Introduction to Chemical Thermodynamics and Kinetics
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Lecture – 35
Chemical Kinetics: Mechanisms – Part 4

We will now discuss the enzyme catalysis. Now, as you know the catalysis by enzymes, as you know that enzymes are biological catalysts. Now, their function is just like any other catalyst, but they are very efficient. And remember that in our body, there are many reactions that do not happen under normal temperature and pressure conditions, but they happen in the temperature of the body which is just around 98 Fahrenheit. And then you can realize that at a very low temperature the enzymes are very efficiently carry out those reactions and without enzymes we would not survive and in our body there are thousands of enzymes that are doing catalysis for many important reactions for which we survive.

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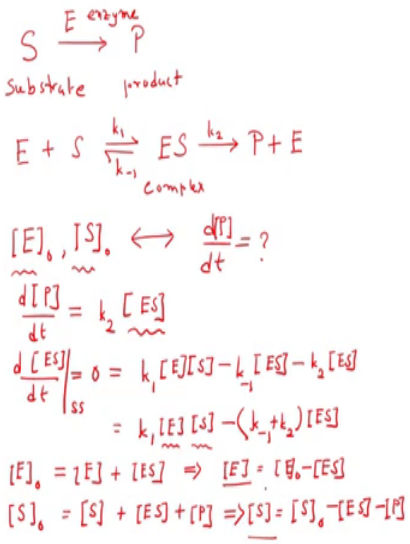


Leonor Michaelis


Maud Leonora Menten

Now, the first model for enzyme catalysis was put forward by Michaelis and Menten, and who provided a very interesting concept of enzyme and how the enzyme actually forms or helps in forming a product from a substrate. So, if you consider that there is a substrate we call it as S and that substrate is going into product. And in presence of the enzyme which you write as E how one can write the kinetic model for this enzyme mediated catalysis or which is known as enzyme catalysis.

Now, what the Michaelis and Menten considered is similar like we just talked about. So, there is a step or enzyme and substrate binds and unbinds together. And there is a formation of a complex which is known as enzyme substrate complex. This is the intermediate. And that complex actually gives rise to the product as before we can think about to rate constant k 1 and k minus 1 and k 2 for the rate of product formation. And we will also consider the steady state approximation for the enzyme substrate complex.

Now, the solution is very easy, but for what Michael and Menten came up with is cleverly manipulating the reaction the amounts of enzyme and the substrate because you cannot measure them. What you can measure is the initial enzyme concentration which you had started with, and the initial substrate concentration we should know, these two things you know. And then what Michaelis and Menten asked is that if we know all these two parameters, how we can write the rate of product formation that is the entire thing.

So, given I have these two information how I can write or how I can express this equation dP dt. So, you follow the usual positive using steady state approximation of for the intermediate which is the enzyme substrate complex or the E s complex. Now, this dP dt is nothing but it will be k 2 into the concentration of these enzyme substrate complex. So, we need to know that this concentration and for that we will use the as usual the steady state approach and that says that d E S dt which is zero under steady state approximation.

And that is nothing but so it is forming at a rate k 1 into E sorry into S it is I mean decomposing at a rate k minus 1 this is giving that the reactant and I mean reactants which are enzyme and substrate. And also it is forming product at a rate like this. So, from that you can easily figure out what is the E S. So, E S will be nothing but k 1 into or we can actually write it slightly differently before we write the equation for the E S. This is nothing but k 1 into E into S minus k minus 1 plus k 2 into E S.

Now, as I said that we have no idea on the E and the S, otherwise we could have solved it all we know that E 0 and S 0. Now, initially if we start with the initial enzyme concentration as E 0, you can always argue that at any time there will be some free enzyme available in the reaction mixture. There will be some enzymes which are which have formed these enzyme substrate complex. So, instead of E, we will write it as E

minus sorry E 0 minus E S that is why I did not eliminate E S at this step, and all wrote the value of E S and put it back.

And similarly for the initial substrate concentration if that is S 0, so at any point it will be there with some free substrate in the reaction mixture. There will be some such enzymes substrate complex in the reaction mixture plus some substrate has been already converted into the product. Here once you have the product, the enzyme actually is you regain that enzyme because it is a catalyst.

Now, from that you can easily figure out what is the we need actually the enzyme as well as the substrate. So, the at anytime the substrate concentration will be nothing but the initial substrate concentration minus E S minus P. So, now you can combine these two, and see what we are getting. So, we will just replace these values of E and S in this equation to get or we can write it in the next page because we need to do some approximation. So, the it will be k 1 into E into S. So, it will be k 1 into E 0 minus E S.

