Integrated rate equations for enzyme-catalysed first-order and second-order reactions

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Generalized rate equations covering all mechanisms giving hyperbolic initial-rate kinetics with stoichiometry $A \rightleftharpoons P$, $A \rightleftharpoons P + Q$, $A + B \rightleftharpoons P$ and $A + B \rightleftharpoons P + Q$ were integrated. The results are regular and reasonably economical.

Kinetic analysis with the use of complete time courses rather than initial rates has several often-quoted advantages: inherently more information is available in each experiment; the need to perform more-or-less subjective calculations of initial rates is eliminated; the data used, primarily in the central portions of the progress curves, should be more reliable; products need not be added in order to obtain product-inhibition effects.

For all these advantages, there appears to have been no attempt to characterize an enzyme's kinetics with the use only of integrated equations, and relatively few attempts to reconcile time-dependent and initial-rate behaviour (see the Discussion section). In effect, we do not yet know that rate equations deduced from the steady-state assumption are adequate to characterize the time course of enzyme-catalysed reactions.

The reason is simple. Rate equations have been integrated entirely on a mechanism-by-mechanism basis (see, e.g., Huang & Niemann, 1951; Alberty & Koerber, 1957; Darvey & Williams, 1964), and the results were mostly formidable. There has been no general integrated equation covering first-order

catalysed reactions, let alone those of second-order, the majority of biochemical interest. Done separately, the cases and the algebra proliferate rapidly. There are many mechanisms, and there are at least three causes (substrate depletion, product accumulation and inhibition, and approach to equilibrium) for the decline of the rate with time.

I have recently described (Boeker, 1984) general integrated equations, applicable to all initial conditions, for reversible uncatalysed bimolecular reactions. For the stoichiometry $A + B \rightleftharpoons P + Q$, the equation is:

$$k_1(1 - 1/K_e)Dt = -\ln \left(1 - \frac{\Delta P}{P_e - P_0} \right) + \ln \left(1 - \frac{\Delta P}{D + P_e - P_0} \right)$$

where $P_e - P_0$ and $D + P_e - P_0$ are the physically possible and physically impossible roots of the quadratic equation for $P_e - P_0$ in terms of the initial concentrations and K_e . D is the discriminant in this quadratic. Both roots can be calculated if the equilibrium constant is known.

For the stoichiometry $A + B \rightleftharpoons P$, the term in brackets on the left-hand side of the equation above is simply 1; for $A \rightleftharpoons P + Q$, it is $-1/K_e$. The corresponding equation for reversible uncatalysed first-order reactions is:

$$k_1(1 + 1/K_e)t = -\ln \left(1 - \frac{\Delta P}{P_e - P_0} \right)$$

In this paper, I extend this analysis to catalysed reactions, using general forms of derivative rate equations. For each stoichiometry, a single inte-

Abbreviations used: A , B , P and Q are the instantaneous concentrations of substrates and products; the subscripts o and e indicate the initial and equilibrium concentrations respectively. K_e is the equilibrium constant, k_1 is the forward rate constant for uncatalysed reactions, V_f is the usual maximum velocity in the forward direction for catalysed reactions, and E_t is the total enzyme concentration. K_A is the Michaelis constant for the substrate, and K_P is the competitive product inhibition constant. For $A \rightleftharpoons P + Q$, $D = -(P_e + Q_e + K_e)$; for $A + B \rightleftharpoons P$, $D = A_e + B_e + 1/K_e$; for $A + B \rightleftharpoons P + Q$, $D = [K_e(A_e + B_e) + P_e + Q_e]/(K_e - 1)$ (Boeker, 1984). The coefficients J and C are defined in the text.

grated equation results that can be used for nearly all unbranched mechanisms, as well as for those random mechanisms where the rapid equilibrium assumption is valid.

