Chapter 12 - Reaction Kinetics

In the last chapter we looked at enzyme mechanisms. In this chapter we’ll see how enzyme kinetics, i.e., the study of enzyme reaction rates, can be useful in learning more about the properties of enzymes. First, we’ll do a brief review of chemical kinetics in general.

Reactions as we normally consider them, such as

\[ A \rightarrow W \rightarrow P \]

represent overall reactions that specify only the stoichiometric relationships between reactants and products. The rate, or \( \textit{velocity} (v) \), of such a reaction will dependent on concentrations of reactants (and possibly products) and can be written

\[ v = \frac{d[P]}{dt} = -\frac{d[A]}{dt} = k[A]^m[B]^n \]

where \( k \) is a proportionality constant (i.e., rate constant) and the sum of exponents, \( m + n \) is called the order of the reaction. Most reactions are either first order (sum of exponents = 1) or second order. Some may also be zero or third order. Mixed order is also possible, in which case the sum of exponents is not an integer.

The overall reaction cannot give us the rate equation because the overall mechanism tells us nothing about the \textit{mechanism} by which reactants are converted to products. The reaction mechanism gives a description of how reactants are converted to products at the molecular level, and consists of one or more \textit{elementary reactions}, which, in the case of a complex mechanism consisting of more than one elementary step, generally show the presence of reaction intermediates (I), all of which eventually cancel out, leaving only reactants and products. An example is:

\[ \text{reactants } W_1 \]
\[ I_1, W_2 \]
\[ \ldots \ldots \]
\[ I_n, W \text{products} \]

The number of molecules involved in a given elementary reaction is called the molecularity of that reaction. Elementary reactions can be unimolecular (one molecule involved in given elementary reaction), bimolecular (two molecules collide with each other in a given elementary reaction), etc.). Commonly, one finds unimolecular and bimolecular reactions. Ternary elementary reactions are very rare. What commonly happens is that for a given mechanism
consisting of one or more elementary reactions, one of these elementary reactions is the rate determining step and determines the overall rate. Unimolecular reactions that determine the overall rate give rise to first order reaction rates, and bimolecular reactions give rise to second order reactions.

Note that the rate equation above is a differential equation. The integrated form of the rate equation depends on the order of the equation, which in turn depends on the molecularity. Consider a first order reaction corresponding to a unimolecular process:

\[
\frac{d[A]}{dt} = -k[A]
\]

To obtain the integrated form of the rate equation, we separate variables and integrate:

\[
\frac{d[A]}{[A]} = -kdt
\]

\[
[A] = [A]_0e^{-kt}
\]

**Enzyme Kinetics**

In the previous chapter we saw that the simplest mechanism we could envision for an enzyme-catalyzed reaction was

\[
E + S \rightleftharpoons E.S \rightarrow E + P
\]

in which the enzyme combines with substrate to form an enzyme-substrate complex, which can then dissociate to simply release substrate, or can lead to catalysis and formation of product. Note that this is not the overall reaction, but a proposed mechanism that predicts what is happening at the molecular level. Based on what we learned in the last chapter, such a mechanism is reasonable. The rates at which the individual steps in an elementary reaction occur depend on rate constants for those steps, depicted with lower case k’s:

\[
E + S \rightleftharpoons E.S \rightarrow E + P
\]

Since formation of product depends on the enzyme-substrate complex, the rate also depends on the concentration of this complex. Since the elementary reaction depicts what is actually happening at the molecular level, we can write for the rate of reaction the following equation:

rate = \(v\) (velocity) = rate of appearance of product = rate of disapearance of \(E.S\)

\[
v = -\frac{d[E.S]}{dt} = k_2[E.S]
\]
This is a first order reaction, implying that rate depends only on the concentration of one species, E S. For a second-order reaction, the sum of exponents in the rate equation would be two, and the reaction would depend on the collision of two species. For example, formation of the enzyme substrate complex, E S, is a second order process.

Our goal in this chapter will be to derive an expression for v that can is useful. So far the rate equation as shown above expresses a dependence of v on [E S]. The problem is that one cannot typically even verify the existence of E S, much less determine its concentration. In order for the rate equation to be useful, v must be expressed in terms of something we can measure, namely [S]. In order to do this we must be able to express [E S] in terms of [S]. Even for the relatively simple mechanism shown above, this is not possible without making some simplifying assumptions. The steady state assumption assumes that once formed, [E S] remains roughly constant. That is,

\[
\frac{d[E S]}{dt} = 0
\]

That is, it is assumed that the rate of formation of [E S] equals its rate of disappearance, or

\[
k_1[E][S] = k_{-1}[E S] + k_2[E S]
\]

