Computer program for the equations describing the steady state of enzyme reactions
R. Varon¹,⁵, F. García-Sevilla¹, M. García-Moreno¹, F. García-Canovas², R. Peyro³ and R. G. Duggleby⁴

Abstract

Motivation: The derivation of steady-state equations is frequently carried out in enzyme kinetic studies. Done manually, this becomes tedious and prone to human error. The computer programs now available which are able to accept reaction mechanisms of some complexity are focused only on the strict steady-state approach.

Results: Here we present a computer program called REFERASS, with a short computation time and a user-friendly format for the input and output files, able to derive the strict steady-state equations and/or those corresponding to the usual assumption that one or more of the reversible steps are in rapid equilibrium. This program handles enzyme-catalysed reactions with mechanisms involving up to 255 enzyme species connected by up to 255 reaction steps, subject to limits imposed by the memory and disk space available.

Availability: REFERASS is available free of charge either on request from the authors or over the EMBL file server (Software@embl-ebi.ac.uk).

Contact: E-mail: fgarcia@iele-ab.uclm.es

Introduction

In spite of the increasing importance that analysis of the kinetic behaviour of enzyme reactions in their transient phase has acquired in recent years, theoretical as well as experimental studies of the steady state of enzyme systems are still the fundamental instrument for their kinetic characterization and for the discrimination between possible reaction mechanisms.

The strict steady-state equations of an enzyme system may be too complex to be of practical interest (Lam, 1981). Thus, many enzyme reactions are simplified by assuming that one or more of the reversible steps in the mechanism is in rapid equilibrium. Cha (1968) proposed a simplifying modification of the King and Altman (1956) method for these mechanisms. Computer implementations of Cha’s method have been described by Cornish-Bowden (1977), Lam (1981) and Ishikawa et al. (1988).

Besides Cha’s method, the steady-state equations of a partial or total equilibrium mechanism can be obtained from the corresponding strict steady-state equations by deleting those terms that are relatively small because of the rapid equilibrium assumption. The elimination of these latter terms can be carried out either manually (Lam, 1981) or by adding suitable subroutines to the computer program which gives the strict steady-state equations. Such a computer program was developed by Kinderleer and Ainsworth (1976). However, in spite of the undoubted merit of this program, it has some important limitations, e.g. it is restricted to mechanisms involving up to 10 enzyme intermediates, with up to six reactions between each enzyme state and with a maximum of eight reactants. Moreover, the printout of the results is not given as a function of the rate constants or, in cases of partial or total equilibrium mechanisms, of the rate and equilibrium constants, but as a table containing vector numbers and symbolic numerical coefficients which require a subsequent translation. More recently, Varon et al. (1995) have developed a computer program which gives the strict steady-state equation as well as the corresponding equation if rapid equilibrium is assumed. Nevertheless, this program has some limitations arising from the programming language employed.

The computer program developed here overcomes the limitations mentioned above: (i) it offers the possibility to derive either the equations when the strict steady-state is assumed or those when one or more of the reversible steps are in rapid equilibrium; (ii) it gives a straightforward printout of the results in an easily understood form; (iii) for the rapid equilibrium assumption, it offers the possibility of delivering results only as a function of the rate constants or as a function of the equilibrium rate constants of all of the steps in rapid equilibrium and the remaining rate constants; and (iv) it handles enzyme-catalysed reactions with mechanisms containing up to 255 enzyme species connected by up to 255 reactions, subject to limits imposed by the memory and disk space available on the computer.

¹Escuela Universitaria Politécnica, Universidad de Castilla-La Mancha, Avda. de España s/n, Campus Universitario, 02071 Albacete, Spain and ²Departamento de Bioquímica y Biología Molecular A, Facultad de Biología, Universidad de Murcia, 30071 Murcia, Spain
³Hospital General de Albacete, 02006 Albacete, Spain and ⁴Centre for Protein Structure, Function and Engineering, Department of Biochemistry, University of Queensland, Brisbane 4072, Australia

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Systems and methods

The computer program REFERASS (an acronym for Rate Equations For Enzyme Reactions At Steady State) has been written in C++ programming language and compiled with the GNU C compiler using the DJGPP GO32 extensor. In the implementation of the present version, the algorithms developed by Varon et al. (1990) have been used. The examples described in this article have been solved in a computer based on a Pentium 133 MHz with 32 Mbytes of RAM and a 1 Gbyte IDE hard disk.

