Computer program for the expression of the kinetic equations of enzyme reactions as functions of the rate constants and the initial concentrations

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A versatile computer program with an easy input method has been developed for the construction of the terms in kinetic equations of enzyme reactions. It allows the expression of the time-dependence of the concentrations of all of the species involved as functions of the kinetic parameters. The mathematical theory used in this paper, the program and examples of its use have been deposited as Supplementary Publication SUP 50159 (41 pages) at the British Library Document Supply Centre, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in Biochem. J. (1990) 265, 5.

INTRODUCTION

The transient-phase equations of enzyme reactions offer the advantage of providing more kinetic parameters than the steady-state rate equation can. A kinetic data analysis can usually be improved by the direct or indirect experimental determination of these parameters, and by the use of their relations to the rate constants and the initial concentrations. Moreover, the transient-phase kinetic equations open new approaches to discrimination between various possible enzyme mechanisms. Once the transient-phase equations have been obtained, the steady-state equations can easily be derived as a particular case by extrapolation to infinite time.

The expression of the transient-phase equations of an enzyme mechanism under the initial condition of limiting enzyme concentration as functions of the rate constants and the initial concentrations from the differential equations or by using the formulae in the literature (Darvey, 1968, 1977; Gálvez & Varón, 1981; Chou, 1989) for a general enzyme mechanism is very laborious and prone to human errors. For this reason it is desirable to compose a simpler procedure for the development of these equations. Other authors (King & Altman, 1956; Wong & Hanes, 1962; Cleland, 1963; Lam & Priest, 1972; Chou, 1980; Chou & Forsén, 1980) have already accomplished this objective for the steady-state rate equations by using graphical methods or computer programs (Hurst, 1969; Cornish-Bowden, 1977; Fromm, 1979; Canela, 1983; Herries, 1984; Ishikawa *et al.*, 1988).

Recently considerable efforts have been made to facilitate the derivation of the transient-phase kinetic equations. Chou (1989) has extended his graphical methods to their use on the transient-phase equations. Also, Varón *et al.* (1990) have suggested a procedure for the derivation of the transient-phase kinetic equations of a mechanism from those of another, more complicated, mechanism. Zhang *et al.* (1989) have proposed a computer program for the transient-phase kinetics, but it requires an input of numerical values of the constants and the initial concentrations. Therefore the problem of finding the relations

between the concentrations of the species, the rate constants and the initial concentrations, regardless of their particular values, remains unsolved.

The aim of the present paper is to show how the kinetic equations of all of the species in the general enzyme system model proposed by Gálvez & Varón (1981) may be obtained as a function of the rate constants and the initial concentrations by using a computer program.

THE MODEL

The model consists of n enzyme species denoted by X_i ($i = 1, 2, \dots, n$) and g ligand species (products, substrates, inhibitors, activators and modifiers) denoted by Y_s ($s = 1, 2, \dots, g$). The reaction steps in the model are of the following types:

The rate constants $k_{i,j}$ or $k_{j,i}$ may be zero in any of the steps [1]–[3]. In steps [4]–[6] the subindex r normally assumes one or two values, except in step [4], in which it has at least two values, since otherwise this process would coincide with step [3].

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Initial conditions and rate constants

We assume that at the onset of the reaction the only enzyme species present is the free enzyme, which we denote by X_1 . Its initial concentration is $[E]_0$. For the other enzyme species, X_i ($i = 2, 3, \dots, n$), the notation may be assigned arbitrarily. We also assume that if a ligand, Y_s , binds to an enzyme form, then its concentration remains nearly constant during the entire course of the reaction, either because $[Y_s]_0 \gg [E]_0$ or as a result of metabolic regulation of the concentration. Under these conditions the corresponding step is of pseudo-first-order, i.e. $[Y_s] \approx [Y_s]_0$.

