Medicinal Chemistry
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Enzyme Kinetics and Inhibition

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Today we will be looking at the various aspects of enzyme kinetics.

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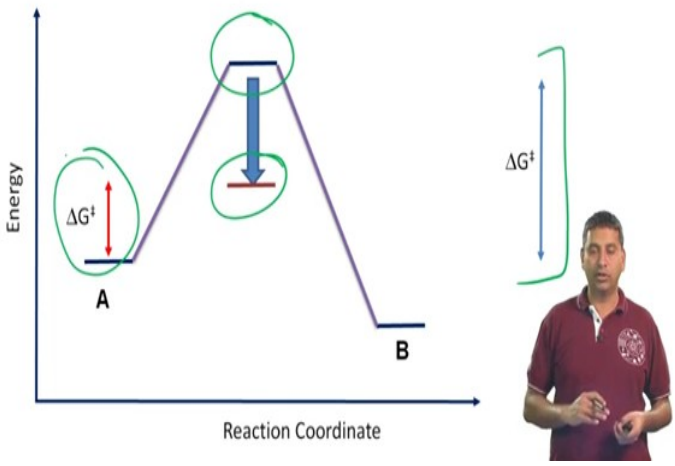

So just to give you a recap we have seen that the way in which the enzyme functions is by if you consider this as the uncatalyzed reaction the delta G double dagger it is possible that it stabilizes the transition state and makes goes through perhaps series of small intermediates

and transition states where the net effect is that it lowers the barrier and the barrier as we know is a function of how fast the reaction proceeds. Now so this delta G double dagger that is now the new delta G double dagger is going to be significantly lower than the uncatalyzed reaction.

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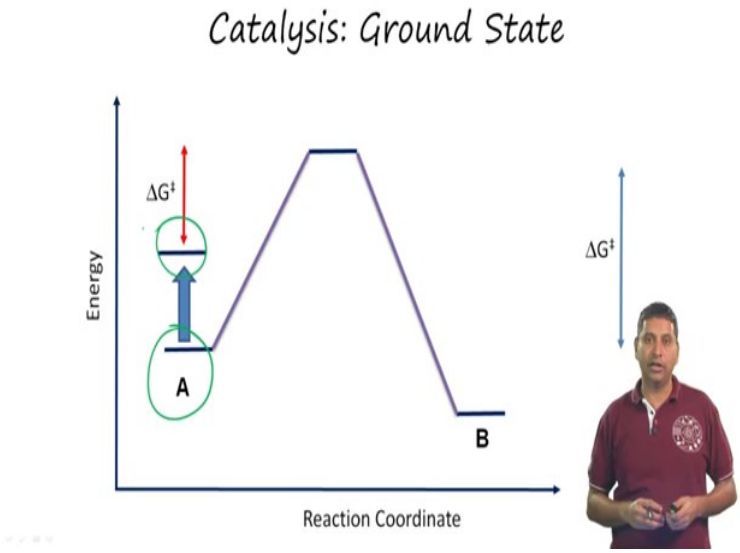

Alternately, we also have found or we have also seen that the ground state energy of the substrate can go up due to binding and so this also results in some sort of a lowering of the barrier so if you imagine that both of these processes are happening then you can think about synergy that occurs and a substantial increase of the rate of the reaction.

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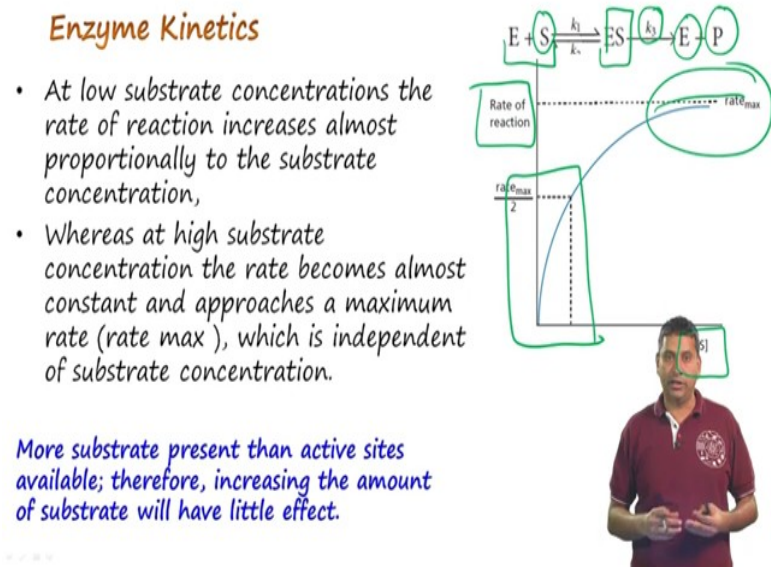

Now let us look at some aspects of enzyme kinetics, so in order to first understand how enzyme kinetics, I mean how this process happens let us propose a reaction of substrate going to a product. We can propose an intermediate where the enzyme binds to the substrate and forms a substrate enzyme complex which is referred to as ES and then the enzyme substrate complex degrades to give you back the enzyme and the product.

