The computerized derivation of steady-state rate equations for enzyme kinetics

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A FORTRAN 77 program is described for the derivation of steady-state rate equations for enzyme kinetics. Input is very simple and consists of the two enzyme forms and the two rate constants for each step in the mechanism. The program may be run interactively or off-line. The results are produced after collecting together the algebraic coefficients of like concentration terms, taking account of sign. A fully interactive BASIC version running on a BBC Microcomputer is also available. Details of the programs have been deposited as Supplementary Publication SUP 50126 (45 pages) with the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies may be obtained as indicated in *Biochem. J.* (1984) 217, 5.

The rate equation for even the most complex mechanism can be derived by writing the individual rate equations for each of the species involved, by setting the rate of concentration change for each enzyme form to zero according to the steady-state assumption, by replacing one of these steady-state equations by the enzyme conservation equation, and finally by solving for the rate of concentration change of one of the substrates or one of the products. Unless one of the reactants is merely a modifier and therefore does not undergo a net chemical transformation, the solutions for the rate of concentration change of each product will be identical, and equal to minus the rate of concentration change for each substrate. As has been pointed out by Cornish-Bowden (1977), this method is extremely tedious for all but the very simplest mechanisms, and hence is prone to human error, even with the major advance provided by King & Altman (1956). Several authors have concentrated on computerized methods for establishing the allowed King–Altman patterns (see, e.g., Lam & Priest, 1972; Lam, 1981), but a systematic solution of the simultaneous equations removes the need to identify the patterns, as terms that would have arisen from patterns containing closed loops automatically cancel. The basis of such a solution has been documented by Silvestri & Zahner (1967), essentially a method for the algebraic expansion of a determinant, with like concentration terms being collected together. The Silvestri & Zahner (1967) program, however, requires considerable preparation of the data for presentation to the computer, again providing

scope for human error, and the purpose of the present paper is to report a new program that combines the very simple format of data presentation used by Cornish-Bowden (1977) with its processing to form the determinant needed for the Silvestri & Zahner (1967) expansion. The program will produce either the numerator of the rate equation or the numerator of the expression for the proportion of any specified enzyme form. Alternatively, it will produce the denominator of the rate equation, which is also the denominator for any of the enzyme expressions. The coefficients of individual concentration terms in any of the above can also be specified for evaluation. This is useful in the unlikely event that processing has not been completed in the time allowed; the concentration terms whose coefficients are missing will have been listed in the first part of the output, and the program can be re-run to evaluate just these coefficients. The program will also handle irreversible steps in a mechanism and hence can be used to derive initial-velocity equations (i.e. those where product concentrations are not considered). Furthermore, if the mechanism involves alternative substrates or products, one can specify the substrate or product whose rate of concentration change is to be evaluated, so as to obtain the flux through just one branch of the mechanism.

The versatility of the program and the fact that it collects together all the terms with the same reactant concentrations is an advance on the program of Cornish-Bowden (1977), which only produces the enzyme proportion expressions and requires further work by hand in order to sort the

terms and obtain, for example, the complete expression for the denominator of the rate equation. The program is written in FORTRAN 77 and makes use of the character-handling capabilities of this recent form of the language. It may be run interactively, but, since the computing time may be substantial for complex mechanisms, the facility is also provided for off-line data handling. The results have been extensively checked, and an earlier version was used in the study of galactosyl-transferase from bovine milk in our laboratory (Khatra *et al.*, 1974; Tsopanakis & Herries, 1978).

Principle

Let us consider the simple example of the Michaelis-Menten mechanism where substrate A is converted reversibly by enzyme E in two steps into product P according to eqn. (1):

Rate equations and the enzyme conservation equation can be written:

$$d[A]/dt = -k_{+1}[A][E] + k_{-1}[EA] \quad (2)$$

$$d[P]/dt = k_{+2}[EA] - k_{-2}[P][E] \quad (3)$$

$$d[E]/dt = -(k_{+1}[A] + k_{-2}[P])[E] + (k_{-1} + k_{+2})[EA] \quad (4)$$

$$d[EA]/dt = (k_{+1}[A] + k_{-2}[P])[E] - (k_{-1} + k_{+2})[EA] \quad (5)$$

$$[E]_T = [E] + [EA] \quad (6)$$

where $d[X]/dt$ is the rate of change of concentration of species X with time, and $[E]_T$ is the total enzyme concentration.

After setting the expressions in eqns. (4) and (5) to zero in accordance with the steady-state assumption, and replacing the former by eqn. (6), we can write the resulting four equations in matrix form:

$$\begin{bmatrix} 1 & 0 & k_{+1}[A] & -k_{-1} \\ 0 & 1 & k_{-2}[P] & -k_{+2} \\ 0 & 0 & 1 & 1 \\ 0 & 0 & (k_{+1}[A] + k_{-2}[P]) & -(k_{-1} + k_{+2}) \end{bmatrix} \begin{bmatrix} d[A]/dt \\ d[P]/dt \\ [E] \\ [EA] \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ [E]_T \\ 0 \end{bmatrix}$$

or

$$G \text{ times } Y = H$$

To solve for $d[A]/dt$, we follow Cramer's rule and replace the first column of the coefficient matrix G by column matrix H and divide the determinant of

this matrix by the determinant of G ($\det G$); to solve for the concentration of enzyme form EA, we replace the fourth column of G by H and similarly divide by $\det G$. Because of the occurrence of units and zeros in these determinants, they can always be reduced to order n , where n is the number of enzyme forms. For example, $\det G$ reduces to the second-order determinant:

$$-\begin{vmatrix} 1 & 1 \\ (k_{+1}[A] + k_{-2}[P]) & -(k_{-1} + k_{+2}) \end{vmatrix}$$

which expands to $k_{-1} + k_{+2} + k_{+1}[A] + k_{-2}[P]$.