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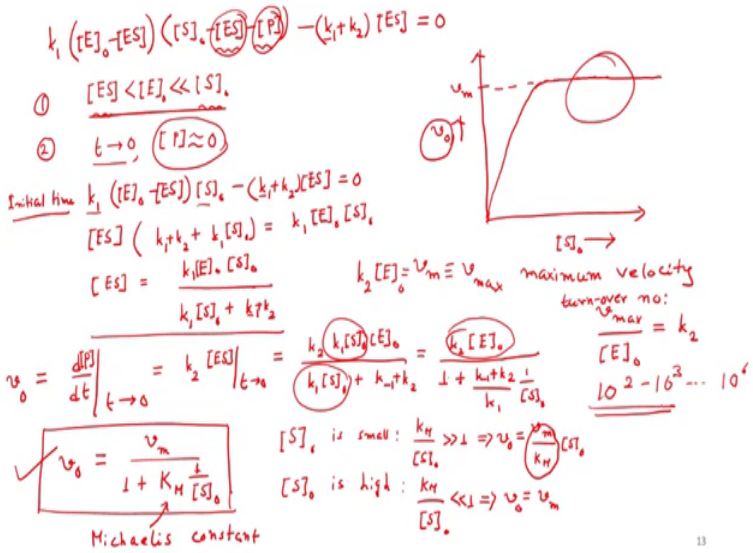

Let us write it. So, it is k 1 into E 0 minus E S. And then we have the substrate concentration which is S. And S is nothing but S 0 minus E S minus P minus P and that should be excuse me so that should be equal to or there is another term it is minus k 1 plus k 2 into E S minus k 1 plus k 2 into E S that is equal to 0 under the steady state approximation.

So, now we will make a further approximation. We know that at any point of time, this enzyme is basically binding to the substrate, but these enzyme substrate concentration is much less than the enzyme concentration itself because the reason is much less than the initial enzyme concentration. The reason is you started with a some amount of enzyme, and then only very initially or at any point actually you will have only a few of them are bound to the are present as enzyme subset complex. Because most of them have if the bind actually they have formed the product or they have actually done something else they are actually gone back to the initial reactants which are enzyme plus substrate.

So, that will be always less than the enzyme concentration. And you usually start with very large excess of the substrate because you know that enzyme is a catalyst and you need it a catalyst you need a for a for a very little amount compared with the reactant. So, this S 0 is pretty high compared to E s. So, we can actually ignore the E S term here so that is assumption number 1.

Assumption number 2 is that if you are asking this question that at t equal to 0, what will happen; so at t equal to 0, the amount of the product that also you can approximate to be nearly equal to 0. So, if you if you add these two things, then we can get k here one into E 0 minus E S and this term will be nothing but S 0 because we ignored both this term you can ignore this term also.

So, it is just times S 0 minus k 1 plus k 2 into E S that is equal to 0, but this is at the beginning meaning and when t tends to 0 meaning it is the initial time, while this is always true because the substrate concentration is always much larger than the enzyme concentration initial enzyme concentration which is of course, larger than the enzyme substrate complex always. So, at initial time which is the second condition, we have this or we can now eliminate E S or evaluate what is the value of E S. So, E S will be nothing but if you call it the terms it is k 1 plus k 2 and then there will be a term which is k 1 into S 0. So, we can write it like this, E S times it is k 1 plus k 2 plus k 1 into S 0 that is equal to k 1 into E 0 into S 0.

So, E S is nothing but k 1 into E 0 into S 0 divided by k 1 S 0 or you can just write it as that is fine, and we can just write k 1 S 0 at the beginning plus k 1 plus k 2. So, just check once more what are should be k minus 1 plus k 2, it should be k minus 1, it should be k minus 1, it is a minus 1 fine. So, the rate of product formation which is dP dt at k

tends to 0, because that is the initial rate which we call as also v o which we can write as now which is nothing but k 2 into E S, E S evaluated at t tends to 0 and which is given by this.

So, I will have basically k 2 into k 1 S 0 I am just writing that S 0 with k 1, and then multiplied by E 0 divided by k 1 S 0 plus k minus 1 plus k 2. And we can divide the numerator and the denominator by k 1 S 0. So, you see there is another k 1 S 0 here. So, what we will get is nothing but k 2 into E 0 divided by 1 plus k 1 sorry k minus 1 plus k 2 divided by k 1 into 1 by S 0, so that we can write in a shorthand form that v 0 is nothing but k 2 into v 0 that we are writing it as v m. The reason will be clear in a while and then divided by 1 plus all these combinations we are writing as a constant which we know which is known as Michaeli's constant it is named after Leonore Michaeli's it is Michaeli's constant into 1 by S 0.