Algorithm

The mechanism schemes of enzyme-catalysed reactions, to which our computer program can be applied, are those which consist of \( n \) enzyme species denoted arbitrarily by \( X_i \) (\( i = 1, 2, ..., n \)) (with the only restriction that \( X_1 \) is the free enzyme, i.e. the unliganded enzyme initiating the reaction) and \( g \) ligand species (products, substrates, inhibitors and activators) denoted by \( Y_s \) (\( s = 1, 2, ..., g \)), such that any of their reaction steps is of one of the following types:

1. \( X_i \xrightarrow{k_{i, j}} X_j \) if the conversion of \( X_i \) into \( X_j \) is of first-order
2. \( X_i \xrightarrow{k_{i, j}} X_j \) if the conversion of \( X_i \) into \( X_j \) is of pseudo-first-order (\( i, j = 1, 2, ..., n; i \neq j \))
3. \( X_i \xrightarrow{k_{i, j}} X_j \) if there is no conversion of \( X_i \) into \( X_j \)

Either \( k_{i, j} \) or \( k_{j, i} \) may be zero in any of steps [1]–[3]. Most mechanisms of enzyme reactions fit the general mechanism described by reaction steps type [1]–[6].

When we want to study a concrete reaction scheme using the computer program presented here, it must be written so that the enzyme species are denoted by \( X_1, X_2, ..., X_n \). As an example, the reaction scheme corresponding to the well-known general modifier mechanism of Botts and Morales (1953) would be:

\[
\begin{align*}
X_1 + S &\xrightarrow{k_{1, 2}} X_2 \\
+ &\xrightarrow{k_{2, 1}} X_1 + P \\
M &\xrightarrow{k_{3, 4}} M \\
X_3 + S &\xrightarrow{k_{5, 6}} X_4 \\
&\xrightarrow{k_{6, 5}} X_3 + P
\end{align*}
\]

Scheme 1

where \( S, M \) and \( P \) are the substrate, the modifier and the product, respectively, and \( X_1, X_2, X_3 \) and \( X_4 \) denote the enzyme species \( E, ES, EM \) and \( ESM \), respectively.

Initial conditions

We assume that at the onset of the reaction, the only enzyme species present is the free enzyme, \( X_1 \), its initial concentration being \( [E]_0 \) and that the concentration of any ligand species remains nearly constant during the entire course of the reaction. Under this condition, any reaction step of the model is either of first or pseudo-first order.

Rate constants and their notation

The conversion of enzyme species \( X_i \) into \( X_j \) is expressed by the constant \( K_{i, j}(i, j = 1, 2, ..., n; i \neq j) \), it is observed:

\[
K_{i, j} = \begin{cases} 
 k_{i, j} & \text{if the conversion of } X_i \text{ into } X_j \text{ is of first-order} \\
 k_{i, j}[Y_s] & \text{if the conversion of } X_i \text{ into } X_j \text{ is of pseudo-first-order (} i, j = 1, 2, ..., n; i \neq j; s = 1, 2, ..., g) \\
 0 & \text{if there is no conversion of } X_i \text{ into } X_j 
\end{cases}
\]

In some mechanisms, there may exist two or more steps between a pair of enzyme forms. In such cases, the involved steps are said to be parallel steps (Cornish-Bowden, 1977). For example, in the mechanism in Scheme 1, the enzyme-substrate complex, \( X_2 \), can be converted into free enzyme, \( X_1 \), either by release of substrate or by release of product. Thus, steps \( X_2 \rightarrow X_1 + P \) and \( X_2 \rightarrow X_1 + S \) are parallel. It is likely that steps \( X_4 \rightarrow X_3 + P \) and \( X_4 \rightarrow X_3 + S \) in the same Scheme 1 are two parallel steps, too.

If there are parallel steps between a pair of enzyme species, \( X_i \) and \( X_j \), then the first or pseudo-first order rate constant involved in each reaction step of the set of parallel steps is distinguished by numbered symbols: \( K_{i, j}(1), K_{i, j}(2), \ldots \), i.e. \( K_{i, j} = K_{i, j}(1) + K_{i, j}(2) + \ldots \) and, in these cases, \( K_{i, j} \) does not mean a first or pseudo-first order rate constant, but a sum of two or more of these rate constants. As an example, in Scheme 1 is observed:

\[
K_{2, 1} = K_{2, 1}(1) + K_{2, 1}(2) \quad K_{4, 3} = K_{4, 3}(1) + K_{4, 3}(2)
\]

where:

\[
K_{2, 1}(1) = k_{2, 1}(1) = k_{-1} \quad K_{2, 1}(2) = k_{2, 1}(1) = k_{s 2} \\
K_{4, 3}(1) = k_{4, 3}(1) = k_{-5} \quad K_{4, 3}(2) = k_{4, 3}(1) = k_{s 6}
\]