So we look at the mathematics of this later but you can think that at low concentrations of the substrate the rate of the reaction almost increases proportionally to the substrate concentration. Whereas at high substrate concentration it goes up and then it plateaus, so the X axis and Y axis of this plot are rate of the reaction is in the Y axis and the substrate concentration in the X axis.

So at high substrate concentrations the rate almost becomes constant and approaches a maximum, one can understand this by thinking about the relationship between the substrate concentration and the enzyme. When more substrate is present than active sites available therefore increasing the amount of the substrate will have no effect.

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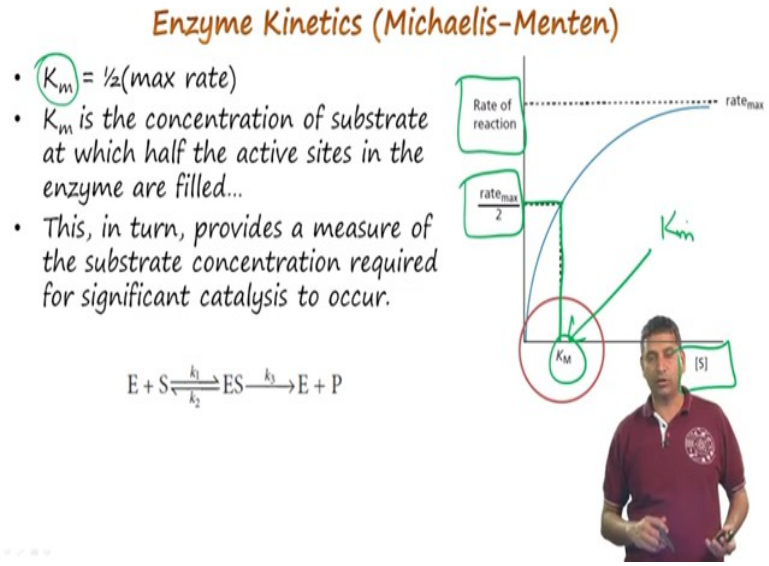

So one of the ways in which we understand enzyme kinetics is to do what is known as the Michaelis-Menten treatment. So we define a term K_M which is half of the maximum rate, it is called the Michaelis constant, K_M is the concentration of the substrate at which half the active sites in the enzyme are filled. So in this plot of rate of reaction versus substrate concentration which we have just looked at, the K_M is defined as the place as the point where half of the rate maximum is obtained.

So the way we obtained this is to draw a line from here to here and then go down and this is the concentration at which you get K_M, this in turn, provides a measure of the substrate concentration required for significant catalysis to occur.

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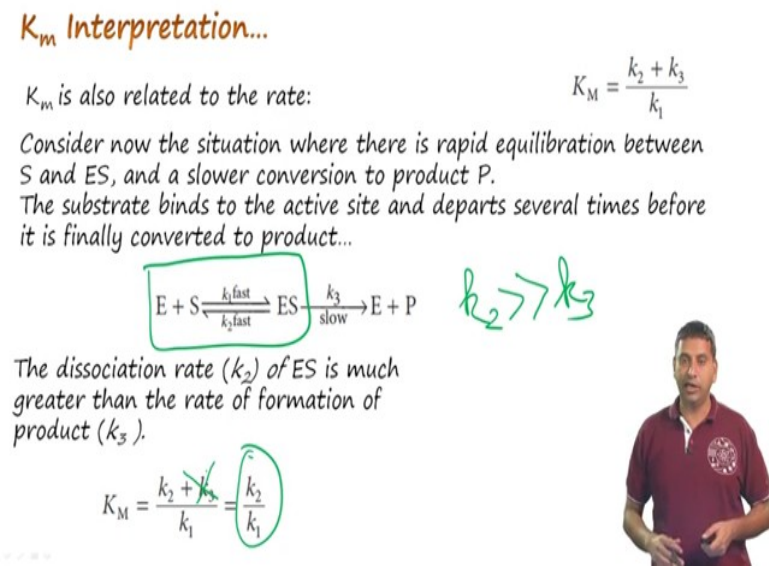

Let us now look at how we can interpret K_M. K_M is also related to the rate so K_M is basically k_2 plus k_3 divided by k_1. So consider now the situation where there is rapid equilibrium between the substrate and enzyme substrate so that means that this process is extremely fast. So the substrate binds to the active site and then departs, binds to the active site departs, does this again and again and again and then in one of these situations it then goes and forms the product.

