Determination of Rapid-Equilibrium Kinetic Parameters of Ordered and Random Enzyme-Catalyzed Reaction A + B = P + Q

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This article deals with the rapid-equilibrium kinetics of the forward and reverse reactions together for the ordered and random enzyme-catalyzed A + B = P + Q and emphasizes the importance of reporting the values of the full set of equilibrium constants. Equilibrium constants that are not in the rate equation can be calculated for random mechanisms using thermodynamic cycles. This treatment is based on the use of a computer to derive rate equations for three mechanisms and to estimate the kinetic parameters with the minimum number of velocity measurements. The most general of these three programs is the one to use first when the mechanism for A + B = P + Q is studied for the first time. This article shows the effects of experimental errors in velocity measurements on the values of the kinetic parameters and on the apparent equilibrium constant calculated using the Haldane relation.
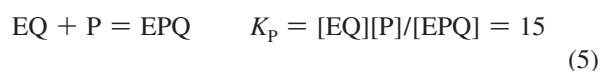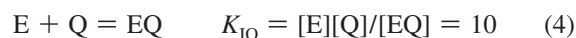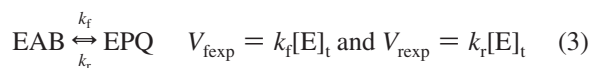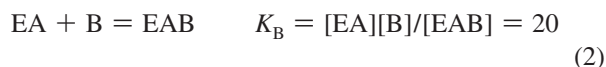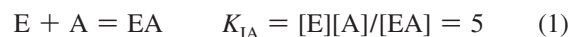
Introduction

This article treats the rapid-equilibrium kinetics of the forward and reverse reactions of the enzyme-catalyzed A + B = P + Q together and emphasizes the importance of reporting the values of the full set of equilibrium constants. A computer is used to derive rate equations for three mechanisms and to estimate the kinetic parameters with the minimum number of velocity measurements. The concept that N kinetic parameters can be calculated from N velocity measurements was introduced by Duggleby[1] in connection with his discussion of the statistics of the determination of the kinetic parameters. Before that, Frieden,[2] in an article about malate dehydrogenase, showed that it was possible to determine four kinetic parameters with four velocity measurements. A recent paper[3] showed how this can be done for A + B → products, and another paper[4] showed how this can be done with five mechanisms for A + B + C → products. The current article shows that there are advantages in treating the forward and reverse reactions at the same time by making calculations on ordered A + B = ordered P + Q, ordered A + B = random P + Q, and random A + B = random P + Q. Three programs are used to estimate the kinetic parameters using the minimum number of velocity measurements. The most general program of these three programs is the one to use first when the mechanism is studied for the first time and the kinetic parameters are unknown. This article shows the effects of experimental errors in velocity measurements on the values of the kinetic parameters and on the apparent equilibrium constant calculated using the Haldane relation.

Derivation of the Rapid-Equilibrium Rate Equation for Ordered A + B = Ordered P + Q. Various mechanisms for A + B = P + Q have been discussed a great deal.[5,6] The objective here is to discuss the estimation of the rapid-equilibrium kinetic parameters of the forward and reverse reactions at the same time using Mathematica. Mulquiney and Kuchel[7] have written a program in Mathematica to derive

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steady-state rate equations for enzyme-catalyzed reactions and have given these rate equations for about 30 reactions.

The rapid-equilibrium mechanism is

$$E + A = EA \qquad K_{IA} = [E][A]/[EA] = 5 \qquad (1)$$

$$EA + B = EAB \qquad K_B = [EA][B]/[EAB] = 20 \qquad (2)$$

$$EAB \underset{k_r}{\overset{k_f}{\rightleftharpoons}} EPQ \qquad V_{fexp} = k_f[E]_t \text{ and } V_{rexp} = k_r[E]_t \qquad (3)$$

$$E + Q = EQ \qquad K_{IQ} = [E][Q]/[EQ] = 10 \qquad (4)$$

$$EQ + P = EPQ \qquad K_P = [EQ][P]/[EPQ] = 15 \qquad (5)$$

The third reaction is rate-determining, and the other four reactions remain at equilibrium. The values of the four dissociation constants are assigned arbitrarily so that numerical calculations can be made. The total concentration of enzymatic sites is given by

$$[E]_t = [E] + [EA] + [EAB] + [EPQ] + [EQ] \qquad (6)$$

There are nine reactants, four equilibrium equations, and five components (A, B, P, Q, and E). Linear algebra[8] shows that the number of reactants N' in an equilibrium calculation must be equal to the number of components C' plus the number of independent reactions $R':N' = C' + R'$. In this case, the number of reactants is given by $9 = 5 + 4$. It is convenient to solve the five equations that have to be satisfied at equilibrium using Solve in Mathematica, Maple, or MatLab to obtain expressions for [EAB] and [EPQ] at equilibrium. The calculations described here were made using Solve[eqs,vars,elims] in Mathematica.

