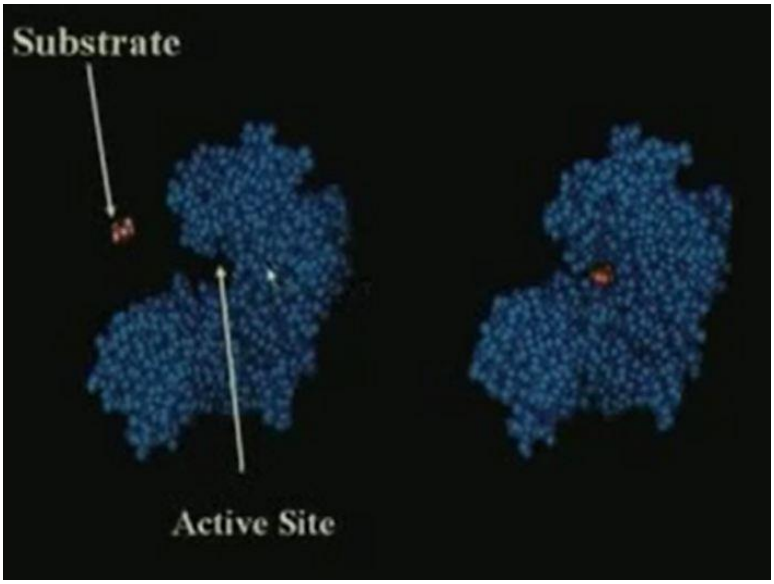

Now what we need to know is see this is a picture of an active site of an actual enzyme and this is a substrate you see how snugly it fits in here. That is showing you how the substrate can actually fit in to the active site of the enzyme.

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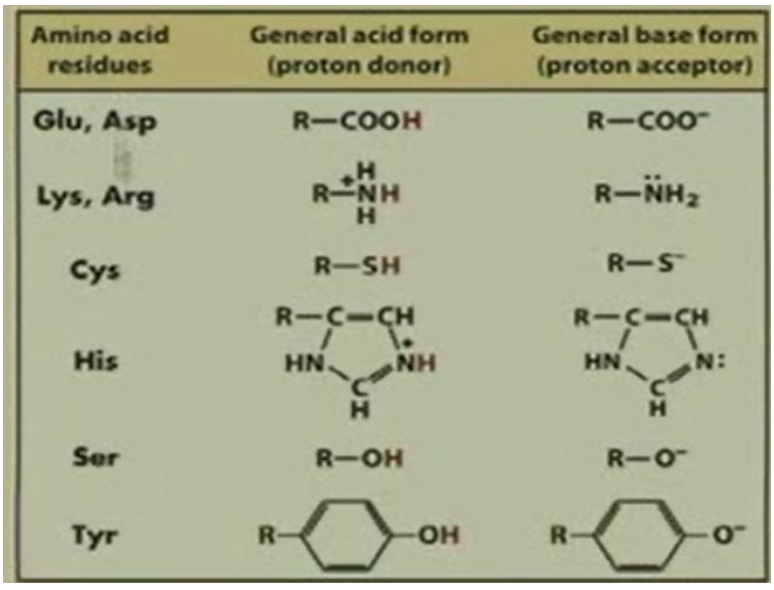

Now what are the amino acid residues that actually can form the active sites of enzymes? Now you recognize that what are interactions that are going to take place? They are going to be non-covalent type interactions because we do not have a covalent linkage that is formed. Why? Because has to lose the substrate or rather it has to lose the product after a transformation. So once we form the enzyme substrate complex there are certain amino acid residues that can actually form this interaction.

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 form this but you release that is going to take place then the pKa is low right. The reaction where this is going to take place has to be at low pH so suppose where can a reaction such as this take place in our bodies? In the stomach where we have an enzyme called pepsin.

This enzyme works at acidic pH okay 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 this if it is in this form it can act as a proton acceptor. So we can have two forms of each of these for cystine we can have R SH or we can have R S minus which is going to act as a proton acceptor.

The most important one you have to remember is Histidine. Why? Because this pKa is around six so what do we have here at some point therefore histidine can act as a proton acceptor and it can also act as a proton donor and this equilibrium is at a pH of six, close to six. Serine R OH what is this R? This is the rest of the amino acid basically or rest of the polypeptide chain let us call this.

Then we have R O minus so what is it doing here? Again, proton donor and if it is this species it can act as a proton acceptor. Again, Tyrosine if this is the proton acceptor then this can be the proton donor. We will see how in certain enzymatic mechanism actually histidine for example we will do one specific example where we have histidine in one of the steps it acts as proton

donor in an another step of the reaction it acts as proton acceptor. Just because you have to remember that it has to get back to it is original form.

