Introduction to Biomolecules
Prof. K. Subramaniam
Department of Biotechnology
Indian Institute of Technology - Madras

Lecture – 9
Enzymes (Part 3/5)

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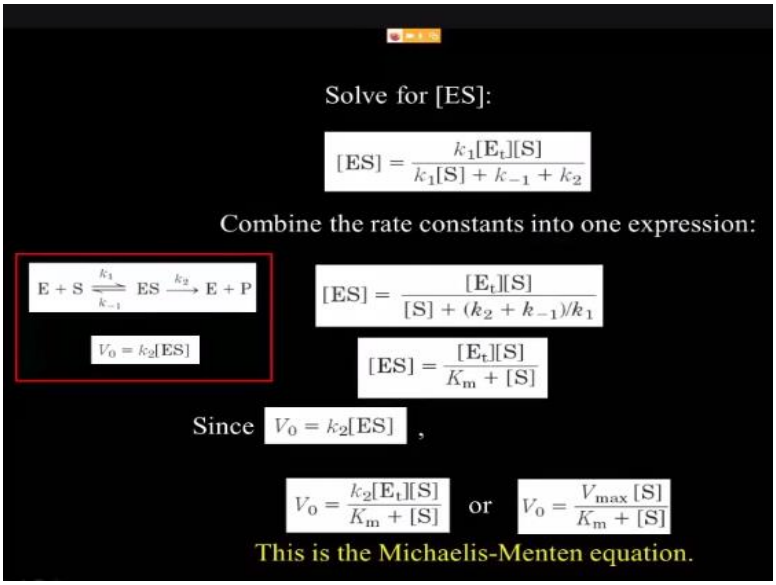

So. let us get started. So yesterday we left at this slide. You know we sort of completed Michaelis-Menten equation. So Michaelis-Menten equation relates the rate of the reaction with these important terms like substrate concentration, the maximum velocity that is possible for a given enzyme at saturating substrate concentration and then the K m okay. So today what we are going to do is look at these two terms V max and K m.

So, the main picture that you need to keep in mind is we are trying to do all of this to get an idea of how good an enzyme is. So how do you define good for an enzyme? How much rate acceleration can it do, compared to the uncatalyzed reaction? So that is one as a catalyst how great it is and second to achieve that sort of a catalytic rate enhancement of what kind of substrate concentration does it need? Can it do even at very low substrate concentration?

So, combining these two is how you measure and define how good an enzyme is okay. So that is what we are getting at once we have understood how to measure the rate of the reaction okay. So, this equation I am trying to understand what K m tells, what V max tells,

etc., is to get an idea of how great an a given enzyme is okay. So, keep that in mind, then our discussions today will make better sense.

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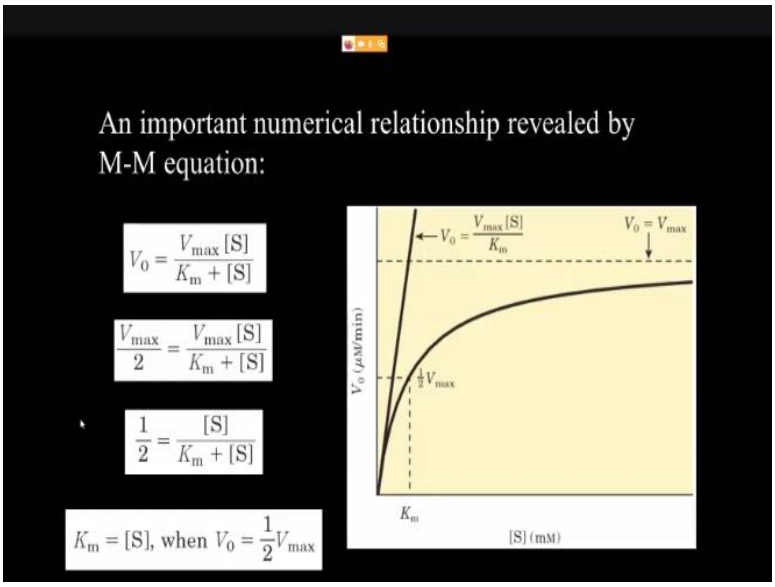

So, first thing is we are trying to see after we saw a simple plot of rate versus the substrate concentration where at the high substrate concentration the rate saturate okay, reaches a maximum, it plateaus off due to enzyme getting saturated with the substrate. So, in this graph we are going to look at how this equation performs in a graph. So, let us look at the early phase. In the early phase your substrate concentration is extremely low.

