

Estimation of Kinetic Parameters When Modifiers Are Bound in Enzyme-Catalyzed Reactions

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Modifiers of enzyme-catalyzed reactions can have various types of effects on the velocity, but the most important effect is that they provide multiple pathways to products. The rapid-equilibrium kinetic effects of modifiers are explored for the enzyme-catalyzed reaction $A \rightarrow \text{products}$. When a single molecule of modifier X is bound, the mechanism involves three independent equilibrium expressions and two rate constants. But when two molecules of X are bound in two reactions, there are five independent equilibria and three paths to products. The advantages of using a computer to derive rapid-equilibrium rate equations are that more complicated rate equations can be derived and the kinetic parameters can be estimated using the minimum number of velocity measurements. The mechanism with three paths to products is of special interest because the effects of cooperativity can be studied. Thermodynamic cycles can be used to estimate additional kinetic parameters.

Introduction

Enzyme mechanisms may involve inhibitors, activators, and modifiers. The feature that characterizes modification is that this leads to more than one path to products. Cornish-Bowden¹ gives a general modifier mechanism in his Figure 5.10 and points out that activation or inhibition may result and that the conditions that determine whether the modifier is an activator or inhibitor may be different in different ranges of substrate concentrations. The rapid-equilibrium kinetic effects of modification are explored here for the enzyme-catalyzed reaction $A \rightarrow \text{products}$, but these effects can also be explored for more complicated enzyme-catalyzed reactions.

General Modifier Mechanism for $A \rightarrow \text{Products}$ When a Single Molecule of Modifier Is Bound and There Are Two Paths to Products. The following mechanism is the rapid-equilibrium version of Cornish-Bowden's general modifier mechanism.



This mechanism involves the binding of one molecule of activator X . The possible reaction $EA + X = EAX$ has been omitted because it is redundant in calculating the equilibrium concentrations of EA and EAX . However, the equilibrium constant for this reaction can be estimated from velocity measurements, as will be shown.

The equilibrium constant expressions for this mechanism are

$$E + A = EA \quad K_A = [E][A]/[EA] \quad (2)$$

$$E + X = EX \quad K_X = [E][X]/[EX] \quad (3)$$

$$EX + A = EAX \quad K_{AX} = [EX][A]/[EAX] \quad (4)$$

The rapid-equilibrium velocity v of the reaction $A \rightarrow \text{products}$ is given by

$$v = k_{f1}[EA] + k_{f2}[EAX] = k_{f1}[E]_t([EA]/[E]_t) + k_{f2}[E]_t([EAX]/[E]_t) \quad (5)$$

The rapid-equilibrium rate equation can be derived by use of Solve in *Mathematica*, Maple, or MatLab. This derivation using *Mathematica* is shown in the Appendix. In making these calculations, $k_{f1}[E]_t$ and $k_{f2}[E]_t$ are considered to be kinetic parameters, and they are represented by `kf1et` and `kf2et` in *Mathematica*. Symbols in *Mathematica* have to start with lowercase letters because the names of operations start with capital letters. To make test calculations, the following values of kinetic parameters are arbitrarily assigned: $k_{f1}[E]_t = 1$, $k_{f2}[E]_t = 2$, $K_A = 10$, $K_X = 20$, and $K_{AX} = 5$. These kinetic parameters are put into `vEAX` (see Appendix) by using the `ReplaceAll` operation (`/x→`) to obtain the following rate equation.

$$v_{ELM1} = \frac{100[A] + 20[A][X]}{1000 + 100[A] + 50[X] + 10[A][X]} \quad (6)$$

The rapid-equilibrium velocity `velM1` with the specified kinetic parameters is plotted versus $[A]$ and $[X]$ in Figure 1. The partial derivatives of `velM1` are also given to show where the velocity is most sensitive to $[A]$, $[X]$, and $[A][X]$.