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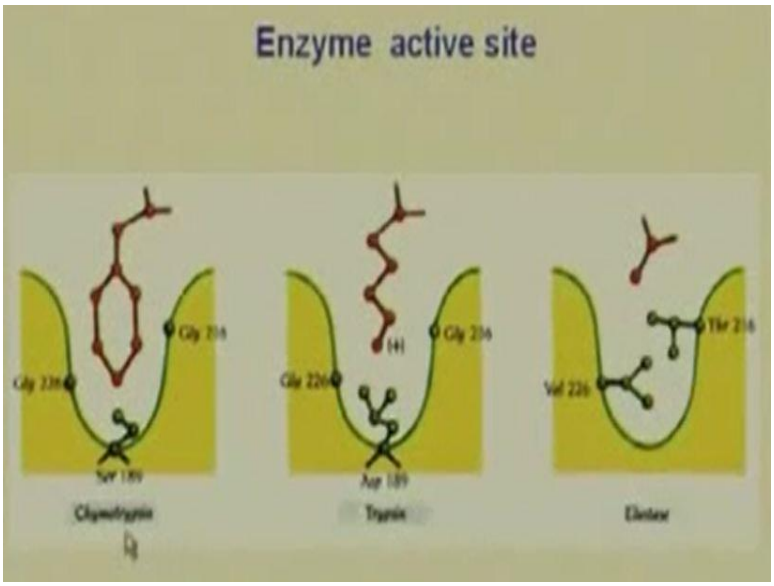

This is what I showed you last time. So, we have a specific serine here that is going to act on an enzyme called chymotrypsin. We will look at the specific mechanism of chymotrypsin and see how the OH of serine actually takes part in this reaction. We have aspartic acid here and a lysine here. What is happening here? 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 here in an interaction where we are going to have another hydrophobic residue.

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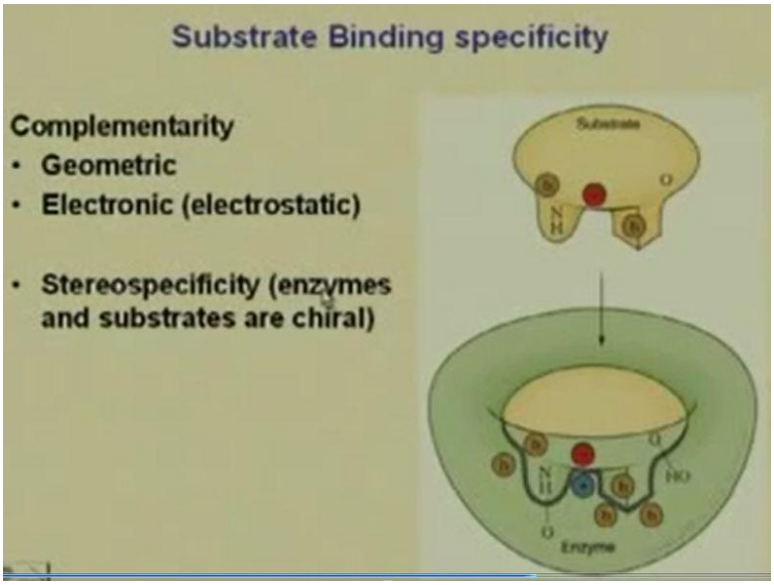

What do we have? We have the complementarity in terms of a geometric complementarity and an electronic complementarity and considering the stereospecificity of the enzymes and the substrate that are usually chiral. Why are the enzymes chiral? Because there are proteins and the proteins are composed of L amino acids. Substrates are also we will see how specific they are later on. So, if we have substrate that looks like this and we have a particular enzyme that looks like this.

This H means it is hydrophobic in nature. The NH means that it has an amine group to it. A minus means it has a negative charge to it. Then we are going to have another hydrophobic group and oxygen say here. Now if this particular substrate where 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 residues close-by because you have to remember that the enzyme substrate complex has to form for the product to form. Close-by the hydrophobic group here we will find another hydrophobic group for the NH we would expect what?

Some sort of hydrogen bonding that might hold it together. For the negative charge that I have here what about I expect at the active site? A positive charge so that I have an electrostatic interaction again a hydrophobic interaction for the other parts of the substrate and so on and so forth. Once the substrate gets in to the active site now if you look at the active site we see we

have a favorable hydrophobic interaction here. We have favorable hydrogen bonding here. We have an electrostatic interaction here.