In this paper, the term rate constant is used indiscriminately for a first or a pseudo-first order rate constant. We denote both the \( k_{i, j} \) and \( k_{j, i} \) (or the corresponding numbered symbols) involved in a reversible reaction step arbitrarily as \( k_{i, q} \) (or \( k_{q, i} \), \( k_{eq} \) and \( k_{\text{-eq}} \), i.e. \( k_{eq} \) may correspond either to the
The strict steady-state concentration, \([X_i]\), of an enzyme species, \(X_i\), and the strict steady-state rate, \(v_{Y_s}\), for a ligand species, \(Y_s\), of a reaction mechanism which fits that given by species, \(X\), and the strict steady-state rate, \(v_{Y_s}\) for a ligand species involved in the reaction step, enclosed in square brackets. Some examples for the expression of the rate constant \(K_{i,j}\) (or a numbered \(K_{i,j}\)) coincides with that of the corresponding \(k_{i,j}\) (or of the numbered \(k_{i,j}\)) in a first-order reaction step. A \(K_{i,j}\) (or a numbered \(K_{i,j}\)) corresponding to a pseudo-first order reaction step must be denoted using the notation for the corresponding \(k_{i,j}\) (or the numbered \(k_{i,j}\)), followed by the string used for the ligand species involved in the reaction step, enclosed in square brackets. The meaning of \(G_i\) is given by the equation:

\[
G_i = (-1)^{n+i}(a_{1,i})_0
\]

This coefficient may or may not be zero. In the latter case, it consists of only one term (positive or negative) or of a sum of terms, either all positive or all negative, depending on whether \(n + i\) is even or odd, respectively. Moreover, it is observed:

\[
G = \sum_{i=1}^{n} G_i
\]

The meanings of the different symbols that appear in equations (1) and (2) are given in the reference above, but it is useful to summarize these meanings here.

**Meaning of \(G_i\).** Let \(D(\lambda)\) be the secular determinant of the set of \(n\) differential linear equations with constant coefficients describing the kinetic behaviour of the enzyme species involved in the reaction mechanism under study. The expansion of this determinant yields:

\[
D(\lambda) = (-1)^n\lambda^n + F_1\lambda^{n-1} + F_2\lambda^{n-2} + \ldots + F_n
\]

where \(c\) and \(u\) are the number of null and non-null roots of \(D(\lambda)\), and their values depend on every concrete reaction mechanism. Obviously, it is observed \(n = c + u\). Because for equation (3) polynomial \(D(\lambda)\) has at least one null root, i.e. the minimal \(c\) value is unity. In most of the enzyme-catalysed reactions \(c = 1\), but there are many other reaction mechanisms where \(c \geq 2\), e.g. those involving two or more irreversible inhibition or inactivation steps. Thus, \(G\) may be defined either as the term of degree zero of the polynomial \(D(\lambda)(-1)^n\lambda^n\) or the coefficient of the term of less degree of the polynomial \(D(\lambda)\), divided by \((-1)^n\).

\(G\) is always positive and it consists of one term or a sum of terms, all of which are a product of \(u K_{i,j}\).
previously developed by us (Varon et al., 1990) so that it now yields the complete equations (1) and (2).

Computerized derivation of the equations for the concentration of enzyme species and ligand rates for steady-state mechanisms in rapid equilibrium

One of the main purposes of this contribution is to carry out the computerized derivation of the equations for the concentration of enzyme species and ligand rates for steady-state mechanisms, when one or more of the reversible reaction steps involved are in rapid equilibrium, i.e. for partial or total equilibrium mechanisms. To achieve this, we have developed subroutines which allow the computerized elimination of those terms of G, G_i (i = 1, 2, ..., n) and G_Ys (s = 1, 2, ..., g) that are much smaller than the others, after taking into account the rapid equilibrium conditions assumed in the mechanism. The resulting expressions for G, G_i and G_Ys after this elimination will be denoted as H, H_i and H_Ys, respectively, so that equations (1), (2) and (5) become:

\[ [X_i] = \frac{H_i[E_o]}{H} \quad (i = 1, 2, \ldots, n) \]  
(6)

\[ \nu_Y = \frac{H_Y}{H} \quad (s = 1, 2, \ldots, g) \]  
(7)

\[ H = \sum_{i=1}^{n} H_i \]  
(8)

To delete terms in G, G_i and G_Ys, we use the fact that the assumption of rapid equilibrium requires the rate constants involved in the reversible reaction steps in rapid equilibrium to be much higher than the others and mutually not very different. For the purpose of this paper, we will name these as high rate constants. So, if a term of G contains fewer high rate constants than any other term of G, then it may be neglected. The same is valid for any G_i and G_Ys. Therefore, all terms in H, H_i and H_Ys have the same number of high rate constants, which are much faster than the steps involving reversible substrate binding. If condition (9) is inserted in expressions of G, G_i and G_Ys, then the corresponding expressions of H, H_i and H_Ys are obtained. That could obviously be carried out manually from the expressions of G, G_i and G_Ys, but the computerized elimination saves time and avoids errors.

