

BL/CH401 Lecture 12 -- Enzyme Kinetics -- Part I

I. Introduction to Enzyme Kinetics.

In chemistry, kinetics has to do with the rate of reactions. In biochemistry, we are most interested in rates of enzyme catalyzed reactions since virtually all biological reactions are catalyzed by enzymes.

Enzyme Kinetics: Rates of enzyme catalyzed reactions

Usefulness of enzyme kinetics:

- Common clinical assays to detect enzymes
- Understanding metabolic pathways
- Measuring binding of substrates and inhibitors to the active site of an enzyme
- Understanding the mechanism of catalysis of an enzyme

Rates of reactions are measured by change in reactant amounts with time. You can measure the disappearance of the substrate or the appearance of the product. Usually, the appearance of the product is easier to keep track of since there should be no product present at the beginning of the reaction.

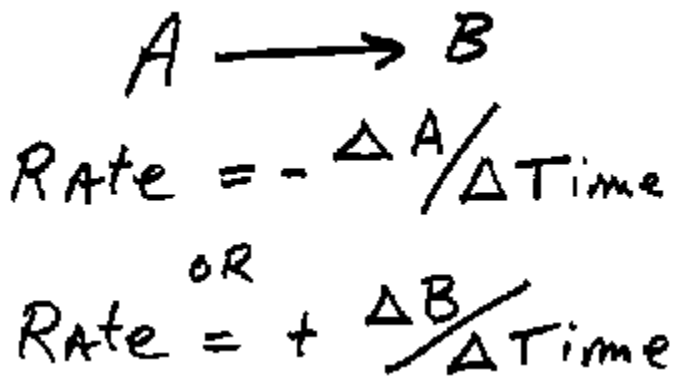


Figure 1. Ways to express a rate for the enzyme catalyzed reaction.

Rates = Reaction Velocity

For enzymes, the initial velocity (before significant product accumulates) is always used. Initial Velocity = V_0

Part II. A Simple Mechanism for the Enzyme Catalyzed Reaction.

For catalysis to begin, the substrate must bind to the enzyme, which results in the formation of the enzyme-substrate complex (ie E-S complex). The E-S complex forms rapidly in the first part of the enzyme catalysis process and the concentration of the E-S stays constant at a steady-state

level. For this reason, this type of kinetics is called *steady-state kinetics*.

A simple mechanism for the enzyme catalyzed reaction helps us to understand and model this process.



Figure 2. A simple enzyme mechanism for a single substrate and product.

This simple mechanism can also be expressed with rates shown for each process in the mechanism:



Figure 2a. Simple enzyme mechanism with rate constants shown for each step.

Part III. Enzyme Catalyzed Rates at Different Substrate Concentrations.

Since the enzyme is used many times to catalyze the same reaction, the concentration of the enzyme is much less than the substrate:

$$[S] \gg [E]$$

Thus, the substrate saturates the enzyme. This is best understood by observing the rate of the reaction or initial velocity at different [S] (ie. substrate concentrations):

[S] mM	V _o μmol product/min
0	0.0
1	0.9
2	1.4
5	1.9
10	2.3
50	2.6
100	2.6

Model data for the enzyme catalyzed reaction. These data show that at low [S], the initial velocity is more or less proportional to the [S]. At high [S], the initial velocity no longer increases as more substrate is added. Thus, at high [S] the enzyme is saturated with substrate and

no increase in the enzyme catalyzed rate is observed.

This model set of data for an enzyme catalyzed reaction shows the initial velocity in terms of the amount of product formed per unit time (ie micromoles of product produced/min) at various substrate concentrations. These data can be plotted in a graphical form to also illustrate the results of an enzyme catalyzed reaction.