The velocity is given by $v = k_f[EAB] - k_r[EPQ]$. The following rapid-equilibrium rate equation is obtained

$$v = \frac{[A][B]V_{fexp}K_{IQ}K_P - V_{rexp}K_BK_{IA}[P][Q]}{[A][B]K_{IQ}K_P + [A]K_BK_{IQ}K_P + K_BK_{IA}K_{IQ}K_P + K_BK_{IA}K_P[Q] + K_BK_{IA}[P][Q]}$$

(7)

This rate equation can be written in various ways,[5,6] but this is the way Mathematica writes it. This method of deriving a rapid-equilibrium rate equation has three advantages: (1) The rate equation is obtained in computer-readable form so that it does not need to be typed. (2) The relation between the mechanism and the rate equation is emphasized. (3) This method can be applied to much more complicated mechanisms,[4] without having to make lengthy derivations by hand.

Equation 7 in a computer with specified values of the six kinetic parameters can be treated like an experimental system in the sense that substituting a quadruplet of substrate concentrations ([A],[B],[P],[Q]) yields a reaction velocity. When the velocity is equal to zero, the Haldane relation for the apparent equilibrium constant is obtained.

$$K' = \frac{V_{fexp}K_{IQ}K_P}{V_{rexp}K_BK_{IA}}$$

(8)

Estimation of the Kinetic Parameters for Ordered A + B = Ordered P + Q. In 2008, Alberty[3] showed that Solve in Mathematica can be used to obtain the kinetic parameters with the minimum number of velocity measurements. The use of Solve to estimate kinetic parameters by solving N simultaneous polynomial equations has been tested by arbitrarily specifying values of the kinetic parameters and calculating velocities. The following values of kinetic parameters are used in this section: $V_{fexp} = 1$, $V_{rexp} = 0.5$, $K_{IA} = 5$, $K_B = 20$, $K_{IQ} = 10$, and $K_P = 15$. In making these calculations, it is not necessary to assign units to these kinetic parameters. Since there are six kinetic parameters, it is necessary to measure six velocities to estimate the kinetic parameters with the minimum number of velocities. To illustrate this calculation, velocities are calculated at six quadruplets of substrate concentrations. The choices of substrate concentrations have been discussed in two previous articles[3,4] and are reviewed in the Discussion. The velocities calculated using eq 7 are $v_1(100,100,0,0) = 0.8264$, $v_2(1,100,0,0) = 0.4545$, $v_3(100,1,0,0) = 0.04545$, $v_4(0,0,100,100) = -0.4292$, $v_5(0,0,100,1) = -0.01887$, and $v_6(0,0,1,100) = -0.02857$.

A program calckinparsordABordPQfr has been written in Mathematica to calculate V_{fexp}, V_{rexp}, K_{IA}, K_B, K_{IQ}, and K_P from six experimental velocities for ordered A + B = ordered P + Q. The program's name indicates that it calculates the kinetic parameters for the forward reaction ordered A + B = ordered P + Q. This program, given in the Appendix, simply provides an orderly way to put six velocities with the corresponding versions of eq 7 into Solve. This yields correct values of the six kinetic parameters, but it is necessary to take experimental errors in the measurements of velocities into account. This is done by multiplying the six velocities by 1.05, one at a time. The results of this calculation are shown in Table 1.