The direct connections between the different enzyme forms are expressed by the rate constants $K_{j,i}$ ($i, j = 1, 2, \dots, n$). If an enzyme form X_j is not converted into another, X_i , then $K_{j,i} = 0$. If the enzyme species X_j combines with Y_s to form X_i , then $K_{j,i} = k_{j,i}[Y_s]_0$, where $k_{j,i}$ is a pseudo-first-order rate constant. If X_j is converted into X_i by a unimolecular step, then $K_{j,i} = k_{j,i}$.

If there are parallel steps between a pair of enzyme species, X_j and X_i , i.e. if more than one step occurs, in which X_j is converted into X_i or vice versa, then both the rate constant for the forward direction and that for the reverse direction (one of these rate constants can be null, according to steps [1]–[6]) in each step of the set of parallel steps must be distinguished by primed symbols: ($K'_{j,i}$, $K''_{j,i}$), ($K'_{i,j}$, $K''_{i,j}$) etc. Then:

$$\begin{aligned} K_{j,i} &= K'_{j,i} + K''_{j,i} + \dots \\ K_{i,j} &= K'_{i,j} + K''_{i,j} + \dots \end{aligned} \quad (1)$$

KINETIC EQUATIONS

The system of differential equations that describe the above model yields the following kinetic equations.

Enzyme species

The concentrations of the enzyme species are represented by the equations:

$$[X_i] = A_{i,0} + \sum_{h=1}^u A_{i,h} e^{\delta_h t} \quad (i = 1, 2, \dots, n) \quad (2)$$

where u is an integer number that always is less than n , and where $\delta_1, \delta_2, \dots, \delta_u$ are the roots of the polynomial $\sum_{q=0}^u F_q \delta^{u-q}$, in which $F_0 = 1$ and the remaining coefficients F_q ($q = 1, 2, \dots, u$) are functions of the rate constants and of the initial concentrations of the ligand species. We shall refer to this polynomial as polynomial $T(\delta)$. Hence:

$$A_{i,0} = \frac{(-1)^{n+i}(a_{1,i})_u [E]_0}{F_u} \quad (i = 1, 2, \dots, n) \quad (3)$$

$$A_{i,h} = \frac{(-1)^{t+c+1} [E]_0 \sum_{q=0}^u (a_{1,i})_q \delta_h^{u-q}}{\delta_h \prod_{\substack{p=1 \\ p \neq h}}^u (\delta_p - \delta_h)} \quad (i = 1, 2, \dots, n; h = 1, 2, \dots, u) \quad (4)$$

where $c = n - u$. If $u = 1$, then the denominator of eqn. (4) is δ_1 , i.e. it is the only root of $T(\delta)$. Note that in this case $\delta_1 = -F_1$.

The coefficients $(a_{1,i})_q$ ($i = 1, 2, \dots, n$; $q = 0, 1, \dots, u$) in eqns. (5) and (6) are functions of the rate constants and of the initial concentrations of the ligand species. $A_{i,0}$ and some of the coefficients $A_{i,h}$ may be zero if certain connections between X_i and the other enzyme species exists.

Ligand species

The kinetic equations for the ligand species are:

$$[Y_s] - [Y_s]_0 = \beta_s + \alpha_s t + \sum_{h=1}^u \gamma_h e^{\delta_h t} \quad (s = 1, 2, \dots, q) \quad (5)$$

where:

$$\alpha_s = \frac{(-1)^n \sum_{(i,j)} [(-1)^j K_{j,i}(a_{1,i})_u - (-1)^i K_{i,j}(a_{1,i})_{u-1}] [E]_0}{F_u} \quad (6)$$

$$\beta_s = \frac{(-1)^n \sum_{(i,j)} [(-1)^j K_{j,i}(a_{1,i})_{u-1} - (-1)^i K_{i,j}(a_{1,i})_{u-1}] [E]_0}{F_u} - \frac{F_{u-1}}{F_u} \alpha_s \quad (7)$$

