More Complex Rate Expressions

16

Henri–Michaelis–Menten equation is the simplest rate law for an isolated enzyme-catalyzed reaction under clearly specified conditions. Not all enzyme reactions are this simple. More complex mechanisms may involve (a) multiple intermediates/complexes (not just one *ES* complex) and (b) more than one substrate and/or product. Indeed most common enzyme mechanisms found in metabolism are reactions with two substrates and two products. The remarkable success of Michaelis–Menten formalism over the last century has led to extension of this classical approach to more complex systems.

16.1 Investigating Enzyme Mechanisms Through Kinetics

Kinetic description of a complex enzyme-catalyzed reaction provides insights into its mechanism. Traditionally, kinetic studies are closely associated with the investigations of enzyme mechanism. They are primarily used to understand reaction mechanisms. The process of elucidating reaction mechanisms through kinetics is an excellent example of the *scientific method* in practice. The sequence of steps, involved in this process of mechanism building, is shown in the box below.

Mechanism Building: The Process

(a) Collate all the available data that describes the enzyme reaction in question.
(b) Postulate a minimal mechanism that accounts for all the enzyme behavior and accommodates available data.
(c) Analyze the proposed mechanism by deriving a rate equation for it.
(d) Predict distinctive kinetic outcomes from the analysis.

(continued)

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(e) Test these predictions by performing critical experiments.
(f) Accept or reject the proposed mechanism based on these results.
(g) If rejected, suitably modify the proposed mechanism and iterate.

The usual general practice in arriving at any enzyme mechanism is to set up a tentative reaction scheme on the basis of initial, often preliminary, evidence and subsequently to test whether this describes the experimental results. If it does not, the initial scheme has to be modified or replaced. The process is repeated until some enzyme mechanism has been developed which accounts for all the experimental results – rates, complexes, substrates, and product stoichiometries. The provisional mechanism is further strengthened by any new supporting data. However, a single piece of contradictory evidence is enough to discredit the mechanism. The mechanism that survives this scrutiny is considered consistent with the experimental data. In this sense, even an attractive mechanism – which is always a hypothesis – can never be proven beyond doubt.

There is no panacea for coming up with the correct model for a complex enzyme mechanism. Model building exercise and mechanism validation are legitimate part of most kinetic analysis. This is iterated till a satisfactory explanation (mechanism!) is in place. Other chemical and physical methods may help bolster the case – but *kinetic data should be the ultimate arbiter of mechanism* – because it reports on the behavior of an enzyme while in action. Rate equations are at the heart of any kinetic study. They capture the essence of a mechanism in a mathematical form. If an enzyme reaction is well described by a rate equation, then the data should fit that equation well. While proposing a kinetic mechanism and describing a rate equation for it, the following points need to be kept in mind.

- Keep the proposed mechanism as simple as possible. Complexity should not be assumed unnecessarily ("Pluralitas non est ponenda sine necessitate" – the Occam's razor, William of Occam) (Wildner 1999). According to this principle of parsimony, hypotheses should not be multiplied beyond necessity ("Entia non sunt multiplicanda praeter necessitatem"). For example, it is meaningless to propose a complex mechanism and invoke many intermediates (*ES* complexes) without actual evidence. This simplicity paradigm of scientific inquiry was also invoked by Newton when he said – "We are to admit no more causes of natural things than such as are both true and sufficient to explain their appearances. To this purpose the philosophers say that Nature does nothing in vain, and *more is in vain when less will serve*; for nature is pleased with simplicity, and affects not the pomp of superfluous causes" (Newton's Principia).
- Process of elucidating enzyme mechanisms works best when alternative mechanisms are postulated. Start with multiple working hypotheses (alternative mechanisms). It is easier to disprove a mechanism (falsification strategy of Popper) than to prove a mechanism with certainty. Kinetics is of great value as it can be used to test and eliminate putative mechanism(s). Beautiful mechanisms

need not necessarily be true. After all, the proposed mechanism is just a model, not data. Also, just because experimental data fits, a particular rate equation is not a proof that the assumptions made to derive that rate equation were correct.