The values of these parameters can be used to calculate K' using the Haldane relation. This is done by simply copying columns in Table 1 and using eq 8. The use of 5 % experimental errors in velocities may be optimistic; however, the errors in

TABLE 1: Kinetic Parameters for Ordered A + B = Ordered P + Q Calculated from Velocities at {100,100,0,0}, {1,100,0,0}, {100,1,0,0}, {0,0,100,100}, {0,0,1,100}, and {0,0,100,1}
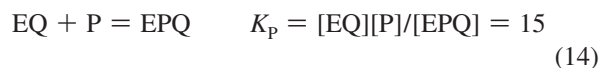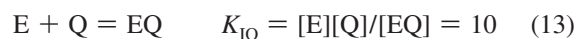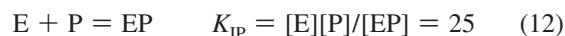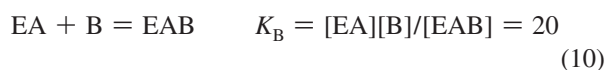
	V_{fexp}	V_{rexp}	K_{IA}	K_{IQ}	K_P	K_B	K'
no errors	1.00	0.50	5.00	10.00	15.00	20.00	3.0
$1.05 \times v_1$	1.06	0.50	5.29	10.00	15.00	21.24	2.8
$1.05 \times v_2$	1.00	0.50	4.45	10.00	15.00	20.11	3.4
$1.05 \times v_3$	0.99	0.50	5.28	10.00	15.00	18.74	3.0
$1.05 \times v_4$	1.00	0.53	5.00	10.37	15.89	20.00	3.1
$1.05 \times v_5$	1.00	0.50	5.00	9.07	15.15	20.00	2.7
$1.05 \times v_6$	1.00	0.50	5.00	10.59	14.04	20.00	3.0

the table can be doubled or tripled, and replicate measurements can be used.

Each of the velocity determinations is targeted on a particular kinetic parameter. The first velocity determination {100,100,0,0} is targeted on V_{fexp}, and the 5 % error in v_1 yields $V_{fexp} = 1.06$. The second velocity determination {1,100,0,0} is targeted on K_{IA}, and the 5 % error in v_2 yields $K_{IA} = 4.45$. The third velocity determination {100,1,0,0} is targeted on K_B, and the 5 % error in v_3 yields $K_B = 18.74$. The fourth velocity determination {0,0,100,100} is targeted on V_{rexp}, and the 5 % error in v_4 yields $V_{rexp} = 0.53$. The fifth velocity determination {0,0,1,100} is targeted on K_{IQ}, and the 5 % error in v_5 yields $K_{IQ} = 9.07$. The sixth velocity determination {0,0,100,1} is targeted on K_P, and the 5 % error in v_6 yields $K_P = 14.04$.

More accurate values of kinetic parameters can be obtained by measuring velocities over a wider range of substrate concentrations. For example, for ordered A + B = ordered P + Q, {200,200,0,0}, {0.1,200,0,0}, {200,0.1,0,0}, {0,0,200,200}, {0,0,0.1,200}, and {0,0,200,0.1} can be used. The recommendations for choices of substrate concentrations are very general: high [A], [B], [P], and [Q] means as high as practical, and low [A], [B], [P], and [Q] means as low as practical, considering the analytical method. When low substrate concentrations are used, rates are low and more difficult to determine, but the enzyme concentration can be increased. When the enzyme concentration is increased 10-fold for a measurement, the velocities have to be divided by a factor of 10 before comparing them with velocities at lower enzyme concentrations.

Estimation of the Kinetic Parameters for Ordered A + B = Random P + Q. The mechanism is

$$E + A = EA \qquad K_{IA} = [E][A]/[EA] = 5 \qquad (9)$$

$$EA + B = EAB \qquad K_B = [EA][B]/[EAB] = 20 \qquad (10)$$

$$EAB \underset{k_r}{\overset{k_f}{\rightleftharpoons}} EPQ \qquad (11)$$

$$E + P = EP \qquad K_{IP} = [E][P]/[EP] = 25 \qquad (12)$$

$$E + Q = EQ \qquad K_{IQ} = [E][Q]/[EQ] = 10 \qquad (13)$$

$$EQ + P = EPQ \qquad K_P = [EQ][P]/[EPQ] = 15 \qquad (14)$$
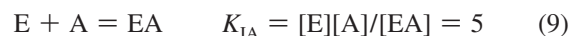
The values of the dissociation constants are assigned arbitrarily so that numerical calculations can be made. The total concentration of enzymatic sites is given by