General expressions for derivative equations

The tedium of integrating rate equations for all possible mechanisms can be avoided if the derivation is performed for a general expression, independent of mechanism, for each stoichiometry. For a first-order ($A \rightleftharpoons P$) reaction that is enzyme-catalysed and reversible, the general steady-state rate equation is (Peller & Alberty, 1959; Cleland, 1963; Taraszka & Alberty, 1964):

$$\frac{dP}{dt} = \frac{V_f J_A (A - P/K_e)}{J_0 + J_A A + J_P P + J_{AP} AP} \quad (1)$$

V_f is the usual maximum velocity in the forward direction and K_e is the equilibrium constant. The coefficients J are collections of microscopic rate constants whose specific definition, for any particular mechanism, can be obtained directly from a derivation done in accordance with King & Altman (1956). When each is defined appropriately (and some may be zero), the equation becomes mechanism-specific.

In ordinary initial-rate kinetic studies, the second term in the brackets of the numerator of eqn. (1) is ignored; otherwise the dependence of the velocity on substrate concentration is not hyperbolic. This assumption is correct regardless of initial conditions if the reaction is irreversible, such that P/K_e is always effectively 0. If the reaction is reversible, the assumption is justified for initial rates if $P_0 = 0$ or if $A_0 \gg P_0/K_e$. For time-course studies where P is not P_0 but instead increases steadily, the term P/K_e can be omitted only if the reaction is strictly irreversible. To omit P/K_e for a reversible reaction is to ignore the approach to equilibrium; the resulting expressions cannot possibly describe the complete time course accurately.

The term $J_P P$ in the denominator of eqn. (1) corresponds to competitive product inhibition; it arises in any mechanism where the substrate and the product combine with the same enzyme form. The term $J_{AP} AP$ corresponds to uncompetitive product inhibition and is uncommon. It arises when the stable enzyme form isomerizes (Cleland, 1963; Taraszka & Alberty, 1964) and in certain other unusual situations (Taraszka & Alberty, 1964).

For the stoichiometry $A + B \rightleftharpoons P + Q$, the general expression that is integrated in the present paper is:

$$\begin{aligned} \frac{dP}{dt} = & \frac{V_f J_{AB} (AB - PQ/K_e)}{(J_0 + J_A A + J_B B + J_P P + J_Q Q} \\ & + J_{AB} AB + J_{AP} AP + J_{AQ} AQ \\ & + J_{BP} BP + J_{BQ} BQ + J_{PQ} PQ \\ & + J_{ABP} ABP + J_{ABQ} ABQ + J_{APQ} APQ \\ & + J_{BPQ} BPQ + J_{ABPQ} ABPQ) \end{aligned} \quad (2)$$

Although their notation is different, Wong & Hanes (1962) use a similar form for second-order rate equations. As they indicate, this expression takes in all linear mechanisms unless a substrate or a product reacts at two separate points in the reaction pathway. Dead-end inhibition by extraneous compounds does not change the form of eqn. (2), only the definition of the appropriate coefficients J . Eqn. (2) does not apply to branched mechanisms unless the rapid-equilibrium assumption is valid.

For linear mechanisms with the stoichiometry $A + B \rightleftharpoons P$, the numerator of the general equation is $V_f J_{AB} (AB - P/K_e)$; the denominator is the same as that in eqn. (2) except that it lacks all terms in Q . For $A \rightleftharpoons P + Q$, the numerator is $V_f J_A (A - PQ/K_e)$ and the denominator lacks all terms in B . The term $J_{ABPQ} ABPQ$ therefore occurs only when the stoichiometry is $A + B \rightleftharpoons P + Q$; it arises, for example, when a stable enzyme form isomerizes.

In the notation used here, not all of the coefficients J are independent. Division of the numerator and denominator of eqn. (2) by $V_f J_{AB}/E_T$ will give a form consistent with Dalziel (1957) coefficients. Division by J_{AB} will give a form consistent with the coefficients given by Alberty (1953) or Bloomfield *et al.* (1962) or Cleland (1963). Although the coefficients are independent in each of these sets, no one choice is best for integrated equations. In the system used in the present paper, the eventual choice of independent constants is discretionary, and the subscripts make each coefficient instantly identifiable. Furthermore, the equation can be made mechanism-specific simply by setting the appropriate coefficients to zero.

