

# 1 A Brief Review of Enzyme Kinetics

Enzymes are proteins which catalyze chemical reactions that usually, but not always, take place within the cell. All proteins of cells, including enzymes, are synthesized by ribosomes. Ribosomes synthesize both “inner-use” proteins (those used within the cell and synthesized by ribosomes randomly distributed in the cell) and “outer-use” proteins. These latter proteins are synthesized by ribosomes which are attached to the membranes of the endoplasmic reticulum. This system of membranes collects these proteins which are eventually exported from the cell.

Enzymes are of primary importance in metabolic pathways which would otherwise require large amounts of energy (heat) to catalyze chains of chemical reactions. Enzymes (which are known to be highly specific for both substrate and reaction type) allow these reactions to take place at a rapid rate at lower temperatures. Roughly speaking, an enzyme joins with its substrate and lowers the energy requirements for activation of the reaction, the reaction occurs, and the enzyme is then released unchanged to be used again. This can be described in the so-called

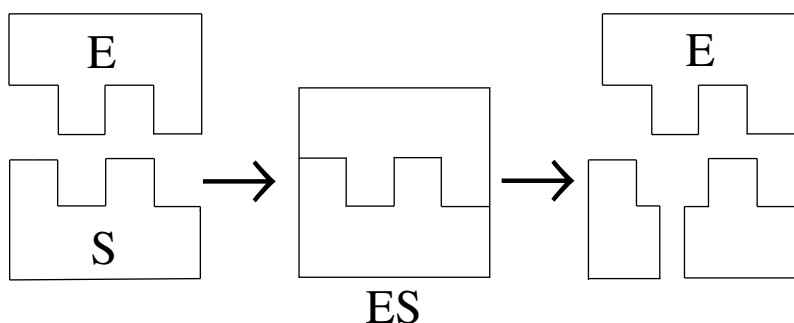


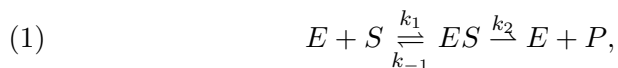
Figure 1:

“lock-and-key” theory which assumes that the structure (shapes) of the enzyme and substrate molecules explain the specificity and inhibition features observed in enzymatic reactions. The schematic in Figure 1 is somewhat misleading since enzyme molecules are usually quite large and exceedingly complex in structure, and may possess a number of “active” or reaction “sites”. For example, the substrate usually occupies only 10% of the enzymes surface during the reaction. In addition, the reactions sometimes requires accessory substances which may be lightly bound to the enzyme

molecule during the reaction. Another important fact that will be recalled in Chapter 3 is that enzymes are usually present at extremely low inter-cellular concentrations (e.g.  $10^{-7}$  molar) and only small quantities of the enzyme are needed to catalyze the reaction. Finally, although we shall not study the control of enzyme levels within the cell in these notes, we point out that there is a highly complex homeostatic system involving synthesis and inhibition which regulates these levels.

The kinetics (dynamics) involved in enzymatic reactions have traditionally been modelled by ordinary differential equations. A very important formulation much used (misused and abused) by modelers involves the *initial reaction velocity* expressions usually associated with the names Henri [7], Michaelis-Menten [9], and Briggs-Haldane [3]. We develop briefly here the theory underlying these expressions (studied in the first quarter of the 20<sup>th</sup> century and subsequent modifications).

We consider a single substrate plus enzyme to product reaction



where it is assumed that an intermediate substrate-enzyme complex  $ES$  is formed. Further, the reaction  $ES \rightarrow E + P$  is assumed *irreversible* (in many cases the back reaction rate is so small that it may be ignored). Additional assumptions sometimes involved which are often not clearly stated are:

- (i) only *initial* reaction rates are considered, the decline of reaction velocity due to decline in substrate concentration being ignored;
- (ii) there is an *excess* of substrate  $S$  in a solution with the enzyme  $E$ ;
- (iii) the rate constant  $k_2$  is small compared to  $k_{-1}$  ( $k_{-1} \gg k_2$ ) and the reaction  $E + S \rightleftharpoons ES$  reaches equilibrium very quickly and maintains it throughout the overall reaction;
- (iv) after a negligible time, the rate of formation and dissociation of the complex  $ES$  becomes and remains very small compared to the rate of changes for  $S$  and  $P$ .