Random Enzyme-Catalyzed Reaction A + B = P + Q

J. Phys. Chem. B, Vol. 113, No. 29, 2009 **10045**

$$[E]_t = [E] + [EA] + [EAB] + [EPQ] + [EQ] + [EP] \quad (15)$$

There are ten reactants, five equilibrium equations, and five components (A, B, P, Q, and E), so that $N' = C' + R'$ is $10 = 5 + 5$. The six equations that have to be satisfied can be solved to obtain expressions for [EAB] and [EPQ] at equilibrium. It is not necessary to include the reaction EP + Q = EPQ because when reactions 12−14 are at equilibrium this reaction is also at equilibrium. The velocity is given by $v = k_f[EAB] - k_r[EPQ]$. When this is done in Mathematica, the following rapid-equilibrium rate equation is obtained

$$v = \frac{[A][B]V_{fexp}K_{IP}K_{IQ}K_P - V_{rexp}K_BK_{IA}K_{IP}[P][Q]}{D_1 + D_2}$$

$$D_1 = [A][B]K_{IP}K_{IQ}K_P + [A]K_BK_{IP}K_{IQ}K_P +$$
$$K_BK_{IA}K_{IP}K_{IQ}K_P$$

$$D_2 = K_BK_{IA}K_{IQ}K_P[P] + K_BK_{IA}K_{IP}K_P[Q] +$$
$$K_BK_{IA}K_{IP}[P][Q] \quad (16)$$

To test the calculation of seven kinetic parameters from seven measured velocities, the following values of the kinetic parameters are chosen: $V_{fexp} = 1$, $V_{rexp} = 0.5$, $K_{IA} = 5$, $K_B = 20$, $K_{IP} = 25$, $K_{IQ} = 10$, and $K_P = 25$. Velocities are calculated at seven quadruplets of substrate concentrations {[A], [B], [P], [Q]}: {100, 100, 0, 0}, {1, 100, 0, 0}, {100, 1, 0, 0}, {0,0, 100, 100}, {0,0,1,100}, {0, 0, 100,1}, and {0,0,5,5} using eq 16. The last quadruplet is new and was recommended in a previous article.[3] The use of eq 16 yields $v_1(100,100,0,0) = 0.8264$, $v_2(1,100,0,0) = 0.4545$, $v_3(100,1,0,0) = 0.04545$, $v_4(0,0,100,100) = -0.4082$, $v_5(0,0,1,100) = -0.04827$, $v_6(0,0,100,1) = -0.05780$, and $v_7(0,0,5,5) = -0.4464$.

Equation 16 is used to write a program calckinparsordABrandPQfr to estimate the seven kinetic parameters. The program name indicates that it calculates the kinetic parameters for the forward reaction ordered A + B = random P + Q. This program has the same basic structure as calckinparsordABordPQfr. This program yields correct values for the seven kinetic parameters, and the effects of 5% errors in the measured velocities, one at a time, are shown in Table 2.

This table gives values for K_Q calculated using the following thermodynamic cycle

$$
\begin{array}{ccc}
K_{IQ} = 10 & & \\
E + Q & = & EQ \\
+ & & + \\
P & & P \\
\|K_{IP} = 25\| & & \|K_P = 15 \\
EP + Q & = & EPQ \\
& K_Q &
\end{array}
\quad (17)
$$
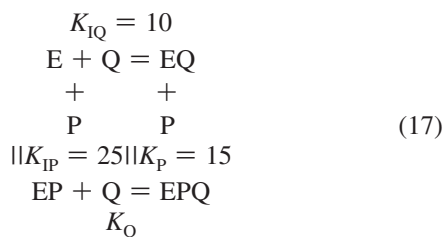
When there are no experimental errors, $K_{IQ}K_P = K_{IP}K_Q$ yields
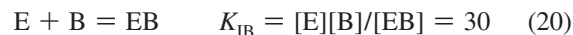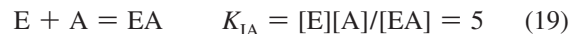
$$K_Q = K_{IQ}K_P/K_{IP} = 10 \times 15/25 = 6 \quad (18)$$

When a reaction with this mechanism is studied experimentally, the value of K_Q should be reported because it is a thermo-
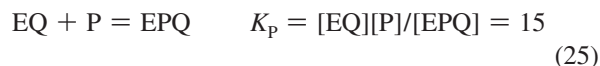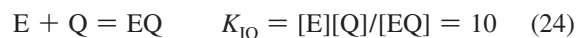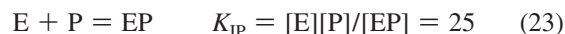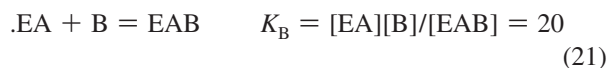
namic equilibrium constant. Another investigator may use a mechanism with K_Q in the mechanism and report the value of K_Q.