Derivation

Reducing the number of concentration variables

A , B , P and Q , the four concentration variables in eqn. (2), are related by stoichiometry. For example, it must always be true that $A_0 + P_0 = A + P = A_e + P_e$. Similarly, $Q_0 - P_0 = Q - P = Q_e - P_e$. If the number of variables is reduced to one by using $A = A_e + P_e - P$, $B = B_e + P_e - P$, $Q = Q_e - (P_e - P)$ and $P = P_e - (P_e - P)$, the numerators and denominators of each general equation become polynomials in $P_e - P$; this is shown in the

next two paragraphs. With these substitutions, the subsequent derivation is manageable and the result is tidy. If initial concentrations are used instead of equilibrium concentrations, the whole business is tedious (cf. Darvey & Williams, 1964).

After substituting, the numerators for each second-order stoichiometry can be re-arranged and factored as previously described for the uncatalysed reactions (Boeker, 1984). This gives:

$A + B \rightleftharpoons P$:

$$V_f J_{AB}(AB - P/K_e) = V_f J_{AB}(P_e - P)(D + P_e - P)$$

where $D \equiv A_e + B_e + 1/K_e$

$A \rightleftharpoons P + Q$:

$$V_f J_A(K_e A - PQ)/K_e = V_f J_A(-1/K_e)(P_e - P)(D + P_e - P)$$

where $D \equiv -(P_e + Q_e + K_e)$

$A + B \rightleftharpoons P + Q$:

$$V_f J_{AB}(AB - PQ/K_e) = V_f J_{AB}(1 - 1/K_e)(P_e - P)(D + P_e - P)$$

where $D \equiv [K_e(A_e + B_e) + P_e + Q_e]/(K_e - 1)$

D is a constant characteristic of a second-order reaction (Boeker, 1984). It depends on the equilibrium constant and the initial concentrations, and can therefore be calculated if K_e is known. For a first-order reaction, the corresponding numerator expression can easily be shown to be:

$$A \rightleftharpoons P: V_f J_A(A - P/K_e) = V_f J_A(1 + 1/K_e)(P_e - P)$$

The denominators of all four first- and second-order equations can be written in terms of $P_e - P$ by substituting appropriately, multiplying out each concentration term and collecting according to dependence on $P_e - P$. The resulting polynomial in $P_e - P$ can be written:

$$\alpha + \beta(P_e - P) + \gamma(P_e - P)^2 + \delta(P_e - P)^3 + \varepsilon(P_e - P)^4$$

ε is J_{ABPQ} and is necessarily zero for $A + B \rightleftharpoons P$ and $A \rightleftharpoons P + Q$. Both δ and ε are zero for $A \rightleftharpoons P$. Complete definitions of α , β etc. are given in the Appendix.

Separation of variables and reduction of the polynomial

If, for a first-order reaction, an additional constant C is defined such that $C = V_f J_A(1 + 1/K_e)$, the rate equation becomes:

$$\begin{aligned} \frac{dP}{dt} &= \frac{C(P_e - P)}{\gamma(P_e - P)^2 + \beta(P_e - P) + \alpha} \\ C \frac{dt}{dP} &= \frac{\gamma(P_e - P)^2 + \beta(P_e - P) + \alpha}{P_e - P} \\ &= \gamma(P_e - P) + \beta + \frac{\alpha}{P_e - P} \end{aligned}$$

For the second-order stoichiometries, a single differential equation can be obtained if $C = V_f J_{AB}$ for $A + B \rightleftharpoons P$, or $C = V_f J_A(-1/K_e)$ for $A \rightleftharpoons P + Q$, or $C = V_f J_{AB}(1 - 1/K_e)$ for $A + B \rightleftharpoons P + Q$. Then:

$$\begin{aligned} \frac{dP}{dt} &= \frac{C(P_e - P)(D + P_e - P)}{\alpha + \beta(P_e - P) + \gamma(P_e - P)^2 + \delta(P_e - P)^3 + \varepsilon(P_e - P)^4} \\ C \frac{dt}{dP} &= \frac{\varepsilon(P_e - P)^4 + \delta(P_e - P)^3 + \gamma(P_e - P)^2 + \beta(P_e - P) + \alpha}{(P_e - P)(D + P_e - P)} \end{aligned}$$