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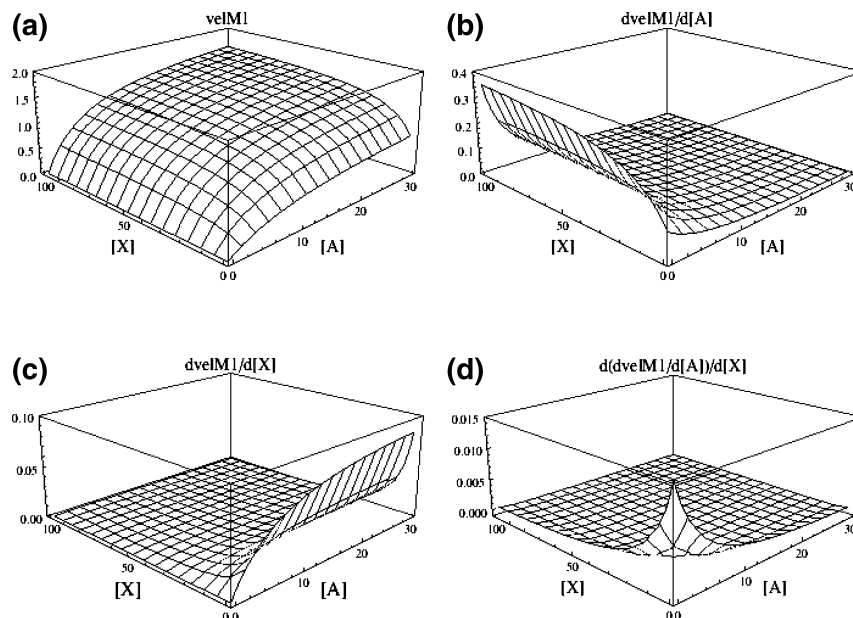


Figure 1. Plots of $velM1$, $\partial velM1/\partial[A]$, $\partial velM1/\partial[X]$, and $\partial(\partial velM1/\partial[A])/d[X]$ versus $[A]$ and $[X]$ for $A \rightarrow$ products for the general modifier mechanism.

The concept that N kinetic parameters can be calculated from N velocity measurements was introduced by Duggleby² in connection with his discussion of the statistics of the determination of the kinetic parameters for enzyme-catalyzed reactions. Before that, Frieden and Fernandez-Sousa,³ in an article about malate dehydrogenase, showed that it was possible to determine four kinetic parameters with four velocity measurements. Recent papers have shown how this can be done for $A + B \rightarrow$ products,⁴ for five mechanisms of $A + B + C \rightarrow$ products,⁵ and for ordered and random $A + B = P + Q$.⁶

Mechanism 1-4 involves five independent kinetic parameters, and so the minimum number of velocity measurements to calculate the kinetic parameters is five. This method can be tested by calculating five velocities using eq 6 and then using these velocities to estimate the kinetic parameters in vEAX given in the Appendix. In the absence of modifier X, Figure 1a shows the Michaelis–Menten plot for $A \rightarrow$ products in the absence of the modifier on the right face. Thus, in the absence of modifier X, the determination of velocities at $\{[A],[X]\} = \{1,0\}$ and $\{100,0\}$ makes it possible to estimate k_{f1et} and K_A . The use of $\{100,100\}$ makes it possible to estimate $k_{f1}[E]_t + k_{f2}[E]_t$. Figure 1c shows that the velocity is very sensitive to the substrate and modifier concentrations at high $[A]$ and low $[X]$. The velocity is very sensitive to both $[A]$ and $[X]$ at very low concentrations of both. Therefore, velocities at the following five pairs of substrate concentrations $\{[A],[X]\} = \{1,0\}$, $\{100,0\}$, $\{100,100\}$, $\{1,100\}$, and $\{5,5\}$ can be used to estimate the five kinetic parameters. The velocities obtained using ReplaceAll (/x→) for these pairs of substrate concentrations using eq 6 are 0.09091, 0.9091, 1.8103, 0.2958, and 0.5000, respectively. The program calckinparsgenmod for estimating the five kinetic parameters by solving five simultaneous polynomial equations is given in the Appendix. This program is really just an orderly way to put velocities and substrate concentrations into Solve in *Mathematica* to obtain the solution to N simultaneous equations for N velocity measurements. This yields the correct values for the five kinetic parameters, but experimental errors in velocity measurements have to be taken into account. That is done by multiplying the velocities by 1.05, one at a time. These calculations yield Table 1. More accurate values of the kinetic parameters can be obtained by using wider ranges of substrate concentrations or different pairs of substrate concentrations.

TABLE 1: Estimation of Five Kinetic Parameters for the General Modifier Mechanism for $A \rightarrow$ Products When One Molecule of Modifier X Is Bound Using Velocities at $\{1,0\}$, $\{100,0\}$, $\{100,100\}$, $\{1,100\}$, and $\{5,5\}$

	$k_{f2}[E]_t$	$k_{f1}[E]_t$	K_X	K_A	K_{AX}
no errors	2.00	1.00	20.00	10.00	5.00
$1.05 \times v(1,0)$	2.01	0.99	21.29	9.42	5.01
$1.05 \times v(100,0)$	1.99	1.06	21.21	10.61	4.94
$1.05 \times v(100,100)$	2.12	1.00	20.34	10.00	5.34
$1.05 \times v(1,100)$	2.00	1.00	22.37	10.00	4.62
$1.05 \times v(5,5)$	1.98	1.00	15.76	10.00	5.09