We have another set of hydrophobic interactions here and again hydrogen bonding here. So all this is going to loosely associate the substrate with the enzyme in to forming the enzyme substrate complex that is eventually going to do what? It is going to then from this complex undergo certain reactions to form the product and the enzyme will retain that is the H, the O, the + the 2H here and the OH it will have to come to exactly back to the same structure if it has to bind another of these substrate molecules. We have to consider a model in which this binding can actual take place.

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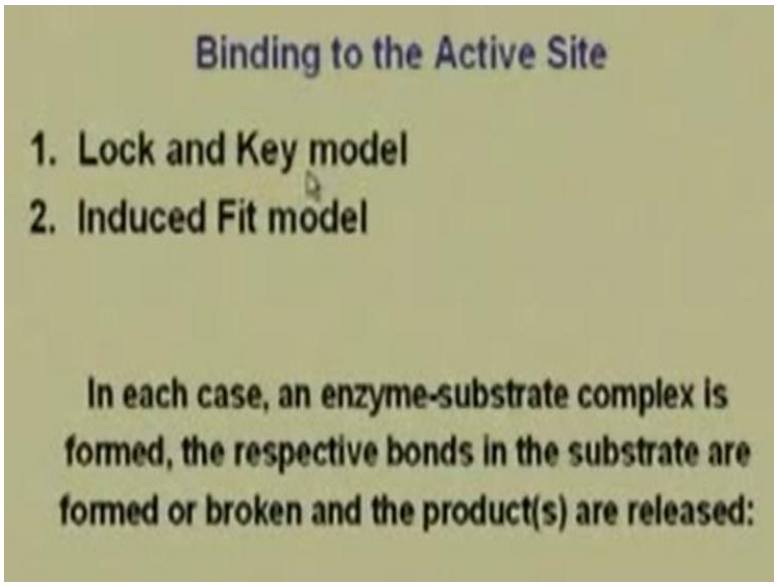

There were two models of binding to the active site. What are we what are talking of? We are talking of the binding of the substrate to the active site. The two models are called the lock and key model and an induced fit model. You understand from the name itself the lock and the key model where the lock is the enzyme and the key is the substrate.

So you have a specific key that is going to fit in to 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 form and the respective bonds in the substrate are formed or broken and the products released in both cases because each of this are talking about how we form the ES complex that is essentially what we

are forming now. How can we form it? We can form it what is lock and key mechanism or an induced fit mechanism.

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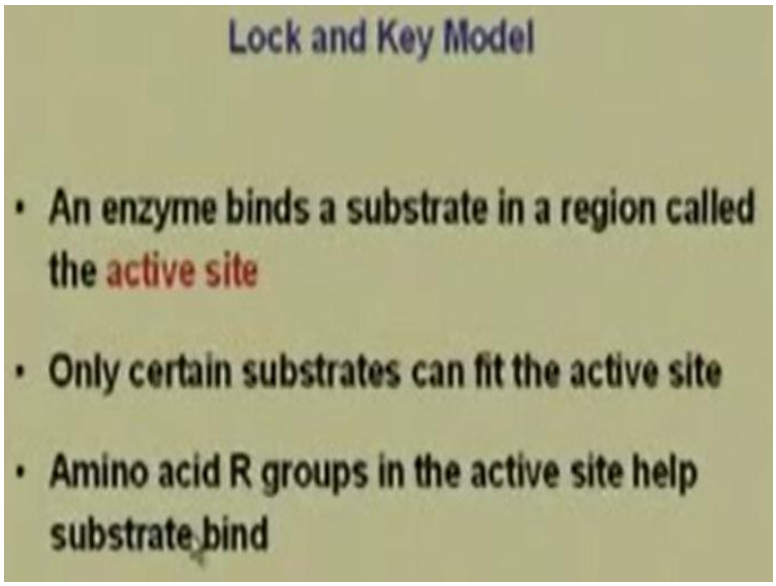

What are the features of this site or this model? The lock and key model the enzyme binds a substrate into the region called the active site which we have seen. Only certain substrate can fit the active site and what helps the active site help the substrate by? What is it? It is the R groups so either the hydrophobicity or the positively charged or the negatively charged or the hydrogen bonding whatever you are talking about it is all the amino acid site chains that are actually eventually taking part in the reaction so to form the enzyme substrate complex we can have a lock and key model.