The fraction on the right-hand side of this equation can be reduced by algebraic long division in which the final two terms are obtained by the method of partial fractions. This gives:

$$\begin{aligned} C \frac{dt}{dP} &= \varepsilon(P_e - P)^2 + (\delta - \varepsilon D)(P_e - P) \\ &\quad + \gamma - \delta D + \varepsilon D^2 + \frac{\alpha}{D(P_e - P)} \\ &\quad - \frac{\alpha - \beta D + \gamma D^2 - \delta D^3 + \varepsilon D^4}{D(D + P_e - P)} \end{aligned}$$

Integration

These equations can be integrated directly. However, as integration will be from P_0 to P , a later rearrangement (which is tedious for the second-order case) can be avoided if the terms that are not fractions are re-expressed by making the substitution $P_e - P = P_e - P_0 - (P - P_0) = P_e - P_0 - \Delta P$. The result is obvious for the first-order case. For a second-order reaction:

$$\begin{aligned} C \frac{dt}{dP} &= \varepsilon(\Delta P)^2 - [\delta - \varepsilon D + 2\varepsilon(P_e - P_0)]\Delta P \\ &\quad + \varepsilon[(P_e - P_0)^2 - D(P_e - P_0) + D^2] \\ &\quad + \delta(P_e - P_0 - D) + \gamma + \frac{\alpha}{D(P_e - P)} \\ &\quad - \frac{\alpha - \beta D + \gamma D^2 - \delta D^3 + \varepsilon D^4}{D(D + P_e - P)} \end{aligned}$$

After integration, the general equation for a first-order reaction is:

$$\begin{aligned} Ct &= -\frac{1}{2}\gamma(\Delta P)^2 + [\beta + \gamma(P_e - P_0)]\Delta P \\ &\quad - \alpha \cdot \ln \left(1 - \frac{\Delta P}{P_e - P_0} \right) \end{aligned}$$

For second-order reactions:

$$Ct = \frac{1}{3}\epsilon(\Delta P)^3 + \frac{1}{2}[-\delta + \epsilon D - 2\epsilon(P_e - P_0)](\Delta P)^2 \\ + \{\epsilon[(P_e - P_0)^2 - D(P_e - P_0) + D^2] + \delta(P_e - P_0 - D) + \gamma\} \Delta P - \frac{\alpha}{D} \cdot \ln \left(1 - \frac{\Delta P}{P_e - P_0} \right) \\ + \frac{\alpha - \beta D + \gamma D^2 - \delta D^3 + \epsilon D^4}{D} \\ \ln \left(1 - \frac{\Delta P}{D + P_e - P_0} \right)$$

This equation has the virtue of being general, but it is almost always unnecessarily complex. ϵ , which is J_{ABPQ} , is necessarily zero for $A + B \rightleftharpoons P$ and $A \rightleftharpoons P + Q$, and is non-zero for $A + B \rightleftharpoons P + Q$ only in unusual mechanisms, for example when a stable enzyme form isomerizes. In these cases, then, an adequate and considerably simpler equation is:

$$Ct = -\frac{1}{2}\delta(\Delta P)^2 + [\gamma + \delta(P_e - P_0 - D)]\Delta P \\ - \frac{\alpha}{D} \cdot \ln \left(1 - \frac{\alpha P}{P_e - P} \right) \\ + \frac{\alpha - \beta D + \gamma D^2 - \delta D^3}{D} \cdot \ln \left(1 - \frac{\Delta P}{D + P_e - P_0} \right)$$

Integrated equations

The integrated equation for first-order reactions has the form:

$$t = \frac{1}{2}C_2(\Delta P)^2 + C_1\Delta P - C_f \cdot \ln \left(1 - \frac{\Delta P}{P_e - P_0} \right) \quad (3)$$

For all three second-order cases, the form is:

$$t = \frac{1}{3}C_3(\Delta P)^3 + \frac{1}{2}C_2(\Delta P)^2 + C_1\Delta P \\ - C_f \cdot \ln \left(1 - \frac{\Delta P}{P_e - P_0} \right) \\ + C_s \cdot \ln \left(1 - \frac{\Delta P}{D + P_e - P_0} \right) \quad (4)$$

For most mechanisms, C_2 in eqn. (3) and C_3 in eqn. (4) will be zero; the only obvious situation where this is not true is when a stable enzyme form isomerizes. Regardless of this, a first-order or second-order time course can now be tested against its equation by attempting to fit the constants, with the use of an appropriate statistical technique (see the Discussion section).

