Organic Chemistry In Biology And Drug Development
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Lecture - 18
Problems on Enzyme Kinetics and Enzyme Inhibition

Welcome back to this course on Organic Chemistry in Biology and Drug Design. Last time, we have discussed the aspects of enzyme inhibition and we have seen that there are different classes of enzyme inhibitors. But broadly speaking they can be classified into reversible and irreversible inhibitors. And then in the reversible side, we have different kinds of inhibitors depending on which active site the inhibitor is binding.

So, accordingly there are competitive inhibition, non-competitive inhibition, uncompetitive inhibition and mixed inhibition. So, before we go on to the next topic, better to clarify some of the doubts. I thought that it is better that we do some problems, so that the concepts become clearer.

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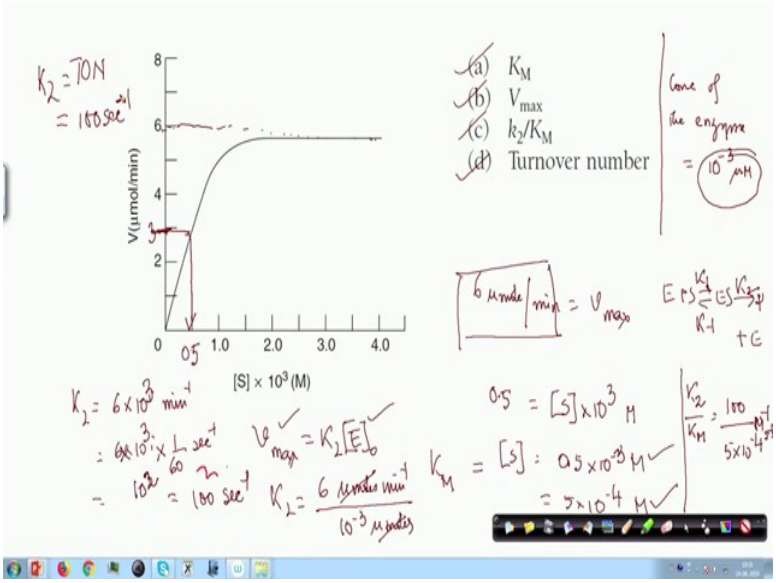

So, here we start with the first problem. There is an enzyme kinetics graph that is given here. And this graph is obtained by plotting the V along the Y axis; actually this is the V_0, the initial velocity, it has a unit that is μM/min. And then on the X axis, you have substrate

concentration, but that was multiplied by 10^3 in order to express it into the in molarity units (M).

So, this is your graph and now it is asked to calculate from the graph the value of K_m? K_m is the Michaelis Menten constant, V_{max} is the maximum velocity that is possible for this enzyme catalyzed reaction. I told you last time k_2 is the rate constant for the breakage of ES complex into the enzyme and the substrate. And K_m gives the dissociation constant of the enzyme substrate complex into the starting substrate and that free enzyme.

However this k_2/K_m is an important parameter, I told you that this actually denotes the catalytic efficiency of the enzyme. And finally it has been asked to calculate the turnover number. So let us go step by step. Now we know that the firstly it is asked to calculate is K_m. Now K_m can be defined in two ways. One, as I told you, it is a measure of the dissociation constant of the enzyme substrate complex into E plus substrate. It is also the concentration needed to reach half of V_{max}.

Now, here I give one more data that is missing here; the initial concentration of the enzyme in this experiment is suppose 10^{-3} (μM); that is needed to calculate some of these parameters. Now we start with V_{max} because, once we have V_{max} then we can draw the lines to calculate the concentration needed to reach half of the V_{max} . So, let us draw a line here. According to the graph, V_{max} is approximately, a little less than 6, but let us draw a line which will ultimately touch the maximum velocity line.

So, we can say that 6 μM/min is your maximum velocity; that is equal to V_{max} according to the graph. Because that is the saturation level. What is saturation level? That means, beyond that level, you cannot increase the rate of the enzyme reaction, unless you add fresh amount of enzyme.

So, in this, the enzyme concentration is specified at 10^{-3}, so the value of V_{max} is 6. Now, how to calculate the value of K_m? K_m is basically the substrate concentration needed to reach half of V_{max}. So, what is half of V_{max}? Half of V_{max} is 3, this is the line denoting the half of V_{max}.

So, you just draw this line and then try to see, what is the value of the X axis? The value of the X axis, as I see is 0.5 now. Remember one thing, this axis is represented by substrate concentration into 10^3. So, this 0.5 is equal to the actual substrate concentration multiplied to 10^3 (M).