The same Haldane relation (see eq 8) applies, and the effects on K' of 5% errors in the measured velocities are shown in Table 2.

Estimation of the Kinetic Parameters for Random A + B = Random P + Q. The mechanism is

$$E + A = EA \qquad K_{IA} = [E][A]/[EA] = 5 \quad (19)$$

$$E + B = EB \qquad K_{IB} = [E][B]/[EB] = 30 \quad (20)$$

This is the new reaction.

$$.EA + B = EAB \qquad K_B = [EA][B]/[EAB] = 20 \quad (21)$$

$$EAB \underset{k_r}{\overset{k_f}{\rightleftharpoons}} EPQ \quad (22)$$

$$E + P = EP \qquad K_{IP} = [E][P]/[EP] = 25 \quad (23)$$

$$E + Q = EQ \qquad K_{IQ} = [E][Q]/[EQ] = 10 \quad (24)$$

$$EQ + P = EPQ \qquad K_P = [EQ][P]/[EPQ] = 15 \quad (25)$$

The value of K_{IB} is assigned arbitrarily so that numerical calculations can be made. The total concentration of enzymatic sites is given by

$$[E]_t = [E] + [EA] + [EAB] + [EPQ] + [EQ] +$$
$$[EP] + [EB] \quad (26)$$

There are eleven reactants, six equilibrium equations, and five components (A, B, P, Q, and E), so that $N' = C' + R'$ is $11 = 5 + 6$. It is convenient to solve the seven equations that have to be satisfied using Solve to obtain expressions for [EAB] and [EPQ] at equilibrium. The velocity is given by $v = k_f[EAB] - k_r[EPQ]$. When this is done, the following rapid-equilibrium rate equation is obtained

$$v = \frac{[A][B]V_{fexp}K_{IB}K_{IP}K_{IQ}K_P - V_{rexp}K_BK_{IA}K_{IB}K_{IP}[P][Q]}{D_1 + D_2}$$

$$D_1 = [B]K_BK_{IA}K_{IP}K_{IQ}K_P + [A][B]K_{IB}K_{IP}K_{IQ}K_P +$$
$$[A]K_BK_{IB}K_{IP}K_{IQ}K_P + K_BK_{IA}K_{IB}K_{IP}K_{IQ}K_P$$

$$D_2 = K_BK_{IA}K_{IB}K_{IQ}K_P[P] + K_BK_{IA}K_{IB}K_{IP}K_P[Q] +$$
$$K_BK_{IA}K_{IB}K_{IP}[P][Q] \quad (27)$$

This rate equation involves eight kinetic parameters, and so eight velocities have to be measured. The following velocities were calculated using eq 27: $v_1(100,100,0,0) = 0.8043$, $v_2(1,100,0,0) = 0.1807$, $v_3(100,1,0,0) = 0.04539$, $v_4(5,5,0,0) = 0.1034$, $v_5(0,0,100,100) = -0.4082$, $v_6(0,0,1,100) = -0.02847$, $v_7(0,0,100,1) = -0.05780$, and $v_8(0,0,5,5) = -0.04464$. A

TABLE 2: Kinetic Parameters for Ordered A + B = Random P + Q Calculated from Velocities at {100,100,0,0}, {1,100,0,0}, {100,1,0,0}, {0,0,100,100}, {0,0,1,100}, {0,0,100,1}, and {0,0,5,5}