If a time course is to be interpreted in terms of mechanism, the meaning of each coefficient C must be defined in terms of the coefficients J . For a first-order reaction, the result is quite straightforward:

$$C_f = \frac{J_0 + J_A A_e + J_P P_e + J_{AP} A_e P_e}{J_A V_f (1 + 1/K_e)} \quad (5) \\ C_1 = [J_A - J_P + J_{AP}(P_0 - A_e)]/J_A V_f (1 + 1/K_e) \\ C_2 = J_{AP}/J_A V_f (1 + 1/K_e)$$

For second-order reactions, the algebra is tedious but eventually leads to much cancelling; the result is tidier than expected and has some interesting regularities. For the two logarithmic terms, it can be shown that:

$$C_f = [J_0 + J_A A_e + J_B B_e + J_P P_e + J_Q Q_e + \dots \\ + J_{ABQ} A_e B_e Q_e + J_{ABPQ} A_e B_e P_e Q_e]/CD \\ C_s = [J_0 + J_A(A_e - D) + J_B(B_e - D) + J_P(P_e + D) \\ + J_Q(Q_e + D) + \dots + J_{ABPQ}(A_e - D) \\ \times (B_e - D)(P_e + D)(Q_e + D)]/CD$$

where the coefficients that do not occur for a particular stoichiometry are zero, and C and D are defined as before for each stoichiometry.

In a mathematical sense, these expressions are symmetrical. A_e and $A_e - D$, for example, are the physically possible and physically impossible roots of the quadratic equation for A_e in terms of K_e and the initial concentrations; so are B_e and $B_e - D$, P_e and $P_e + D$, and Q_e and $Q_e + D$ (Boeker, 1984). Thus, if the denominator on the right of the equation for C_f is cleared, CDC_f can be seen to be the sum of all the coefficients J multiplied by the appropriate physically possible equilibrium concentrations; in first-order reactions, CC_f is this sum. In CDC_s , the coefficients are multiplied by the physically impossible concentrations.

C_f and C_s can be obtained in terms of initial concentrations and K_e by substituting for D , A_e , B_e , P_e and Q_e . Expressions for D and P_e are given in Boeker (1984), and expressions for the other equilibrium concentrations can easily be obtained from the stoichiometric relationships. The resulting expressions for C_f and C_s do not appear to have a simple form, and they obscure the meaning of the coefficients.

On the contrary, the simplest forms for C_1 , C_2 and C_3 appear to result when they are expressed in terms of initial concentrations. The substitutions must be carried out independently for each stoichiometry. The results are shown in Table 1, along with the original definitions of C . In particular for reactions with only one substrate or one

Table 1. Expressions for C , C_1 , C_2 and C_3

Stoichiometry	C	$C_3 \cdot C$	$C_2 \cdot C$
$A = P + Q$	$V_f J_A (-1/K_e)$	0	J_{APQ}
$A + B = P$	$V_f J_{AB}$	0	J_{ABP}
$A + B = P + Q$	$V_f J_{AB} (1 - 1/k_e)$	J_{ABPQ}	$J_{ABP} + J_{ABQ} - J_{APQ} - J_{BPQ}$
			$+ J_{ABPQ} \left(\frac{A_0 + B_0 + K_e(P_0 + Q_0)}{K_e - 1} \right)$
Stoichiometry	$C_1 \cdot C$		
$A = P + Q$	$J_{AP} + J_{AQ} - J_{PQ} - J_{APQ}(A_0 + K_e)$		
$A + B = P$	$J_{AB} - J_{AP} - J_{BP} + J_{ABP}(P_0 + 1/K_e)$		
$A + B = P + Q$	$J_{AB} + J_{PQ} - J_{AP} - J_{BP} - J_{AQ} - J_{BQ}$		
	$+ J_{ABP} \left(P_0 + \frac{A_0 + B_0 + P_0 + Q_0}{K_e - 1} \right) + J_{ABQ} \left(Q_0 + \frac{A_0 + B_0 + P_0 + Q_0}{K_e - 1} \right)$		
	$+ J_{APQ} \left(A_0 - \frac{K_e(A_0 + B_0 + P_0 + Q_0)}{K_e - 1} \right) + J_{BPQ} \left(B_0 - \frac{K_e(A_0 + B_0 + P_0 + Q_0)}{K_e - 1} \right)$		
	$+ J_{ABPQ} \left[K_e \left(\frac{A_0 + B_0 + P_0 + Q_0}{K_e - 1} \right)^2 + \frac{K_e P_0 Q_0 - A_0 B_0}{K_e - 1} \right]$		