$$\gamma_{s,h} = (-1)^{c+1} [E]_0 \frac{\sum_{q=0}^u \left(\sum_{(i,j)} [(-1)^j K_{j,i}(a_{1,i})_q - (-1)^i K_{i,j}(a_{1,i})_{q-1}] \right) \delta_h^{u-q}}{\delta_h^2 \prod_{\substack{p=1 \\ p \neq h}}^{p-1} (\delta_p - \delta_h)} \quad (8)$$

where (i,j) means that the corresponding sum is extended to all pairs of values (i,j) , i and j being the subindices of the enzyme species X_i and X_j that participate in one or more of the steps of types [2]–[6]. If $u = 1$, then the denominator of the eqn. (8) is δ_1^2 , i.e. F_1^2 .

The kinetic equations (2)–(8) given here are valid for a great variety of enzyme reactions, including those that contain parallel steps, irreversible inhibition steps, inactivation steps, reactions with suicide substrates or unstable enzymes. The kinetic equations obtained are valid for the whole course of the reaction, i.e. for both the transient phase and the steady state.

If in the scheme of a reaction there is a set of parallel steps between two enzyme species X_j and X_i and Y_s is involved in some of them, then the (i,j) for each of the steps, as well as $K_{j,i}$ and $K_{i,j}$ in eqns. (6)–(8), must be replaced by the corresponding primed symbols. The primed symbol to be used for a pair (i,j) of a parallel step must correspond to the primed symbol used for the rate constants in the same step (see eqns. 1), e.g. in a parallel step, in which the symbols $K'_{j,i}$ and $K''_{j,i}$ have been used, the label $(i,j)'$ must be used in eqns. (6)–(8).

COMPUTER PROGRAM FOR ACQUISITION OF THE TERMS INVOLVED IN THE KINETIC EQUATIONS

Once a reaction scheme has been written, the quantities that we need to obtain the kinetic equations for the enzyme and ligand species in the mechanism are the following. (1) n , the number of enzyme species (free enzyme and its intermediates), which is read from the scheme of the reaction. (2) The expressions of the coefficients $F_1, F_2, \dots, F_u$ as functions of the kinetic parameters. (3) The numbers u and/or c . (4) $\delta_1, \delta_2, \dots, \delta_u$, i.e. the roots of the polynomial $T(\delta)$. Therefore previous knowledge of the coefficients $F_1, F_2, \dots, F_u$ gives all the necessary information about the parameters $\delta_1, \delta_2, \dots, \delta_u$. (5) The expressions of all of the coefficients $(a_{1,i})_q$ ($i = 1, 2, \dots, n$; $q = 0, 1, \dots, u$).

Hence the kinetic equations for all the enzyme and ligand species (eqns. 2–8) may be obtained from knowledge of the expressions for $F_1, F_2, \dots, F_u$ and of those for $(a_{1,i})_q$ ($i = 1, 2, \dots, n$; $q = 0, 1, \dots, u$). We have developed a computer program that delivers all these expressions for any enzyme reaction that fits the model. The only requirement is the entry of the number of enzyme species and of the rate constants.

The execution of this program is divided into the following steps: the input of the data, the choice of the option and the printing of the results.

Input of the data

The program begins with the request for the number of enzyme species present. Next, it asks for an arbitrary numbering

of these species as $X_1, X_2, \dots, X_n$, on the only condition that the free enzyme is X_1 .

Then, the program needs the $n(n-1)$ expressions for the rate constants $K_{1,2}, K_{1,3}, \dots, K_{n,(n-1)}$. All characters in the expressions for the rate constants, including the subindex, must be written on the same line, e.g. a rate constant such as $k_{+1}[A]_0$ must be entered as $k+1[A]0$. If a rate constant both has a subindex and a superindex, e.g. k'_1 , then it may be entered either as $k'1$ or as $k1'$. If a rate constant is zero, then the input must be 0 or the key 'enter' must be pressed. When the rate constants have been entered, the program allows the correction of possible errors in the input.