Assumption (iii) is sometimes referred to as the “equilibrium” assumption while (iv) is often termed the “steady-state approximation” or “steady-state” assumption.

We may, under assumption (i), write the nonlinear kinetic velocity expressions

$$\begin{aligned}
 \frac{d[S]}{dt} &= -k_1[E][S] + k_{-1}ES \\
 \frac{dES}{dt} &= -(k_{-1} + k_2)ES + k_1[E][S] \\
 \frac{d[P]}{dt} &= k_2ES
 \end{aligned}
 \tag{2}$$

where  $[E]$  is the concentration (in molars) of free enzyme,  $[S]$  the concentration of free substrate, and  $ES$  the concentration of the enzyme of the enzyme-substrate complex. Also, we have the conservation law ( $at = 0, [P] = 0$ )

$$\begin{aligned}
 E_T &= [E] + ES \\
 S_T &= [S] + ES
 \end{aligned}
 \tag{3}$$

where  $E_T, S_T$  represent fixed concentration of the total (free and bound) amounts of enzyme and substrate present, respectively. A fourth nonlinear expression for  $\frac{d[E]}{dt}$  follows immediately from the first conservation law and the second equation in (2) and thus need not be written. The assumption (ii) allows one to approximate, replacing the second equation in (3) by  $S_T \approx [S]$ .

The Michaelis-Menten derivation else uses assumption (iii), which allows to write

$$\begin{aligned}
 0 &= -k_1[E][S] + k_{-1}ES \\
 &= -k_1(E_T - ES)[S] + k_{-1}ES.
 \end{aligned}$$

So that one finds

$$ES = \frac{E_T[S]}{\frac{k_{-1}}{k_1} + [S]}.$$

One thus obtains the familiar expression for the initial velocity of product formation

$$\frac{d[P]}{dt} = v = \frac{k_2 E_T [S]}{K_M + [S]} = \frac{V_{\max} [S]}{K_M + [S]}$$

or

$$v = \frac{V_{\max} S_T}{K_M + S_T}
 \tag{4}$$

where  $V_{\max} \equiv k_2 E_T$  and  $K_M \equiv k_{-1}/k_1$ .

In the Briggs-Haldane modification (in which one replaces assumption (iii) by (iv)) one uses the steady-state approximation to write

$$\frac{dES}{dt} = 0$$

or

$$0 = -(k_{-1} + k_2)ES + k_1[E][S],$$

which, upon use of the first equation in (3), yields

$$(5) \quad ES = \frac{E_T[S]}{\frac{k_{-1}+k_2}{k_1} + [S]}.$$

The initial velocity expression is thus found to be

$$(6) \quad v = \frac{V_{\max} S_T}{K_M + S_T}$$

where once again  $V_{\max} \equiv k_2 E_T$ , but now the ‘‘Michaelis constant’’  $K_M$  is given by

$$(7) \quad K_M = \frac{k_{-1} + k_2}{k_1}.$$

The initial velocity expression (4), (6) and its relation to transient terms can also be discussed using more sophisticated arguments involving singular perturbations. We shall not present these here, but instead refer the interested reader to [5, 8, 10, 11].

We note that at maximum velocity one has no free enzyme so that  $E_T = ES$  and hence  $v = k_2 ES = k_2 E_T$ , thus justifying the definition  $V_{\max} \equiv k_2 E_T$  made above. We also point out that assumption (iii) may be considered a special case of (iv) and, as Briggs and Haldane have observed, one may in fact have (iv) obtaining even though  $k_{-1} \gg k_2$  (this is a statement about certain, perhaps unobservable, parameters characteristic of the particular reaction under consideration) cannot be verified.

In the Michaelis-Menten derivation, the constant  $K_M$  is an approximate value of the dissociation constant for  $S + E \rightleftharpoons ES$ , while in the Briggs-Haldane modification the Michaelis constant (being a function of the kinetic

parameters  $k_{-1}, k_1, k_2$ ) has no simple theoretical significance. However,  $K_M$  does have practical significance in either situation. At  $v = \frac{1}{2}V_{\max}$  the expressions (4), (6) yield  $S_T = K_M$ . Indeed, one interpretation (which is sometimes used as a definition in the derivation of the velocity expressions) of the Michaelis constant  $K_M$  is that  $K_M$  is that value of substrate concentration  $S_T$  which yields a reaction velocity one-half the maximum velocity (i.e.  $v = \frac{1}{2}V_{\max}$ ).

