The computerized derivation of rate equations for enzyme reactions on the basis of the pseudo-steady-state assumption and the rapid-equilibrium assumption

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A computer program is developed for the derivation of the rate equation for enzyme reactions on the basis of the pseudo-steady-state assumption and the combination of the pseudo-steady-state and the rapid-equilibrium assumptions. The program not only has an easy input method, but also can obtain a complete rate equation in itself on only one run. The usefulness of the program is demonstrated by deriving the rate equations for some typical enzyme reactions. Details of the program have been deposited as Supplementary Publication SUP 50141 (42 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7QB, U.K., from whom copies may be obtained as indicated in Biochem. J. (1988), 249, 5.

INTRODUCTION

The rate equation of an enzyme reaction is usually derived by the following procedure. First, we write the individual rate of the concentration change for each of the species involved, and set the rate of concentration change for each enzyme form to zero according to the pseudo-steady-state assumption. By replacing one of these pseudo-steady-state equations by the enzyme conservation equation, we can obtain the concentrations for all the enzyme forms in terms of the measurable concentrations of the substrates and the products, the total enzyme concentration and the individual rate constants. Finally, we substitute these relations into the rate of the concentration change of one of the substrates or the products. The above step in which the concentrations of each enzyme are derived reduces to solving \( n \) simultaneous algebraic equations, the number \( n \) being equivalent to that of the enzyme forms. This can be easily done by applying Cramer’s rule. However, if \( n \) and the number of reaction rate constants increase, solving the equations mentioned above is extremely tedious and prone to human error. Therefore a lot of methods that can derive rate equations with ease have been proposed.

King & Altman (1956) developed a schematic technique, based on the determinant method, for obtaining the rate equations for enzyme-catalysed reactions with any number of intermediate enzyme complexes. This is, in effect, a set of geometric rules designed to simplify an algebraic procedure. However, if the mechanism is complicated and the number of enzyme forms is considerable, even the King–Altman method is likely to be tedious and prone to human error. The above method can be further simplified by the application of the theory of graphs, as shown by Volkenstein & Goldstein (1966).

Theoretically the rate equation of enzyme-catalysed reactions can be derived on the basis of the pseudo-steady-state assumption even if the mechanisms are extremely complicated or the number of enzyme forms involved is large. However, the pseudo-steady-state treatment of the complicated reaction mechanisms leads to equations and constants so complex that the basic kinetic properties of the mechanism may be obscured. Therefore rate equations derived on the basis of the rapid-equilibrium assumptions are sometimes more useful than the pseudo-steady-state equations. For this reason Cha (1968) proposed a simplified method to derive rate equations under equilibrium assumptions or the combination of the pseudo-steady-state and the equilibrium assumptions.

Fully automatic versions of the King–Altman method were published by Lam & Priest (1972) and Cornish-Bowden (1977). Other computerized methods involving expansion of determinants by applying Cramer’s rule have already been reported by Silvestri & Zahner (1967), Rhoads & Pring (1968), Hurst (1969) and Herries (1984). Since in these programs determinants are expanded in a systematic manner, no errors occur, however complicated the mechanisms. Unfortunately, however, these computerized programs produce either the numerator or the denominator of the rate equation or the numerator of the expression for the proportion of any specified enzyme form (Silvestri & Zahner, 1967), or the numerator or the denominator of the rate equations (Herries, 1984). None of them produces a complete rate equation. Furthermore, a computerized version of the Cha method has not been published yet.

In the present work a versatile program, which has an easy input method and can obtain a complete rate equation in itself on the basis of either the pseudo-steady-state assumption or the combination of the pseudo-steady-state and the rapid-equilibrium assumptions on only one execution, was written in BASIC for a desk-top digital computer, NEC PC 9800 series, which runs on the operating system of MS-DOS.

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In the present reaction scheme, the total chemical species are nine. Therefore nine of the ten equations, eqns. (6)–(15), are independent. Five species of the nine are free or complex forms of the enzyme and their concentrations cannot be measured. Therefore the rate equations should be expressed only in terms of the measurable concentrations of the two substrates, the two products and the total enzyme. This can be easily done by applying the pseudo-steady-state assumption to any four out of the five enzyme forms, all of which concentrations cannot be measured.

For example, let us apply the pseudo-steady-state assumption to the four enzyme complexes EA, EQ, EAB and EPQ of the five. This is equivalent to setting the left-hand side of the equations, namely eqns. (10)–(13), to zero. Combining the resulting equations with the conservation equation of enzyme, we can obtain the concentrations for all these enzyme forms in terms of the measurable concentrations of the species A, B, P and Q, the total enzyme concentration [E]₀ and the reaction rate constants k₁–k₁₀.