So, S is now 0.5 x 10^{-3}(M). You can write it as 5 x 10^{-4} (M). So, this is basically the concentration that is needed to reach half of the V_{max}. So, this value should be equal to K_m. Now, we know what is K_m and the V_{max}. The third parameter that you are to calculate is k_2/ K_m. We already have the K_m value, but we do not have the k_2 value yet.

Remember k_2 is the rate constant corresponding to E plus S going to ES. And then ES going to the product P plus E. So, we need the value of k_2. So, k_2 is related to V_{max} by this equation, that if you multiply k_2 by the enzyme concentration, you get V_{max}. You have V_{max} and you have E_0 (that is mentioned 10^{-3}). Hence k_2 will be equal to V_{max} (6 μM/min), divided by your enzyme concentration (10^{-3} μM). Better write these units, so that you know that you are using the same units for the concentration.

So, here this is micro moles so that crosses out. So, ultimately k_2 becomes equal to 6 x10^3 x 1/60, so that will come out to be 100 sec^{-1}.

Then k_2/K_m is equal to 100sec^{-1} divided by 5x10^{-4}M; overall unit will be $M^{-1}sec^{-1}$. So, you can calculate this and then find out what is the ultimate value. And the last one is the turnover number.

Now, what is turnover number? Turnover number was basically the value of k_2. k_2 has different names; k_2 is sometimes called k_{cat} (catalytic constant), but it is also known as the turnover number. Now turnover number is the number of substrate molecules, which are turned over into the products by each single enzyme molecule per unit time. It could be per second or per minute.

So, whatever k_2 you have here, that is equal to the turnover number (TON). So that will be 100 sec^{-1}. So, what does it mean? That means, in 1 second, 100 molecules of the substrate are converted into the product by a single molecule of the enzyme.

However, there is one assumption here, that the enzyme has only one active site for the substrate. Again remember what I said, the turnover number is the number of substrate molecules turned over into the products per unit of time, in this case second, so per second 100 molecules are converted into the product, by 1 molecule of the enzyme. But there is one assumption that the enzyme should have only one active site. Because, some enzymes may be

in dimeric form and they can have two active sites, so that is also possible. Those cases are excluded; it is only the general case where we consider that each enzyme has one active site for the substrate to bind and then convert it into the product.

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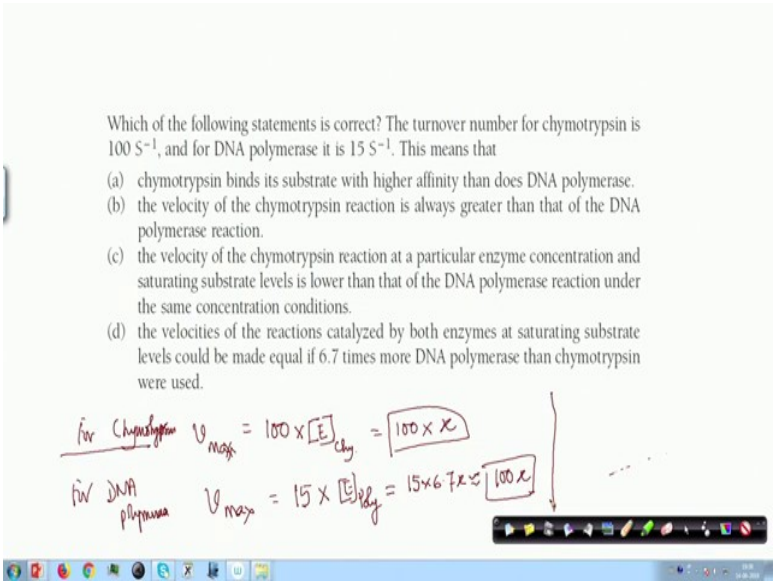

We go to a second problem. The second problem is that which of the following statements is correct? There are several statements given where the turnover number (TON) for an enzyme chymotrypsin are given. We are now quite conversant with chymotrypsin; chymotrypsin hydrolyzes peptide bonds, specific to aromatic amino acids. And like the earlier problem, the turnover number came to be 100 sec^{-1}. And for another enzyme which is called DNA polymerase, the turnover number is 15 sec^{-1}. So the turnover number here is more. We have not yet read the nucleic acids, but DNA polymerase is the enzyme which actually synthesizes the polymeric DNA molecules. . Now what does it mean when something has a higher turnover number? We are comparing with an enzyme which is having lower turnover number. Now you have to find out the correct statement. Chymotrypsin binds its substrate, with higher affinity then does the DNA polymerase. We have to decide whether it is correct or not.Now, turnover number is basically the k_2; that means how quickly the ES is converted into the product; it does not have any connection with your higher affinity of binding. . Higher affinity does not have any connection with k_2 because, affinity means the stability of the ES complex. Affinity is directly related to the K_m value. If the K_m value is low; that

means, the dissociation is low, the association will be more, in that case, the binding affinity is more for the substrate.