So therefore the formation of the product is significantly slower than the rapid equilibrium that happens between the enzyme and the substrate. So the dissociation rate k_2 of the enzyme substrate is much much greater than the rate of formation of the product which is k_3. So you can approximate this equation to k_2 divided by k_1, so how do we get this? So k_2 is much much greater than k_3, so in that case you can remove this term because it does not add much value to the numerator and you end up getting k_2 divided by k_1.

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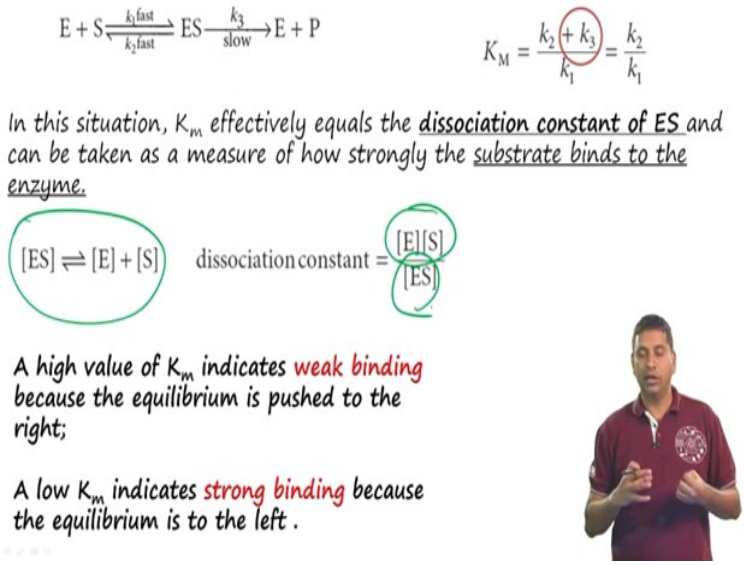

So in this situation the Michaelis-Menten constant K_M effectively equals the dissociation constant of the enzyme substrate complex and it can be taken as a measure of how strongly the substrate binds to the enzyme. So for the equation that we are looking at here ES going to give you this is the equilibrium that we are looking at, the dissociation constant is nothing but the concentration of the enzyme multiplied by the concentration of the substrate divided by the concentration of the enzyme substrate complex. So a high value of K_M indicates weak binding because the equilibrium is pushed towards the right.

Whereas a low value of K_M indicates strong binding because the equilibrium is towards the left. So therefore the K_M is a very good measure of how well the substrate binds to the enzyme.

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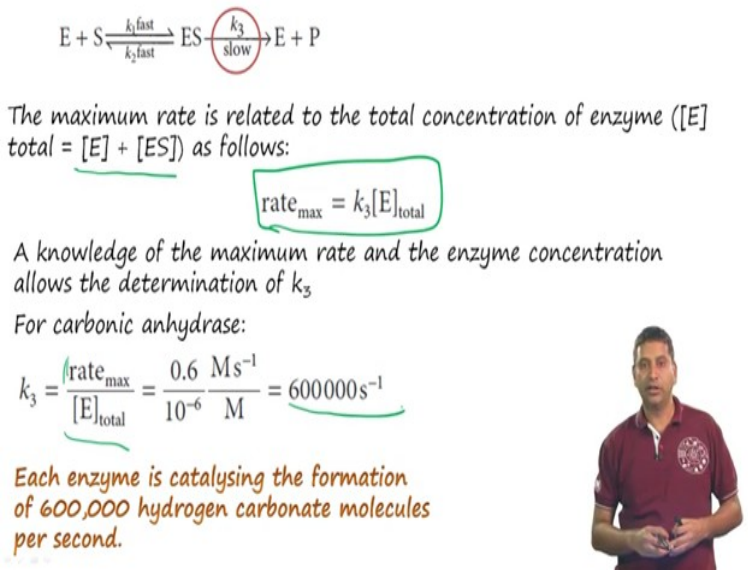

So the maximum rate is related to the total concentration of the enzyme which is basically nothing but the total concentration of the enzyme plus the enzyme substrate complex. So the rate max that we can attain is basically k_3 times the concentration of the enzyme in total. So the knowledge of the maximum rate and the enzyme concentration allows us to determine k_3, so k_3 is the rate max divided by enzyme total.

