ENZYMES

A. Definitions

1) Catalysts -- A material that accelerates the velocity of a thermodynamically feasible reaction without itself undergoing a net chemical change.
2) Enzymes -- protein catalysts.
   a) Cells contain inorganic as well as organic compounds that can act as catalysts but the term enzyme is restricted to proteins that catalyze metabolic reactions. For instance chloride ions catalyze the hydrolysis of starch and riboflavin can catalyze the photoreduction of cytochromes but neither is an enzyme.

B. Classification and nomenclature

1) Classification of enzymes is based on the nature of the chemical reaction catalyzed and the name generally describes the specific reaction. The suffix -ase is restricted solely for the names of enzymes but a few discovered before a more systemic nomenclature was introduced do not bear the suffix -ase (i.e., pepsin, renin, ...).
2) Specific classes
   a) Oxidoreductases -- catalyze oxidation-reduction reactions.
   b) Transferases -- catalyze the transfer of a functional group between two compounds.
   c) Hydrolases -- catalyze the cleavage of certain functional groups by water.
   d) Lyases -- catalyze the elimination of groups from a compound leaving double bonds or conversely adding groups to double bonds.
   e) Isomerases -- catalyze isomeric interconversions.
   f) Ligases -- catalyze joining of two molecules coupled to the hydrolysis of pyrophosphate bonds.

C. Descriptive terms

1) Substrate -- a reactant in an enzyme catalyzed reaction.
2) Activity -- rate acceleration produced by the enzyme usually expressed in molar quantities of substrate converted per unit time (eg. µmoles/min.).
3) Specific activity -- catalytic activity of an enzyme, usually expressed as umoles per min per mg protein.
4) k_{cat} -- moles substrate converted to product per second by one mole of enzyme.
5) Specificity -- number of different substrates accepted by the enzyme. Many are highly specific and catalyze the transformation of only a single substrate whereas others will accept hundreds of structurally different compounds.
6) Active site -- that region of the enzyme that interacts with substrate.
7) Cofactors -- Nonprotein compounds required in large molar excess over the enzyme for activity.
8) Prosthetic groups -- Nonprotein components tightly bound in a fixed molar ratio with enzyme protein (often covalently linked to the enzyme).
9) Coenzyme -- A term approved only for the name of coenzyme A. All other uses should be avoided.
10) Catalytic cosubstrates -- Cellular metabolites consumed in some reactions and continuously regenerated by others.
11) V_{max} -- the velocity of an enzyme catalyzed reaction at saturating (infinite) substrate concentrations.
12) K_m (Michealis constant) -- The concentration of substrate when the velocity = $V_{max}/2$. This constant has no intrinsic mechanistic significance and is usually not, a measure of enzyme-substrate affinity.
13) Inhibitors -- compounds that inhibit enzyme-catalyzed reactions. Many drugs, either directly or as metabolic products, fall in this category. Most useful are those selective for a single enzyme.
14) Activators -- Compounds, often metabolites, that stimulate an enzyme-catalyzed reaction.

D. Mechanism(s) of enzyme action

1) Enzymes accelerate a reaction by lowering the energy barrier (activation energy) of a feasible reaction. This is accomplished by formation of an enzyme-substrate complex as shown by the following equation.

$$E + S \Leftrightarrow E{\bullet}S \Rightarrow E + P$$

2) Positioning of substrate at the active site is directed by amino acid side chains, and/or prosthetic groups that form a three dimensional structure complementary with the activated (transition) state of the substrate.

E. Factors that affect enzyme activity

1) pH -- changes in pH can influence activity in three different ways.
   a) Reactions where protons are either a substrate or product.
   b) Changing the concentration of active substrate by protonation/deprotonation of acidic or basic groups on the substrate.
   c) Changes in pH can also alter the charge of enzyme ionizable groups which may modify the tertiary structure of the enzyme.
2) Temperature -- The velocities of chemical reactions increase with temperature and the rate of an enzyme-catalyzed reaction will increase with temperature as long as the enzyme is not denatured. Experimentally determined optimal temperatures are strongly influenced by the assay method and have no particular physiological significance.
3) Concentration of enzyme and substrate -- The velocity is usually directly proportional to enzyme concentration but because the concentration of enzyme is usually much less than substrate, the enzyme becomes limiting at saturating substrate concentrations. Thus, at high concentrations of substrate, activity no longer changes with increasing substrate.