So, turnover number has nothing to do with the binding affinity for the substrate. The given statement says that chymotrypsin binds its substrate with higher affinity than does the DNA polymerase. In the equation, you have seen V_{max} equal to k_2 multiplied to your enzyme concentration; there is no mention of any K_m in that equation. So, first statement is incorrect; you cannot say that. The second one says that the velocity of the chymotrypsin reaction is always greater than that of DNA polymerase reaction. We have to decide whether this is true or not.

If we use the same enzyme concentration for chymotrypsin as well as for DNA polymerase, then this statement becomes true. But nothing is written about that. It says that it is always greater than that of DNA polymerase; that may not be true. If you start with more DNA polymerase (more enzyme to start with), then what will happen? Then the DNA polymerase can have higher velocity because you have higher concentration of the enzyme. So the second statement is also not true.

Let us consider the third statement. The velocity of chymotrypsin reaction at a particular enzyme concentration and saturating substrate levels is lower than that of DNA polymerase reaction under the same concentration conditions. Saturation means the V_{max} value when you have lot of substrate; that means, all the active sites are blocked there. This third statement is not correct. Actually you are now using the same enzyme concentration. Since chymotrypsin has a higher turnover number, so that cannot be lower than that of DNA polymerase.

So; obviously, the fourth one has to be correct because all these are incorrect. The fourth statement says that the velocities of reactions catalyzed by both the enzymes, at saturating substrate levels could be made equal if 6.7 times more DNA polymerase than chymotrypsin were used. It is known that at saturating substrate levels, the velocity of the reaction is equal to the V_{max}; saturating means when you have saturated the active sites.

So, V_{max} for chymotrypsin will be the turnover number of chymotrypsin (which is 100) multiplied to the concentration of chymotrypsin; Suppose x is the strength in molarity of the enzyme. So, for DNA polymerase what will happen? You are using enzyme concentration (DNA polymerase) which is 6.7 times concentrated than the chymotrypsin. It has got a turnover number of 15 this means k_2=15; and then the enzyme concentration of polymerase is

6.7 x (where x is the concentration of chymotrypsin). And if you calculate, V_{max} for DNA polymerase will be approximately equal to 100 x.

So, turnover number for both the enzymes is 100 x; that means, the velocities of the reactions at saturating substrate levels could be made equal provided you use 6.7 times more enzyme concentration of DNA polymerase as compared to the chymotrypsin. So, you cannot just say that one enzyme having a higher turnover number will always be faster as compared to others. Because, what you need apart from turnover number is the enzyme concentration that you are using for the reactions to carry out.

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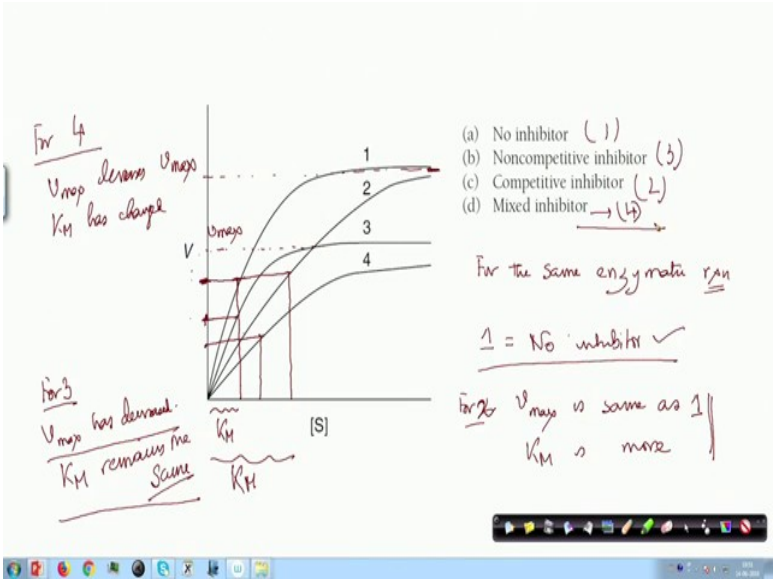

So, next we go to the third problem. Third problem is basically an inhibition problem; earlier it was just Michaelis Menten equation that we used to determine the various parameters. And the second problem was quite interesting as it clears some doubts because lots of people have doubts that which factor really says that an enzyme efficiency is very high. I told you that enzyme efficiency is inversely proportional to K_m. Because higher the K_m more will be the dissociation of ES complex; that means, less will be the concentration of the ES complex and it is only the ES complex concentration from which the product is generated.