The above procedure is equivalent to solving the following matrix equation:

\[
\begin{pmatrix}
0 & 0 & 0 & 0 & 0 \\
0 & k₁[B] & 0 & k₁₀[Q] & 0 \\
-(k₂[k₈] + k₅) & k₈[P] & 0 & k₈[Q] & 0 \\
0 & k₄ & 0 & -(k₆[P] + k₉) & 0 \\
-1 & 1 & 1 & 1 & 1
\end{pmatrix}
\begin{pmatrix}
0 \\
0 \\
0 \\
0 \\
0
\end{pmatrix}
= \begin{pmatrix}
[EPQ] \\
[E]₀ \\
[EQ] \\
[EA] \\
[E]
\end{pmatrix}
\]

Eqn. (16) can be solved by applying Cramer's rule, and the expressions for all the enzyme forms can be derived. Substituting these resulting expressions into the fundamental differential equations for the concentration changes of species A to Q yields the rates of decrease in the concentrations of the substrates A and B or the rates of increase in the concentrations of the products P and Q.

Usually, except for species such as modifier that do not change chemically, the consumption rates of all the substrates agree with each other, and furthermore with the production rates of all the products, which also agree with each other (Herries, 1984), when the pseudo-steady-state assumption holds. Therefore it is sufficient to derive the rate equation for one of the substrates or products.

To solve the above matrix equation by using a computer is simple in principle and is not subject to error. Therefore, from the point of accuracy, it is superior to the King–Altman method, because in the latter method it is often difficult for us to judge whether to take into consideration or reject the individual terms. However, from the point of the computer time required for execution, the former method may be inferior to the latter one. However, this defect is not serious.

In this type of program, the input and output methods are especially important. The input should be as simple and easy as possible, and not be prone to error. As to the output, the final form of the rate equation should be printed out.

Deriving a matrix equation such as eqn. (16) from the differential equations is not usually difficult, and therefore one can start the program by inputting the matrix elements into the computer as the data. However, in the present work, we wrote a program in which we can choose either of two input methods, inputting the matrix
Computerized derivation of rate equations for enzyme reactions

15000 DATA 5: ' Number of the Elementary Reaction Steps
15010 ' "Reactants","Products","Rate Constant of the Forward Reaction"
15020 ' "Rate Constant of the Backward Reaction"
15030 DATA "E+A","EA","k01","k02"
15040 DATA "EA+B","EAB","k03","k04"
15050 DATA "EAB","EPQ","k05","k06"
15060 DATA "EPQ","EQ+P","k07","k08"
15070 DATA "EQ","E+Q","k09","k10"
15080 DATA "F" : ' Name of Species For which Reaction Rate is Derived.

Fig. 1. Input data

The number of the elementary reaction steps, the reactants and the products and the rate constants are given as the input data, and the species on which the rate equation is based is designated.

\[
\begin{align*}
\text{Fig. 2. Print-out of the elementary reaction steps with their rate constants} \\
\text{[ Individual Reaction Steps ]} \\
k_{01} & \quad \text{E+A} \rightarrow \text{EA} \quad (1) \quad k_{02} \\
& \quad \text{EA+B} \rightarrow \text{EAB} \quad (2) \\
k_{07} & \quad \text{EPQ} \rightarrow \text{EQ+P} \quad (4) \quad k_{08} \\
& \quad \text{EQ} \rightarrow \text{E+Q} \quad (5) \\
\end{align*}
\]

\[
\begin{align*}
k_{03} & \quad \text{EA} \rightleftharpoons \text{EPQ} \quad (3) \\
& \quad \text{EP} \rightleftharpoons \text{E} \quad (17) \\
& \quad \text{EPQ} \rightleftharpoons \text{EP+Q} \quad (24) \\
& \quad \text{EP} \rightleftharpoons \text{E}+\text{Q} \quad (25) \\
& \quad \text{EPQ} \rightleftharpoons \text{EQ+P} \quad (26) \\
& \quad \text{EQ} \rightleftharpoons \text{E+Q} \quad (27) \\
& \quad \text{EP} \rightleftharpoons \text{E}+\text{Q} \quad (28) \\
& \quad \text{E}+\text{B} \rightleftharpoons \text{E} \rightleftharpoons \text{E}+\text{B} \quad (29)
\end{align*}
\]

As to the output, we wrote the program so that the complete rate equation can be printed out on one run.

Fig. 1 shows the input data according to the input method in which the elementary reaction steps with their rate constants are given as the input statement, and these are confirmed to be correct by Fig. 2, which shows the print-out of the elementary reaction steps. Fig. 3 shows the typical output, in which the rate equation derived agrees completely with the one derived by Cleland (1963).