Treatment of those loops whose reaction steps are all reversible and in rapid equilibrium

For the purpose of this contribution, we will name any loop with all its reaction steps in rapid equilibrium an \( \alpha \)-loop. Our program checks the reaction scheme entered to find possible \( \alpha \)-loops. Once an \( \alpha \)-loop has been found, the program derives the corresponding relationship arising from the application of the mass-action law to each of the reversible reaction steps in the \( \alpha \)-loop and which involves all the forward and reverse rate constants of these steps, in which, generally, some ligand concentrations can be cancelled on both sides of the equation. Thus, if we assume that in Scheme 1 all the reversible steps of the only loop are in rapid equilibrium, then the loop is an \( \alpha \)-loop, and if we apply the mass-action law to each reversible reaction step in the above loop, then we have the relationship:

\[ k_{+,1}k_{+,5}k_{-,3} = k_{-,1}k_{-,5}k_{+,3} \]

The program is designed in such a way that it furnishes the minimal set of independent relationships among the rate constants arising from the existence of \( \alpha \)-loops. In those cases in which the number of these relationships given by the program is less than the number of \( \alpha \)-loops in the reaction scheme, all the other relationships can be obtained, if desired, by combining two or more of those given by the program.

Modification of equations (6)–(8) including the equilibrium constants of the reversible reaction steps in rapid equilibrium

The steady-state equations of partial or total equilibrium mechanisms can also be given as a function of the equilibrium constants, \( K_{eq} \), of the reversible reaction steps which are assumed to be in rapid equilibrium. In this contribution, any equilibrium constant, \( K_{eq} \), is always defined as \( k_{-,q}/k_{+,q} \) irrespective of whether \( k_{eq} \) corresponds to the forward or reverse reaction. If we divide both the numerator and denominator of equations (6) and (7) by the product of all the high \( k_{eq} \)-s type \( k_{eq} \), then the latter ones which appear in the expressions of \( H, H_i \), and \( H_Ys \), cancel out and equations (6)–(8) become:

\[ [X_i] = \frac{L_i[E_o]}{L} \quad (i = 1, 2, \ldots, n) \]  
(10)

\[ \nu_Y = \frac{L_Y[E_o]}{L} \quad (s = 1, 2, \ldots, g) \]  
(11)

\[ L = \sum_{i=1}^{n} L_i \]  
(12)

where \( L, L_i \), and \( L_Ys \) contain the equilibrium constants of the reversible steps in rapid equilibrium. The above procedure to express the steady-state equations as a function of the equilibrium constant can obviously be carried out manually from the expressions of \( H \) and \( H_i \), however, we have
developed a subroutine which allows the computer to carry out this task from the expressions of \( H, H_i \) and \( H_{Y_s} \) for any partial or total reaction mechanism, thus avoiding errors and saving time.

The computer program presented here is designed in such a manner that if there are one or more \( \alpha \)-loops in a reaction mechanism, it transforms the relationships among the rate constants, so that the equilibrium constants \( K_{q_s} \), corresponding to the reaction steps, replace the respective quotients \( k_{-q}/k_{+q} \). Obviously, in the expressions of \( L, L_i \) and \( L_{Y_s} \), for those reaction mechanisms involving one or more \( \alpha \)-loops, one or more equilibrium constants are missing. These missing equilibrium constants may be included in the above expressions if the relationships involving the equilibrium constants in the \( \alpha \)-loops are used, but then other equilibrium constants previously present in \( L, L_i \) and \( L_{Y_s} \), will now be missing.

**Flow diagram**

A simplified flow diagram of the computer program is shown in Figure 1.

**Implementation**

The computer program REFERASS represents a considerable improvement of a previous program written by us using Microsoft Visual Basic for MS-DOS V1.0 (Varon et al., 1995). The main characteristics of the present version are:

(i) It admits reaction mechanisms containing up to 255 enzyme species and up to 255 reaction steps, subject to limits imposed by the memory and disk space available on the computer. These numbers can easily be increased if necessary (see Results and Discussion).