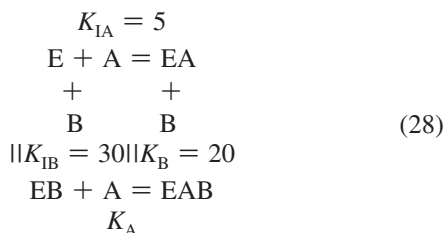|	V_{fexp}	V_{rexp}	K_{IA}	K_{B}	K_{IQ}	K_{P}	K_{IP}	$K_{\text{Q}}{}^a$	K'
no errors	1.00	0.50	5.00	20.00	10.00	15.00	25.00	6.00	3.0
$1.05 \times \nu_1$	1.06	0.50	5.29	21.24	10.00	15.00	25.00	6.00	2.8
$1.05 \times \nu_2$	1.00	0.50	4.45	20.11	10.00	15.00	25.00	6.00	3.4
$1.05 \times \nu_3$	0.99	0.50	5.28	18.74	10.00	15.00	25.00	6.00	3.0
$1.05 \times \nu_4$	1.00	0.53	5.00	20.00	10.03	16.00	24.96	6.43	3.0
$1.05 \times \nu_5$	1.00	0.50	5.00	20.00	10.95	13.99	25.93	5.91	3.1
$1.05 \times \nu_6$	1.00	0.50	5.00	20.00	10.16	14.91	27.36	5.54	3.0
$1.05 \times \nu_7$	1.00	0.50	5.00	20.00	8.93	15.17	22.00	6.16	2.7

^{a} Calculated using thermodynamic cycle 17.

TABLE 3: Kinetic Parameters for Random A + B = Random P + Q Calculated from Velocities at {100,100,0,0}, {1,100,0,0}, {100,1,0,0}, {5,5,0,0}, {0,0,100,100}, {0,0,1,100}, {0,0,100,1}, and {0,0,5,5}

	V_{fexp}	V_{rexp}	K_{IA}	K_{IQ}	K_{P}	K_{IP}	K_{IB}	K_{B}	$K_{\text{Q}}{}^a$	$K_{\text{A}}{}^b$	K'
no errors	1.00	0.50	5.00	10.00	15.00	25.00	30.00	20.00	6.00	3.33	3.0
$1.05 \times \nu_1$	1.06	0.50	5.04	10.00	15.00	25.00	29.86	21.34	6.00	3.60	3.0
$1.05 \times \nu_2$	1.00	0.50	5.07	10.00	15.00	25.00	33.21	19.93	6.00	3.04	3.0
$1.05 \times \nu_3$	0.99	0.50	5.59	10.00	15.00	25.00	32.21	18.68	6.00	3.24	2.8
$1.05 \times \nu_4$	1.00	0.50	4.33	10.03	15.00	25.00	25.21	20.15	6.00	3.46	3.4
$1.05 \times \nu_5$	1.00	0.53	5.00	10.95	16.00	24.96	30.00	20.00	6.43	3.33	3.0
$1.05 \times \nu_6$	1.00	0.50	5.00	10.16	13.99	25.93	30.00	20.00	5.91	3.33	3.1
$1.05 \times \nu_7$	1.00	0.50	5.00	8.93	14.91	27.36	30.00	20.00	5.54	3.33	3.0
$1.05 \times \nu_8$	1.00	0.50	5.00	8.93	15.17	22.00	30.00	20.00	6.16	3.33	2.7

^{a} Calculated using thermodynamic cycle 17. ^{b} Calculated using thermodynamic cycle 28.

computer program calckinparsrandABPQfr was written to estimate eight kinetic parameters from eight measured velocities. The program name indicates that it calculates the kinetic parameters for the forward reaction random A + B = random P + Q.

K_{Q} was calculated using the thermodynamic cycle in eq 17, and K_{A} was calculated using the following thermodynamic cycle.

$$
\begin{array}{ccc}
& K_{\text{IA}} = 5 & \\
\text{E} + \text{A} & = & \text{EA} \\
+ & & + \\
\text{B} & & \text{B} \\
||K_{\text{IB}} = 30 & & ||K_{\text{B}} = 20 \\
\text{EB} + \text{A} & = & \text{EAB} \\
& K_{\text{A}} &
\end{array} \qquad (28)
$$

Since $K_{\text{IA}}K_{\text{B}} = K_{\text{A}}K_{\text{IB}}$, it is possible to calculate K_{A} when there are no experimental errors

$$K_{\text{A}} = K_{\text{IA}}K_{\text{B}}/K_{\text{IB}} = 5 \times 20/30 = 3.333$$

Table 3 gives the values of ten kinetic parameters and values of the apparent equilibrium constants calculated using the Haldane relation. Table 3 shows the effect of experimental errors on the value of K_{A}. The Haldane relation in eq 8 was used to calculate apparent equilibrium constants when there are 5% errors in the velocities, one at a time.