- Different mechanisms may display the same kinetic behavior. Therefore kinetic methods alone cannot be used to unambiguously identify a mechanism. For example, a mechanism with one intermediate (*ES* complex) with four rate constants (Fig. 14.1) and a mechanism with two intermediate complexes (and six rate constants) result in the same general rate expression. Their V_{max} and K_M terms may however be composed of different individual rate constants. Steady-state kinetics (and Michaelis–Menten formalism) therefore cannot establish the nature and number of intermediates. For that, other methods/techniques (such as pre-steady-state tools) are required.

16.2 Notations and Nomenclature in Enzyme Kinetics

Rate equations and reaction equilibria are two complementary bits of information describing the kinetic mechanism of an enzyme. Representing both these becomes a challenge with increasing mechanistic complexity. A major difficulty in following the enzymology literature is the variety of abbreviations and notations used by different research groups. Beginners are bound to be confused by the diversity of nomenclature. A comprehensive and uniform nomenclature was set forth by WW Cleland in three landmark papers published in *Biochimica Biophysica Acta* (in 1963) (Cleland 1989). The following table lists (Table 16.1) the most common notations in the enzyme kinetics literature, and these are used throughout this book.

An enzyme mechanism may involve a number of different enzyme forms and various steps of reaction equilibrium. These need to be properly represented for clear

Table 16.1 Notations commonly used in enzyme kinetics

Item	Notation
Substrates	*A*, *B*, *C*, and *D*, in the order of their addition to the enzyme (*S*, for a single substrate)
Products	*P*, *Q*, and *R*, in the order of their release from the enzyme
Inhibitors	*I*, *J*, etc.
Enzyme forms	*E*, *F*, etc.
Forward rate constants	k_1, k_2, k_3, etc.
Reverse rate constants	k_{-1}, k_{-2}, k_{-3}, etc.
Michaelis constants	K_A, K_B, K_C, etc. (K_M, in general)
Dissociation constants	K_{iA}, K_{iB}, K_{IC}, K_I, K_J, K_P, etc. (K_S or K_D, in general)