ENZYME KINETICS

Enzyme kinetics lies at the heart of drug design and metabolic regulation. It also has many industrial and medical applications including widely used diagnostic tests for tissue damage. Yet despite its fundamental importance, enzyme kinetics is one of the more misunderstood (and often misused) tools in biochemistry. The presentation in introductory texts is often more complex than necessary. In addition, underlying basic concepts and units are not defined or simply ignored on the assumption that students are familiar with these topics. While perhaps not always true, every class has a substantial number of students that fail to retain (or make a deliberate effort to forget) basic definitions and concepts covered in introductory courses. The following sections describe the simplest approach possible to the principles of enzyme kinetics. This handout is intended to supplement, not replace, the chapters on enzymes in the text.

The preceeding section of this handout summaries other facets of enzymes along with a glossary of terms used to describe properties of enzymes. The definitions listed follow recommendations of the Nomenclature Committee of the International Union of Biochemistry. A few differ from those used in the text and I would recommend that you learn definitions recommended by IUB. It may take a few years, but these will undoubtedly be adopted by most texts in the next 10-15 years.

Derivation of the Michaelis-Menten Rate Equation

By the turn of the century, several investigators had shown that the velocity (**v**) of enzyme-catalyzed reactions at constant substrate [S] was directly proportional to the concentration of enzyme as illustrated in Figure 1. That is, doubling the amount of enzyme doubled the velocity. On the other hand, at fixed concentrations of enzyme, the velocity as a function of substrate concentration produced a hyperbolic curve (Figure 2).

The equation for a rectangular hyperbola is

$$y = \mathbf{a}x/\mathbf{b}+x \qquad \textbf{(1)}$$

where the constant **a** is the value of y at infinite x and **b** is the value of x when y = **a**/2.

Based on the earlier suggestions of Fischer and of Henri that enzyme catalyzed reactions proceed in two discrete stages as shown in Equation **2**, Leonor Michaelis and Maud Menten derived a rate equation defining the constants **a** and **b** in the equation for a rectangular hyperbola (Equation **1**)

$$E + S \Leftrightarrow E{\bullet}S \Rightarrow P + E \quad \textbf{(2)}$$

In deriving the rate equation for the model shown in Equation **2**, they assumed that the concentration of [E] was always much less than [S] and that formation of the E•S complex was an obligatory intermediate in the conversion of S to P. It therefore follows that at very high concentrations of S, all of E will be saturated with S and adding more substrate will not make the reaction go any faster. For any fixed concentration of enzyme, the velocity at saturating substrate will be the maximum possible and will be equal to **a** in Equation **1**. It is usually denoted as V_{max} or simply as capital V.

To define the other constant in Equation **1**, Michaelis and Menten assumed that the velocity of the reaction as a function of substrate concentration was only dependent on the equilibrium constant for the dissociation of the E•S complex into E and S which they designated as K_s. Substituting this term for **b** in Equation **1** and S and **v** for the variables x and y gives the well known Michaelis-Menten rate equation

$$\mathbf{v} = \frac{V}{1 + K_s/S} \quad \textbf{(3)}$$

Please note that when **v** = V/2, the concentration of S is equal to K_s.

Although Equation **3** fits the experimental data in the vast majority of cases, this does not prove that assumptions used in its derivation are correct. In fact, the constant **b** in Equation **1** is almost never equal to K_s. This point will be taken up later in a section on the interpretation of constants in Equation **3**. However, Michealis and Menten were the first to derive an equation consistent with most of the experimental data available at that time. To honor this achievement, K_s in Equation **3** is called the Michaelis constant -- abbreviated as K_m. Rearranging Equation **3** gives Equation **4,** which is the form of the Michaelis-Menten rate equation listed in most texts.

$$\mathbf{v} = \frac{V_{max}\,[S]}{K_m + [S]} \quad \textbf{(4)}$$

Briggs-Haldane Steady-State Derivation – This is the graph we drew in class with [S], [E]. [ES], and [P]

Briggs and Haldane provided a kinetic approach for the derivation of Equation **4** that expresses the constants K_m and V_{max} in terms of the rate constants [1] in Equation **5**.