So higher K_m means lower ES concentration. So, velocity is inversely proportional to K_m and velocity is directly proportional to k_2. k_2 is the turnover number or k_{cat}, as also called. So, more k_{cat} means more number of ES molecules will be turned over into the products in a

smaller time. So; that means, enzyme efficiency is directly proportional to k_{cat} or k_2 or turnover number. So, ultimately the efficiency is given by the value of k_{cat}/K_m.

This is not a Lineweaver Burk plot, this is the normal plot from the enzyme Michaelis Menten equation, V versus S; you will get a curve like this that goes up and finally it reaches the saturation level.

There are four curves that are given here; this is for the same enzymatic reaction; for the same enzyme concentration and all conditions are kept similar. In one of the experiments, there was no inhibitor and one of these curves represents that kinetics.

Another one is where a non-competitive inhibitor is added; another one represents the kinetics with competitive inhibitor; and finally one represents the kinetics with a mixed inhibitor.

So, all these four conditions are given: no inhibitor, a non-competitive inhibitor, a competitive and a mixed inhibitor. You have to find out which curve actually fits to these specific cases. First of all, out of these four curves, you can see that the first curve has the highest velocity when the substrate concentration is low. This is the fastest growing; this has got the highest gradient.

So; that means, this curve represents the highest value of the velocities. So, if that be the case then, this is the one where there is no inhibitor because, inhibitor slows down the reaction. So, if there is no inhibitor, then the normal enzyme kinetics will be followed. Normal means the fastest growing curve. So, in the first curve, basically there is no inhibitor present. I hope this is clear; just from the gradient, I can decide because this has the highest value of the velocity followed by third one, followed by second one and this is the fourth one. So, you are slowly decreasing the value; that means, these (2-4) are the curves for the enzyme kinetics in presence of inhibitor.

Now, we have to find out which type of inhibitor gives which curve. So, we have decided that the number one does not have any inhibitor; no inhibitor is present. So, now, let us see what is the V_{max}? So, this is the V_{max} without inhibitor. No inhibitor because this is number one, this is the maximum velocity that you get under saturating conditions.

Now, you look at the number two curve, let us go one by one. Number two curve started slowly, but then as you increase the substrate concentration more and more, ultimately it reaches a phase where curve one and two are almost same. The V_{max} value is almost same for 1 and 2, there is minor discrepancy, but you can neglect that.

V_{max} is same as 1 (where there is no inhibitor). Now this is the number 1 curve, which has no inhibitor. What will be the K_m value? So, the K_m value will be the half of V_{max} and you draw a line. So, your K_m will be somewhere here on the X axis.

So, this gives your K_m ok. So, if you consider the number 2 curve, the V_{max} is same, but what about the K_m? V_{max} is same for curve 1 and 2. So, you have to now extend this up to 2. So, this is where the line is crossing; the half V_{max} line is cutting this graph and now you draw a perpendicular to see the value of K_m.

So, for 2, V_{max} is same; but, K_m is more as compared to 1. So, now, you see that for which type of inhibitors, V_{max} remains the same, but K_m is more? Remember what is competitive inhibition? It is when both the substrate and the inhibitor goes for the same active site.

And if you make the substrate concentration very large as compared to the inhibitor then, ultimately inhibitor becomes insignificant. So, you reach almost the same V_{max} like the earlier one as if there is no inhibitor. But there is no change in the value of V_{max} because you will reach the same V_{max} using higher concentration of S. So, to reach half of V_{max}, now you need more concentration of S.

So, more concentration of S means your K_m increases. The number two graph must be for competitive inhibition. Now let us consider the third graph; in third graph, I could see V_{max} has changed; this is the value of V_{max} the third graph.

So, I can write here, for curve number 3, V_{max} has decreased.What about K_m? If this is the V_{max} then half of V_{max} is somewhere here. So, this is the half of V_{max} for the curve 3; and then you draw the line connecting the Y axis to the curve. And then you drop a perpendicular to the X axis; as was drawn for the absence of any inhibitor.