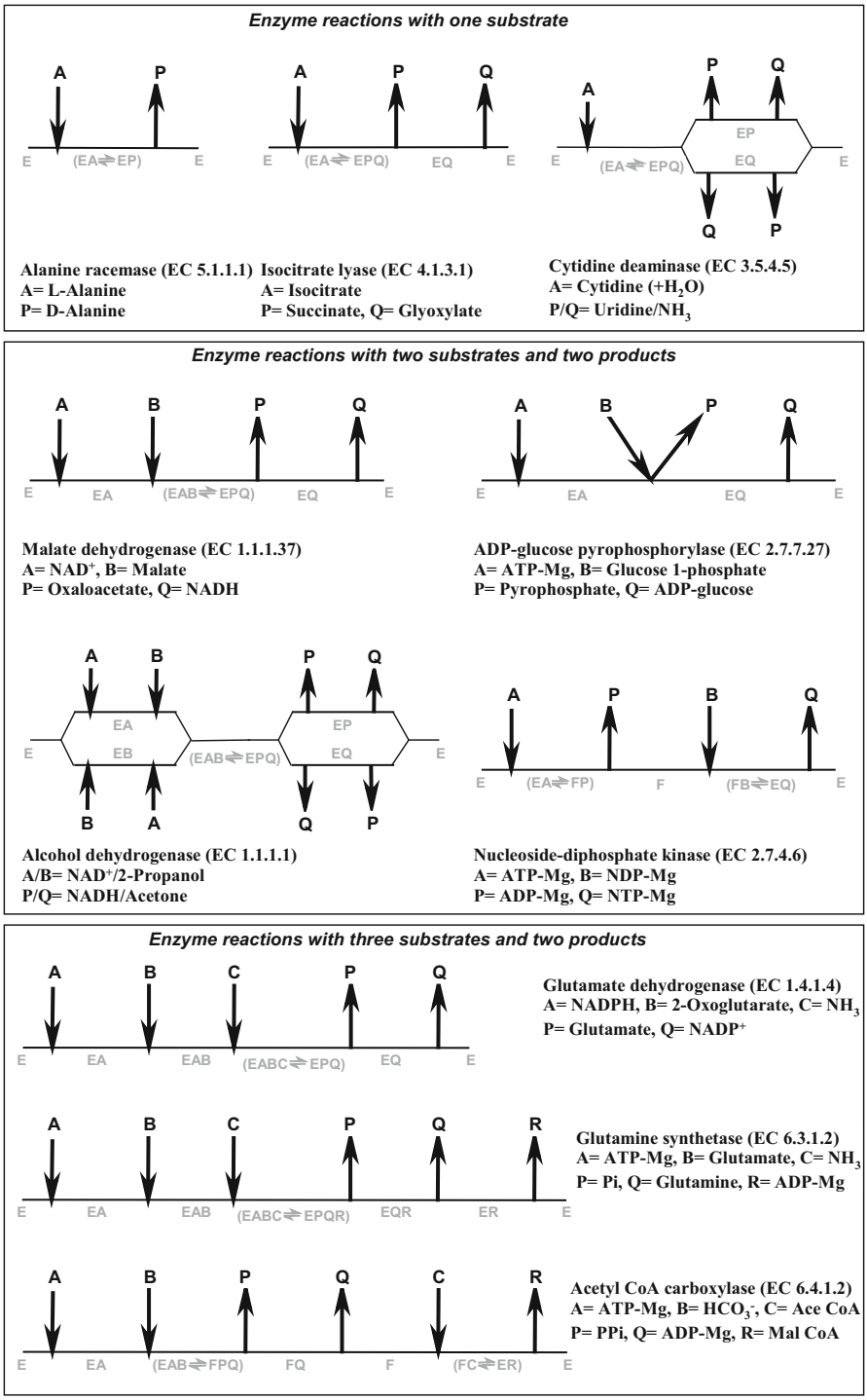


Fig. 16.1 Common enzyme mechanisms and their equilibria according to Cleland notations. All enzyme forms are in gray, and central complexes are in brackets. Enzymes with their substrates/

understanding. The mechanistic scheme indicating various enzyme forms and the rate constants for individual step(s) can be shown in different ways. Correctly displaying a complex mechanism, with large number of enzyme forms and/or steps, poses a challenge. *Cleland notations* are often preferred for their simple yet clear presentation of enzyme mechanisms. In this depiction, (a) the reaction sequence is written from left to right; (b) the enzyme surface is denoted by a horizontal line; (c) various enzyme forms are denoted below this horizontal line, while the central complexes (where bond breaking/forming chemistry takes place) are given in brackets; (d) vertical downward arrows represent substrate addition to that enzyme form; and (e) upward arrows from the enzyme surface indicate product dissociation. Even though the binding and release arrows are single-headed, these steps are viewed as reversible steps. For reactions in aqueous solutions, water is in large excess (55.5 M) and its concentration usually remains constant. Therefore water is not explicitly shown (either as substrate or product) in these mechanisms.

Many enzyme reactions are freely reversible. In these cases, the reactants (substrates) become products, and products become substrates for the reverse direction. For instance, NADP-glutamate dehydrogenase (EC 1.4.1.4) in the forward (reductive amination) reaction has three substrates (viz., NADPH, 2-oxoglutarate, and NH_3) and two products (viz., glutamate and $NADP^+$) (Fig. 16.1). However, the same enzyme is an example of two substrate and three product reaction in the reverse (oxidative deamination). A few more common enzyme equilibria are depicted in Fig. 16.1 according to Cleland notations. The first case (and also the simplest) corresponds to a single substrate – single product equilibrium, used earlier to derive the Michaelis–Menten equation (see Fig. 14.1). All others are more complex. Addition of multiple substrates (or release of more than one product) may occur in various ways as shown. In a *sequential mechanism*, all the substrates must add on to the enzyme before any product can leave. This addition may be either *ordered* (e.g., malate dehydrogenase and glutamine synthetase) or *random* (e.g., alcohol dehydrogenase). In a *ping–pong mechanism* (also known as substituted-enzyme mechanism or double-displacement reaction), the substrate addition sequence is broken by the release of one or more products (e.g., nucleoside-diphosphate kinase and acetyl CoA carboxylase).

Appropriate rate expressions can be derived for every mechanism shown in Fig. 16.1 (and for many others that may be proposed!). How to study these mechanisms with the help of suitable rate equations is discussed later (see Part III). Different methods to derive rate equations, starting with postulated enzyme equilibria, will be discussed next.