In discussing the parameters  $K_M, V_{\max}$  which are taken as characteristic parameters for a specific reaction, biochemists often use the so-called Lineweaver-Burk plot. In this graph one takes reciprocals in (6), obtaining  $\frac{1}{v} = \left(\frac{K_M}{V_{\max}}\right) \frac{1}{S_T} + \frac{1}{V_{\max}}$ , and then plots  $1/v$  vs.  $1/S_T$  and abscissa intercept  $-1/K_M$ . Values for  $K_M, V_{\max}$  are usually determined for a particular reaction by measuring reaction velocities at a fixed enzyme concentration and at various substrate concentrations, plotting the resulting Lineweaver-Burk curve, and reading the intercept values.

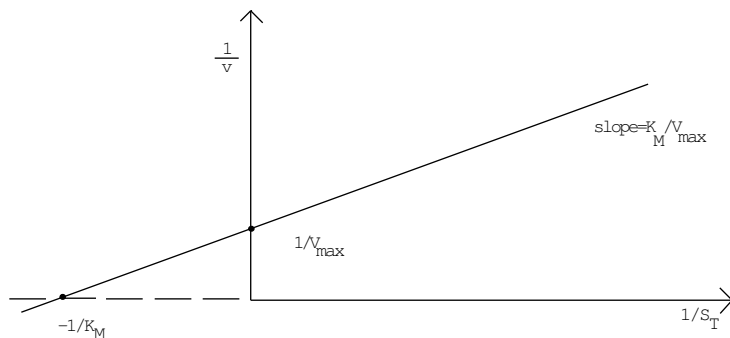


Figure 2:

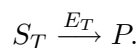
Numerical values for  $K_M$  are found to range from 1 to  $10^{-8}\text{M}$  (molar). Since in some cases (see assumption (iii)) the value of  $K_M$  is an approximation to the extent of dissociation for the ES complex, biochemists often equate “low  $K_M$ ” and “high degree of affinity of enzyme for substrate” when discussing this characteristic parameter.

Another useful concept often found in the literature is that of “turnover number”. If one calculates the hypothetical maximum conversion rate to be expected per unit molar concentration of enzyme, one obtains the turnover number  $TN$  for an enzyme. That is,  $TN = V_{\max}/E_T$  and hence from our above discussion we see that  $TN = k_2$ , where the rate constant is now expressed in terms of the maximum number of moles of substrate converted

per minute per mole of enzyme. Numerical values of  $TN$  have been found to range from 6 to  $17 \times 10^6$ .

For many investigations, the underlying assumptions detailed above in deriving (4), (6) are much too stringent and there have thus been a number of modifications proposed (see, for example, [2, 4, 6]). In particular, assumption (ii) is often quite objectionable. In our work on enzyme cascades detailed in substrates only roughly ten times those of the enzymes at each stage in the cascade. Therefore velocity expressions derived under assumption (ii) above are inadequate for use in such instances.

We further note that in many mathematical uses of the reaction velocity expressions one wishes to ignore the formation of the intermediate  $ES$  complex and consider the reaction (1) as one simply of the form



Use of the expressions (4), (6) as velocity terms then involves either very crude approximations or an implicit assumption of the form (ii), which in some cases is undesirable.

For the modifications discussed here, we drop the assumption (ii), retaining only (i) and (iv), which again imply  $dES/dt = 0$ . Defining  $K_M$  as in (7), we obtain

$$ES = \frac{[E][S]}{K_M} = \frac{(E_T - ES)(S_T - ES)}{K_M}.$$

This can be written

$$(8) \quad (ES)^2 - (K_M + S_T + E_T)ES + E_T S_T = 0$$

which yields

$$(9) \quad ES = \frac{1}{2} \left\{ (K_M + S_T + E_T) - \sqrt{(K_M + S_T + E_T)^2 - 4E_T S_T} \right\},$$

where we have chosen the smaller root (minus sign) so that at  $E_T = 0$ ,  $S_T = 0$  the expression yields  $ES = 0$ . Then we obtain

$$(10) \quad v = \frac{V_{\max}}{2E_T} \left\{ (K_M + S_T + E_T) - \sqrt{(K_M + S_T + E_T)^2 - 4E_T S_T} \right\}.$$