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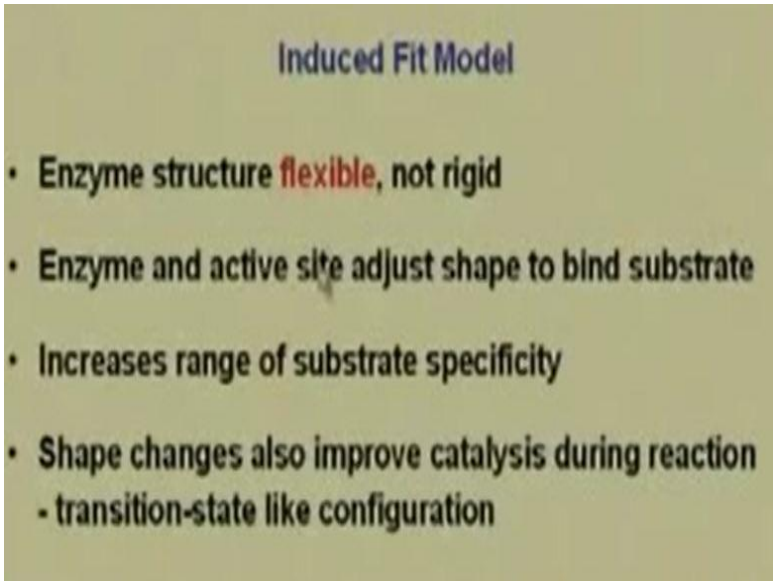

We can have the induced fit model. What does it say? It says that the enzyme structure is actually not as rigid as a lock. It is slightly flexible so what it does is the enzyme and subsequently its active site can adjust its shape to bind the substrate. There is a slight modification that can occur due to the flexibility. Why will I say that this is flexible? Why can I say that the enzyme structure is flexible?

Because we have all those torsional angles we have all the bending that can occur a slight change in what in the polypeptide chain that can occur. If this happens to occur near the active site just to accommodate the substrate, then we call it an induced fit model. The enzymes 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 sub-transition state like configuration.

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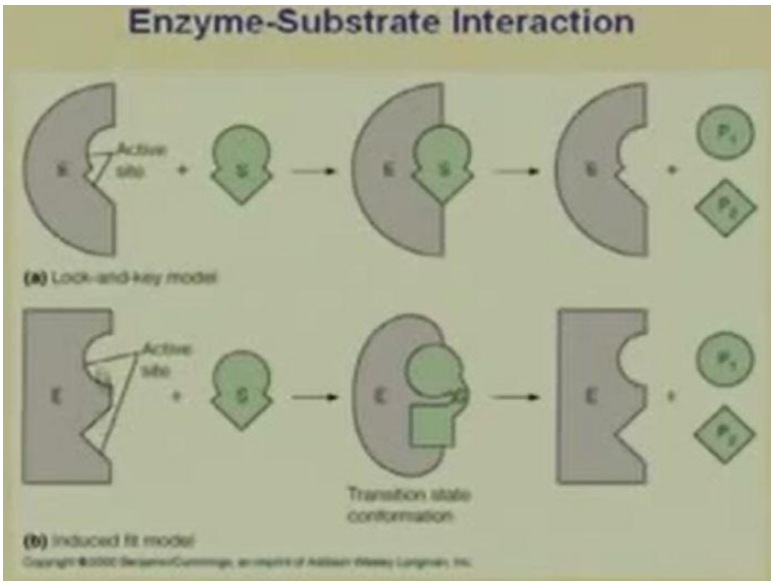

This is what we mean by a lock and key model. This is our active site where we have our enzyme and this substrate so what is happening? We have this is our key that fits exactly into the cleft of the active site. So it fits exactly in here then you have your product formation whatever say this could be analogous to the sucrose where we have the glucose and fructose. What are the features of this? We have our enzyme we have a substrate.

The enzyme substrate complex is formed that considers this enzyme to actually be rigid in nature slightly rigid in nature where this 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 absent okay. Now as the substrate approaches the enzyme what happens? There is an accommodation.

An accommodation of the enzymes so there is a slight change flexibility associated with this that is going to result in an induced fit so now we see that the shape of this is slightly changed why has it changed to accommodate the substrate. The substrate also basically this is formed so that it can be cleaved in this case it is being cleaved okay so now we have the enzyme the transition state conformation that is formed and we have the enzyme form the products okay so we have these two models.