So, if we plot the initial rate remember v it is not v, actually it is v 0 which means it is the rate of product formation at a very early time versus the substrate concentration. And you will see that at a very very I mean a small substrate concentration, what will happen, so this will be a very large this is very a large number right. So, at a if S 0 is very large so what you will have is that k m by S 0 is a very small number that you can actually ignore.

So, if we plot it in terms of the substrate initial substrate concentration, and then you will see that let us just consider at a very high value of the substrate concentration, so if it is a very high value, so then 1 by S 0 will be very small number. So, we can ignore this k m divided by S 0 that term with respect to 1. And then v 0 will be nothing but equal to some velocity of the reaction which we are calling as say v m. And at a very early very small substrate concentration when S 0 is very small, then we cannot write it. So, so then let us just do it like this.

So, when S 0 is small, then we can write that k m by S 0 that is much greater than 1. So, what you have is nothing but it will be vm divided by km into S 0. So, v 0 will be nothing but v m divided by k m and then this S 0 goes up. So, it is I see that this v m by k m, if we consider it to be constant is a constant because v m is nothing but k 2 into v 0, k 2 is a constant and E 0 is initial enzyme concentration which is also constant. So, it is a

constant times S 0 so which means that early time it will vary linearly with respect to the substrate concentration.

So, we will have a just a linear slope. And at a very large substrate concentration, when this is very high, so you can write that k m by S 0 is much less than one which means v 0 is nothing but I can ignore this term compared to 1, it is just equal to v m. So, then it will basically be a constant which is k 2 into E 0. Now, that is why it says that the initial velocity can have a very maximum velocity and that is why we wrote it as a v m, sometimes book you instead of v m they write it as v max, because it is known as which is basically k 2 times the E 0 is sometimes known as the v max and which is nothing but the maximum velocity or the maximum rate.

Now, what happens is that there are some enzymes in the in your system. And when you start with a very, very small subset concentration, then there are more number of enzymes than the substrate, but if you think about that if we keep on increasing the substrates, so then actually all the enzymes although all the active site of the enzyme will be now occupied by the surface and there is no free enzyme. So, you cannot have a rate which is now dependent on the substrate concentration. Even if you keep on adding the substrate all the enzymes right now are occupied by the substrate.

So, you basically saturate your system and that exactly what you see here in the following the Michaeli's Menten equation. But always keep in mind that here we are not plotting velocity versus substrate concentration, it is the initial velocity because we made an assumption where we showed that this product concentration is almost 0. So, what we were doing in the experiment is that you are mixing the substrate with the enzyme with a known concentration of the enzyme, you are basically adding the substrate and you are measuring the rate at a very early time. And then you change the substrate concentration again you measure the rate at a very early time and that way you are measuring keeping the initial enzyme concentration same, because you have to keep the k 2 into E 0 same, otherwise v max will also differ if you vary the E 0 also.

So, then you see that it basically gives you a saturation like behavior. Now, in this equation is known as the Michaeli's Menten equation, but you could actually write the equation in a slightly different way because always you would like to fit any equation in a linear fashion. So, you can easily recovered our linear equation if you take the

reciprocal of this and that was first shown by two scientists the Lineweaver and Burk, who just took this Michael's equation and plotted the inverse of v 0 which is 1 over v 0. So, if you see if I write it as 1 over v 0, it will be on the right hand side, it will be 1 over v m plus k m by v m into 1 over S 0.

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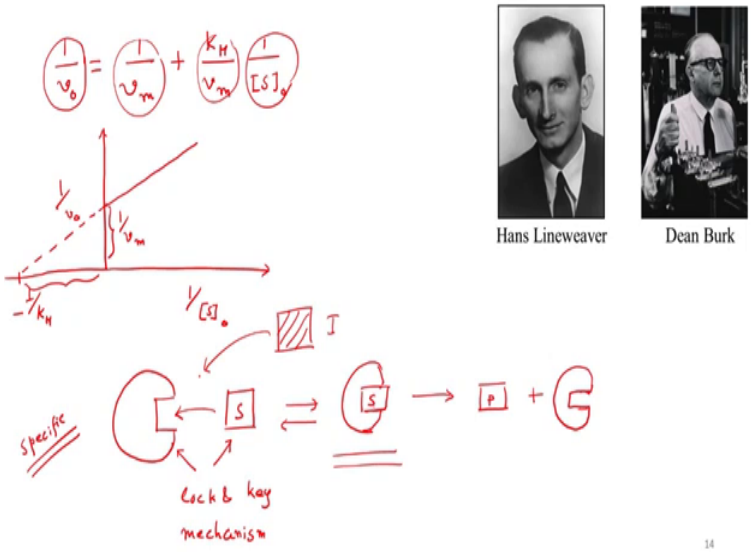