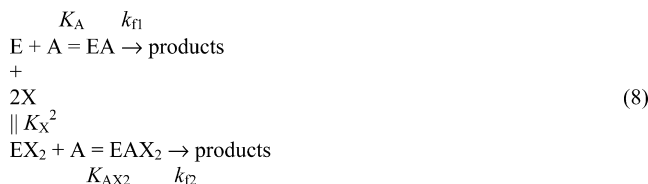
Calculation of a Fourth Equilibrium Constant When a Single Molecule of X Is Bound and There Are Two Paths to Products. Mechanism 1 can be extended to include $EA + X = EAX$ with equilibrium constant K_{XA} .



When the three reactions in mechanism 1 are at equilibrium, the reaction $EA + X = EAX$ is also at equilibrium because of the thermodynamic cycle in eq 7 for which $K_A K_X = K_{AX} K_{XA}$. Since K_A , K_X , and K_{AX} can be estimated from five measurements of velocities, K_{XA} can be estimated by using $K_A K_X / K_{AX}$. These values of K_{XA} can be added to Table 1 to obtain Table 2.

When experimental results are published, values of K_{XA} should be reported because they are equilibrium constants and because another investigator might use a mechanism that includes $EA + X = EAX$, rather than $E + X = EX$.

General Modifier Mechanism for $A \rightarrow$ Products When Two Molecules of Modifier Are Bound in a Single Reaction and There Are Two Paths to Products. The mechanism in the preceding section can be changed to include the binding of $2X$ in a single reaction.



The equilibrium constant expressions are

$$E + A = EA \quad K_A = [E][A]/[EA] \quad (9)$$

$$E + 2X = EX_2 \quad K_X^2 = [E][X]^2/[EX_2] \quad (10)$$

$$EX_2 + A = EAX_2 \quad K_{AX2} = [EX_2][A]/[EAX_2] \quad (11)$$

The rapid-equilibrium velocity is given by

$$v = k_{f1}[EA] + k_{f2}[EAX_2] \quad (12)$$

The rate equation v_{EAX2} can be derived by using Solve, as shown in the Appendix.

To make test calculations, the following values of kinetic parameters are arbitrarily assigned: $k_{f1}[E]_t = 1$, $k_{f2}[E]_t = 2$, $K_A = 10$, $K_X = 20$, and $K_{AX2} = 5$. These kinetic parameters can be put into v_{EAX2} in the Appendix by using the ReplaceAll operation (/x→) to obtain the following rate equation.

$$velM2 = \frac{2000[A] + 20[A][X]^2}{20000 + 2000[A] + 50[X]^2 + 10[A][X]^2} \quad (13)$$

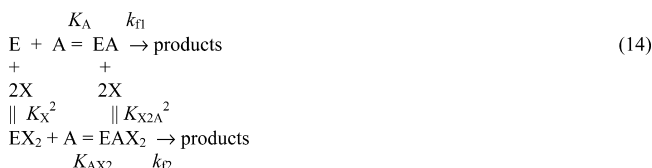
The rapid-equilibrium velocity $velM2$ is plotted versus [A] and [X] in Figure 2. Note that the plots of velocity versus [X] at constant [A] are sigmoid.

There are five kinetic parameters, and so five velocities have to be measured. Velocities are calculated at the following pairs of substrate concentrations: {[A],[X]} = {1,0}, {100,0}, {100,100}, {1,100}, and {5,5}. The velocities obtained using ReplaceAll (/x→) for these pairs of substrate concentrations using eq 13 are 0.09091, 0.9091, 1.8843, 0.3248, and 0.3846, respectively. A program like calckinparsgenmod in the Appendix was written to estimate the five kinetic parameters. The correct values of the five kinetic parameters are obtained using these velocities, but experimental errors in velocity measurements can be taken into account by multiplying the velocities by 1.05, one at a time. These calculations yield Table 3.