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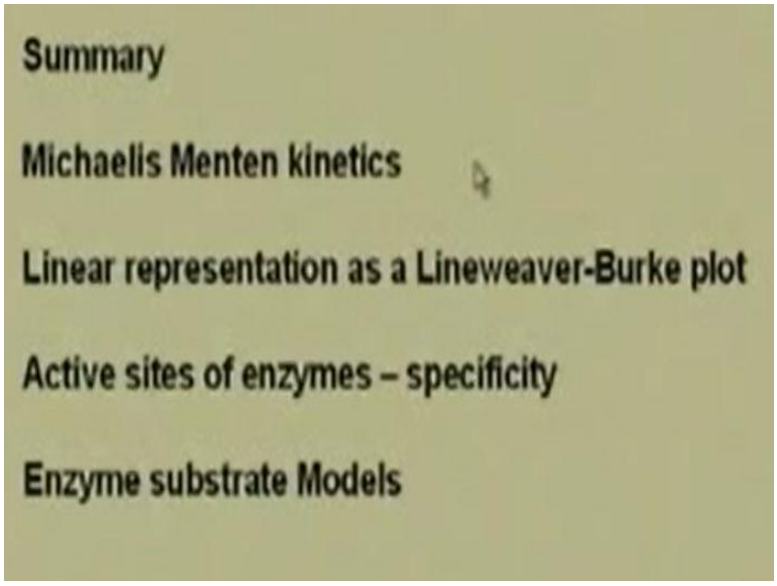

So, what we learnt here is we have summary. The summary here is the Michaelis-Menten kinetics that we considered today. What did we have? We had the Vmax what is this Vmax? It is the k2 or the Kcat versus the total enzyme concentration. We looked at a linear representation as a Lineweaver–Burke plot. What is this Lineweaver–Burke plot? It tells us how we can calculate Vmax from Vmax what can we get? We can get k2 that means we can get Kcat and we can also get Km.

Kcat and Km will tell us what? It will give us an idea about the catalytic efficiency of our enzyme and we consider the active sites of the enzymes what are the active sites telling us the specificity of the particular enzyme. How this specific amino acid site change can form or can act rather as proton donors and proton acceptors in the active sites to bring about the particular complex formation or the particular reaction.

We also have the different enzyme substrate models that tell us how the substrate going to bind the enzyme active site and what are the two models we considered here 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 substrate complex is going to form and then 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.

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 Vmax of a particular reaction or a particular enzyme substrate complex.

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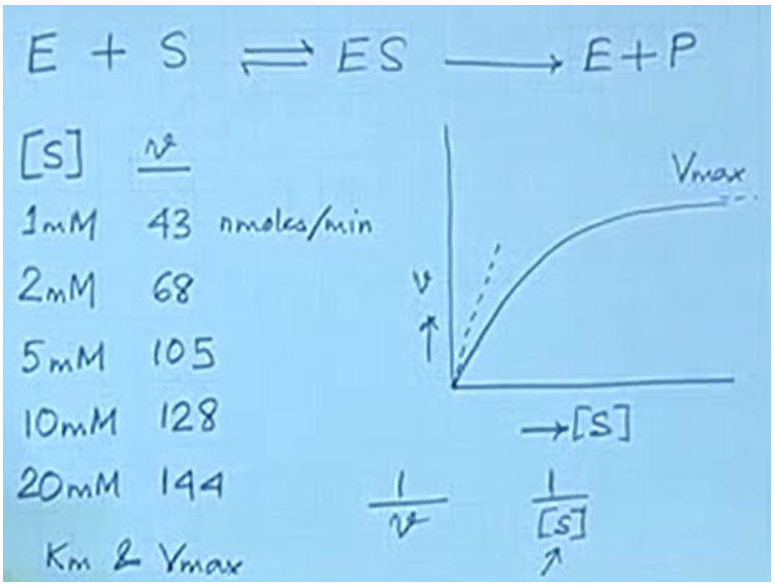

So, we have our enzyme concentration, we have our enzyme + the substrate that is giving us the enzyme substrate complex that eventually gives us the E + P. Now if I have a set of substrate concentrations what do I need now if I want to calculate? So, I know that when I do this for a particular substrate concentration this is going to be my Vmax right. Now if I want to plot the Lineweaver-Burke plot what do I need?

I need one by V, I need one by S right. Now 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 what is called my initial velocity. Say for substrate concentration one millimolar, my velocity is 43 this velocity is given in nanomoles per minute.

Now I have a particular velocity what is this velocity? It is the initial velocity associated with the substrate concentration of one millimolar. Now I repeat the reaction with two millimolar substrate then my velocity increases to 68 nanomole per minute. I now go to five millimolar where I have 105 nanomoles per minute, 10 millimolar where I have a 128 nanomoles per

minute and 20 millimolar where I have a 144 nanomoles per minute. If I want to determine Km and Vmax from the data that I have here what I need to do is?