If, in a reaction scheme, parallel steps between a pair of enzyme forms, e.g. X_i and X_j , exist, then the expression for the corresponding rate constant $K_{i,j}$ must be entered as a sum of rate constants (see eqn. 2), e.g. $K_{2,1}$ in the Michaelis-Menten scheme must be entered as the sum $k_{-1} + k_{-2}$ etc.

This program only needs the number of enzyme species and the expressions for the first-order or pseudo-first-order rate constant as input. Moreover, it admits a wide freedom of expression of the rate constants to be entered. Hence, in the example given in Scheme 1 below, the rate constant $k_{+1}[A]_0$ may be entered as $k+1[A]0$, $k+1[A]$, $k+1A$, $k+1a$, $K12$, $K0102$, $K1.2$ etc.

Choice of the option

After the input of the rate constants, the program offers the choice between gaining the expression of the terms involved in both the transient-phase and the steady-state equations or those involved in the steady-state equations only.

(1) The option of both the transient-phase and the steady-state kinetic equations. The kinetic equations (4) and (7) are valid for both the transient phase and the steady state. If these equations are desired, then the number 1 must be entered.

(2) The option of the steady-state equations. For the steady state ($t \rightarrow \infty$), eqns. (2) and (5) are simplified to:

$$[X_i] = A_{i,0} \quad (i = 1, 2, \dots, n) \quad (9)$$

$$[Y_s] - [Y_s]_0 = \beta_s + \alpha_s t \quad (s = 1, 2, \dots, g) \quad (10)$$

The parameter $A_{i,0}$ (given by eqn. 3) means that the concentration of the enzyme species X_i in this state and the parameter α_s (given by eqn. 5) signify the steady-state rate of the ligand species Y_s . These two parameters are the only ones needed for a steady-state study of an enzyme reaction. If only the steady state is of interest, the number 2 must be entered.

Printing of the results

When the option has been chosen, the computer begins to process the information entered and simultaneously the printer delivers the corresponding results.

(a) Results when option (1) is chosen. In this case the printer produces the expressions for $(a_{1,i})_0$ ($i = 1, 2, \dots, n$), F_1 , $(a_{1,i})_1$ ($i = 1, 2, \dots, n$), ..., F_u and $(a_{1,i})_u$ ($i = 1, 2, \dots, n$). Then, the u and c values are printed. When the above results are inserted into the corresponding general kinetic equations (2)–(5), the particular

kinetic equations, valid from $t = 0$, for the reaction studied are complete.

(b) Results when option (2) is chosen. The results printed are the expressions for F_u , those for the coefficients $(a_{1,i})_u$ ($i = 1, 2, \dots, n$) and the u and c values. They furnish both the numerator and the denominator on the right-hand side of eqns. (3) and (6) for any enzyme or ligand species. Note that the expression given for F_u always is the denominator of the equation for any $A_{i,0}$.

In both of the cases (a) and (b), the period elapsed in the process initiated by the choice of the option is printed.

The program has been written in BASIC, which is a language that is applicable to most of the computers available and that is easily understood by the layman. The program runs on computers, the operating system of which is MS-DOS. It is a short program, which only occupies a memory space of 6193 bytes. The present version accepts enzyme systems with up to 132 different enzyme forms, even if they all are directly connected. The only limitations are the storage capacity of the disc used and the requirement that no terms of the coefficients F_q ($q = 1, 2, \dots, u$) possess more than 255 characters. Normally this limit will not be reached. The execution time depends on the computer memory, the complexity of the mechanism, the length for the expressions of the rate constants and the printing velocity.