So, what we will have is 1 over v 0 is 1 over v max plus k m by v m into 1 over S 0. So, how this plot will look like, if we plot instead of v 0 versus S 0, if we plot 1 over v 0 versus 1 over S 0, of course, that will be a linear plot. But let us consider the slope and the at the intercept, now think about it the slope of this reaction are first let us consider that the intercept, if we consider the at what point it cuts the say y-axis cutting y-axis means actually that x position will be 0, so 1 by S 0 that is 0 at that 1, so it will cut it at 1 over v m. So, it will be a linear plot because we can see from here. But what about the intercept at the x-axis, so x-axis intercept means actually the y value will be 0. So, you can put 0 here, and we can ask what is the value of 1 by S 0.

So, 1 by S 0 will be nothing but we can take this on the left hand side minus 1 over v m and then you can multiply it by v m into k m. So, it will be nothing but 1 over k m with a negative sign. So, it just cuts the x-axis at negative 1 over k m. So, this is negative corner 1 over k m. And this plot is known as Lineweaver-Burk plot. And it is often used and it will be very useful particularly when you consider the enzyme inhibition.

Now, what is enzyme inhibition, suppose in every engine has some kind of ship because enzyme is very particular for a particular substrate. And a very specific substrate can actually get bound to this enzyme. And then you form an enzyme substrate complex which is like this and then so this is the equilibrium which we showed. And then the this substrate actually forms some different product, and then the enzyme releases the substrate and we get the free enzyme back. And then the enzyme is ready to bind to another substrate and that is how it works.

Now, you can see that for a particular enzyme, there is a particular substrate. So, it is a kind of known as the lock and key mechanism. So, you can see it that there is one key for one particular lock. So, at the binding of the substrate to the enzyme is very specific. So, enzymes are very specific in nature. It will not work on different many different molecules. Sometimes it may, but usually enzymes are very specific to a particular molecules or a particular set of molecules; and that molecular shape and the three-dimensional structure determines which enzyme is acting which enzyme is acting upon this molecule.

Now, once we have this situation once we have a very, very large number of substrate, then there is will be no free enzyme in your system, and then you will reach a saturation condition which is here. But a very high enzyme concentration you have already reached the maximum velocity. Now, sometimes there is another quantity that you would like to measure that what is the maximum number of enzyme to substrate conversion per minute what are the number of concern number of this thing per minute per enzyme per unit enzyme concentration. Because if you have more enzyme concentration then you will have more maximum velocity, so that will be nothing but so what you were asking is basically v max by E 0.

What is the v max by E 0 and that will be nothing but equal to k 2. So, this number this number is known as the turnover number meaning how many times the enzyme is turning over substrate into product, it is basically doing this catalytic cycle per unit time, because there is a time already involved here, it is a v max because it is a k 2 there it is a rate constant. So, if there is a first order rate constant which means it is how many times it is doing, but we know that it is dependent on the initial enzyme concentration. So, we are asking this question per unit enzyme concentration.

So, if I take one molar theoretically or say 1 micromolar if it is the concentration then we ask this question what is this number. And usually this number if you take a one enzyme molecule, this number usually for most of the enzymes varies from 10 sorry 10 to the power 2 to 10 to the power 3 which means it does this substrate to product substrate to product cycle, but about say 100 to 1000 per second, but sometimes you can actually go to very high limit like 10 to the power 6 also. So, every one microsecond it does a conversion which means in 1 second, it does 10 to the power 6 or 1 million substrate to product conversion. Now, it it gives you a feeling how enzymes are so efficient how and why enzymes are so efficient and in converting the substrate and how basically it has a very, very efficient rate in the process.

Now, the next thing we will just do is that it will just build up our discussion on this Lineweaver-Burk plot and we will discuss how enzyme inhibition happens. By enzyme inhibition we will see that if there is some other substrate which actually has some shape or some other molecule which has the same shape just like the substrate which we call as an inhibitor. If that actually binds to the enzyme, what will happen to the kinetics of the of these enzyme, what will happen to this enzyme kinetics and what will happen if we now plot 1 over v 0 by versus 1 over S 0 in the Lineweaver-Burk seems.

So that will discuss in the next part.