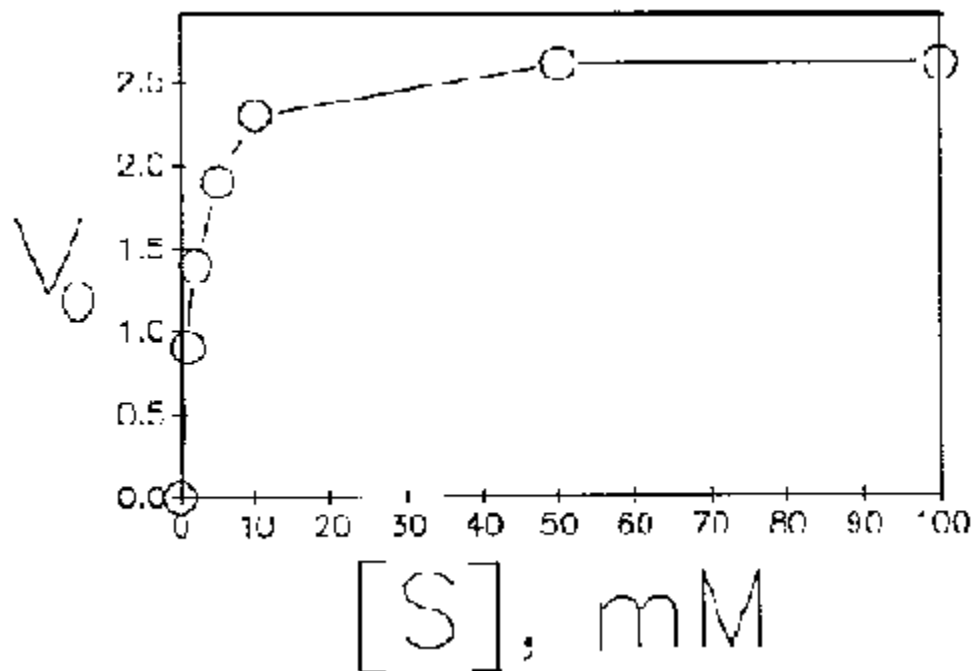


Figure 4. Plot of initial velocity of the enzyme catalyzed reaction (V_0) versus the $[S]$ (ie substrate concentration). Initial velocity is always given in units of amount of product formed per unit time and the substrate concentration is given in molar units (ie mM).

Here it is easy to see the saturation of the enzyme at high $[S]$ where the initial velocity approaches a limiting value. The plot has the shape of a square hyperbola.

Part IV. The Michaelis-Menten Equation.

The plot of V_0 versus $[S]$ can be represented by an equation, which is known as the Michaelis-Menten equation in honor of the scientist who first described it. This equation, sometimes called the M-M equation, is an important one for you to know and understand.

$$v_0 = \frac{V_{\max} [S]}{K_M + [S]}$$

Figure 5. The Michaelis-Menten equation which describes the change in V_o as $[S]$ increases.

The constants in this equation, K_m and V_{max} , are defined:

V_{max} = Maximum velocity catalyzed by a fixed $[E]$

K_m = the $[S]$ which gives $1/2 V_{max}$

These definitions are illustrated below:

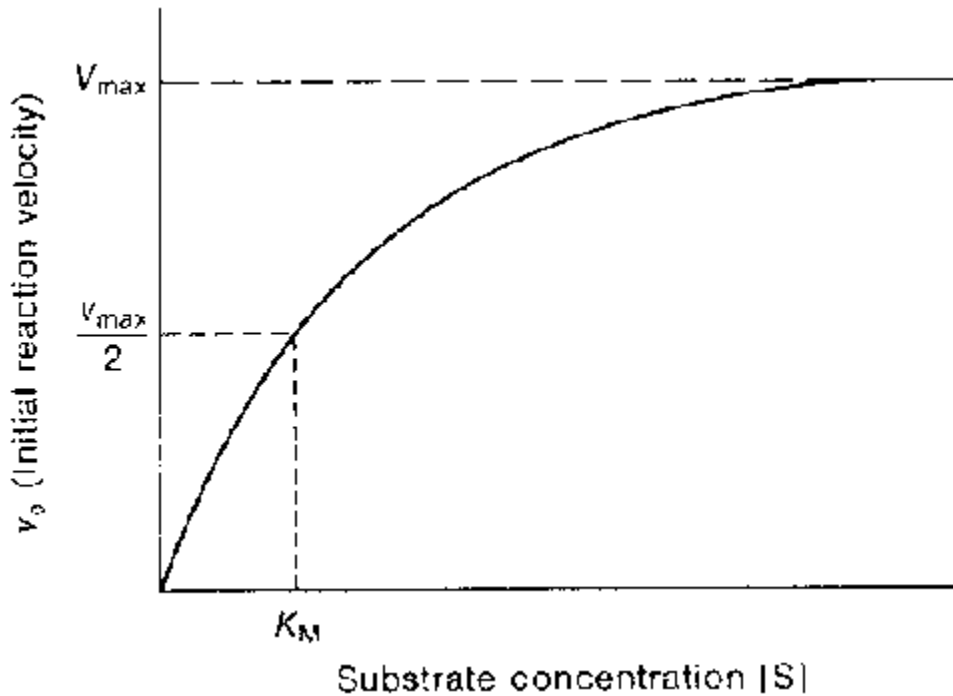


Figure 6. V_o versus $[S]$ plot illustrating the operational definitions of V_{max} and K_m .

Thus, the limit approached in the V_o versus $[S]$ plot is the V_{max} .

Part V. Definition of K_m and V_{max} and Their Ratio - V_{max}/K_m .

The K_m is sometimes called the Michaelis Constant. The K_m is an intrinsic property of an enzyme related to the binding constant for forming the ES complex, which is an equilibrium and can be defined by the rate constants for its formation and breakdown using the simple enzyme



mechanism shown above in Figure 2a:

$$K_M \approx K_S$$

$$K_S = \frac{[E][S]}{[ES]} = \frac{k_2}{k_1}$$

Figure 7. The approximate relationship between the K_M and the K_S for the binding of the substrate to the enzyme which leads to the formation of the E-S complex. K_S is defined by the equilibrium formed between the enzyme (E) and substrate (S) and the E-S complex, as shown above. K_S is also defined by the ratio of the rate of breakdown of the E-S complex divided by its rate of formation.