Fig. 16.1 (continued) products and EC numbers are shown. Note that the same enzyme from a different organism, tissue, or organelle could have a different kinetic mechanism

16.3 Deriving Rate Equations for Complex Equilibria

The first step in obtaining a suitable rate expression for any reaction is to set up appropriate equilibria with relevant steps and corresponding rate constants. Once the equilibria with various steps and enzyme forms are set up to represent the mechanism, derivation of an appropriate rate equation is straightforward. Recall that *deriving the rate equation is an exercise in evaluating the concentration of the productive (ES) complex* (Chap. 15). It is necessary to obtain [*ES*] in terms of [*S*] at any given instance. As the *ES* form alone breaks down to products, the velocity is proportional to [*ES*]. The fraction of total enzyme ([E_t]) that is present in the ES form is the key.

Fraction of the total enzyme in the ES form,

$$f = [ES]/[E_t] = v/V_{max}$$

Apart from the direct algebraic method (as originally used to derive the Michaelis–Menten equation), there are other ways of deriving rate equations for more complex equilibria. A few of these approaches (with relevant short cuts and simplifications) are briefly described below.

16.3.1 Algebraic Method

This method involves the following steps: (1) Set up proper equilibria for various reaction steps and enzyme forms, (2) make use of steady-state assumption and conservation equations, (3) evaluate the concentration of the *ES* complex, and finally, (4) present it in terms of [E_t]. As an example, we will derive the rate equation for the equilibria involving two enzyme forms (Fig. 14.1); however, we will also consider the reversible reaction, with $[P] \neq 0$.

Rate Equation for the Equilibria Involving Two Enzyme Forms
Assuming steady state, we get

$$\frac{d[ES]}{dt} = k_1[E][S] + k_{-2}[E][P] - (k_{-1} + k_2)[ES] = 0$$

Rearranging for [*E*] in terms of [*ES*],

$$[E] = \left(\frac{k_{-1} + k_2}{k_1[S] + k_{-2}[P]}\right)[ES]$$

Substituting for [*E*] in the enzyme conservation equation ([E_t] = [*E*] + [*ES*]) and then solving for [E_t], we get

(continued)

$$[E_t] = \left(\frac{k_{-1} + k_2}{k_1[S] + k_{-2}[P]} + 1\right)[ES] \text{ and then } [ES] = \frac{[E_t]}{\left(\frac{k_{-1}+k_2}{k_1[S]+k_{-2}[P]} + 1\right)}$$

From the above two equations, we have both $[E]$ and $[E_t]$ expressed in terms of $[ES]$. Now consider the reaction velocity "v" expressed as substrate disappearance. This may be written as

$$v = -\frac{d[S]}{dt} = k_1[E][S] - k_{-1}[ES]$$

Substituting for $[E]$ in terms of $[ES]$ and then rearranging, we obtain,

$$v = k_1[S]\left(\frac{k_{-1} + k_2}{k_1[S] + k_{-2}[P]}\right)[ES] - k_{-1}[ES]$$

$$v = \left(\frac{k_1[S](k_{-1} + k_2)}{k_1[S] + k_{-2}[P]} - k_{-1}\right)[ES]$$

Substituting for $[ES]$ in terms of $[E_t]$, we get

$$v = \frac{\left(\frac{k_1[S](k_{-1}+k_2)}{k_1[S]+k_{-2}[P]} - k_{-1}\right)}{\left(\frac{(k_{-1}+k_2)}{k_1[S]+k_{-2}[P]} + 1\right)}[E_t]$$

Simplifying this equation one obtains,

$$v = \left(\frac{k_1k_2[S] - k_{-1}k_{-2}[P]}{k_{-1} + k_2 + k_1S + k_{-2}P}\right)[E_t]$$

Further simplification by dividing both the numerator and the denominator by $(k_{-1} + k_2)$, and then rearranging, we get

$$v = \frac{\frac{k_2[E_t][S]}{\left(\frac{k_{-1}+k_2}{k_1}\right)} - \frac{k_{-1}[E_t][P]}{\left(\frac{k_{-1}+k_2}{k_{-2}}\right)}}{1 + \frac{[S]}{\left(\frac{k_{-1}+k_2}{k_1}\right)}} + \frac{[P]}{\left(\frac{k_{-1}+k_2}{k_{-2}}\right)}$$

$$v = \frac{\frac{V_{maxf}[S]}{K_{MS}} - \frac{V_{maxr}[P]}{K_{MP}}}{1 + \frac{[S]}{K_{MS}}} + \frac{[P]}{K_{MP}}$$