So what we would get here is if you look at the rate max for the turnover of a particular enzyme, you get a value of 0.6 molar per second which is in the numerator and the concentration of the enzyme that we have started with is 10 powers minus 6 molar which is 1 micro molar. So if I divide these two numbers then I get a value of 600000 per second, okay. So this is quite a large number of turnover. So the example that looking at here is carbonic anhydrase which basically converts carbon di oxide to hydrogen carbonate.

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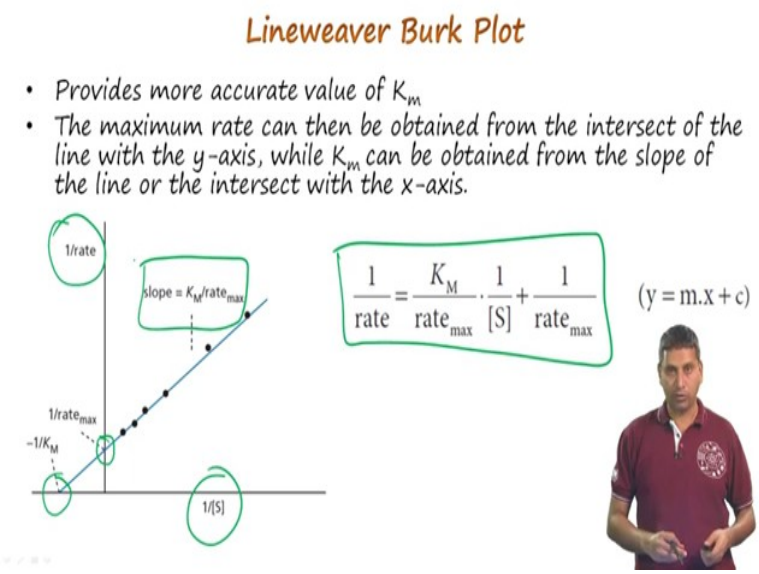

A very important and convenient way of looking at enzyme kinetics is to use the Lineweaver Burk Plot. So this plot actually provides more accurate value of the Michaelis constant K_M. The maximum rate can then be obtained from the intercept of this line. So now let us look at what this equation is, so we take 1 by rate equals this term over here. So now if we plot 1 by rate versus 1 by substrate concentration, this is called the Lineweaver Burk Plot.

So you can think about this equation here that I have shown up as y equals mx plus c and you will end up with a term with a straight line and there are number of important parts of this straight line that we need to understand. I am not going to go through the derivation of this now, but this is something that you could do in your own free time. So here we will look at certain parts of this plot and try to figure out what is going on.

So the intercept over here is minus 1 by K_M and the point here where the line meets the y axis is minus 1 by rate max and the slope is nothing but the Michaelis constant K_M divided by rate max. So a number of important parameters about the enzyme can be obtained by this Lineweaver Burk Plot.

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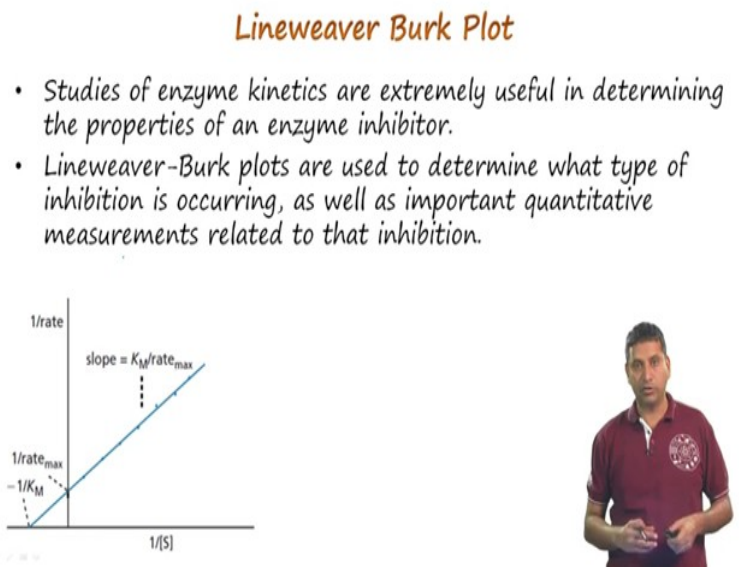