product, C_1 and C_2 have quite straightforward interpretations.

For $A + B \rightleftharpoons P + Q$, C_1 and C_2 appear to be unavoidably complex. There are a number of possible algebraic forms; one of the simpler is given. Although it is by no means obvious, the concentration terms in these expressions do have physical interpretations. In C_1 , for example, the concentration term for J_{ABP} can also be written as $-(A_0 + B_0) + P_0 + [K_e(A_0 + B_0) + P_0 + Q_0]/(K_e - 1)$. The last term in this expression is $P_e - P_0$ when the discriminant in the quadratic equation is zero, i.e. when the reaction has a large K_e and $A_0 = B_0$ (Boeker, 1984). Similarly, the term in J_{ABQ} is $-(A_0 + B_0) + Q_0 +$ this same term; the term in J_{APQ} is $A_0 - (P_0 + Q_0) -$ the term; and that in J_{BPQ} is $B_0 - (P_0 + Q_0) -$ the term. In C_2 , the concentration for J_{ABPQ} is $-(A_0 + B_0) + P_0 + Q_0 +$ this term.

Discussion

Integrated equations have been presented previously for a number of irreversible first-order mechanisms: in the absence of any product inhibition (Henri, 1902), with competitive product inhibition only (Huang & Niemann, 1951; Schönheyder, 1952), with uncompetitive inhibition only or with both types (Laidler & Bunting, 1973; Orsi & Tipton, 1979). The general equation for an irreversible first-order reaction can be ob-

tained from the analysis in the present paper either by integrating eqn. (1) directly, after noting that P/K_e becomes zero, or from eqn. (5), by noting that $1/K_e$ and A_e become zero and P_e becomes $A_0 + P_0$. Either method gives:

$$V_f J_A t = \frac{1}{2} J_{AP} (\Delta P)^2 + (J_A - J_P + J_{AP} P_0) \Delta P - [J_0 + J_P(A_0 + P_0)] \cdot \ln(1 - \Delta P/A_0) \quad (6)$$

Eqn. (6) is consistent with all of the equations presented previously for irreversible first-order reactions.

For a reversible first-order reaction, Alberty & Koerber (1957) gave an integrated equation that includes competitive product inhibition. This equation is consistent with eqn. (5) when J_{AP} is zero. Only the presence of uncompetitive product inhibition (the term in J_{AP} in eqn. 1) introduces $(\Delta P)^2$ into eqn. (5) or (6). Although they do not derive an explicit integrated equation, Taraszka & Alberty (1964) suggest this result in attempting to rationalize progress curves obtained for fumarase.

Walter (1963) and Darvey & Williams (1964) have presented integrated rate equations for several second-order mechanisms. Walter (1963) considers only the stoichiometry $A + B \rightleftharpoons P + Q$, and assumes that the overall reaction does not approach equilibrium. His equations are presented in terms of microscopic rate constants. Darvey & Williams (1964) consider all three second-order

stoichiometries and take into account the approach to equilibrium, but also present their equations in terms of microscopic rate constants. Both sets of results therefore depend explicitly on the original choice of mechanism and are extraordinarily complex.