As mentioned before (see Haldane relationship, Chap. 15), this rate equation is symmetric with respect to $S \rightarrow P$ and $P \rightarrow S$. It is a more general form of the Michaelis–Menten equation. If we put $[P] = 0$, then the equation collapses to the classic form of Michaelis–Menten equation, as derived in the previous chapter.

The algebraic method, exemplified above, is good for a mechanism with few enzyme forms. The complexity of derivation increases with more enzyme forms, and the algebra involved becomes daunting. One needs to solve "*n*" simultaneous equations for as many enzyme forms – to get their unknown concentrations. This is best achieved by solving the simultaneous algebraic equations by *determinants* (of *n*th order) method. While the method is time-consuming for complex enzyme mechanisms, it is quite useful in computer-assisted derivation of rate equations. Further details may be found in specialist books on enzyme kinetics.

16.3.2 King–Altman Procedure

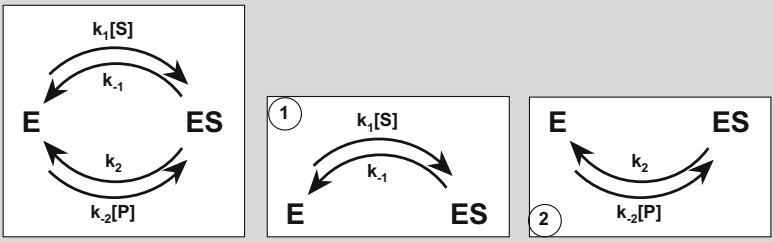
Complex equilibria with many enzyme forms are best handled by this method. The King–Altman method exploits the topological approach (King and Altman 1956). Various enzyme forms are set up with proper equilibria in the form of a figure. Care is taken to ensure that each enzyme form occurs only once in this figure. The fraction of $[E_t]$ present in each enzyme form is then evaluated using this representation. To do this, one lists all the possible patterns that interconnect all enzyme forms, but without forming closed loops. For example, for "*n*" enzyme species, each pattern should contain "$n-1$" lines. A partition equation can now be written for each form – which defines the proportion of the enzyme in that form, in terms of individual rate constants and relevant concentrations. A partition equation for any enzyme form (En) can be written in terms of $[E_t]$ generally as

$$[En] = \frac{Dn}{D1 + D2 + \cdots + Dn}\,[E_t]$$

where $D1$ through Dn are numerators for respective enzyme forms while their sum ($\sum$) is the denominator. Thus, for each enzyme form, there is an expression which when divided by the sum of all such expressions ($\sum$) gives the partition equation – describing the fraction of that enzyme form present in steady state. Suitable partition equations are then used to evaluate the rate in the forward direction. Derivation of the rate equation, for equilibria involving two enzyme forms (Fig. 14.1), by this approach is shown in the box below.

King–Altman Procedure for Equilibria with Two Enzyme Forms

The equilibria shown in Fig. 14.1 may be rewritten in the form of a figure as shown.



Two possible patterns that interconnect both enzyme forms, without forming closed loops, are shown in 1 and 2. With their help, partition equations corresponding to E and ES forms can now be written. Consider the formation of E for instance. It gets formed with a rate constant of k_{-1} (in box 1) and k_2 (in box 2). Accordingly its partition equation may be written as

$$\frac{[E]}{[E_t]} = \frac{k_{-1} + k_2}{\Sigma}$$

Similarly, for ES we get

$$\frac{[ES]}{[E_t]} = \frac{k_1[S] + k_{-2}[P]}{\Sigma}$$

From enzyme conservation equation ($[E_t] = [E] + [ES]$), we observe that $\sum$ is the sum of all the numerator terms.

$$\frac{[E]}{k_{-1} + k_2} = \frac{[ES]}{k_1[S] + k_{-2}[P]}$$
$$= \frac{[E]_t}{\Sigma} \quad (\text{and hence } \Sigma = k_{-1} + k_2 + k_1[S] + k_{-2}[P])$$