So studies of enzyme kinetics are extremely useful in determining the properties of an enzyme inhibitor. So let us say you want to set up a large scale screen (for determining) for identifying a new inhibitor for an enzyme, we need to first be able to understand the kinetics of the enzyme and then and only then we will be able to find out the inhibitor and which inhibitor is more efficient or which inhibitor is less efficient. So Lineweaver Burk Plots are used to determine what type of inhibition is occurring as well as other important quantitative measurements that are related to that inhibition.

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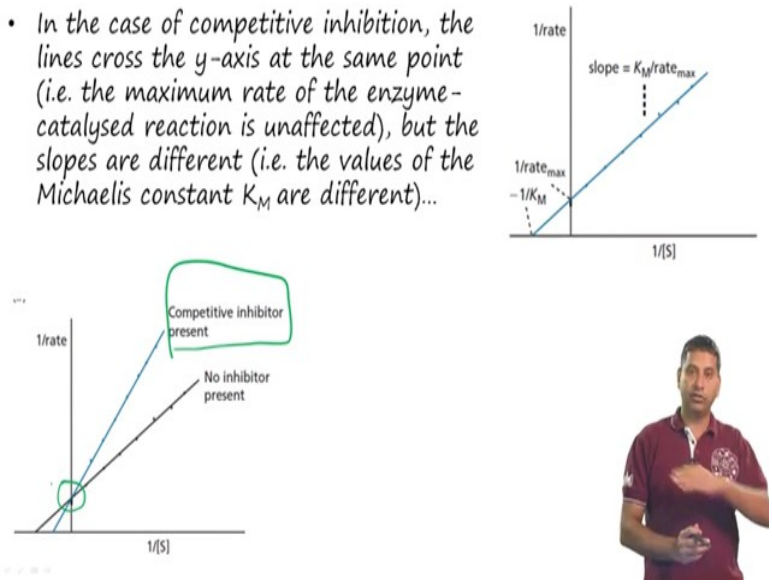

So in the case of a competitive inhibition which we have looked at previously, the line crosses the Y axis at the same point that is the maximum rate of the enzyme is unaffected, but

the slopes are different. So if you were to look at how a competitive inhibitor functions you will find that this point here is going to remain the same, whereas the slope has changed.

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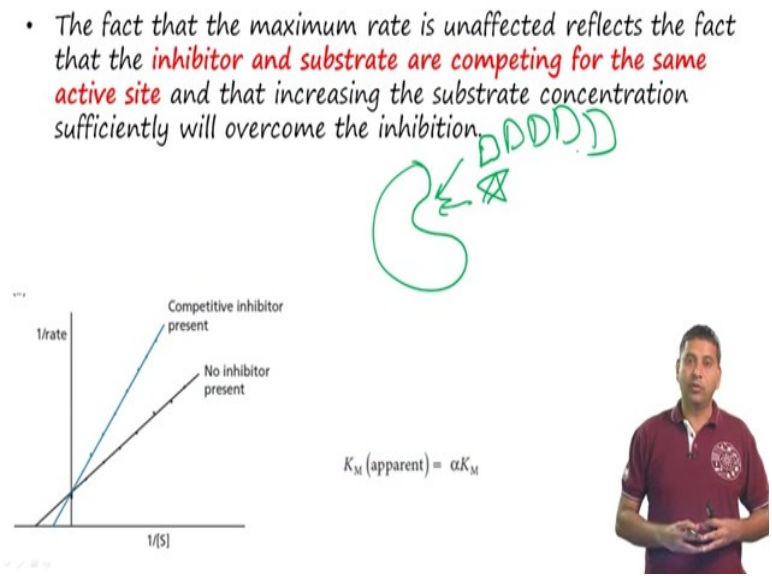

So the fact that the maximum rate remains unaffected reflects the fact that the inhibitor and the substrate are competing for the same active site. So remember when we are looking at an enzyme and a competitive inhibitor, so here let us say this is the natural substrate of the molecule and this is our inhibitor they both go and bind to the same site. So when you have an inhibitor it is going to go and compete for the same site for binding. So therefore increasing the substrate concentration, so if I have more of this substrate what it does is that it will overcome the inhibition and therefore the rate max does not change.