Like if you take this portion and therefore K m is probably a lot greater than substrate and we can compare to the K m value S maybe ignored and if you are going to ignore S then this equation becomes like this. So, these two are going to be constants. That means velocity is going to vary linearly with respect to substrate. As substrate increases V 0 increases. So, you see linear relationship in this region of this graph okay and this is extrapolation.

But we need not worry about the extrapolation for us in this low substrate concentration it does increase linearly and that is what this equation tells us. Okay now what happens when it goes to the other end? In the other end you are going to have substrate lot higher than K m and therefore K m can be ignored and if you do not have K m then these two S cancels then V 0 = V max and these two being constant it is going to remain the same.

So now velocity is no longer going to be a function of the substrate okay because that does not figure in the equation anymore. So at least in this graph for a simple enzyme this

Michaelis-Menten equation holds well okay. So, having done that now we look at another important relationship that the Michaelis-Menten equation reveals and that is the value of K m itself. So now imagine the velocity is roughly about half of the V max that is possible.

So, if V 0 therefore equals half of V max, then if you substitute that value here as well then you are going to find that so if you cancel these two then half equals S by K m + so that eventually tells you that K m is actually S right, so then only it will be half. So, therefore K m actually is equal into the concentration of the substrate when rate is about half of the maximum rate possible.

When V0 equals half V max, whatever be the substrate concentration for that like for example here at this substrate concentration you reach half the V max and that concentration of substrate equals K m. So, therefore K m has units in terms of concentration okay equals moles. So, this is an important relationship revealed by MM. So, when people ask you what is K m you should be able to tell this, K m equals substrate concentration when V 0 equals half V max okay.

So, this is an important relationship and it also helps us to calculate K m very ready. So now this equation can be transformed in multiple ways to focus on one term or the other or for making accurate calculation of one term or the other in this equation. So, one such transformation is shown in the next slide where we are actually taking double reciprocal of these two terms on both sides and that is what is here.

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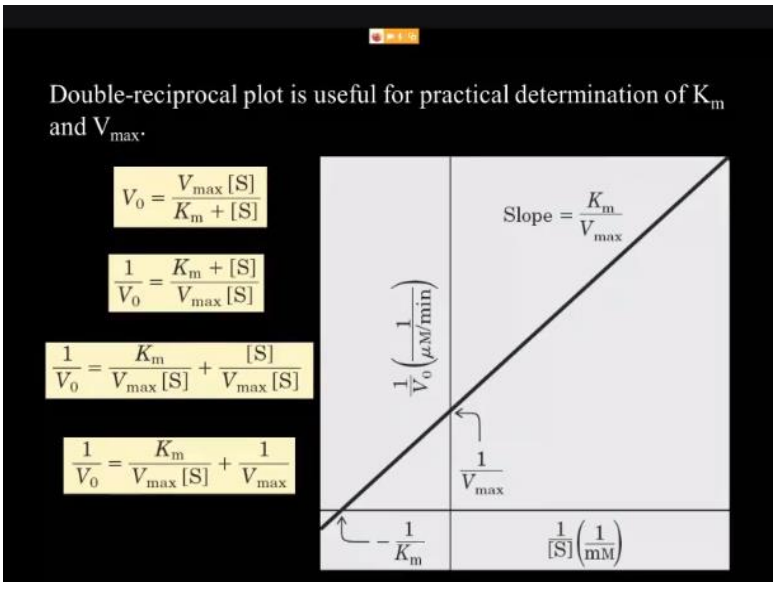

So, you take a reciprocal. So, 1 by V 0, so this will become K m + S divided by V max okay. Now if you split this up it becomes like K m by V max S + S by this. So, this cancel then it becomes 1 by V max. So, this becomes like the equation for a straight line. So, y = mx + c, so this is constant this is c this is m K m by V max will be S, sorry m and this will be 1 by S will be the x. So, y = mx + c that is the equation for a straight line.

Now going by that we know the slope, slope for the straight line will be m and therefore it is K m by V max. The slope of this line is K m by V max. And this the y axis intercept will be c in the equation for a linear line and therefore it is 1 by V max. So, we do not worry about this interceptor and –1 by K m. So, these two are the really main points and this is what helps us to measure the V max as well as K m very readily.

So this double reciprocal plot was proposed by Line Weaver and Burk and this is also called Lineweaver-Burk equation okay, 1 by V 0 = K m by V max S + 1 by V max this is called double reciprocal equation because you are taking a reciprocal on both sides of the Michaelis-Menten equation. And this makes it easier to plot and very accurately because it is a linear line calculating slope is very easy and it gives an accurate measurement of K m and V max.