General mechanism for two-substrate systems based on the combination of the pseudo-steady-state and the rapid-equilibrium assumptions

Let us consider Scheme 2, which is the same scheme as that considered by Cha (1968). Here \(k_1\)–\(k_{26}\) express the first-order or second-order rate constants. The single-lined double-headed arrows \(\rightleftharpoons\) indicate the equilibrium steps and the double-lined single-headed arrows \(\rightarrow\) express the slow reaction steps. The mechanism involves ten enzyme-containing species and the following interconversion steps:

\[
\begin{align*}
\text{EAB} & \rightleftharpoons \text{EPQ} \\
& \quad (17)
\end{align*}
\]

...
forms in terms of the concentrations of the measurable species A, B, P and Q and the total enzyme concentration \([E]_0\). ten equations are required.

First of all, we derive the following relations from eqns. (20)–(29), all of which are in equilibrium:

\[
\begin{align*}
[E] & = K_s[B][E], & K_s = k_s/k_8 & (30) \\
[EAB] & = K_{s1}[A][EB], & K_{s1} = k_{s1}/k_{10} & (31) \\
[EA] & = K_{s2}[A][E], & K_{s2} = k_{s2}/k_{12} & (32) \\
[EAB] & = K_{s3}[B][EA], & K_{s3} = k_{s3}/k_{14} & (33) \\
[EPQ] & = K_{s4}[Q][EP], & K_{s4} = k_{s4}/k_{15} & (34) \\
[EP] & = K_{s5}[P][E], & K_{s5} = k_{s5}/k_{17} & (35) \\
[EPQ] & = K_{s6}[P][EQ], & K_{s6} = k_{s6}/k_{19} & (36) \\
[EQ] & = K_{s7}[Q][E], & K_{s7} = k_{s7}/k_{21} & (37) \\
[E'] & = K_{s8}[P'][E'], & K_{s8} = k_{s8}/k_{23} & (38) \\
[E'B] & = K_{s9}[B'][E'], & K_{s9} = k_{s9}/k_{25} & (39)
\end{align*}
\]

Here \(K_s\)–\(K_{s9}\) express the equilibrium constants. Since the concentrations of the complexes \(EAB\) and \(EPQ\) are both given by two equations, either relation can be used. Therefore eight of the ten equations are independent.

The conservation of enzyme is given by:

\[
[E]_0 = [E] + [E'] + [EA] + [EB] + [EP] + [EQ] + [E'B] + [E'P] + [EAB] + [EQP] \quad (40)
\]

Nine of the ten required equations have been derived above. In general, if there are some enzyme forms that are connected to other enzyme species only by slow steps, we can easily obtain equations relating the change in concentration of the enzyme species in terms of the concentrations of other species on the basis of the pseudo-steady-state assumption, as mentioned in the preceding section. In the present scheme there is no such enzyme form. Therefore we must derive an appropriate equation related to the enzyme complexes by using a different method.

If the existence of the rapid-equilibrium reaction steps is temporarily ignored, the concentration changes for all the species involved are given by:

\[
d[A]/dt = -k_{10}[A][EB] - k_{11}[A][E] + k_{12}[EA] \\
d[B]/dt = -k_{16}[B][E] + k_{18}[B][EA] + k_{14}[EAB] - k_{25}[B][E'] + k_{26}[E'B] \quad (41)
\]
When the pseudo-steady-state assumption is applied to [X] (Cha, 1968), eqn. (56) yields:

\[ -k_6[E] + k_4[E'P] + k_4[E'B] - k_6[EQ] = 0 \]  

Eqn. (58) can also be obtained from eqns. (46), (51) and (52).

Combining eqns. (30)–(32), (34), (35) and (37)–(39) with eqns. (40) and (58) reduces to the matrix equation, as shown in Fig. 4. By solving it, one can obtain the concentrations for all the enzyme forms in terms of the measurable concentrations of the species A, B, P and Q, the total enzyme concentration [E]_o, the rate constants \( k_1 \)-\( k_8 \) and the equilibrium constants \( K_r \)-\( K_{eq} \).

Next, we derive the rate equation. We define the reaction rate \( v \) by the rate of production of the product \( P \):

\[ v = \frac{d[P]}{dt} \]  

Therefore \( v \) is given by eqns. (43) and (59). The right-hand side of eqn. (43) is indeterminate, because all the terms are concerned with the rapid-equilibrium steps. These terms can be eliminated by adding each side of eqns. (43), (49), (52) and (54), and the resultant equation is as follows:


Under usual conditions where the concentration of the enzyme is much lower than that of substrates or products, the following relation holds:

\[ d[P]/dt \gg d[EP]/dt \]  

Therefore eqns. (59) and (60) reduce to:

\[ v = k_1[EAB] - k_4[EPQ] + k_6[EA] - k_6[E'P] \]  

Eqn. (62) coincides with the equation used by Cha (1968).