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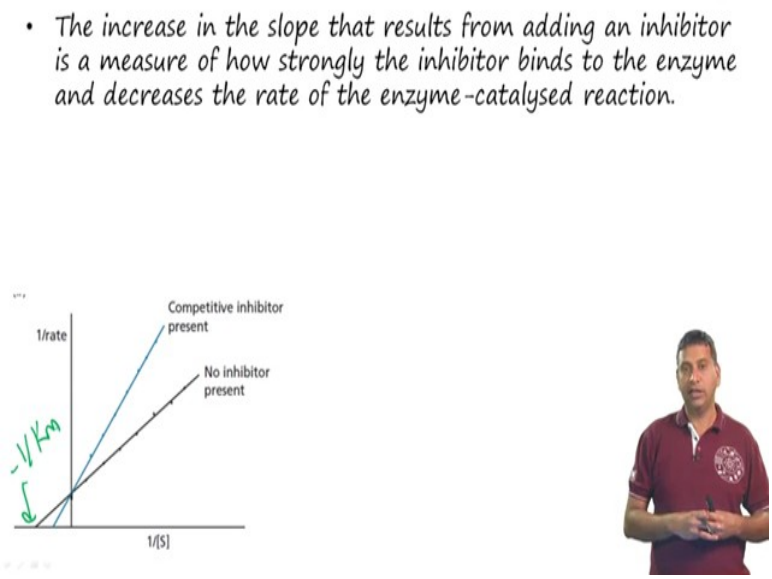

Now the increase in the slope that results from adding an inhibitor is a measure of how strongly the inhibitor binds to the enzyme and decreases the rate of the enzyme catalysed reaction. So we have already looked at that this value is nothing but minus 1 by K_M and the interpretation that we made about K_M is how tightly the enzyme binds to the substrate and so in the presence of a competitive inhibitor, the value of K_M is going to change and this means that the inhibitor is going to bind strongly or weakly, we can find that out by this plot.

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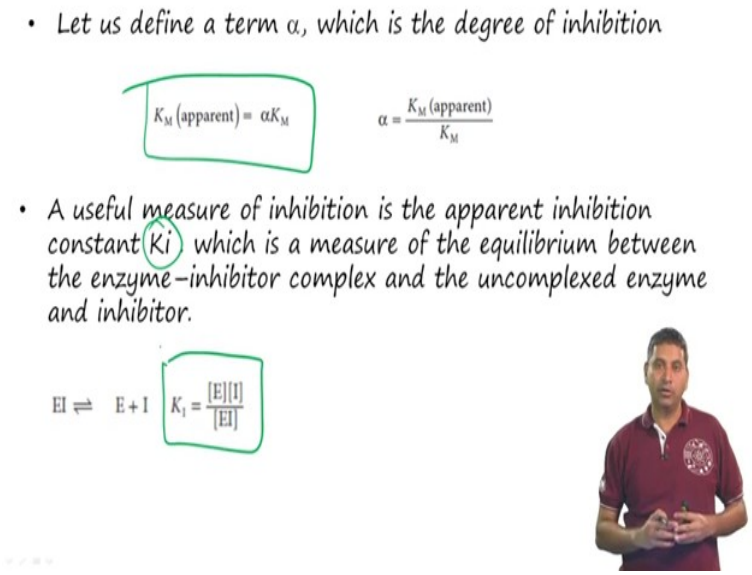

Now let us define a term alpha, which is the degree of inhibition. So in the presence of the inhibitor you have a K_M which is K apparent which is nothing but alpha times K_M. Now a useful measure of the inhibition is the apparent inhibition constant K_i. So K_i which is an equilibrium constant is a measure of how well the enzyme inhibitor dissociates and becomes associated again. So this K_i is defined as the concentration of the enzyme multiplied by the concentration of the inhibitor divided by the concentration of the enzyme inhibitor complex.