This establishes a relationship between [E S] and the observable, [S]. [E], however is not an observable because it refers to concentration of free enzyme. We can only measure total enzyme. Total enzyme concentration, [E]_T, by conservation of total enzyme, must be either [E S] or [E], or

\[
[E] = [E]_T - [E S]
\]

Now we substitute the right hand side for [E] in the equation that results from the steady state assumption and rearrange to solve for [E S]:

\[
[E S] = \frac{[E]_T}{K_M + [S]}
\]

where the algebra involved results in the grouping of rate constants \((k_{-1} + k_2)/k_1\), which we define as \(K_M\), the Michaelis constant.. Finally, we substitute the right hand side of this equation for [E S] in the expression for v = k_2[E S],

\[ v_0 = \frac{k_2[E][S]}{K_M + [S]} = \frac{V_{\text{max}}[S]}{K_M + [S]} \]

This is the Michaelis-Menten equation. Note the similarity between this expression and that for the saturation of myoglobin. It should come as no surprise that the curve of this equation resembles the oxygen saturation curve for myoglobin (Figure 12-3):

The parameters \( V_{\text{max}} \) and \( K_M \) can be estimated as shown above. Since \( K_M \) is seen to be equal to the substrate concentration at which the reaction rate is half-maximal, an enzyme with a small value of \( K_M \) is clearly more efficient than an enzyme with a large \( K_M \) at a given \([S]\). This can also be seen by considering that \( K_M \) is defined as \( (k_{-1} + k_2)/k_1 \). For many enzymes, formation of the enzyme-substrate does not typically lead to product formation, and \( k_{-1} \) is much larger than \( k_2 \), in which case \( K_M \) is approximately equal to \( k_{-1}/k_1 \) the dissociation constant for enzyme substrate complex, \( K_S \). Smaller values of \( K_S \) indicate a stronger affinity of enzyme for substrate. Thus, in
many cases, $K_M$ is a measure of affinity of enzyme for substrate. Lower values of $K_M$ indicate greater affinity of enzyme for substrate. Since $V_{\text{max}}$ is the limiting value of $v$ at large $[S]$, the magnitude of $V_{\text{max}}$ is seen to be a direct measure of enzyme efficiency. An even better measure of catalytic efficiency is the ratio $V_{\text{max}}/K_M$, or, since $V_{\text{max}} = k_2[E]_T$, $k_2 / K_M$ (Note also that in more complex mechanisms $k_2$ is replaced by $k_{\text{cat}}$). This can be seen by considering typical conditions, such that $[S] \ll K_M$. In such cases the denominator in the Michaelis-Menten equation is approximately equal to $K_m$, and the equation reduces to

$$v_0 = V_{\text{max}} [S]/K_m = k_2/K_m [E]_T[S] - k_2/K_m [E][S],$$

since at low $[S]$ most of the enzyme is free and not present as enzyme substrate complex. This is a second order elementary reaction, with second order rate constant $k_2/K_m$. Thus, $k_2/K_m$ is a measure of how frequently encounters of enzyme with substrate lead to product formation.

More accurate values for the parameters $K_M$ and $V_{\text{max}}$ can be obtained by linearizing the equation for $v_0$, which can be done by taking the reciprocal of both sides of the above rate equation and rearranging to give

$$\frac{1}{v_0} = \left( \frac{K_M}{V_{\text{max}}} \right) \frac{1}{[S]} + \frac{1}{V_{\text{max}}}$$

which has the appearance of a straight line, $y = mx + b$, where $y = 1/v_0$ and $x = 1/[S]$. Graphically we get Figure 12-4. The slope of this plot is $K_m/V_{\text{max}}$, the $y$ $(1/v)$ intercept is $1/V_{\text{max}}$ and the $x$ $(1/[S])$ intercept is $-1/K_M$. 

This is called a double reciprocal, or **Lineweaver-Burke** plot.

Recall that in the above analysis we used the steady-state approximation (concentrations of all intermediates remain approximately constant) to simplify the math. The downside is that because of this simplification, we can never be sure of the exact nature of the intermediates. The best one can do is come up with a reasonable mechanism that is consistent with the kinetic data. If a proposed mechanism is not consistent with the data, it must be rejected.

**Bisubstrate Reactions**

Many enzyme mechanisms are more complex than the simple model described above (Michaelis-Menten). Enzymatic reactions requiring more than one substrate and yielding more than one product are more plentiful. A bisubstrate reaction is depicted below.

\[
A + B \rightleftharpoons WP + Q
\]

These reactions typically involve transfer of a specific group, X, such as a phosphate group, from substrate to another, or, in the case of oxidation reduction reactions, transfer of a pair of electrons.