So this is the equation that practically people use in a lab to evaluate the catalytic efficiency of an enzyme. So now let us look at what the K m and V max can tell about an enzyme.

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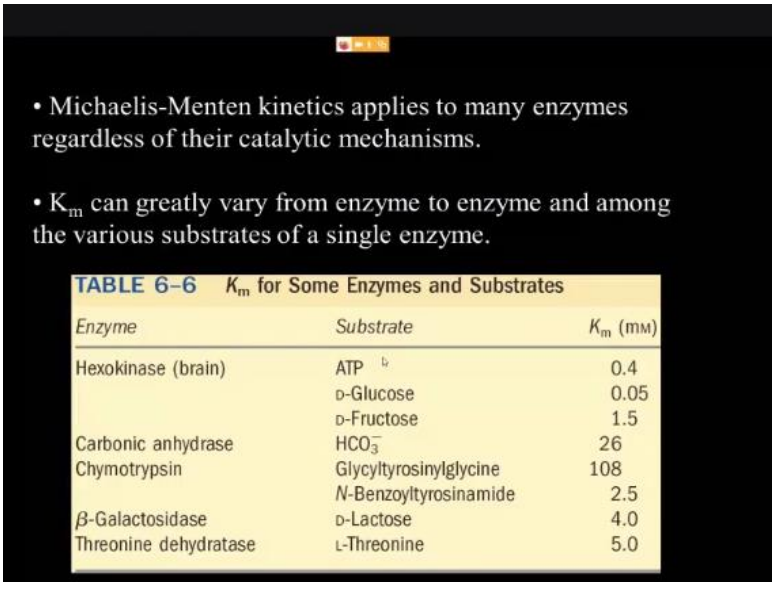

So before going into that here is one small discussion. So Michaelis-Menten kinetics applies to many enzymes regardless of their catalytic mechanisms that actually is the limitation as

well of this steady state kinetics. So, I will tell you in a minute about that. So then K m can vary greatly from enzyme to enzyme and among various substrates for a single enzyme. Suppose a given enzyme has some flexibility in substrate specificity and can actually work on two or three different substrates.

One substrate to another substrate K m can vary. So here are K m for some of the commonly known enzymes with the given substrate. So, this hexokinase is the one that phosphorylates glucose to glucose 6-phosphate that is the starting step in breaking down glucose to make ATP. So here ATP itself is a substrate because it transfers the phosphate group from ATP to glucose to make glucose 6-phosphate.

The version of this enzyme present in brain is hexokinase and the one that works else are called glucokinase as well and that has this sort of concentration for K m value. So, let me see what I have. So, one of the limitations of the steady state kinetics that is the Michaelis-Menten equation is a steady state equation because we make that assumption at the beginning. We are measuring initial velocity.

And then we are measuring at a point where the rate of formation of enzyme substrate complex equals the rate of breakdown of the enzyme substrate complex that is ES. So here regardless of the number of steps involved okay, it does not tell how many intermediate steps are involved. Remember we have considered that enzyme catalyzed reaction can have multiple intermediate steps like for example ES formation is an intermediate step.

Probably in some cases ES becomes EP that is one more intermediate step, then you have the EP dissociating as enzyme plus product. So, there could be multiple intermediate steps. So this does not tell you about how many intermediate steps are there and what is the rate of individual intermediate steps and it does not tell what is the chemical nature of the conversions that are going on. So, it does not tell any of that.

It depends primarily on the rate limiting steps which we will see in a minute, but nevertheless it is still very useful to measure the property of an enzyme, so it is still always used. So all other technologies like side directory mutagenesis, crystallographic structural determination of enzyme, active site, etc., they are always used in conjunction with this Michaelis-Menten

kinetics. So, therefore it really is still being used as a major way of measuring enzyme activity. So, therefore it is still very valuable.