TABLE 2: Estimation of Six Kinetic Parameters for the General Modifier Mechanism for A → Products When One Molecule of Modifier X Is Bound Using Velocities at {1,0}, {100,0}, {100,100}, {1,100}, and {5,5} and the Thermodynamic Cycle in eq 7

	$k_{f2}[E]_t$	$k_{f1}[E]_t$	K_X	K_A	K_{AX}	K_{XA}
no errors	2.00	1.00	20.00	10.00	5.00	40.00
$1.05 \times v(1,0)$	2.01	0.99	21.29	9.42	5.01	40.03
$1.05 \times v(100,0)$	1.99	1.06	21.21	10.61	4.94	45.55
$1.05 \times v(100,100)$	2.12	1.00	20.34	10.00	5.34	38.09
$1.05 \times v(1,100)$	2.00	1.00	22.37	1.00	4.62	48.42
$1.05 \times v(5,5)$	1.98	1.00	15.76	10.00	5.09	30.96

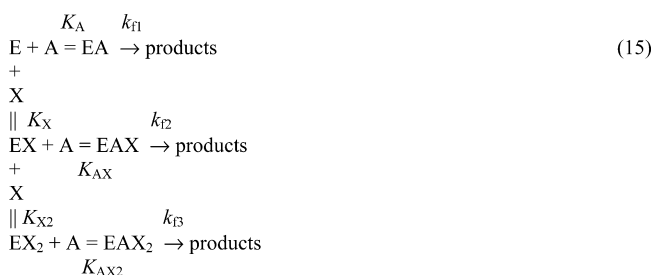
Calculation of a Fourth Equilibrium Constant When 2X Are Bound in a Single Reaction and There Are Two Paths to Products. Mechanism 8 can be extended to include $EA + 2X = EAX_2$ with equilibrium constant K_{X2A} .



When the three reactions in mechanism 8 are at equilibrium, the reaction $EA + 2X = EAX_2$ is also at equilibrium because of the thermodynamic cycle for which $K_A K_X^2 = K_{AX2} K_{X2A}^2$. Since K_A , K_X , and K_{AX2} can be determined from five measurements of velocities, K_{X2A} can be calculated by using $(K_A K_X^2 / K_{AX2})^{0.5}$. These values of K_{X2A} can be added to Table 3 to obtain Table 4.

Determination of the Number of Modifier Molecules Bound. The effect of having two X bound rather than one X bound is best seen at high [A] and low [X]. The effect of having more X bound is striking. Figure 3a shows that plots of measured velocities at high [A] over a range of [X] can be used to determine the number of modifier molecules bound. The number of modifier molecules bound is even clearer when the derivative of the plot with respect to [X] is taken.

General Modifier Mechanism for A → Products When 2x Is Bound in Two Reactions and There Are Three Paths to Products. This is an extension of mechanism 1 to a mechanism with three paths to products. Since 2X are bound in two reactions, there is an opportunity to discuss positive and negative cooperativity⁷ in the binding of X. Cooperativity is positive when the second molecule of X is bound more tightly than the first. This occurs in hemoglobin where the second molecule of molecular oxygen is bound more tightly than the first. Cooperativity is negative when the second molecule of X is bound less tightly than the first.



The equilibrium constants in this mechanism are

$$E + A = EA \quad K_A = [E][A]/[EA] \quad (16)$$

$$E + X = EX \quad K_X = [E][X]/[EX] \quad (17)$$

$$EX + A = EAX \quad K_{AX} = [EX][A]/[EAX] \quad (18)$$

$$EX + X = EX_2 \quad K_{X2} = [EX][X]/[EX_2] \quad (19)$$

$$EX_2 + A = EAX_2 \quad K_{AX2} = [EX_2][A]/[EAX_2] \quad (20)$$

The rapid-equilibrium velocity v of the reaction $A \rightarrow \text{products}$ is given by

$$v = k_{f1}[EA] + k_{f2}[EAX] + k_{f3}[EAX_2] = k_{f1}[E]_t([EA]/[E])_t + k_{f2}[E]_t([EAX]/[E])_t + k_{f3}[E]_t([EAX_2]/[E])_t \quad (21)$$

The rapid-equilibrium rate equation vM derived using Solve is given in the Appendix.

The first set of calculations is made for a case of positive cooperativity, and the following values for the equilibrium constants are used: $K_A = 20$, $K_X = 20$, $K_{AX} = 20$, $K_{X_2} = 5$, and $K_{AX_2} = 10$. The second set of calculations is made for a case of negative cooperativity, and the following values for the equilibrium constants are used: $K_A = 20$, $K_X = 5$, $K_{AX} = 20$, $K_{X_2} = 20$, and $K_{AX_2} = 10$. In both sets of calculations, $k_{f1}[E]_t = 1$, $k_{f2}[E]_t = 2$, and $k_{f3}[E]_t = 3$.