The data required for the program consist of a title, followed by instructions governing the type of calculation to be made, and then by the reaction equations of the mechanism. In the above example, these equations, which give the reactant, first-order or pseudo-first-order rate constant, the same for the reverse, and the product, will be as follows:

$$\begin{array}{llll} (1) & [E] & k_{+1}[A] & k_{-1} & [EA] \\ (2) & [EA] & k_{+2} & k_{-2}[P] & [E] \end{array}$$

No difference is made to the result by presenting any reaction step in the reverse order or by altering the order of the reaction steps. Thus reaction (1) could be given as:

$$(1) [EA] \quad k_{-1} \quad k_{+1}[A] \quad [E]$$

Replacing $k_{-2}[P]$ by blanks in reaction (2) would result in the initial-velocity equation being generated, corresponding to irreversibility of the second step in the mechanism.

The program constructs matrix G and modifies it according to which result it has been instructed to produce, reducing the order of the determinant before expanding it. Fig. 1 shows the main part of the output for the data given above, assuming that derivation of the denominator expression has been asked for.

Parallel steps occur in the mechanism used here as an example, i.e. E and EA are linked by two steps with rate constants k_{-1} and k_{+2} , but, in contrast with the program of Cornish-Bowden (1977), no preliminary addition of the rate constants needs to be done. On the other hand, in mechanisms with a large number of steps, where the number of rate constants exceeds the limit, parallel steps can deliberately be given the same rate constant; this will reduce the total number of rate constants, and the output will show the common rate constant, which must be interpreted as the sum of the rate constants that it has replaced. Taking the above mechanism as a trivial example, we find that, on replacing k_{+2} by k_{-1} , the program produces a coefficient for the constant term in the denominator of $2k_{-1}$ (cf. Fig. 1). The factor of 2 correctly preserves the number of constant terms at

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CONSTANT TERM WITH 2 POSITIVE AND 0 NEGATIVE TERMS WHICH ARE
      1      k-1
      1      k+2
A1      TERM WITH 1 POSITIVE AND 0 NEGATIVE TERMS WHICH ARE
      1      k+1
P1      TERM WITH 1 POSITIVE AND 0 NEGATIVE TERMS WHICH ARE
      1      k-2

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Fig. 1. Part of the program output showing the denominator of the mechanism in eqn. (1)

two, originally k_{-1} and k_{+2} . This result arises from the fact that the program will recognize identities among the rate constants, and will, if required, show squared or higher-degree rate constants in the output.

Implementation

The method described above has been implemented in an earlier FORTRAN IV version on both the International Computers Ltd. 1906A mainframe computer and the Amdahl (U.K.) Ltd. model 470 V/7 mainframe computer at the University of Leeds. The present version in FORTRAN 77 runs on the Amdahl 470 V/7 and should be capable of running on any computer that can accommodate integer numbers up to seven digits long. As many as ten enzyme forms, ten reactants and 28 rate constants are allowed; error messages are issued if these numbers are exceeded. Array sizes can easily be increased if there is a need for more than these limits. With the IBM VS FORTRAN compiler and optimization at level 3, no mechanism has been encountered whose equations cannot be produced by a single run of the program. With only minor modifications, this version also runs on the Systime 8780 mainframe (equivalent to a Digital VAX 11/780), where limitations on the amount of nesting, imposed by the VAX-11 FORTRAN compiler, prevent acceptance of more than eight enzyme forms.

A second version written in BASIC for the BBC Microcomputer (32 kilobytes) has also been shown to be capable of handling quite complex mechanisms, though execution time is far greater, but this is not necessarily a problem with a personal computer.

Both these language versions of the program, together with data and output examples and running instructions, have been deposited as Supplementary Publication SUP 50126 at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7QB, U.K., from whom copies may be obtained on the terms indicated in *Biochem. J.* (1984) **217**, 5.

Discussion

The main advantage of the present program is that it expands determinants in a systematic manner, each term in the expansion having the correct algebraic sign. The coefficients of each substrate or product concentration term are then collected together, and those that are equal but opposite in sign automatically cancel. These correspond to disallowed King–Altman patterns. The complications of determining valid King–Altman patterns are thus avoided. Programs involving expansion of determinants have already been reported by Rhoads & Pring (1968) and by Hurst (1969). The former method is linked to determining valid King–Altman patterns, and the latter does not go further than the numerators of the enzyme proportion expressions. Cornish-Bowden (1977) has given a useful review of other computer programs designed to derive enzyme kinetics rate equations; none of these presents the results in as succinct a manner as the present program, nor is any as versatile.

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