Eqn. (62) is also derived by the following procedure. First, overall rate of production of \( P \) is equated to the summation of the rate of each step that releases the product \( P \). Then, if any of the product-releasing steps are in rapid equilibrium, we trace up the reaction steps until a slow step is encountered. Thus, in the present example, \( v \) is derived as:

\[ v = v_p = v_{EPQ \rightarrow EQ + P} + v_{EP \rightarrow E + P} + v_{E'P \rightarrow E' + P} \]

\[ v_{EPQ \rightarrow EQ + P} = v_{EPQ \rightarrow EQ + P} + v_{EPQ \rightarrow EQ + P} + v_{E'P \rightarrow E' + P} \]

\[ v_{EAB \rightarrow EPQ + EA \rightarrow EQ} = k_1[EAB] - k_4[EPQ] + k_6[EA] - k_6[E'P] \]  

\[ \text{Mat}\text{rix }\text{Equ}\text{ation}\]

\[
\begin{array}{cccccccc}
  1 & 0 & 0 & 0 & -k_{235} & 0 & 0 & 0 \\
  0 & 1 & 0 & 0 & 0 & -k_{150} & 0 & 0 \\
  0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
  0 & 0 & 0 & 1 & 0 & 0 & -k_{109} & 0 \\
  0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
  0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
  0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\
  0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
  0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
  0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
\end{array}
\]

\[ E'P \]

\[ 0 \]

\[ \text{Fig. 4. Matrix equation derived by the computer} \]

\( K \) expresses the equilibrium constant \( K \) and its definition is given in Fig. 5; \( k \) expresses the rate constant \( k \).
Fig. 5. Print-out of the elementary reaction steps with their rate constants

The reactions designated by 'equil.' are in the rapid equilibrium. The definition of the equilibrium constants is given.

Substitution of [EAB], [EPQ], [EA] and [E'P] derived by solving the matrix equation into eqn. (62) or eqn. (63) yields the required rate equation.

The procedure mentioned above can easily be computerized, and is actually processed by the same program mentioned in the above section. However, in this case we have to input information into the computer concerning whether the scheme involves the rapid-equilibrium steps and which steps are in equilibrium.

Fig. 5 shows the print-out of the inputted reaction steps, in which the rapid-equilibrium steps are designated by 'equil.' As already shown in Fig. 4, the matrix equation is correctly derived in the computer. Fig. 6 shows the final print-out, in which the rate equation derived agrees with the one derived by Cha (1968).

IMPLEMENTATION

The above-mentioned procedure was programed in BASIC and was implemented on a desk-top computer, NEC PC 9800 series, of which the operating system is MS-DOS. This program can be rewritten in FORTRAN 77 in order to reduce the computer time. In the present program as many as 50 enzyme forms are theoretically allowed if many of the matrix elements are zero, except as limited by the memory storage requirements of the computer employed. From the point of memory storage requirements, up to 2000 terms that are produced by expanding the determinant can be processed with the NEC PC 9801E computer (512 kilobytes), and up to 20000 terms with the same computer with an external memory storage (a disc-drive with two 1-megabyte discs). If the memory required for processing exceeds the

Fig. 6. Definition of the rate equation and the derived rate equation for the generalized two-substrate system (Cha, 1968) on the basis of the combination of the pseudo-steady-state and the rapid-equilibrium assumptions
available one, the implementation is to stop after printing the message 'Memory over'.

We have successfully derived the rate equation for a reaction with nine enzyme forms and 20 kinetic constants on the basis of the pseudo-steady-state assumption. If the scheme involves some rapid-equilibrium steps, the rate equation becomes simpler. Therefore in this case the number of enzyme forms that can be processed will approach the limit mentioned above.

The program, together with input and output examples, and running instructions, has been deposited as Supplementary Publication SUP 50141 at the British Library Lending Division.

CONCLUSION

The program developed in the present work is based on the determinant method, in which the determinant is expanded in a systematic manner. Therefore there is no ambiguity in the derivation of the rate equations.

All the past computerized programs except the one developed by Cornish-Bowden (1977) cannot derive the rate equation when the mechanism involves the rapid-equilibrium steps. The Cornish-Bowden program, however, requires some preparation of the data for presentation to the computer, which may cause some mistakes when the mechanism is complicated.

Some more features of our program can be summarized as follows.

1. The program is versatile and can derive the rate equation for the reactions irrespective of whether the scheme involves the rapid-equilibrium steps or not.

2. The program was written so that we can choose either of the two input methods, inputting the matrix elements and inputting the elementary reaction steps with their rate constants, the latter method being especially simple and easy and not prone to human error.

3. A complete rate equation can be obtained on one run.

REFERENCES


Received 27 July 1987/29 September 1987; accepted 27 November 1987