Example

We use the reaction scheme of a two-substrate Bi Bi enzyme reaction (Scheme 1) as an example of the application of the computer program. The following notation is used in this example:

$$E \equiv X_1, EA \equiv X_2, EAB \equiv X_3 \text{ and } EQ \equiv X_4 \quad (11)$$

The 12 expressions for the rate constants $K_{1,2}, K_{1,3}, \dots, K_{4,3}$ are:

$$\begin{aligned} K_{1,2} &= k_{+1}[A]_0 \\ K_{2,1} &= k_{-1} \\ K_{2,3} &= k_{+2}[B]_0 \\ K_{3,2} &= k_{-2} \\ K_{3,4} &= k_{+3} \\ K_{4,1} &= k_{-4} \\ K_{1,3} &= K_{1,4} = K_{2,4} = K_{3,1} = K_{4,2} = K_{4,3} = 0 \end{aligned} \quad (12)$$

All characters in the expressions for the rate constants must be written on the same line. When all of the expressions for the rate constants have been entered, one of the options must be chosen. Assuming that we select the option (1), the printer presents the results shown in the Appendix. The time-dependence for all of the species involved can be obtained. If the kinetic equation for the product Q is needed, then, according to eqn. (5), the following equation must be used:

$$[Q] - [Q]_0 = \beta_Q + \alpha_Q t + \gamma_{Q,1} e^{\delta_1 t} + \gamma_{Q,2} e^{\delta_2 t} + \gamma_{Q,3} e^{\delta_3 t} \quad (13)$$

where δ_1 , δ_2 and δ_3 are the roots of the polynomial $\delta^3 + F_1 \delta^2 + F_2 \delta + F_3$. Note that the expressions for F_1 , F_2 and F_3 are among the results offered by the computer. Hence, according to the eqns. (6)–(8) and taking into account that $n = 4$, that $u = 3$ and $c = 1$ (given by the computer) and that the product Q is liberated in the step, in which X_4 forms X_1 [i.e. there is only one

Scheme 1.

pair (i, j) , namely $(1, 4)$, we obtain:

$$\alpha_q = \frac{k_{+4}(a_{1,4})_3[E]_0}{F_3} \quad (14)$$

$$\beta_q = \frac{k_{+4}(a_{1,4})_2[E]_0}{F_3} - \frac{F_2}{F_3}\alpha_q \quad (15)$$

$$\gamma_{q,h} = [E]_0 \frac{k_{+4}[(a_{1,4})_0\delta_h^3 + (a_{1,4})_1\delta_h^2 + (a_{1,4})_2\delta_h + (a_{1,4})_3]}{\delta_h^2 \prod_{p=h}^{p-1} (\delta_p - \delta_h)} \quad (h = 1, 2, 3) \quad (16)$$

Insertion of the expressions for the coefficients $(a_{1,4})_q$ ($q = 0, 1, 2, 3$) into the above eqns. (14)–(16) yields:

$$\alpha_q = \frac{k_{+1}k_{+2}k_{+3}k_{+4}[A]_0[B]_0[E]_0}{F_3} \quad (17)$$

$$\beta_q = -\frac{F_2}{F_3}\alpha_q \quad (18)$$

$$\gamma_{q,h} = \frac{k_{+1}k_{+2}k_{+3}k_{+4}[A]_0[B]_0[E]_0}{\delta_h^2 \prod_{p=h}^{p-1} (\delta_p - \delta_h)} \quad (h = 1, 2, 3) \quad (19)$$

Note that α_q in the transient-phase equation (13) and given by eqn. (17) coincides with the steady-state rate of formation of Q. However, if only the steady state is of interest, then option (2) should be chosen and the results given by the computer will be

those presented in the Appendix. In this example an IBM Personal System/2 computer and a Panasonic KX-P1080 printer in the high-quality mode were used.