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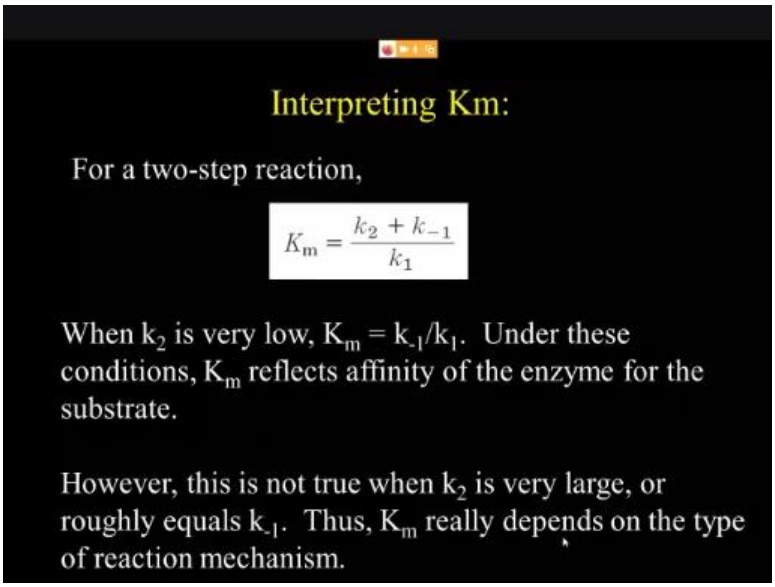

So, let us look at K m under different conditions. So, many times if you particularly look at old textbooks and some really preliminary introductory textbooks what they will say K m equal is a measure of the affinity of the enzyme. So higher the affinity, lower the K m. Meaning at a lower substrate concentration itself the enzyme can readily bind the substrate. Like 50% of the enzyme will be bound with substrate even at low concentration of S that is what would be low K m indicating.

So therefore, K m indicates affinity of the enzyme for the substrate. This is true under certain conditions so that is what this slide is trying to tell you. So, for example where k 2 is very low, let us say you are looking at the very initial reaction where ES formation and breakdown is the main thing, ES has not yet started producing E + P. So, the k 2 will not be a major factor. So, if you take out the k 2 in this equation, then it is simply this is the rate of formation of ES, this is the rate of breakdown of ES.

So, this is E + S in a reversible equilibrium with ES which would simply be like a ligand receptor binding dissociation constant is what K m will be. Under that condition obviously this will indicate the affinity okay. But this relationship will not be true if k 2 is a significant value, if it is significantly large then it will be a function of all three. And suppose if you have an intermediate step like for example you need not even have intermediate step.

You will have K – 2 as well like E + P combining to form E + S, sorry ES enzyme substrate complex. So, like this or the other side of ES. So, the K m then will reflect all of those rates and therefore it will be a complex function. It will not simply be reflecting the affinity of the enzyme that is the major interpretation of the K m. And in many situations this value being large or this being equal to this etc. will depend on a given reaction mechanism.

So, therefore K m will not simply be equal to affinity of the enzyme for the substrate. So, it is true only under certain conditions. But still it is good idea to remember that K m is an indicative of affinity of the enzyme for substrate because in many situations that is true and that assumption helps us to understand many aspects of enzyme kinetics because after we are defining K m as equal to substrate concentration when V = half V max.

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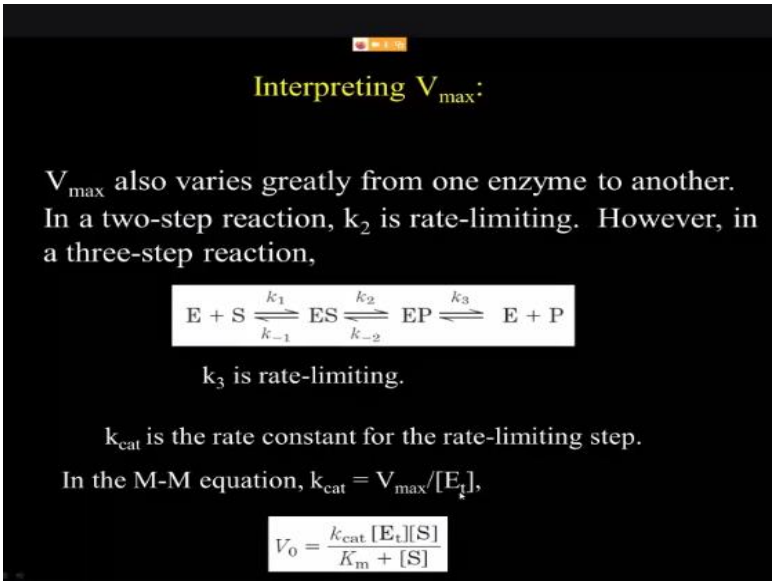

Next let us look at similarly about V max as well. What V max tries to tell us? So yesterday we saw this k 1 that is the forward reaction of E combining with substrate is faster and readily happens and this step is the rate limiting step that is ES getting converted into E + P. Now under such situations k 2 will be rate limiting step okay and therefore V max will actually depend on k 2. So V0 actually reflects this reaction step and not all of them.