When the kinetic parameters for positive cooperativity are used, the following rate equation is obtained:

$$v_{MP2} = (20000[A] + 2000[A][X] + 1200[A][X]^2)/(400000 + 20000[A] + 20000[X] + 1000[A][X] + 4000[X]^2 + 400[A][X]^2) \quad (22)$$

When the kinetic parameters for negative cooperativity are used, the following rate equation is obtained:

$$v_{MN2} = (20000[A] + 8000[A][X] + 1200[A][X]^2)/(400000 + 20000[A] + 80000[X] + 4000[A][X] + 4000[X]^2 + 400[A][X]^2) \quad (23)$$

The 3D plots of v_{MN1} and v_{MN2} do not look very different, but the differences between positive and negative cooperativity can be seen in cross sections of the usual 3D plots. The plots shown in Figure 4 confirm the statement by Cornish-Bowden¹ that “the conditions that determine whether the modifier is an

TABLE 3: Estimation of Five Kinetic Parameters for the General Modifier Mechanism for $A \rightarrow$ Products When 2X are Bound in One Reaction Using Velocities at {1,0}, {100,0}, {100,100}, {1,100}, and {5,5}

	$k_{f2}[E]_t$	$k_{f1}[E]_t$	K_A	K_{AX_2}	K_X
no errors	2.00	1.00	10.00	5.00	20.01
$1.05 \times v(1,0)$	2.01	0.99	9.42	4.98	22.62
$1.05 \times v(100,0)$	2.00	1.06	10.61	4.98	21.27
$1.05 \times v(100,100)$	2.11	1.00	10.00	5.32	20.04
$1.05 \times v(1,100)$	1.99	1.00	10.00	4.68	20.82
$1.05 \times v(5,5)$	1.99	1.00	10.00	5.03	16.68

TABLE 4: Estimation of Six Kinetic Parameters for the General Modifier Mechanism for $A \rightarrow$ Products When Two Molecules of Modifier X are Bound in a Single Reaction Using Velocities at {1,0}, {100,0}, {100,100}, {1,100}, and {5,5} and the Thermodynamic Cycle in eq 14

	$k_{f2}[E]_t$	$k_{f1}[E]_t$	K_X	K_A	K_{AX_2}	K_{X_2A}
no errors	2.00	1.00	20.00	10.00	5.00	28.30
$1.05 \times v(1,0)$	2.01	0.99	22.62	9.42	4.98	31.11
$1.05 \times v(100,0)$	2.00	1.06	21.27	10.61	4.98	31.05
$1.05 \times v(100,100)$	2.11	1.00	21.04	10.00	5.32	27.48
$1.05 \times v(1,100)$	1.99	1.00	20.82	10.00	4.68	30.43
$1.05 \times v(5,5)$	1.99	1.00	16.68	10.00	5.03	23.52

activator or inhibitor may be different in different ranges of substrate concentrations.”

Discussion

These calculations demonstrate the usefulness of deriving rapid-equilibrium rate equations with a computer and testing new methods for estimating kinetic parameters using the minimum number of velocity measurements. With a computer, it is very easy to calculate velocities for a given set of kinetic parameters and substrate concentrations. When kinetic parameters have been estimated, it is very easy to calculate velocities to check agreement with the experimental velocities.