Further, solving for $[E_t]$ in terms of $[ES]$ we get

$$[E_t] = \left(\frac{k_{-1} + k_2}{k_1[S] + k_{-2}[P]}\right)[ES] + [ES] = \left(1 + \frac{(k_{-1} + k_2)}{k_1[S] + k_{-2}[P]}\right)[ES]$$

On rearranging, this equation allows us to present $[ES]$ in terms of $[E_t]$ as

(continued)

$$[ES] = \left(\frac{k_1[S] + k_{-2}[P]}{k_{-1} + k_2 + k_1[S] + k_{-2}[P]}\right)[E_t]$$

Note that the denominator (i.e., k_{-1} + k_2 + $k_1[S]$ + $k_{-2}[P]$) of the above equation is the sum of all numerators ($\sum$) mentioned above. Next, we write an analogous expression for $[E]$ in terms of $[E_t]$ as

$$[E] = \left(\frac{k_{-1} + k_2}{k_{-1} + k_2 + k_1[S] + k_{-2}[P]}\right)[E_t]$$

Considering the reaction velocity "v" in terms of product formed, we write

$$v = \frac{d[P]}{dt} = k_2[ES] - k_{-2}[E][P]$$

Upon substituting for $[E]$ and $[ES]$ obtained from the above partition equations,

$$v = k_2\left(\frac{k_1[S] + k_{-2}[P]}{k_{-1} + k_2 + k_1[S] + k_{-2}[P]}\right)[E_t] - k_{-2}\left(\frac{k_{-1} + k_2}{k_{-1} + k_2 + k_1[S] + k_{-2}[P]}\right)[E_t][P]$$

Simplifying further,

$$v = \frac{k_1k_2[S][E_t] - k_{-1}k_{-2}[P][E_t]}{k_{-1} + k_2 + k_1[S] + k_{-2}[P]}$$

Dividing both the numerator and the denominator by (k_{-1} + k_2) and rearranging, we get

$$\frac{v = \frac{k_1}{k_{-1}+k_2}k_2[E_t][S] - \frac{k_{-2}}{k_{-1}+k_2}k_{-1}[E_t][P]}{1 + \frac{k_1}{k_{-1}+k_2}[S] + \frac{k_{-2}}{k_{-1}+k_2}[P]}$$

By appropriate substitutions for V_{max} and K_M terms, the equation takes the following form:

$$v = \frac{\frac{V_{maxf}[S]}{K_{MS}} - \frac{V_{maxr}[P]}{K_{MP}}}{1 + \frac{[S]}{K_{MS}}} + \frac{[P]}{K_{MP}}$$

Notice that the above equation is identical to the one derived by the algebraic method. King–Altman procedure is schematic in nature, and one can write down the rate equation by inspecting patterns connecting the different enzyme forms. It can be used for more complex schemes than the example described above. The procedure however becomes complicated with multi-substrate random mechanisms as it gives

squared substrate terms. Also, if more than one closed loop is present, the precise number of unique patterns to be considered becomes nontrivial.

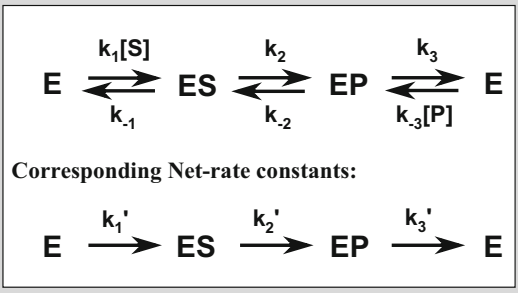
16.3.3 Net Rate Constant Method

This method is a useful shortcut developed by W Cleland (1975). It is ideal to derive rate equations for simple kinetic mechanisms without branched pathways. The protocol involves the following steps:

1. Set up appropriate equilibria with all enzyme forms.
2. Represent steady-state flux at each step as unidirectional (net rate) constants (denoted by k' values) such that flux values in each step along with the distribution of enzyme species remain the same.
3. Begin with an irreversible step in the original scheme, where the net rate constant and the real rate constant are the same ($k_n' = k_n$), and evaluate each net rate constant by going backward.
4. Substitute net rate constants in a suitable equation to obtain the rate expression. How this procedure works is shown for a linear mechanism with three enzyme forms (see box below).