But on the other hand, let us assume you have one more intermediate step like on the enzyme substrate actually gets converted into product and you have an enzyme product complex and that is going to dissociate into E + P and that dissociation is slower than all these 4 reactions then this k 3 will be rate limiting and therefore V max will be affected by k 3. So what is

determining V max therefore will vary which step is rate limiting and where this leads us to a term called the k cat.

So, in this particular experiment k 3 is k cat and if you have more k 4, k 5 and so on, then whichever one is rate limiting that rate limiting steps rate constant would be the cat okay, k cat. So, k cat is the rate constant for the rate limiting step, in this particular case k 3, in the earlier examples we considered it is k 2 which is the rate limiting. So, the rate limiting steps rate constant is what is k cat and this is an important one.

So, you know the higher the value of this then the higher the overall rate of the reaction. So, in Michaelis-Menten equation if we go back, so k cat will be equal to V max by E t that is simply because let me think, so in Michaelis-Menten equation so you have a k 2 and that k 2 is V max by E t and that if you substitute then you get this situation. So V 0 equals, so now k cat equals going by this relationship it will be k cat time E t will be V max and in Michaelis-Menten equation you substitute that.

So, in any case V max is always going to be equal to this because it is not going to increase beyond the total enzyme available and the rate constant for that this would always be the V max in any situation. And when you have this substituted in the Michaelis-Menten equation, then if you look at different situations suppose when you are looking initially where S is really low then it will be V 0 = k cat by K m. So, this ratio times E t S.

So now the substrate concentration will be directly related to this linearly related to V 0. And this term k cat indicates the number of substrate molecules that is converted into product on a single enzyme molecule when the substrate is on a saturating concentration okay. So that is another definition.

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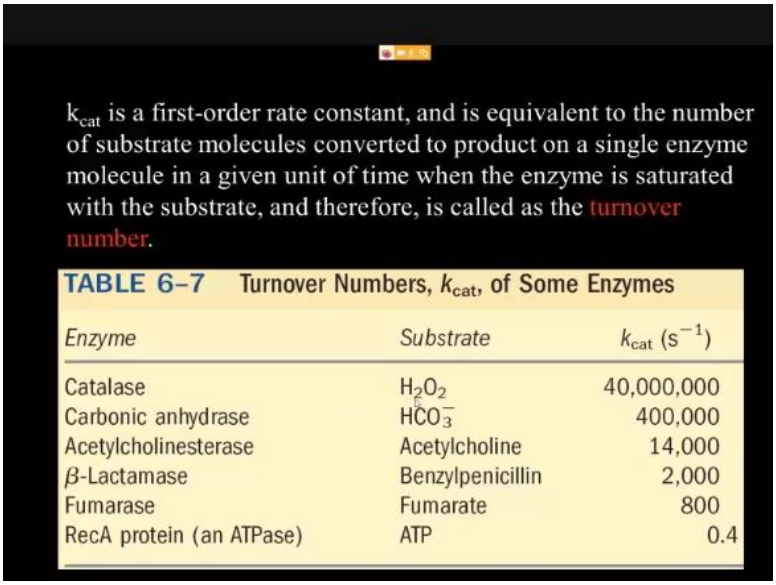

So, it is a first order rate constant because it is not dependent on substrate concentration. It is simply V max by E t and as a result it is a first order rate constant not depending on the concentration of the substrate. And this is what you need to really remember. It is equal to the number of substrate molecules converted to product on a single enzyme per unit time when the enzyme is saturated with the substrate this is an essential condition under which this relationship holds good.

So, this is also called the turnover number which is because how many substrates are turned over per unit time. The only additional thing you need to remember is where this is true when substrate is at saturating concentration. So, this helps us to measure essentially the rate at which substrate is being converted and that if you look at for some of the enzymes and it is extraordinarily high like for catalyst that breaks down hydrogen peroxide into water and oxygen.

So, this one acts at a rate of 40 million hydrogen peroxide molecules per enzyme molecule okay that is what you need to remember. One enzyme molecule converts 40 million H 2 O 2 into products per second okay. So that is one of the highest turnover number known okay. This catalase and along with an enzyme called superoxide dismutase both of them are important in scavenging free radicals generated in many reactions in our cell.

Otherwise, the breakdown of H 2 O 2 generating O 2– the superoxide radical reacts with many molecules and causes damage particularly to membranes bio membranes and this is a

major issue in the brain and to take care of it a subsequent enzyme superoxide dismutase converts that into water molecules. So essentially H 2 O 2 becomes water molecules.