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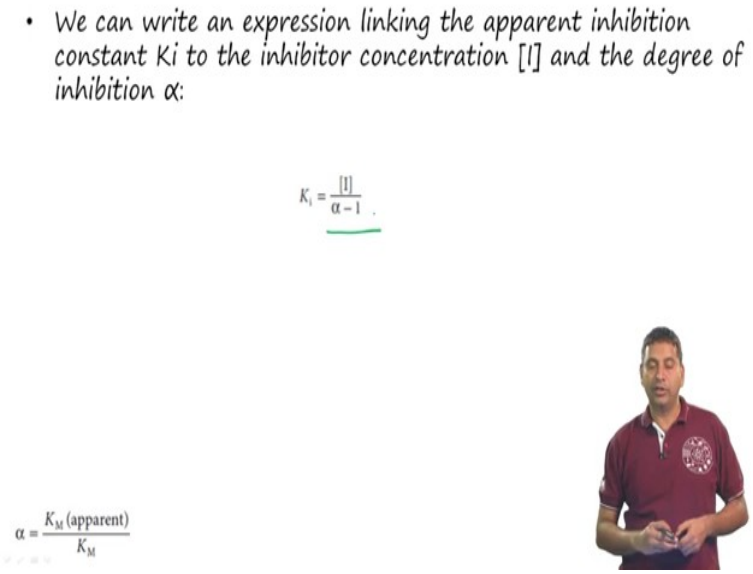

So we can write an expression K_i is equal to concentration of [I] which is the inhibitor divided by alpha minus 1 and alpha as we recall is the degree of inhibition.

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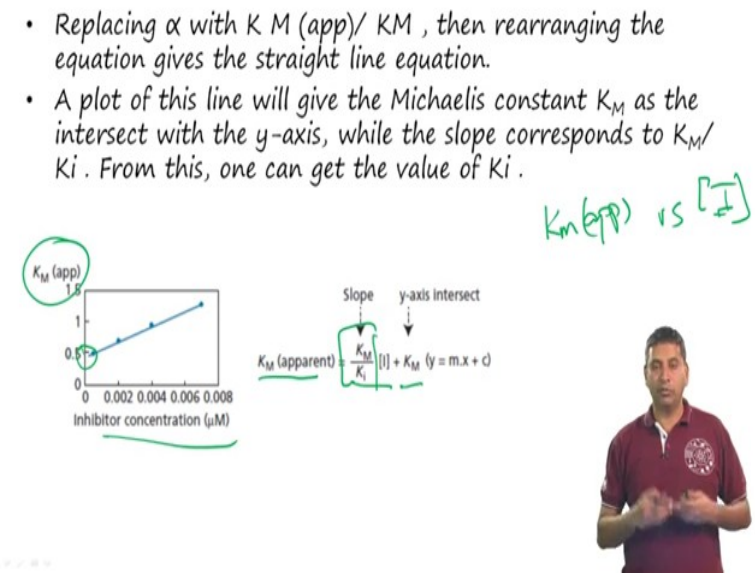

Now replacing alpha with K_M apparent by K_M and then rearranging the equation gives us a straight line equation, the straight line equation is nothing but K_M apparent equals this term K_M by K_i times concentration of [I] plus K_M. So if we were to plot K_M apparent versus the concentration of the inhibitor then we could get some important values from this plot. So what we would expect is we would get if the Y axis here is K_M and inhibitor concentration is the X axis, now we would expect to see a straight line with a slope and the slope is nothing but K_M divided by K_i and the Y axis intercept should be K_M when the inhibitor concentration

is 0 we would expect no inhibition and that should give us the Michaelis-Menten constant of the enzyme with a natural substrate.

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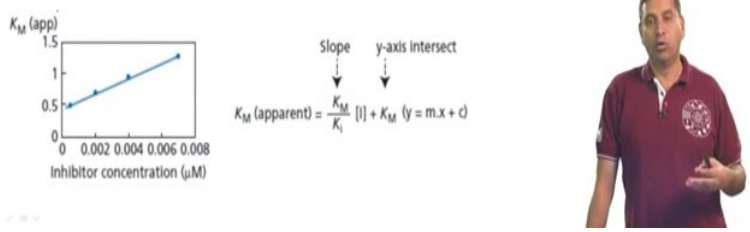
- To create this plot, a series of Lineweaver-Burk plots is first created in order to get values of K_M (apparent) at different inhibitor concentrations.
- The plot of K_M (apparent) versus [I] is then drawn, allowing Ki to be calculated from the slope of the line.
- The lower the value of K_i, the more potent the inhibitor.



To create this plot what we need would be a series of Lineweaver Burk Plots and then we would get the values of K_M apparent at different inhibitor concentrations, then the plot of K_M apparent versus [I] gives us a K_i to be calculated from the slope of the line. Now K_i is a measure of how good the inhibitor is the lower the value of K_i, the more potent the inhibitor.