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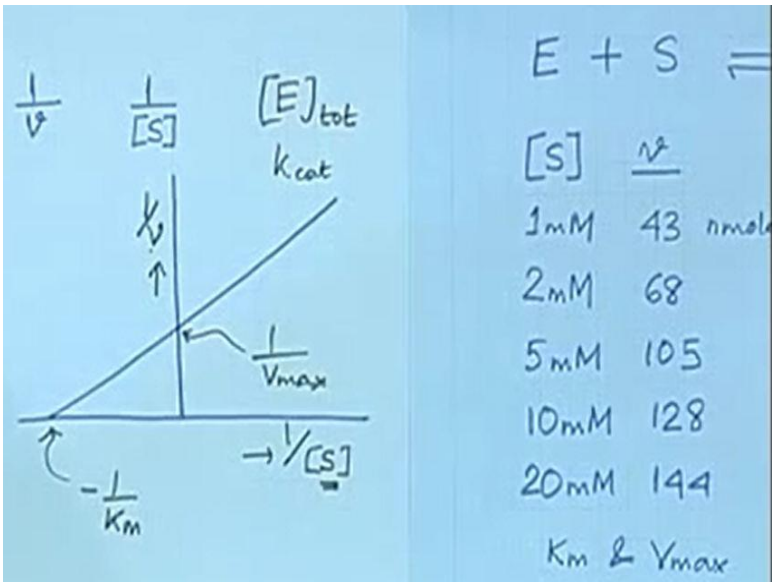

I have to calculate one by V and one by [S] now once I calculate one by V and one by [S] what am I going to get. I can plot the data on a Lineweaver-Burke plot and what is that going to give me? Something that looks like this so what have I plotted one by V one by [S] what have I done? I have conducted the reaction at a variety of substrate concentrations and calculate the initial velocity for each of the substrate concentrations.

Then what I do is I plot one by V versus one by [S] what is this here? I can calculate what the maximal velocity is and I can calculate what the Km value is. If I knew what the total enzyme concentration is what else could I calculate? Kcat right so this completes our discussion on enzyme kinetics for reactions where we do not have any inhibition. In the next we will see how enzyme inhibition can occur and what are the effects on the different plots that we have and what are the different types of inhibition that can occur? Thank you.

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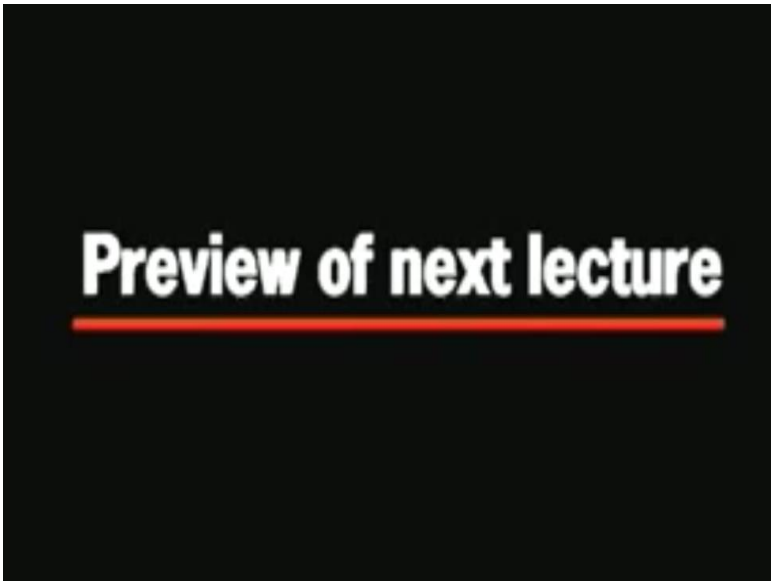

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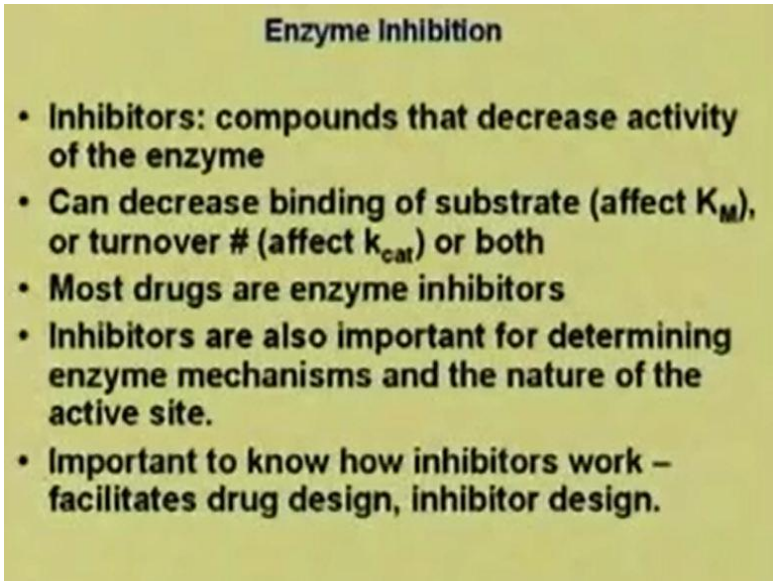

In the last class, we spoke about enzyme kinetics and how we have the specific active site of the enzyme and how we form the enzyme substrate complex. What we going to speak about in this class is Enzyme inhibition. Because enzyme inhibition is a very important factor in determining drugs or specific compounds that are going to inhibit the action of definite or specific enzymes so apart from knowing the structure and once we know the function.