Variations of bisubstrate reactions include **sequential reactions**, in which all substrates must combine with the enzyme before a reaction can occur and products be released, and **ping pong reactions**, in which one or more products are released before all substrates bind. Using a notation developed by Cleland, different types of sequential and ping pong reactions can be easily identified. For example, an ordered sequential mechanism is shown below:
In this case, substrate A binds before B and P is released before Q. In a random sequential mechanism, either substrate can bind first, followed by release of either P or Q.
An example of a ping pong reaction is shown below in which product P is released prior to binding of the second substrate B:

![Ping Pong Reaction Diagram]

**Enzyme Inhibition**

Enzyme inhibitors are substances that reduce an enzyme's activity. Many drugs are inhibitors. Consider AIDS, for example. This disease is caused by the HIV virus, a retrovirus that injects RNA rather than DNA into the target cell. In order for the viral genome to be incorporated into the host cell DNA, the viral RNA must be converted into DNA, which requires the viral enzyme reverse transcriptase. One of the early drugs used for treatment of AIDS was AZT, an inhibitor of reverse transcriptase. The viral DNA, when expressed, initially results in a viral polyprotein, a single long polypeptide chain which is subsequently cleaved into active viral proteins by the virally encoded HIV protease. More recently, protease inhibitors have proven to be effective in the treatment of AIDS.

Enzyme inhibitors can bind covalently (irreversibly) or noncovalently (reversibly). We briefly considered a covalent inhibitor when we looked at the mechanism of action of serine proteases. Recall that various insecticides and nerve gases irreversibly target the active site serine of such enzymes. Drugs typically bind reversibly and noncovalently. Included in this category are inhibitors whose structures closely resemble the enzyme substrate, and also
inhibitors whose structure do not. Those inhibitors in the former category are competitive inhibitors and we will consider only these (this is a long section and you are not responsible for the rest of it).

As an example of a competitive inhibitor, consider the citric acid cycle enzyme succinate dehydrogenase, which catalyzes the oxidation of succinate to fumarate:

Malonate is structurally similar to succinate, but has one less methylene group, hence cannot be oxidized: Malonate, being structurally similar to succinate, competes with succinate for the active site of the enzyme, but its binding leads to no reaction. Malonate is an effective citric acid cycle inhibitor and you should probably resist any temptation to ingest it.

As a final example of competitive inhibition, consider the methanol - ethanol story. Methanol, as you may know, is toxic not because methanol is itself toxic, but because it is converted to the highly toxic formaldehyde by the liver enzyme alcohol dehydrogenase (see page 373). Ethanol is used to treat methanol poisoning because it competes with methanol for binding to alcohol dehydrogenase, thus inhibiting the production of formaldehyde.

A general model for competitive inhibition is shown below:
Kᵢ is the enzyme inhibitor dissociation constant. In this kinetic scheme, binding of inhibitor leads to no reaction, or inhibition. Since substrate and competitive inhibitor compete for the same binding site, namely the enzyme active site, the effects of the inhibitor can be effectively countered by increasing the substrate concentration, thus Vₘₐₓ should not be affected by an competitive inhibitor. However, the presence of a competitive inhibitor will effectively reduce the affinity of enzyme for substrate, thereby increasing Kₘ (recall that affinity of enzyme for substrate varies indirectly with Kₘ). A Lineweaver-Burke plot showing the effects of a competitive inhibitor, namely increasing Kₘ leaving Vₘₐₓ unchanged, is shown in Figure 12-7.
In noncompetitive inhibition the inhibitor does not bind to the active site. In this case the affinity of the enzyme for the substrate is unchanged because substrate and inhibitor bind to different sites. Inhibition occurs via a decrease in enzyme efficiency. In pure noncompetitive inhibition, $V_{\text{max}}$ is decreased but $K_m$ is unchanged. Compare to the effects of purely competitive inhibition. You should be able to sketch a Lineweaver Burke plot for purely noncompetitive inhibition.

Finally, recall the structural and functional differences between myoglobin and hemoglobin. Hemoglobin is an allosteric protein exhibiting cooperativity and subject to modulation of its oxygen binding properties by allosteric modulators such as $H^+$, $CO_2$ (Bohr effect) and BPG. Allosteric enzymes are similar to hemoglobin in that they two are multiple subunit proteins that exhibit sigmoid activity curves rather than the hyperbolic curve shown in Figure 12-3, and are subject to changes in their activity by allosteric modulators. Allosteric proteins play important roles in regulation of metabolic pathways.

Problems: 1, 2, 5, 10, 19