But K_M also involves the breakdown of the E-S complex to E and P, which is not a component of the K_S . Thus, the rate of the breakdown of the E-S complex to make product (P) is also defined in the simple enzyme mechanism shown in Figure 2a:



$$K_M = \frac{k_2 + k_3}{k_1}$$

Figure 8. The definition of K_M by using rate constants for simple enzyme mechanism. The point of this graphic is to emphasize that the K_M constant of the enzyme catalyzed reaction includes more than just the formation of the E-S complex, but also its breakdown to form product, which is of course the key to an enzyme catalyzed reaction.

So K_M reflects both binding of E to S but also the catalytic constant (shown as k_3 above, but also defined as k_{cat}) of the enzyme catalyzed reaction.

The V_{max} is also dependent on the catalytic constant:

$$V_{max} = k_{cat} [E]$$

So both V_{max} and K_M are properties of individual enzymes and not very useful for comparing enzymes.

However, the ratio V_{max}/K_M can be used to compare enzymes. This ratio (V_{max}/K_M) measures the efficiency of the enzyme. The efficiency of the enzyme is ultimately limited by the rate of diffusion of the substrate to the enzyme - thus the diffusion of substrates, which is very rapid,

sets an upper limit. The most efficient enzymes like Triose-P Isomerase are limited by how fast their substrates get to them. But most enzymes are not this efficient and more limited by chemical events in the active site of the enzyme.

Part VI. Finding the K_M and V_{max} by the Graphical Solution Method.

To calculate the K_M and V_{max} , the Michaelis-Menten equation is converted into a linear form by taking the reciprocal of both sides of the equation. This is called the Lineweaver-Burk equation in honor of the first scientists to describe it.

$$\frac{1}{v_0} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

↓

$$y = b x + c$$

Figure 9. The Lineweaver-Burk equation linearizes the M-M equation by taking the reciprocal of both sides of the equation. This equation then takes on the form of the equation of a line. The y values are $1/v_0$, the x values are $1/[S]$. The b value in the line equation is the slope and equal to K_M/V_{max} , while the c value is the y-intercept and equal to $1/V_{max}$.

The double reciprocal plot is useful for deriving K_M and V_{max} by plotting kinetic data for an enzyme and you should use it to find the K_M and V_{max} via graphing for the problem set you got today.

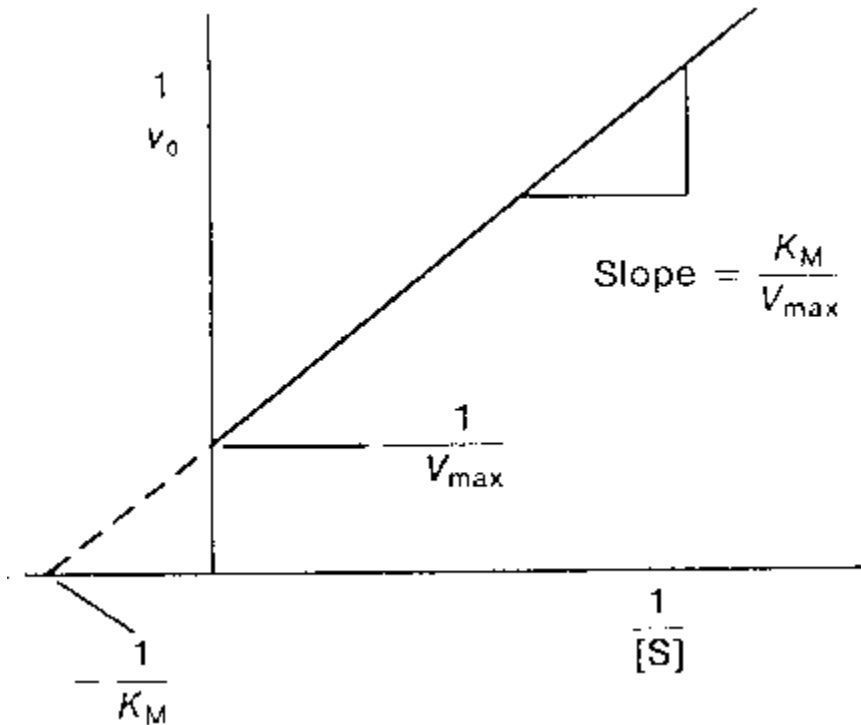


Figure 10. The double reciprocal plot for enzyme kinetic data.

This plot must be used to find K_M and V_{max} for enzyme kinetic data in this class as shown on the graphic. The y-intercept is the $1/V_{max}$. The x-intercept, which is found in the 4th quadrant, is $-1/K_M$. Alternatively, the K_M value can be found from the slope using the V_{max} value found from the y-intercept.

However, there are statistical problems with the Lineweaver-Burk equation and double reciprocal plots, so today in research, one derives K_M and V_{max} using other methods such as the direct linear plot using a computer program. However, the Lineweaver-Burk equation makes the clearest representation of kinetic data and makes it easy to understand the results, so it is most often used to illustrate the data even when the K_M and V_{max} are derived by other methods.

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