The test of integrated rate equations is whether they can be used experimentally to determine kinetic constants. A number of attempts have been made at this in the past. If they lack uncompetitive product inhibition, first-order equations can be re-arranged to the form:

$$V^{\text{app.}}t = \Delta P - K^{\text{app.}} \cdot \ln \left(1 - \frac{\Delta P}{P_e - P_0} \right)$$

If the reaction is irreversible:

$$V^{\text{app.}} = \frac{V_f J_A}{J_A - J_p} = \frac{V_f}{1 - K_A/K_p}$$

$$K^{\text{app.}} = \frac{J_0 + J_p(A_0 + P_0)}{J_A - J_p} = \frac{K_A[1 + (A_0 + P_0)/K_p]}{1 - K_A/K_p}$$

A further re-arrangement gives:

$$\frac{\Delta P}{t} = \frac{V^{\text{app.}}}{1 - \frac{K^{\text{app.}}}{\Delta P / \ln(1 - \Delta P/A_0)}}$$

which has exactly the same form as the Michaelis-Menten equation and therefore the same three linear transformations. If, for example, a progress curve is plotted in accordance with $\Delta P/t$ versus $[-\ln(1 - \Delta P/A_0)]/t$ (corresponding to v_0 versus v_0/S_0), it should produce a straight line from which $K^{\text{app.}}$ and $V^{\text{app.}}$ can be extracted. Walker & Schmidt (1944) first used a plot of this type for histidine ammonia-lyase. By assuming that the reaction was irreversible and was not inhibited by product, they were able to interpret $K^{\text{app.}}$ and $V^{\text{app.}}$ in terms of K_A and V_f .

Jennings & Neimann (1953, 1955) pointed out that this same type of plot can be used in the presence of competitive product inhibition provided that $P_0 = 0$. In effect, their method recognizes that the limit, as t approaches zero, of $\Delta P/t$ is v_0 , and that of $-\Delta P / \ln(1 - \Delta P/A_0)$ is A_0 . Points corresponding to v_0 can be found for a set of progress curves by extrapolating each back to the point at which $-\Delta P / \ln(1 - \Delta P/A_0) = A_0$. The resulting set of points can then be treated like an ordinary set of values of v_0 and A_0 in order to obtain K_A and V_f . Alternatively, Orsi & Tipton (1979) have pointed out that replots can be used to obtain K_A , K_p and V_f , much as with the usual initial-rate studies.

Atkins & Nimmo (1973) determined K_A and V_f from simulated progress curves with normally distributed errors by running a regression of $\Delta P/t$ on $[\ln(1 - \Delta P/A_0)]/t$, i.e. on the plot suggested by Walker & Schmidt (1944). While recognizing that this procedure is theoretically invalid, they found that it gave good parameter estimates when A_0 was at least $1.4 \times K_A$.

It is clear that eqn. (3), for a reversible reaction, will also give a linear plot of this sort provided that $J_{AP} = 0$, i.e. in the absence of uncompetitive product inhibition. The two apparent constants will be:

$$V^{\text{app.}} = \frac{V_f J_A (1 + 1/K_e)}{J_A - J_p} = \frac{V_f (1 + 1/K_e)}{1 - K_A/K_p}$$

$$K^{\text{app.}} = \frac{J_0 + J_A A_e + J_p P_e}{J_A - J_p}$$

$$= \frac{K_A \left[1 + \left(\frac{A_0 + P_0}{K_e + 1} \right) \left(\frac{K_e}{K_p} + \frac{1}{K_A} \right) \right]}{1 - K_A/K_p}$$

If K_e approaches infinity, these expressions approach those given above for an irreversible reaction. This plot could be used to test for the presence of uncompetitive product inhibition.

Schwert (1969a,b) obtained parameter values for the second-order lactate dehydrogenase reaction by varying the substrate concentration and solving the integrated equations at identical fractional reactions. This is effective only if the mechanism is known. Bates & Frieden (1973a,b), also by knowing in advance the mechanism and empirical rate equation, were able to obtain kinetic constants by calculating progress curves by numerical integration and fitting actual data for fumarase and glutamate dehydrogenase by eye.

Alberty & Koerber (1957) used a polynomial expansion of the logarithm term in order to fit fumarase data. Philo & Selwyn (1973), using simulated first-order progress curves, showed that polynomials in t give poor fits even with eleventh-order terms, whereas third-order polynomials in P fit well. They used this technique to obtain parameter estimates for the soluble mitochondrial ATPase from ox heart, an enzyme that is difficult to study by initial-rate methods because K_A/K_p is 25.