Application of calckinparsrandABPQfr to Data for Ordered A + B = Ordered P + Q. When a mechanism is being studied for the first time, the limiting velocities and Michaelis constants are not known. As recommended earlier,[3] when the kinetics of A + B = P + Q is studied for the first time, there is an advantage in using a more general program first since the mechanism is unknown. When the general program yields reasonable values, that shows that the more general program

applies. When the general program yields unreasonable values, that shows that the simpler mechanism applies.

For ordered A + B = ordered P + Q, six velocities had to be measured (see Table 1), but to use the more general program calckinparsrandABPQfr, two more velocities are needed. These velocities can be measured at {5,5,0,0} and {0,0,5,5}. These velocities calculated for ordered A + B = ordered P + Q are $\nu_4(5,5,0,0) = 0.1034$ and $\nu_8(0,0,5,5) = -0.05000$. When these velocities are put in calckinparsrandABPQfr, the results in Table 4 are obtained.

All of the K_{IP} and K_{IB} values are unreasonable, and this shows that using the most general program can identify the mechanism and yield correct values for the kinetic parameters in the actual mechanism. Notice that the effects of 5% errors in measured velocities on K_{IP} and K_{IB} are highly variable as well as being unreasonable; this is another sign that the velocity data are not from a random A + B = random P + Q mechanism but are from an ordered A + B = ordered P + Q mechanism.

Application of calckinparsrandABPQfr to Data for Ordered A + B = Random P + Q. Seven velocities were calculated earlier using the rate equation for ordered A + B = random P + Q. To apply calckinparsrandABPQfr, it is necessary to have eight velocities. The following velocity was calculated: $\nu(5,5,0,0) = 0.1111$. Including this velocity makes it possible to calculate eight kinetic parameters. When these velocities are put in calckinparsrandABPQfr, the results in Table 5 are obtained.

The values for K_{IB} are unreasonable and very sensitive to 5% errors, so E + B = EB is not in the mechanism. The conclusion that the more general program should be used first is confirmed.

Table of Velocities Calculated for the Three Mechanisms. It is of interest to consider the eight velocities for the three mechanisms that are given in Table 6. There are some small differences between the columns that are not really significant, but ordered A + B = ordered P + Q and ordered A + B = random P + Q differ mainly at {0,0,100,1}, where there is a

TABLE 4: Kinetic Parameters for Ordered A + B = Ordered P + Q Calculated by Applying the More General Program calckinparsrandABPQfr

	V_{fexp}	V_{rexp}	K_{IA}	K_{IQ}	K_P	K_{IP}	K_{IB}	K_B
no errors	1.00	0.50	5.00	10.00	15.00	10×10^6	-3×10^6	20.00
$1.05 \times v_1$	1.06	0.50	5.04	10.00	15.00	10×10^6	2×10^3	21.30
$1.05 \times v_2$	1.00	0.50	5.03	10.00	15.00	10×10^6	-9×10^2	19.97
$1.05 \times v_3$	0.99	0.50	5.59	10.00	15.00	10×10^6	-2×10^3	18.68
$1.05 \times v_4$	1.00	0.53	4.38	10.00	15.00	10×10^6	7×10^2	20.14
$1.05 \times v_5$	1.00	0.50	5.00	10.03	15.95	3×10^3	-3×10^6	20.00
$1.05 \times v_6$	1.00	0.50	5.00	10.05	14.97	-1×10^3	-3×10^6	20.00
$1.05 \times v_7$	1.00	0.50	5.00	10.94	13.99	-3×10^3	-3×10^6	20.00
$1.05 \times v_8$	1.00	0.50	4.38	9.04	15.15	-1×10^3	-3×10^6	20.00

TABLE 5: Kinetic Parameters for Ordered A + B = Random P + Q Calculated by Applying the More General Program calckinparsrandABPQfr

	V_{fexp}	V_{rexp}	K_{IA}	K_{IQ}	K_P	K_{IP}	K_{IB}	K_B
no errors	1.00	0.50	5.00	10.00	15.00	25.00	-4×10^5	20.00
$1.05 \times v_1$	1.06	0.50	5.04	10.00	15.00	25.00	2×10^3	21.30
$1.05 \times v_2$	1.00	0.50	5.03	10.00	15.00	25.00	-9×10^2	19.97
$1.05 \times v_3$	0.99	0.50	5.59	10.00	15.00	25.00	-2×10^3	18.68
$1.05 \times v_4$	1.00	0.53	5.00	10.03	16.00	24.96	-4×10^5	20.00
$1.05 \times v_5$	1.00	0.50	5.00	10.95	13.99	25.93	-4×10^5	20.00
$1.05 \times v_6$	1.00	0.50	5.00	10.16	14.91	27.36	-4×10^5	20.00
$1.05 \times v_7$	1.00	0.50	5.00	8.93	15.17	22.00	-4×10^5	20.00
$1.05 \times v_8$	1.00	0.50	4.38	10.00	15.00	25.00	7×10^2	20.14