On the other hand, if  $ES \ll K_M + E_T + S_T$  (which is true if  $K_M \gg E_T > ES$ ), we may approximate the equation (8) by

$$-(k_M + S_T + E_T)ES + E_T S_T = 0$$

or

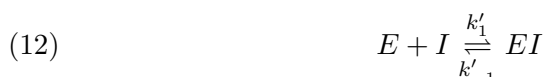
$$ES = \frac{E_T S_T}{K_M + S_T + E_T}.$$

With  $V_{\max}$  defined as above, this yields

$$(11) \quad v = \frac{V_{\max} S_T}{K_M + S_T + E_T}.$$

This expression will be used in several aspects of the investigation of cascade models discussed in Chapter 3 below.

We return to the Briggs-Haldane formulation and indicate the changes involved if a “competitive inhibitor” is added to the reaction represented by equation (1). A competitive inhibitor is an inhibitor (chemical reagent which inhibits the catalytic action of the enzyme) whose action can be reversed by increasing the concentration of the substrate. That is, one may consider that the inhibitor and substrate “compete” for the “active site” of the enzymes, with inhibition taking place if the inhibitor occupies the site. To the equation (1) we must adjoin



and the first equation in (3) must be replaced by

$$(13) \quad E_T = [E] + ES + EI,$$

where  $[I]$  is the concentration of the inhibitor and  $EI$  the concentration of the enzyme-inhibitor complex. The kinetic equations (2) are still valid, but must be supplemented with another equation

$$\frac{d}{dt} EI = k'_1 [E][I] - k'_{-1} EI.$$

A steady-state assumption (iv) for (1) and an equilibrium assumption for (12) yield the approximations

$$\frac{d}{dt}ES = 0, \quad \frac{d}{dt}EI = 0$$

from which it follows that

$$(14) \quad ES = \frac{[E][S]}{K_M}, \quad EI = \frac{[E][I]}{K_I}$$

where  $K_I = k'_{-1}/k'_1$ . Using (13),

$$E_T = [E] + ES + \frac{[E][I]}{K_I} = ES \left( 1 + \frac{[I]}{K_I} \right) [E]$$

we obtain from the first equation in (14)

$$K_M ES = [S] \left\{ \frac{E_T - ES}{1 + [I]/K_I} \right\}$$

or

$$\left\{ K_M + \frac{K_M[I]}{K_I} + [S] \right\} ES = E_T[S].$$

We thus find

$$ES = \frac{E_T[S]}{K_M + \frac{K_M[I]}{K_I} + [S]}$$

and hence

$$(15) \quad v = \frac{V_{\max}[S]}{K_M + \frac{K_M[I]}{K_I} + [S]}.$$

If we again approximate by  $S_T \approx [S]$ , we finally have



$$(16) \quad v = \frac{V_{\max}S_T}{K_M \left(1 + \frac{[I]}{K_I}\right) + S_T}$$

In a similar manner, one may derive modified velocity expressions under relaxed assumptions (see (8)-(11) above) in the case of the presence of a competitive inhibitor. For example, if one assumes only (i) and (iv) and ignores terms  $(ES)^2$  as in the derivation of the (1) above, one obtains

$$ES = \frac{E_T S_T}{K_M \left(1 + \frac{[I]}{K_I}\right) + S_T + E_T}$$

which then implies

$$(17) \quad v = \frac{V_{\max}S_T}{K_M \left(1 + \frac{[I]}{K_I}\right) + S_T + E_T}$$

Velocity expressions for reactions in the presence of other types of inhibitors are also easily derived. For a so-called “noncompetitive inhibitor”  $I$  (the inhibitor binds to both free enzyme and the enzyme-substrate complex) one must add to equations (1) and (12) the equation



The conservation laws become

$$\begin{aligned} E_T &= [E] + ES + EI + IES \\ S_T &= [S] + ES + IES. \end{aligned}$$

Making a steady-state assumption (iv) for (1) and equilibrium assumption for (12) and (18), one can carry out arguments similar to those already detailed to obtain a Briggs-Haldane-type expression ( $S_T \approx [S]$ )

$$(19) \quad v = \frac{V_{\max}S_T}{K_M \left(1 + \frac{[I]}{K_{iE}}\right) + S_T \left(1 + \frac{[I]}{K_{iC}}\right)}$$

where  $K_M$  is again given by (7),  $K_{iE} = k'_{-1}/k'_1$ ,  $K_{iC} = \tilde{k}_{-1}/\tilde{k}_1$ .

