

## 5.3: Michaelis-Menten Kinetics

### Introduction

The general reaction scheme of an enzyme-catalyzed reaction is as follows:



The enzyme interacts with the substrate by binding to its active site to form the enzyme-substrate complex, ES. That reaction is followed by the decomposition of ES to regenerate the free enzyme, E, and the new product, P.

To begin our discussion of enzyme kinetics, let's define the number of moles of product (P) formed per time as V. The variable, V, is also referred to as the rate of catalysis of an enzyme. For different enzymes, V varies with the concentration of the substrate, S. At low S, V is linearly proportional to S, but when S is high relative to the amount of total enzyme, V is independent of S. **Concentrations is important in determining the initial rate of an enzyme-catalyzed reaction.**

To understand Michaelis-Menten Kinetics, we will use the general enzyme reaction scheme shown below, which includes the back reactions in addition the the forward reactions:

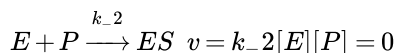
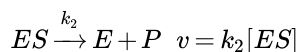
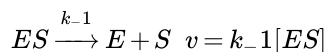


The table below defines each of the rate constants in the above scheme.

**Table 1:** Model parameters

Rate Constant	Reaction
$k_1$	The binding of the enzyme to the substrate forming the enzyme substrate complex.
$k_2$	Catalytic rate; the catalysis reaction producing the final reaction product and regenerating the free enzyme. This is the rate limiting step.
$k_{-1}$	The dissociation of the enzyme-substrate complex to free enzyme and substrate .
$k_{-2}$	The reverse reaction of catalysis.

### Substrate Complex



The ES complex is formed by combining enzyme E with substrate S at rate constant  $k_1$ . The ES complex can either dissociate to form  $E_F$  (free enzyme) and S, or form product P at rate constant  $k_2$  and  $k_{-1}$ , respectively. The velocity equation can be derived in either of the 2 methods that follow.

## Method 1: The Rapid Equilibrium Approximation

E, S, and the ES complex can equilibrate very rapidly. The instantaneous velocity is the catalytic rate that is equal to the product of ES concentration and  $k_2$  the catalytic rate constant.

$$v = k_2[ES] \quad (5.3.4)$$

The total enzyme concentration ( $E_T$ ) is equal to the concentration of free enzyme E ( $E_F$ ) plus the concentration of the bound enzyme in ES complex:

$$[E]_T = [E_F] + [ES] \quad (5.3.5)$$

$$K_s = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} \quad (5.3.6)$$

$$K_s \frac{([E_o] - [ES])[S]}{[ES]} \quad (5.3.7)$$

$$[ES] = \frac{[E_o][S]}{K_s + [S]} \quad (5.3.8)$$

$$v_o = \left( \frac{dP}{dt} \right)_o = k_2[ES] \quad (5.3.9)$$

$$v = \left( \frac{dP}{dt} \right)_o = k_2[E_o] = V_{max}[E_o][S]K_s + [S]$$

At high substrate concentrations,  $[S] \gg K_s$  we get:

$$v = \left( \frac{dP}{dt} \right)_o = k_2[E_o] = V_{max}$$

## Method 2: The Steady-State Approximation

The figure below shows the relatively low and constant concentration of the enzyme-substrate complex due to the complex's slow formation and rapid consumption. Note the falling substrate concentration and the rising product concentration.

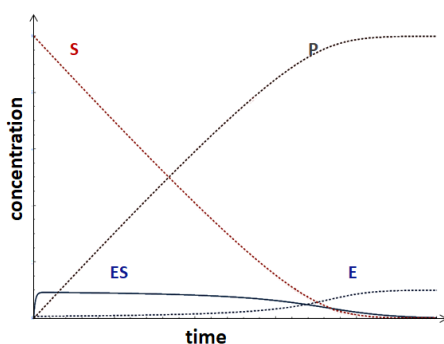


Figure 5.3.1: Change in concentrations over time for enzyme E, substrate S, complex ES and product P.

The rates of formation and breakdown of the E - S complex are given in terms of known quantities:

- The rate of formation of ES =  $k_1[E][S]$

(with the assumption that  $[P] = 0$ )

- The rate of breakdown of ES =  $k_2[ES] + k_{-1}[ES] = (K_2 + K_{-1})[ES]$

At steady state,

$$\frac{d[ES]}{dt} = k_1[E][S] + k_2[ES] + k_{-1}[ES] = 0 \quad (5.3.10)$$

**Therefore, rate of formation of [ES] is equal to the rate of breakdown of [ES]**

So,

$$k_1[E][S] = (k_2 + k_{-1})[ES] \quad (5.3.11)$$

Dividing through by  $k_1$ :

$$[E][S] = \frac{(k_2 + k_{-1})}{k_1} [ES] \quad (5.3.12)$$

Substituting  $\frac{(k_2 + k_{-1})}{k_1}$  with  $k_M$ :

$$[E][S] = K_M [ES]$$

$$k_M = \frac{\text{breakdown}[ES]}{\text{formation}[ES]} \quad (5.3.13)$$

$K_m$  implies that half of the active sites on the enzymes are filled. Different enzymes have different  $K_m$  values. They typically range from  $10^{-1}$  to  $10^{-7}$  M. The factors that affect  $K_m$  are:

- pH
- temperature
- ionic strengths
- the nature of the substrate

Substituting  $[E_T]$  with  $[E_T] - [ES]$ :  $E_T = [ES] + [E_T]$

$$([E_T] - [ES])[S] = k_M [ES]$$

$$[E_T][S] - [ES][S] = k_M [ES]$$

$$[E_T][S] = [ES][S] + k_M [ES]$$

$$[E_T][S] = [ES]([S] + k_M)$$

Solving for [ES]:

$$[ES] = \frac{([E_T][S])}{([S] + k_M)}$$

The rate equation from the rate limiting step is:

$$v_o = \frac{dP}{dt} = k_2[ES]$$

Multiplying both sides of the equation by  $k_2$ :

$$k_2[ES] = k_2 \left( \frac{([E_T][S])}{(K_M + [S])} \right) \quad (5.3.14)$$

$$v = k_2 \left( \frac{([E_T][S])}{(K_M + [S])} \right) \quad (5.3.15)$$

When  $S \gg K_M$ ,  $v_o$  is approximately equal to  $k_2[E_T]$ . When the  $[S]$  great, most of the enzyme is found in the bound state ( $[ES]$ ) and  $V_o = V_{\max}$

We can then substitute  $k_2[E_T]$  with  $V_{\max}$  to get the **Michaelis Menten Kinetic Equation**:

$$v = \frac{v_{max}[S]}{(k_M + [S])}$$

### Reaction Order Note

When  $[S] \ll K_m$ ,

$$v = \frac{V_{max}[S]}{K_m}$$

This means that the rate and the substrate concentration are directly proportional to each other. The reaction is first-order kinetics.

When  $[S] \gg K_m$ ,

$$v = V_{max} \quad (5.3.16)$$

This means that the rate is equal to the maximum velocity and is independent of the substrate concentration. The reaction is zero-order kinetics.

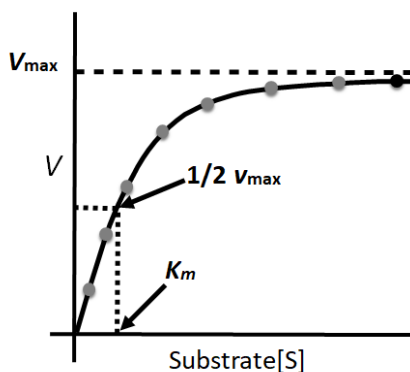


Figure 5.3.2: Diagram of reaction velocity and Michaelis-Menten kinetics.

$$v = \frac{V_{max}}{2}, K_m = [S] \text{ n}$$

$$v = \frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]} \quad (5.3.17)$$

Therefore,  $K_m$  is equal to the concentration of the substrate when the rate is half of the maximum velocity. From the Michaelis Menten Kinetic equation, we have many different ways to find  $K_m$  and  $V_{max}$  such as the Lineweaver-Burk plot, Hanes-Woolf plot, and Eadie-Hofstee plot, etc.

### Lineweaver-Burk Plot

For example, by taking the reciprocal of the Michaelis Menten Kinetics Equation, we can obtain the Lineweaver-Burk double reciprocal plot:

$$v = \frac{V_{max}[S]}{(K_M + [S])} \quad (5.3.18)$$

$$\frac{1}{v} = \frac{(k_M + [S])}{v_{max}[S]} \quad (5.3.19)$$

$$\frac{1}{v} = \left( \frac{K_m}{V_{max}} \right) \left( \frac{1}{[S]} \right) + \frac{1}{V_{max}} \quad (5.3.20)$$

Apply this to equation for a straight line  $y = mx + b$  and we have:

$$y = \frac{1}{v} \quad (5.3.21)$$

$$x = \frac{1}{[S]} \quad (5.3.22)$$

$$m = \text{slope} = \frac{K_m}{V_{max}} \quad (5.3.23)$$

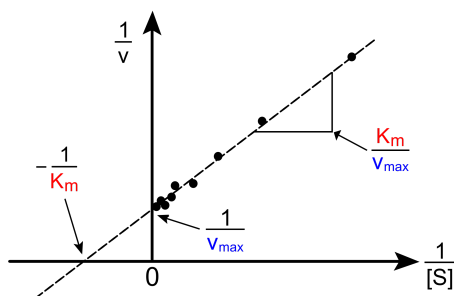
$$b = y - \text{intercept} = \frac{1}{V_{max}} \quad (5.3.24)$$

When we plot  $y = \frac{1}{v}$  versus  $x = \frac{1}{[S]}$ , we obtain a straight line.

$$x - \text{intercept} = \frac{-1}{K_m} \quad (5.3.25)$$

$$y - \text{intercept} = \frac{1}{V_{max}} \quad (5.3.26)$$

$$\text{slope} = \frac{K_m}{V_{max}} \quad (5.3.27)$$



**Figure 5.3.3.** An example of a Lineweaver-Burke plot.

## References

1. Chang, Raymond. *Physical Chemistry for the Biosciences*. Sansalito, CA: University Science, 2005. Page 363-371.
2. Atkins, Peter and de Paula, Julio. *Physical Chemistry for the Life Sciences*. New York, NY: W. H. Freeman and Company, 2006. Page 309-313.
3. Stryer, Lubert. *Biochemistry (Third Edition)*. New York, NY: W.H. Freeman and Company, 1988. Page 187-191.

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