There are certain designs to the drugs that can be made to determine how we can design what is called an inhibitor of a particular enzyme now what we want to do is it means that we want to stop the particular reaction that the enzyme is catalyzing. So, these inhibitors are compounds that

decrease the activity of the enzyme. Now how can they decrease the activity of the enzyme they have to affect the efficiency of the enzyme?

They decrease the efficiency of the enzyme by doing what? They can affect the Km value or the Kcat value or both of the values. We have to decrease the binding of the substrate if an inhibitor of an enzyme has to act so we want to stop the enzyme from forming the enzyme substrate complex as simple as that. So, if I want to form or I want to deter the formation of the enzyme substrate complex I have to have the inhibitor bind to the substrate somehow I bind to the enzyme somehow.

Now most drugs are enzymes inhibitor okay because there is certain bodily function that goes wrong which is why you have a specific activity of an enzyme in the wrong fashion so what would you want? You would want some drug that is going to inhibit the enzyme so it does not act in a fashion that is acting right now for example if you have antibiotics. What does it do? It inhibits the enzymatic reactions of the bacteria that have formed the infection.

Inhibitors are also used to determine enzyme mechanism and also for the nature of the active site okay and of course it is extremely important to know how these inhibitors work because this can facilitate drug design and inhibitor design.

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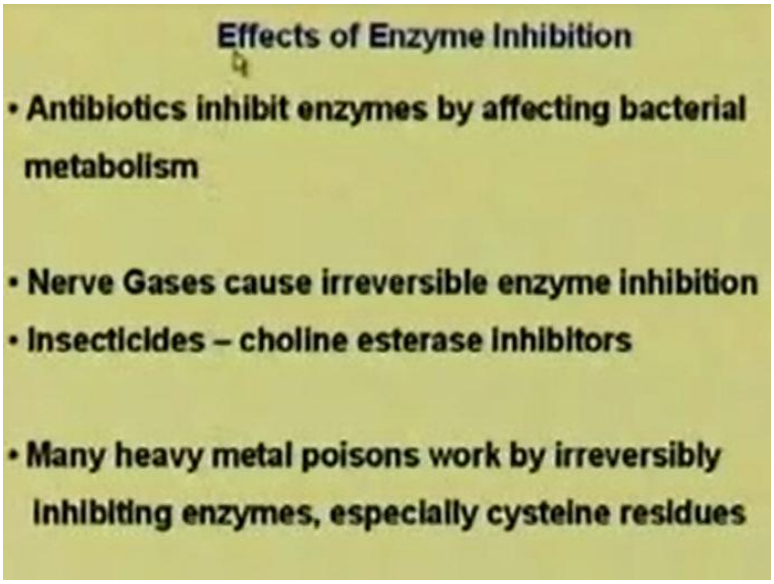

For example, what are the effects of enzyme inhibition the antibiotics. What do they do? They inhibit the enzymes by affecting bacterial metabolism.

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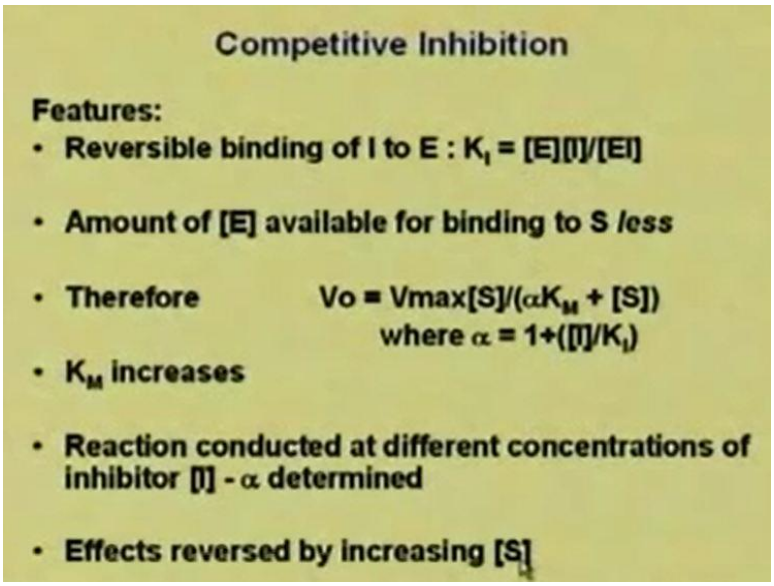

If I increase the substrate concentration to a (()) (55:50) value then the free enzyme can react with the substrate to form the normal products in the normal way it would.