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Uncompetitive Inhibition

- The inhibitor binds to the enzyme–substrate complex rather than the free enzyme. Enzyme inhibition studies result in Lineweaver-Burk plots where the lines are parallel and cross the y-axis at different points, indicating that the maximum rate for the enzyme has been reduced

We have already looked at the other cases of inhibition other than competitive inhibition which is uncompetitive inhibition. Uncompetitive inhibition happens when the inhibitor binds

to the enzyme substrate complex rather than the free enzyme. So enzyme inhibition studies which result in Lineweaver Burk Plots where the lines are parallel and cross the Y axis at different points. So you have this is parallel to the first line that we have drawn in the absence of the inhibitor then you can infer that it is an uncompetitive inhibition, what actually happens here is that because the inhibitor is going to bind to the enzyme substrate complex, the maximum rate is going to reduce.

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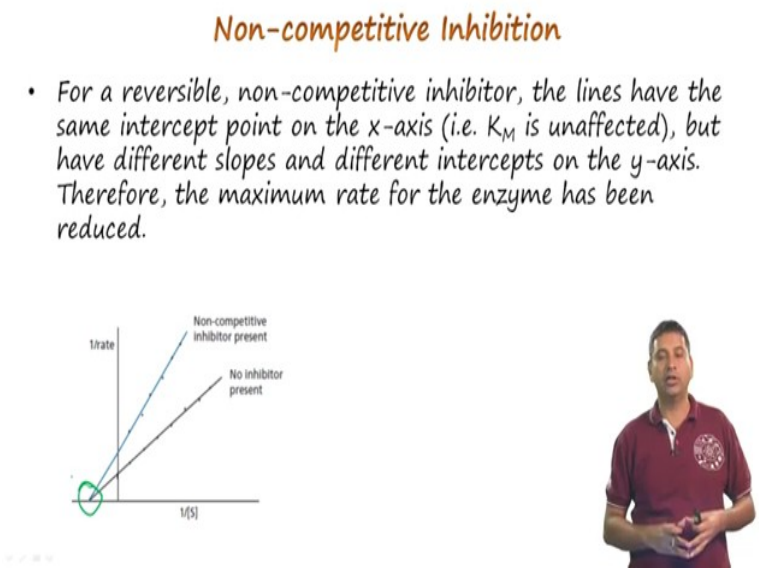

Now let us look at the last case which is a non-competitive inhibition, so for a reversible, non-competitive inhibitor, the lines have the same intercept on the x axis that is the Michaelis-Menten constant remains unaffected, but they have a different slope at different intercept, the intercept is different because the rate is going to change the enzyme is going to be inhibited in a non-competitive manner.

So therefore this Lineweaver Burk Plot if it shows situation like this where you have the same K_M but you have slopes which are different than one could infer that non-competitive inhibition is actually happening.

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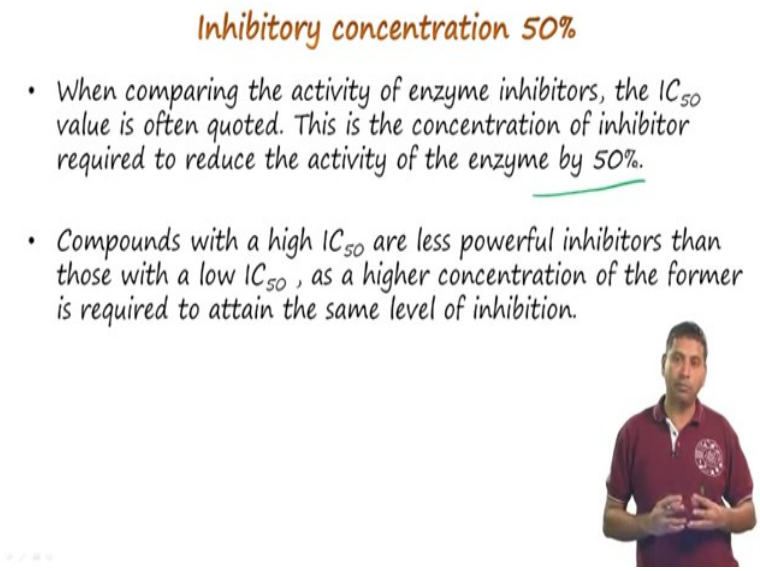

Now let us look at another term which is very useful which is IC_{50} or inhibitory concentration 50%, so when we want to compare across various enzyme inhibitors the value of IC_{50} is very often quoted. This is nothing but the concentration of the inhibitor required to reduce the activity of the enzyme by 50%. So compound with high IC_{50} are less powerful inhibitors and those with the low IC_{50} are better inhibitors.