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APPENDIX

Results for Scheme 1 when option (1) is chosen

$$\begin{aligned} (a_{1,1})_0 &= -1 \\ (a_{1,i})_0 &= 0 \quad (i = 2, 3, \dots, 4) \\ F_1 &= (k + 1[A]_0) + (k - 1) + (k + 2[B]_0) + (k - 2) + (k + 3) + (k + 4) \\ (a_{1,1})_1 &= -[(k - 1) + (k + 2[B]_0) + (k - 2) + (k + 3) + (k + 4)] \\ (a_{1,2})_1 &= (k + 1[A]_0) \\ (a_{1,3})_1 &= 0 \\ (a_{1,4})_1 &= 0 \\ F_2 &= (k + 1[A]_0)(k + 2[B]_0) + (k + 1[A]_0)(k - 2) + (k + 1[A]_0)(k + 3) + (k + 1[A]_0)(k + 4) + (k - 1)(k - 2) + (k - 1)(k + 3) + (k - 1)(k + 4) + (k + 2[B]_0)(k + 3) + (k + 2[B]_0)(k + 4) + (k - 2)(k + 4) + (k + 3)(k + 4) \\ (a_{1,1})_2 &= -[(k - 1)(k - 2) + (k - 1)(k + 3) + (k - 1)(k + 4) + (k + 2[B]_0)(k + 3) + (k + 2[B]_0)(k + 4) + (k - 2)(k + 4) + (k + 3)(k + 4)] \\ (a_{1,2})_2 &= (k + 1[A]_0)(k - 2) + (k + 1[A]_0)(k + 3) + (k + 1[A]_0)(k + 4) \\ (a_{1,3})_2 &= -(k + 1[A]_0)(k + 2[B]_0) \\ (a_{1,4})_2 &= 0 \\ F_3 &= (k + 1[A]_0)(k + 2[B]_0)(k + 3) + (k + 1[A]_0)(k + 2[B]_0)(k + 4) + (k + 1[A]_0)(k - 2)(k + 4) + (k + 1[A]_0)(k + 3)(k + 4) + (k - 1)(k - 2)(k + 4) + (k - 1)(k + 3)(k + 4) + (k + 2[B]_0)(k + 3)(k + 4) \\ (a_{1,1})_3 &= -[(k - 1)(k - 2)(k + 4) + (k - 1)(k + 3)(k + 4) + (k + 2[B]_0)(k + 3)(k + 4)] \\ (a_{1,2})_3 &= (k + 1[A]_0)(k - 2)(k + 4) + (k + 1[A]_0)(k + 3)(k + 4) \\ (a_{1,3})_3 &= -(k + 1[A]_0)(k + 2[B]_0)(k + 4) \\ (a_{1,4})_3 &= (k + 1[A]_0)(k + 2[B]_0)(k + 3) \\ u &= 3 \\ c &= 1 \end{aligned}$$

Time employed (in minutes) = 0.4999

Results for Scheme 1 when option (2) is chosen

$$\begin{aligned} F_3 &= (k + 1[A]_0)(k + 2[B]_0)(k + 3) + (k + 1[A]_0)(k + 2[B]_0)(k + 4) + (k + 1[A]_0)(k - 2)(k + 4) + (k + 1[A]_0)(k + 3)(k + 4) + (k - 1)(k - 2)(k + 4) + (k - 1)(k + 3)(k + 4) + (k + 2[B]_0)(k + 3)(k + 4) \\ (a_{1,1})_3 &= -[(k - 1)(k - 2)(k + 4) + (k - 1)(k + 3)(k + 4) + (k + 2[B]_0)(k + 3)(k + 4)] \\ (a_{1,2})_3 &= (k + 1[A]_0)(k - 2)(k + 4) + (k + 1[A]_0)(k + 3)(k + 4) \\ (a_{1,3})_3 &= -(k + 1[A]_0)(k + 2[B]_0)(k + 4) \\ (a_{1,4})_3 &= (k + 1[A]_0)(k + 2[B]_0)(k + 3) \\ u &= 3 \\ c &= 1 \end{aligned}$$

Time employed (in minutes) = 0.2398