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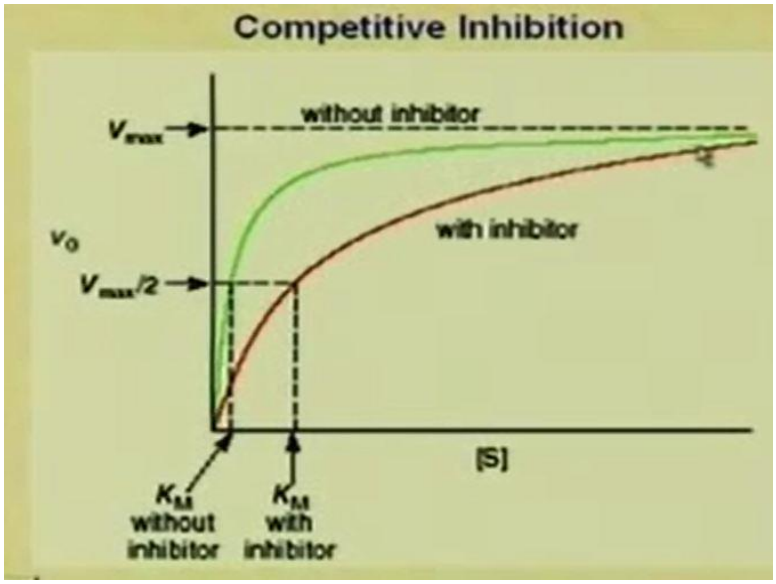

So this is what we have. This is the case the green line is what it is without the inhibitor the normal Michaelis Menten constant that you would see or the Michaelis Menten kinetics that you would see which eventually gets to Vmax. With the inhibitor, we have a slow reaction because some of the enzyme has been taken up by the inhibitor and we know that if we want to calculate

Km I have to consider half of Vmax so this Vmax corresponds or this Km corresponds to what without the inhibitor and this Km corresponds to with the inhibitor now how does this chain change our Lineweaver-Burke plot. What is the Lineweaver-Burke plot? We have one by V0 versus a one by [S]. Let us work on that now.

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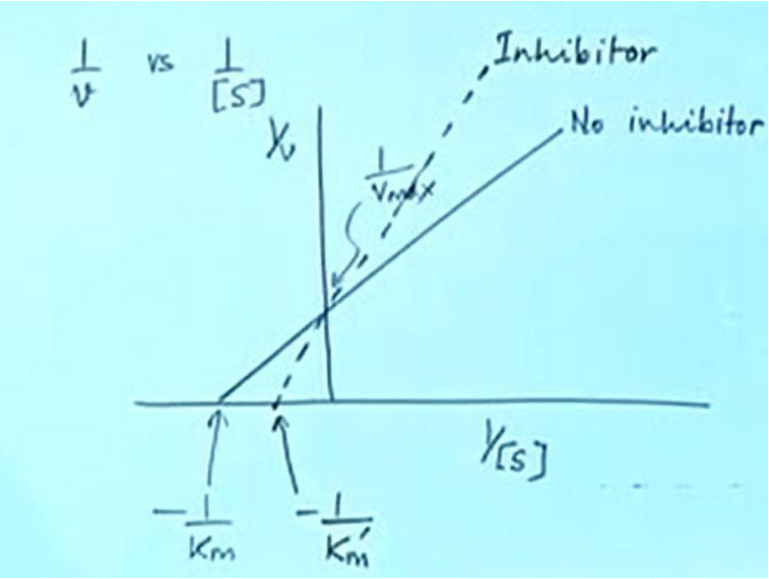

So I have now a one by V versus one by [S] one by V one by [S]. What is this? No inhibitor. What is my Y intercept? One by Vmax. What is this? Minus one by Km. Now tell me? Slope will but the Vmax is the same. The slope increases but the Vmax is the same. So I have inhibitor now I have the same Vmax one minus Km right. So, I have no inhibitor in one case in the next case so what can I now determine? I can determine my Km values for the uninhibited enzymes, the Km prime value for the presences of the inhibitor.