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} E{\bullet}S \overset{k_2}{\Rightarrow} E + P \quad \textbf{(5)}$$

This derivation assumes that upon mixing enzyme and substrate, the concentration of E•S rises rapidly and reaches a steady state where its rate of formation, $\mathbf{v}_1$, (Equation **6**) is equal to its rate of decomposition, $\mathbf{v}_2$, (Equation **7**). Although many enzymes also catalyze the conversion of P to S, if the rate of the forward reaction is measured before a significant amount of S is converted to P, the back reaction can be ignored.

$$\mathbf{v}_1 = k_1[E][S] \quad \textbf{(6)}$$

$$\mathbf{v}_2 = k_{-1}[E{\bullet}S] + k_2[E{\bullet}S] \quad \textbf{(7)}$$

While the concentration of free enzyme [E] cannot be measured, the total amount added $[E_t]$ is known and at any time

$$[E] = [E_t] - [E{\bullet}S] \quad \textbf{(8)}$$

Substituting this value for [E] in Equation **6** gives and assuming that upon reaching steady state, $\mathbf{v}_1 = \mathbf{v}_2$

$$k_1[E][S] = k_{-1}[E{\bullet}S] + k_2[E{\bullet}S] \quad :$$

$$k_1([E_t] - [E{\bullet}S])[S] = (k_{-1} + k_2)[E{\bullet}S]$$

Rearranging to collect the rate constants gives Equation **9.**

$$\frac{([E_t] - [E \bullet S])\ [S]}{[E \bullet S]} = \frac{k_{-1} + k_2}{k_1} = K_m \qquad \textbf{(9)}$$

Solving Equation **9** for $[E \bullet S]$ gives:

$$[E \bullet S] = \frac{[E_t]\ [S]}{K_m + [S]} \qquad \textbf{(10)}$$

The model (Equation **5**) states that at any concentration of [S]

$$\mathbf{v} = k_2[E \bullet S] \qquad \textbf{(11)}$$

and at saturating [S]

$$V_{max} = k_2[E_t] \qquad \textbf{(12)}$$

Substituting these values for $[E_t]$ and $[E \bullet S]$ in Equation **10** gives a rate equation identical to the one derived by Michealis and Menten.

$$\mathbf{v} = \frac{V_{max}[S]}{K_m + [S]} \qquad \textbf{(13)}$$

This is the fundamental rate equation in enzyme kinetics. Although the equation was derived for a unimolecular reaction (a single substrate), the equation is readily adapted for enzymes catalyzing di- and tri-molecular reactions.

You do not have to remember how to derive Equation **13** but memorize it and try to understand its applications and limitations. (The same equation can be used to describe any biological process that follows saturation kinetics, but interpretation of the constants will change.)

The concentration of enzyme is buried in the V_{max} term in Equation **13** which can be a source of confusion. This can be avoided by simply substituting $E_t \bullet k_2$ (or $E_t \bullet k_{cat}$) for V_{max}. This will simply remind you that the **v** is always directly proportional to E_t -- the total amount of enzyme present.

Interpretations of V_{max} and K_m

The meaning of V_{max} is easy. It is the velocity at infinite substrate concentration. While infinite concentrations cannot be reached experimentally, 90-95% saturation are usually possible and if enough measurements are carried out above and below K_m, V_{max} (or k_{cat}) can be calculated by extrapolation. [2]

The definition of K_m is also straight forward. K_m is always equal to the concentration of substrate when **v** = $V_{max}/2$. You can prove this by substituting $V_{max}/2$ for **v** in Equation **13**. K_m is an experimental constant that depends heavily on the conditions used to measure **v**. Change the conditions (i.e. pH, ionic strength, nature of the buffer, temperature, etc.) and K_m will change. It is, therefore, wrong to express this constant as an apparent K_m [K_m(app)]. This implies that somewhere in its natural environment there is a "true" K_m that can be approximated but never measured. Such mythical K_m's do not exist. Even within the cell where you have a constantly changing environment, K_m for many enzymes is not constant. Remember that K_m is only an experimentally determined value which, if you are a careful worker and describe assay conditions exactly, can be repeated by others.