Net Rate Constant Method for Linear Equilibria

Consider the mechanism with three enzyme forms *E*, *ES*, and *EP*, as shown. Although the actual rate constants are different, for each step we substitute a net rate constant (k_n') such that $k_n' = k_n \times$ partition ratio. Note that k_n is the true forward rate constant for the step in question.



If steady-state conditions operate, then by definition net rates for all the steps are equal. That is, at steady state:

$$v = k_1'[E] = k_2'[ES] = k_3'[EP]$$

Therefore,

(continued)

$$\frac{[E]}{v} = \frac{1}{k_1'} \text{ and } \frac{[ES]}{v} = \frac{1}{k_2'} \text{ and } \frac{[EP]}{v} = \frac{1}{k_3'}$$

Since $[E_t]=[E]+[ES]+[EP]$, we know that

$$\frac{[E]}{v} + \frac{[EA]}{v} + \frac{[EP]}{v} = \frac{1}{k_1'} + \frac{1}{k_2'} + \frac{1}{k_3'} = \frac{[E_t]}{v}$$

And this can be suitably rearranged to get

$$v = \frac{[E_t]}{\frac{1}{k_1'} + \frac{1}{k_2'} + \frac{1}{k_3'}}$$

The concept of "net rate constant" is thus similar to "conductance" in electrical systems. Reciprocal of net rate constant then becomes resistance, and the sum of resistances (denominator term above) dictates what fraction of $[E_t]$ is in the productive from.

It now remains to plug in the values of individual net rate constants and simplify to obtain the rate expression. If we consider initial velocity conditions (i.e., $[P] = 0$), then the last step of the above linear mechanism becomes irreversible. And therefore $k_3' = k_3$. We now go backward sequentially, to evaluate the net rate constant for the previous steps. For instance,

$$k_2' = k_2 \times \text{Partition ratio for } EP$$

The net rate constant thus is the real forward rate multiplied by the partition ratio for that enzyme form. For EP form,

$$\text{Partition ratio} = \frac{\text{Rate of } EP \text{ going forward}}{\text{Rate of } EP \text{ going forward} + \text{Rate of } EP \text{ returning to } EA}$$

This can be represented as,

$$\text{Partition ratio for } EP = \frac{k_3'}{k_{-2} + k_3'} = \frac{k_3}{k_{-2} + k_3} \quad (\text{because } k_3' = k_3)$$

We now substitute this value of partition ratio to obtain k_2' as shown below:

$$k_2' = k_2 \times \frac{k_3}{k_{-2} + k_3} = \frac{k_2 k_3}{k_{-2} + k_3}$$

In a similar manner k_1' (step previous to k_2' step) may now be evaluated. Finally, by substituting for k_2' from above and simplifying,

(continued)

$$k_1{}' = k_1[S] \times \frac{k_2{}'}{k_{-1} + k_2{}'} = \frac{k_1[S]\frac{k_2k_3}{k_{-2}+k_3}}{k_{-1} + \frac{k_2k_3}{k_{-2}+k_3}} = \frac{k_1k_2k_3[S]}{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3}$$

This is how all the net rate constants ($k_1{}'$ through $k_3{}'$, in the given mechanism) are evaluated. These can now be substituted in the general form of the rate expression obtained earlier:

$$v = \frac{[E_t]}{\frac{1}{k_1{}'} + \frac{1}{k_2{}'} + \frac{1}{k_3{}'}}$$

The following equation is thus obtained.

$$v = \frac{[E_t]}{\frac{1}{\frac{k_1k_2k_3[S]}{k_{-1}k_{-2}+k_{-1}k_3+k_2k_3}} + \frac{1}{\frac{k_2k_3}{k_{-2}+k_3}} + \frac{1}{k_3}}$$

This equation can now be rearranged and simplified into the rate expression as shown below.