So, in this case what happens for curve 3? V_{max} has decreased, but K_m remains the same. So, which type of inhibition does this correspond to? That means, where the binding of the

inhibitor and the substrate are independent on each other, when they do not have any relation; substrate binds to its active pocket and the inhibitor goes and binds to another pocket.

So, there is no sequence of binding that it may be substrate first, followed by inhibitor or it could be inhibitor first followed by substrate. That is called the non-competitive inhibitor, they are not competing at all.

So, in that case what happens? K_m remains the same because, the binding of the substrate is independent of the binding of the inhibitor, but your V_{max} will decrease. Because, no reaction happens when both substrate and inhibitor are bound to the enzyme. So, your effective ES concentration is decreased, consequently your value of V_{max} will also decrease.

So, this is a case of non-competitive inhibition; that means, curve 3 represents noncompetitive inhibition. And if you draw the 4^{th} one, you will see that here V_{max} has changed as well as the K_m. So, for curve 4, V_{max} decreases and K_m has increased. Because, if you draw the V_{max} here, so V max will be somewhere here and then half of V_{max} will be somewhere here; and then you draw the line it cuts 4 here and then you drop the perpendicular to the X axis.

So, K_m has increased and V max has also increased; so both are changed. Hence this is the case of mixed inhibition; mixed inhibition is represented by curve number 4; where both K_m and V_{max} have changed.

So, for these things, if you do more and more practice, you will be more comfortable with all these parameters and how to calculate them, if you enter into any enzymology lab. In mechanistic enzymology, you need to know that whether the inhibitor is a competitive inhibitor, non-competitive or a mixed inhibitor.

But one other type of inhibition belonging to reversible inhibition is not shown here in the graph which is called the uncompetitive inhibition; where the binding of the inhibitor depends on the substrate binding. So, substrate goes and binds and then the inhibitor follows; the active site is created for the inhibitor and inhibitor goes and binds.

In that case, what happens is that the K_m is decreased.

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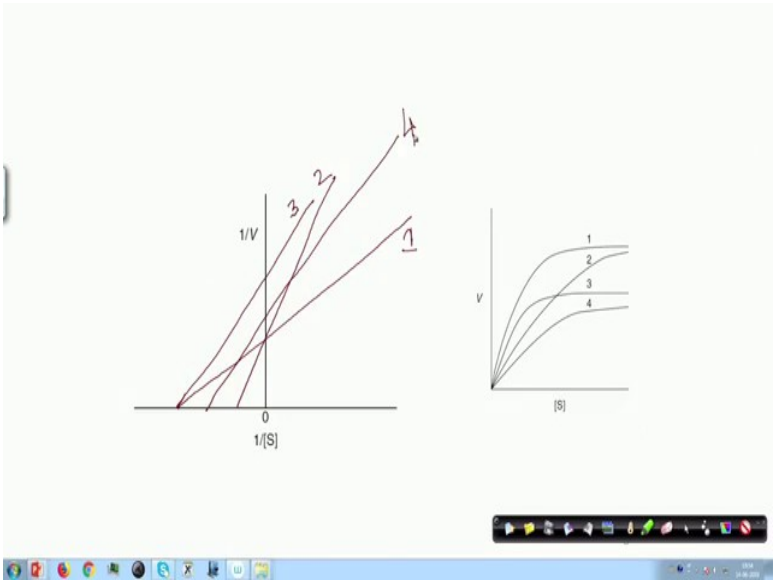

Actually for the same problem, you have been also asked to draw the Lineweaver Burk plot; that means the corresponding double reciprocal plot for each curve (1-4). You know double reciprocal plot is something like this, suppose this is the normal reaction no inhibitor so, this is the corresponding graph for curve 1.

For competitive inhibition, we know that V_{max} remains the same. So, for competitive inhibition (curve number 2) the Y intercept will remain the same. So, this is now transferring this graph into a Lineweaver Burk plot.

So, you see that here the K_m changes, V_{max} remains the same.. Curve 3 represented non-competitive inhibition. In noncompetitive inhibition, , what does not change is K_m. So, the graph will be something like this. Remember this is reciprocal so; that means, if V decreases, then 1/V increases.

So, it has to be like this, it cannot be lower than the straight line for 1 because, these are reciprocals. So, this is 2, this is your 3 and number 4 is difficult to draw. Since it is a mixed inhibition, so both K_m and V_{max} are changing. So, something like this is your number 4. So, that I think concludes this problem session on enzyme kinetics.

Thank you.