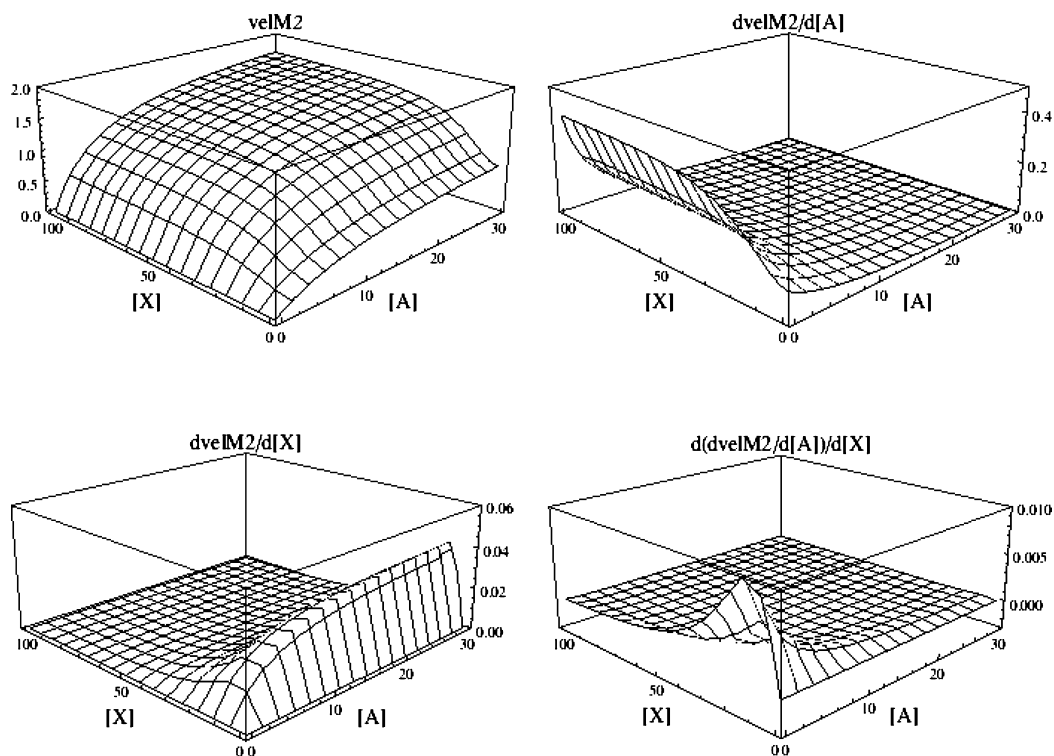


Figure 2. Plots of v_{M2} , $\partial v_{M2}/\partial[A]$, $\partial v_{M2}/\partial[X]$, and $\partial(\partial v_{M2}/\partial[A])/ \partial[X]$ versus $[A]$ and $[X]$ for $A \rightarrow$ products for the general modifier mechanism when 2X are bound in one reaction.

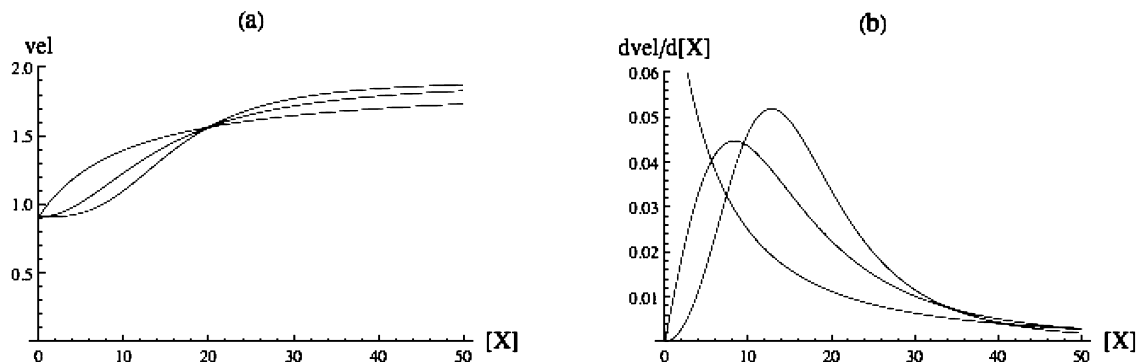


Figure 3. (a) Velocities at $[A] = 100$ and various concentrations of $[X]$. The numbers of modifier molecules bound are 1, 2, and 3. (b) Derivatives of velocities with respect to $[X]$ at $[A] = 100$ and various concentrations of X .