The other more serious misinterpretation of K_m goes all the way back to the original assumption by Michaelis and Menten that K_m (K_s is their derivation) was equal to the enzyme-substrate dissociation constant and that breakdown of the E•S complex to product and free enzyme did not affect the velocity of the reaction. This assumption repeated in many texts has bedeviled much of our biochemical literature ever since. As long as you do not have to think seriously about the meaning(s) of K_m, it is rather satisfying to equate K_m with enzyme-substrate affinity or binding.

However, Equation **9** in the Briggs-Haldane derivation shows that K_m is a kinetic constant not a thermodynamic equilibrium constant. If you examine Equation **9** and the kinetic model in Equation **5**, it is evident that k_2 is always present in K_m. Only when k_2 is much less than k_{-1} does K_m approach K_s.

However this is never true for enzymes catalyzing essentially irreversible reactions which are usually highly regulated and at the center of metabolic control. They are also the favorite targets for drug design. For such enzymes, the back reaction does not take place and k_{-1} can be ignored. K_m for these, and perhaps for most enzymes, is the product of two forward rate constants as shown in Equation **14**.

$$K_m = k_2/k_1 \qquad \textbf{(14)}$$

The rate constant k_2 is usually identical (or very close) to the turnover number written as k_{cat} which has the dimensions of a first order rate constant min^{-1} or sec^{-1}. This constant is in fact the first order rate constant for the breakdown of E•S to E and P and describes the reaction velocity in Figure 2 where substrate approaches

$$E{\bullet}S \overset{k_{cat}}{\Rightarrow} E + S \qquad \textbf{(15)}$$

saturation and the rate only depends upon the concentration of E•S. However, at very low substrate, the velocity depends on <u>both</u> [E] and [S] and we need a second order rate constant which in this portion of the curve is equal to the slope (k_{cat}/K_m). The dimensions of this constant (M^{-1}, sec^{-1}) are those of a second order reaction. According to this model, the leveling off of v with increasing [S] (Figure 2) represents the transition from the second order dependence on <u>both</u> [E] and [S] to a first order dependence on [E] alone. K_m is simply the midpoint in this transition.

While K_m can have a number of different mechanistic interpretations, it is, along with k_{cat} , a useful number for calculating other facets of enzyme activities. These applications along with methods for measuring K_m and k_{cat} are adequately treated in most texts and will not be taken up here.

Footnotes:

(1) Rate constants are among some of the first things some students would like to forget. However, you cannot function in every day life without them. So what are they and why are they important? In first grade you probably learned that you cannot add, subtract, or equate apples and paper (or plastic). However, you probably do this every time you go to the grocery store. Rate constants called price makes this possible. Rate constants allow you to equate all kinds of things that are inherently unequal and they come with different dimensions. Here we will be concerned with only two -- first and second order rate constants. First order rate constants describe the rate at which a single reactant goes to products (i.e. the breakdown of E•S to E and P). First order rate constants have the dimensions sec^{-1} or min^{-1}. On the other hand, second order rate constants contain a molar term in addition to reciprocal time and describe the velocity at which two reactants get together to form products. The dimensions of a second order rate constant are M^{-1}, sec^{-1}.

Some texts also discuss zero order reactions. This can be confusing and is best ignored. It does not mean that the reaction does not require a reactant, but only that there is already so much reactant present that adding a little bit more won't make much of a difference.

Also always remember that the subscript specifies a specific reaction and is valid only for that reaction.

(2) Enzyme kineticists are a hardy breed quite happy to live in a world of approximations and rarely carry out calculations requiring more than two significant figures. While this facilitates progress in kinetics, you do not want an enzyme kineticist to balance your check book. The bean counter at banks and at other equally nefarious institutions take a dim view of approximations.