If one doesn't assume excessive substrate (i.e. uses the conservation law instead of  $S_T \approx [S]$ ), the modified velocity expression obtained is

$$(20) \quad v = \frac{V_{\max} S_T}{K_M \left(1 + \frac{[I]}{K_{iE}}\right) + (S_T + E_T) \left(1 + \frac{[I]}{K_{iC}}\right)}.$$

In the case of an ‘‘uncompetitive inhibitor’’ I (the inhibitor binds only to the enzyme-substrate complex, one uses equations (1) and (18) with the conservation laws

$$\begin{aligned} E_T &= [E] + ES + IES \\ S_T &= [S] + ES + IES. \end{aligned}$$

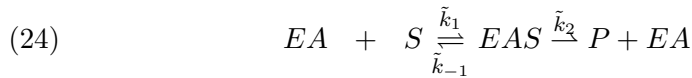
The usual arguments (steady-state assumption on (1), equilibrium assumption on (18), and  $S_T \approx [S]$ ) yield a Briggs-Haldane-type velocity expression

$$(21) \quad v = \frac{V_{\max} S_T}{K_M + S_T \left(1 + \frac{[I]}{K_I}\right)}$$

with  $K_M$  as in (7) and  $K_I \equiv \tilde{k}_{-1}/\tilde{k}_1$ . The velocity expression under the modified assumptions becomes

$$(22) \quad v = \frac{V_{\max} S_T}{K_M + (S_T + E_T) \left(1 + \frac{[I]}{K_I}\right)}.$$

In addition to inhibitors, one may also have ‘‘activators’’ which combine with the enzyme to promote the reaction. Suppose, for example, that one has a activator  $A$  which combines with the enzyme  $E$  to form an enzyme-activator complex  $EA$  which may also act as an ‘‘enzyme’’ for the substrate  $S$ . Then the stoichiometric equations are (1) plus



and the velocity of product formation is

$$(25) \quad v = \frac{d[P]}{dt} = k_2 ES + \tilde{k}_2 EAS.$$

Under the steady-state assumption for (1) and (24), and equilibrium assumption for (23), and the excessive substrate assumption ( $S_T \approx [S]$ ), one obtains the Briggs-Haldane-type velocity expression

$$(26) \quad v = \frac{k_2 S_T E_T}{\left(1 + \frac{[A]}{K'_{eq}}\right) K_M + \left(1 + \frac{[A]K_M}{K'_{eq}\tilde{K}_M}\right) S_T} + \frac{\tilde{k}_2 [A] S_T E_T}{\left([A] + K'_{eq}\right) \tilde{K}_M + \left([A] + K'_{eq} \frac{\tilde{K}_M}{K_M}\right) S_T}$$

where  $K_M \equiv \frac{k_{-1}+k_2}{k_1}$ ,  $\tilde{K}_M \equiv \frac{k'_{-1}}{k'_1}$ , and  $[A]$  is the concentration of free activator. Here  $k_2, \tilde{k}_2$  can be interpreted as the turnover numbers  $TN_E, TN_{EA}$  for the enzyme and enzyme-activator complex respectively.

If one does not make the excessive substrate assumption but uses instead the conservation law

$$S_T = [S] + ES + EAS,$$

the modified velocity term obtained (again ignoring certain higher-order terms) analogous to (11) is

$$(27) \quad v = \frac{k_2 S_T E_T}{\left(1 + \frac{[A]}{K'_{eq}}\right) K_M + \left(1 + \frac{[A]K_M}{K'_{eq}\tilde{K}_M}\right) (S_T + E_T)} + \frac{\tilde{k}_2 [A] S_T E_T}{\left([A] + K'_{eq}\right) \tilde{K}_M + \left([A] + K'_{eq} \frac{\tilde{K}_M}{K_M}\right) (S_T + E_T)}$$

If one makes the assumption of excessive activator (often useful in utilizing the expressions (26), (27) in modelling) so that  $A_T \approx [A]$ , where  $A_T =$  concentration of total (free and bound) activator present, then of course the velocity terms (26), (27) are the same except that  $[A]$  is replaced by  $A_T$  in these expressions.

In the next two chapters we shall use the velocity expressions developed above to discuss two areas of modelling where optimality ideas have been fruitfully employed.

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