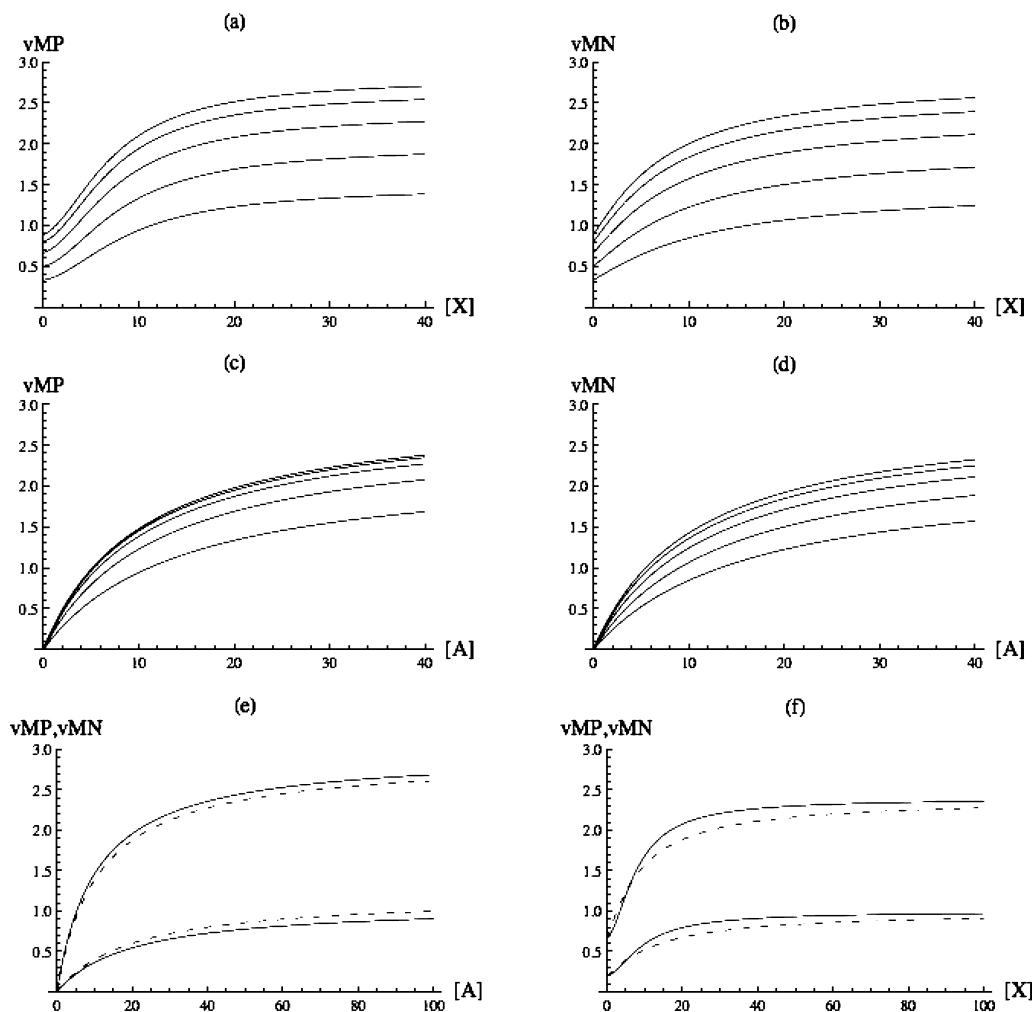


Figure 4. (a) Plot of v_{MP} (velModificationPositive) versus modifier concentration $[X]$ when $[A] = 10, 20, 40, 80$, and 160 . This shows sigmoid plots because $K_X = 20$ and $K_{X2} = 5$. (b) Plot of v_{MN} (velModificationNegative) versus modifier concentration $[X]$ when $[A] = 10, 20, 40, 80$, and 160 . (c) Plots of v_{MP} at $[X] = 10, 20, 40, 80$, and 160 versus $[A]$. (d) Plots of v_{MN} at $[X] = 10, 20, 40, 80$, and 160 versus $[A]$. (e) The solid lines are for v_{MP} at two concentrations of modifier X ; the upper solid line is for $[X] = 40$, and the lower solid line is for $[X] = 5$. The dashed lines give the velocity v_{MN} for the same two concentrations of modifier X . The top pair of lines shows that when $K_X = 20$ and $K_{X2} = 5$, the velocity is higher than when $K_X = 5$ and $K_{X2} = 20$. The bottom pair of lines shows that when $K_X = 20$ and $K_{X2} = 5$, the velocity is higher than when $K_X = 5$ and $K_{X2} = 20$. (f) Plots of v_{MP} at $[A] = 5$ and 40 (solid line) and v_{MN} at 5 and 40 (dashed line) as functions of $[X]$. In both cases, v_{MP} is higher than v_{MN} at higher concentrations of modifier X , but not at lower concentrations of modifier X .

The accuracy of estimation of kinetic parameters can always be improved by the use of a wider range of substrate concentrations, especially at the low concentrations. These calculations emphasize the special importance of velocities at the lowest substrate concentrations. It is much easier to measure velocities accurately near the limiting velocity because these velocities are not as sensitive to small errors in substrate concentrations

and do not drop off rapidly. To increase the accuracy of velocities at low substrate concentrations, replicate measurements can be used or the enzyme concentration can be increased. When the enzyme concentration is increased by a factor of 10, the measured velocity needs to be decreased by a factor of 10.

Solve can also be used to derive rapid-equilibrium rate equations to estimate the pK 's of the enzymatic site, enzyme—

substrate complexes, substrates, and the number of hydrogen ions consumed in the rate-determining reaction. When this is done, the number of velocities that have to be measured increases. At some point, it becomes impractical to use calckinpars programs to determine very large numbers of kinetic parameters. The investigation of pH effects can be carried out in two steps. In the first step, the rate equations with pH-dependent kinetic parameters are determined at three or more pHs. Measurements at three pHs is enough when the enzymatic site and enzyme-substrate complexes each have two pK 's and one or more hydrogen ions are consumed in the rate-determining reaction. This is because the pH-independent kinetic parameters can all be obtained from bell-shaped plots.⁸

In modification, there can be activation or inhibition, but only activation is considered here. The number of modifier molecules bound can be determined without determining all the kinetic parameters. In treating systems of enzyme-catalyzed reactions, it is important to know about inhibition, activation, and modification in addition to the usual kinetic parameters.¹

Calckinpars programs can be written for steady-state rate equations as well as rapid-equilibrium rate equations. For example, the formation of dead-end complexes can be included in the rapid-equilibrium mechanism. The only requirement of the use of Solve is that the rate equation be a polynomial.