Fernley (1974) (see also Nimmo & Atkins, 1974) appears to have been the first to suggest the use of non-linear techniques to fit integrated equations directly. In these procedures, initial estimates of the parameters such as K_A are used to obtain calculated values of ΔP . An iterative non-linear regression technique is then used to refine the para-

meter values until they give the best fit to the observed values of ΔP , i.e. until the sum of the squares of the differences is minimized. Fernley (1974) outlines this procedure for irreversible first-order reactions in the absence of product inhibition. Darvey *et al.* (1975) use it to fit fumarase data at low substrate concentrations to a reversible mechanism that includes competitive, but not uncompetitive, product inhibition. Duggleby & Morrison (1977, 1978) extend the technique to second-order reactions under pseudo-first-order conditions.

These non-linear techniques appear to be the most appropriate for the equations presented above. For a particular time course, the coefficients C that, in eqn. (3) or (4), minimize the squared differences between ΔP observed and ΔP calculated could be obtained by non-linear regression. And, at least in principle, the fundamental kinetic constants of the reaction (V_i and ratios of the coefficients J) could then be determined from the dependence of the coefficients C on the initial conditions. However, it remains to be seen in practice just how precise the original parameter estimates must be in order to achieve convergence, how well the technique will work for ordinary, error-containing, data when many parameters are to be estimated, and what effect errors in the equilibrium constant will produce. It is apparent that irreversible reactions will be simpler than reversible ones, both in theory and practice; such reactions are probably a good place to start.

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APPENDIX

Definitions of α , β , γ , δ and ε

The definitions are consistent for all of the four stoichiometries. For $A \rightleftharpoons P$, all coefficients con-

taining B or Q in the subscript are zero. For $A \rightleftharpoons P + Q$, those containing B are zero, and for $A + B \rightleftharpoons P$, those containing Q are zero.

$$\varepsilon = J_{ABPQ}$$

$$\delta = -J_{ABP} - J_{ABQ} + J_{APQ} + J_{BPQ} + J_{ABPQ}(A_e + B_e - P_e - Q_e)$$

$$\gamma = J_{AB} + J_{PQ} - J_{AP} - J_{AQ} - J_{BP} - J_{BQ} + J_{ABP}(-A_e - B_e + P_e) + J_{ABQ}(-A_e - B_e + Q_e) \\ + J_{APQ}(A_e - P_e - Q_e) + J_{BPQ}(B_e - P_e - Q_e) + J_{ABPQ}(A_e B_e + P_e Q_e - A_e P_e - A_e Q_e - B_e P_e - B_e Q_e)$$

$$\beta = J_A + J_B - J_P - J_Q + J_{AB}(A_e + B_e) + J_{PQ}(-P_e - Q_e) + J_{AP}(-A_e + P_e) + J_{AQ}(-A_e + Q_e) \\ + J_{BP}(-B_e + P_e) + J_{BQ}(-B_e + Q_e) + J_{ABP}(-A_e B_e + A_e P_e + B_e P_e) + J_{ABQ}(-A_e B_e + A_e Q_e + B_e Q_e) \\ + J_{APQ}(-A_e P_e - A_e Q_e + P_e Q_e) + J_{BPQ}(-B_e P_e - B_e Q_e + P_e Q_e) \\ + J_{ABPQ}(-A_e B_e P_e - A_e B_e Q_e + A_e B_e Q_e + A_e P_e Q_e + B_e P_e Q_e)$$

$$\alpha = J_O + J_A A_e + J_B B_e J_P P_e + J_Q Q_e + J_{AB} P_e Q_e + J_{AP} A_e P_e + J_{AQ} A_e Q_e + J_{BP} B_e P_e + J_{BQ} B_e Q_e \\ + J_{ABP} A_e B_e P_e + J_{ABQ} A_e B_e Q_e + J_{APQ} A_e P_e Q_e + J_{BPQ} B_e P_e Q_e + J_{ABPQ} A_e B_e P_e Q_e$$