TABLE 6: Velocities Calculated for Three Different Mechanisms at Eight Quadruplets of Substrate Concentrations

([A],[B], [P],[Q])	ord A + B = ord P + Q	ord A + B = rand P + Q	rand A + B = rand P + Q
(100,100,0,0}	0.8264	0.8265	0.8043
{1,100,0,0}	0.4545	0.4545	0.1807
{100,1,0,0}	0.04545	0.04545	0.04539
{5,5,0,0}	0.1111	0.1111	0.1034
{0,0,100,100}	−0.4292	−0.4081	0.4082
{0,0,1,100}	−0.02857	−0.02847	−0.02847
{0,0,100,1}	−0.1887	−0.05780	−0.05780
{0,0,5,5}	−0.05000	−0.04464	−0.04464

3-fold difference in velocities. The main difference between ordered A + B = random P + Q and random A + B = random P + Q is at {1,100,0,0}, where there is a 2.5-fold difference in velocities. It is these differences that make it possible to differentiate between the three mechanisms.

Discussion

In general, rapid-equilibrium rate equations are simpler than steady-state rate equations, and so they are the place to start when an enzyme-catalyzed reaction is studied for the first time. If more kinetic parameters are required to fit the kinetic data, steady-state rate equations can be used and even arbitrary terms can be added. When rate equations are polynomial, Solve can be used to determine the kinetic parameters.

The choice of substrate concentrations for the determination of the rapid-equilibrium kinetic parameters with the minimal number of velocity measurements has been discussed earlier[3] by using partial derivatives of the velocity with respect to substrate concentrations. These choices, which have been described in this article, emphasize using the highest and lowest practical concentration. The highest concentrations provide information about V_{fexp} and V_{rexp}, and the lowest concentrations provide information about the Michaelis constants. The accuracy of the measurements at low concentrations of substrates is very dependent on the equipment for the measurements of velocities. When the values of kinetic parameters have been determined

with one minimal set of measured velocities, it is recommended that the calculation be repeated with a second set of substrate concentrations to be sure the same values (within experimental errors) are obtained. Replicate measurements can be used, especially for the low velocities.

This article has emphasized the use of a more general Mathematica program to determine kinetic parameters when an enzyme-catalyzed reaction is being studied for the first time. The calculations described here show that a program written to estimate the kinetic parameters for random A + B = random P + Q can be used to identify the mechanism and to determine correct kinetic parameters when the mechanism is actually ordered A + B = ordered P + Q or ordered A + B = random P + Q.

The kinetic parameters discussed in this paper are usually functions of pH, and when the velocities are measured at a small number of pHs, the pKs of the enzymatic site and the enzyme−substrate complexes can be determined.[9] Some enzyme-catalyzed reactions require metal ions so that the kinetic parameters depend on the free concentration of the metal ion.

Appendix

The following Mathematica program uses Solve to calculate the six kinetic parameters for ordered A + B = ordered P + Q from six measured velocities for mechanism 1−5:

```
calckinparsordABordPQfr[v1_, a1_, b1_, p1_, q1_, v2_, a2_,
b2_, p2_, q2_, v3_,
  a3_, b3_, p3_, q3_, v4_, a4_, b4_, p4_, q4_, v5_, a5_, b5_,
p5_, q5_, v6_,
  a6_, b6_, p6_, q6_]:=
  Module[{},
  Solve[{v1 == (a1 b1 kIQ kP vfexp − kB kIA p1 q1 vrexp)/
(a1 b1 kIQ kP + a1 kB kIQ kP + kB kIA kIQ kP + kB kIA
kP q1 + kB kIA p1 q1), (The next five lines are just like this
one, but with subscripts 2, 3, 4, 5, and 6 on a, b, p, and q)},
{vfexp, vrexp, kIA, kB, kIQ, kP}]]
```

References and Notes

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