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Appendix

Derivation of the Rapid-Equilibrium Rate Equation When a Single Modifier Molecule Is Bound Using Mathematica

In:

Solve[{ $k_A = e*a/ea$, $k_X = e*x/ex$, $k_{AX} = ex*a/eaX$, $et = e+ea+ex+eaX$ },{ ea , eaX },{ e , ex }]

Out:

{{ $ea \rightarrow (a \text{ et } k_{AX} k_X)/(a k_{AX} k_X + k_A k_{AX} k_X + a k_A x + k_A k_{AX} x)$, $eaX \rightarrow (a \text{ et } k_A x)/(a k_{AX} k_X + k_A k_{AX} k_X + a k_A x + k_A k_{AX} x)$ }}

Since the denominators for [EA] and [EAX] are the same, the rate equation is given by $v_{EAX} = (a k_{f1et} k_{AX} k_X + a k_{f2et} k_A x)/(a k_{AX} k_X + k_A k_{AX} k_X + a k_A x + k_A k_{AX} x)$. This rate equation can be used to calculate the velocity *velMI* for specified values for the kinetic parameters by use of

$velMI = v_{EAX}/k_{fet} \rightarrow 1/k_{f2et} \rightarrow 2/k_A \rightarrow 10/k_X \rightarrow 20/k_{AX} \rightarrow 5$. This rate equation is eq 6 in the text.

Program to Estimate Five Kinetic Parameters from Five Velocity Measurements When a Single Molecule of Modifier Is Bound

calckinparsgenmod[v1_, a1_, x1_, v2_, a2_, x2_, v3_, a3_, x3_, v4_, a4_, x4_, v5_, a5_, x5_] := Module[{ } (this program calculates k_{f1et} , k_{f2et} , k_A , k_X , and k_{AX} from 5 measured velocities for the general modifier mechanism for $A \rightarrow$ products), Solve[{ $v1 == (a1 k_{f1et} k_{AX} k_X + a1 k_{f2et} k_A x1)/(a1 k_{AX} k_X + k_A k_{AX} k_X + a1 k_A x1 + k_A k_{AX} x1)$ (there are 5 lines like this with subscripts 2, 3, 4, and 5.), { k_{f1et} , k_{f2et} , k_A , k_X , k_{AX} }]}

Derivation of the Expression for the Rapid-Equilibrium Velocity Using Mathematica When Two Modifier Molecules Are Bound in a Single Reaction

Solve[{ $k_A = e*a/ea$, $k_X^2 = e*x^2/ex^2$, $k_{AX^2} = ex^2*a/eaX^2$, $et = e+ea+ex^2+eaX^2$ },{ ea , eaX^2 },{ e , ex^2 }] {{ $ea \rightarrow (a \text{ et } k_{AX^2} k_X^2)/(a k_{AX^2} k_X^2 + k_A k_{AX^2} k_X^2 + a k_A x^2 + k_A k_{AX^2} x^2)$, $eaX^2 \rightarrow (a \text{ et } k_A x^2)/(a k_{AX^2} k_X^2 + k_A k_{AX^2} k_X^2 + a k_A x^2 + k_A k_{AX^2} x^2)$ }}

The rate equation is given by $v_{EAX^2} = (a k_{f1et} k_{AX^2} k_X^2 + a k_{f2et} k_A x^2)/(a k_{AX^2} k_X^2 + k_A k_{AX^2} k_X^2 + a k_A x^2 + k_A k_{AX^2} x^2)$.

Expression for the Rapid-Equilibrium Velocity When Two Modifier Molecules Are Bound in Two Reactions

$v_M = (a k_{AX} k_{AX^2} k_{f1et} k_X k_X^2 + a k_A k_{AX^2} k_{f2et} k_X^2 x + a k_A k_{AX} k_{f3et} x^2)/(a k_{AX} k_{AX^2} k_X k_X^2 + k_A k_{AX^2} k_X k_X^2 + a k_A k_{AX^2} k_X^2 x + k_A k_{AX} k_{AX^2} k_X^2 x + a k_A k_{AX} x^2 + k_A k_{AX} k_{AX^2} x^2)$.

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