$$v = \frac{[E_t]}{\frac{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3}{k_1k_2k_3[S]} + \frac{k_{-2} + k_3}{k_2k_3} + \frac{1}{k_3}}$$

$$= \frac{k_1k_2k_3[E_t][S]}{k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3 + (k_2 + k_{-2} + k_3)k_1[S]}$$

$$v = \frac{\left(\frac{k_2k_3}{k_2+k_{-2}+k_3}\right)[E_t][S]}{\left(\frac{k_{-1}k_{-2}+k_{-1}k_3+k_2k_3}{(k_2+k_{-2}+k_3)\,k_1}\right) + [S]}$$

We recognize that the final form of the rate equation derived by net rate constant method resembles the typical Michaelis–Menten equation. Remarkably the expression contains all the individual rate constants and thus allows us to obtain V_{max}/K_M and V_{max} in terms of these individual rate constants. The method therefore (a) is best suited for deriving rate expressions for isotope exchange, isotope partitioning, and positional isotope exchange studies and (b) shows good promise in interpreting isotope effects on V_{max}/K_M and V_{max} of the enzyme.

Another advantage of net rate constant method is that expressions for V_{max}/K_M or V_{max} may be obtained without deriving the entire rate equation. Consider V_{max}/K_M first. From the basics of Michaelis–Menten formalism, we know that $v = (V_{max}/K_M) \times [S]$, at low $[S]$. By inspecting the above linear mechanism, we see that $k_1{}'$ is rate limiting at low $[S]$. And therefore,

$$v = k_1{}' \times [E_t] = \frac{k_1 k_2 k_3 [S]}{k_{-1}k_{-2} + k_{-1}k_3 + k_2 k_3} \times [E_t]$$

In comparison with the equation "$v = (V_{max}/K_M) \times [S]$," we can write

$$\frac{V_{max}}{K_M} = \frac{k_1 k_2 k_3 [E_t]}{k_{-1}k_{-2} + k_{-1}k_3 + k_2 k_3}$$

Similarly now consider V_{max}. At saturating $[S]$, $v = V_{max}$ and $k_1{}'$ can be neglected. Therefore,

$$v = \frac{[E_t]}{\frac{1}{k_2{}'} + \frac{1}{k_3{}'}} = \frac{[E_t]}{\frac{1}{\frac{k_2 k_3}{k_{-2}+k_3}} + \frac{1}{k_3}} = \frac{k_2 k_3 [E_t]}{k_2 + k_{-2} + k_3} = V_{max}$$

We can compare these expressions for V_{max}/K_M and V_{max} with the full rate expression above and identify the relevant terms contributing to them.

16.3.4 Other Methods

There are a few other variations, in addition to the three methods described above, to derive a rate expression. A method described by Sangman Cha simplifies the rapid equilibrium segment containing many enzyme forms as though it were a single enzyme species (Cha 1968). A single lumped up rate constant is then used to represent this segment, and a rate equation is derived. This is a useful tool when random addition of substrates occurs in the mechanism – as it avoids squared terms. The assumption of rapid equilibrium is a useful simplification and need not actually be true for the method to work.

16.4 Enzyme Kinetics and Common Sense

It should be obvious from the general theme of this chapter that enzyme kinetic analysis provides valuable mechanistic insights. A minimal mathematical ability is required to meaningfully appreciate and use this tool. Although an added advantage, mathematical proficiency is not mandatory to apply kinetic methods to enzyme mechanisms. As ascribed to Einstein: *We should make things as simple as possible, but not simpler*. Derivation of a few rate equations was deliberately included to bring home this point. However it is not a prerequisite to appreciate the subject matter of this book.

The kinetic methods described above allow us to derive and appreciate the connection between a mechanism and its corresponding rate equation. But the correctness of such an equation is only as valid as the assumptions made in deriving it. Some mechanisms may be quite complex and equations formidable. Nevertheless

doing kinetics can be fun so long as one understands what these equations mean. For most examples, the hard work (of deriving them!) has already been done; a lot can be accomplished by judicious use of these equations found in the literature. One needs only to develop a sense of discrimination and understand the conceptual meaning of the equation to be used. Appropriate use of equations found in the enzyme literature is as important as deriving new ones. As Cleland stated, "All the mathematics in the world is no substitute for a reasonable amount of common sense."

This common sense approach coupled with elementary mathematical ability forms the basis of enzyme mechanisms described in this book. The emphasis will therefore be more on conceptual framework of kinetic description and analysis.

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