(ii) The computation time is very short, even for complex mechanisms. Obviously, this time depends on the computer used. Thus, when a 133 MHz Pentium was used, the computer time was <12 s for all examples, whereas using a 33 MHz 486 machine the computation times were <65 s.

(iii) All available RAM in the computer is used and, in addition, a virtual memory of up to 128 Mb on a hard disk can be used.

(iv) The input of the data is very easy and versatile.

(v) It can furnish simultaneously the steady-state concentration equation of one or more (even all) of the involved enzyme species and/or the steady-state rate of one or more (even all) of the involved ligand species.

(vi) It provides the results in the most simplified form.

**Hardware requirements**

CPU 80386 or higher.

Memory. This program needs at least 512 kbytes of memory; the greater the memory, the better the program runs.

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Fig. 1. Simplified flow diagram of the computer program.
Hard disk. The required free space depends on the complexity of the reaction scheme under study.

Operating system. MS-DOS 3.3 or higher

Input syntax and structure

The interaction between the program and the user is made by creating a text file, with any valid (according to the operating system) name, and having the following syntax and structure. There are five possible types of text lines: comment lines; species notation lines; rate constants notation lines; rapid equilibrium steps lines; user option lines.

Comment lines. To enter a comment, type a number sign (#) in the first column, then type the comment to the right of the number sign. If the comment requires more than one line, repeat the number sign on each line before continuing the comment, e.g.:

```
# Title: The mechanism under study
# User: The one who writes the file
# ...
```

Species notation lines. For each enzyme species, type X in the first column, followed by an index number between 1 and 255, which must be different for each one; then write the equals symbol (=) and finally the original notation of the corresponding enzyme species used in the reaction scheme. XI must always correspond with the free enzyme. The numbering of the other enzyme species is arbitrary, e.g. for Scheme 1:

```
X1 = E
X2 = EM
X3 = ES
X4 = EP
X5 = EMP
X6 = MES
```

An alternative notation of the enzyme species is to type the letter n, then the equals symbol and finally the number of enzyme species, e.g. n = 6 instead of the above six lines. In this case, the enzyme species will be shown in the output as X1 to Xn. If both syntaxes are used simultaneously, then the second form (i.e. where the n value is entered) takes precedence.

Rate constants notation lines. You must type only the non-null Ka,j. For each first or pseudo-first order rate constant Ka,j, type a capital K in the first column, followed by the numbers i and j separated by a comma (e.g. K2,7); then write the equals symbol (=) and finally the expression of the corresponding rate constant. Any expression for the rate constant of a reaction step consists of a lower-case k (or k') followed by a plus or a minus sign and the subindex (one or more characters). The plus sign can be omitted. If the rate constant is of pseudo-first order, then the notation of the corresponding ligand species must be written in square brackets. The two rate constants involved in the same reversible reaction step must be denoted using the same subindex (one of them preceded necessarily by the minus sign) and the two expressions must begin with either k or k', e.g.:

```
K1, 2 = k+1[S]
K1, 3 = k+3[M]
K2, 4 = k+4[M]
K3, 1 = k−3
K3, 4 = k+5[S]
K4, 2 = k−4
```

In those cases in which two or more parallel steps exist between two enzyme species XI and Xj, the rate constant Ki,j corresponding to each step must be distinguished, writing at the right side of the Ki,j a different arbitrary number (1–255) in parentheses for each step, e.g.:

```
K2,1(1) = k−1
K2,1(2) = k+2
K4,3(2) = k+6
```

If there is an irreversible step, parallel or not, in which one or more ligand species are released, then you must write on the right of the corresponding Ki,j the symbol for implication (=>) followed by the expression(s) used in the reaction mechanism for the ligand species released. If more than one ligand species are released, then their expressions must be separated by at least one blank space, e.g.:

```
K2,1(2) = k+2 => P1 P2
K4,3(2) = k+6 => P
```

Rapid equilibrium steps lines. If there are one or more different pairs of enzyme species connected by at least one reversible reaction step in rapid equilibrium, then type, for each of the above pairs, RE in the first two columns of the file followed by one or more blank spaces, and finally the index numbers corresponding to each enzyme species notation in rapid equilibrium, separated by a comma. For example, if the pairs of enzyme species X7 and X8 and X3 and X4 are in rapid equilibrium, then this would be specified:

```
RE 7,8
RE 3,4
```

User option lines. You can select which results you want to obtain and the form of the output file. To do this, there are four option lines, all of them beginning with a dot. These options are:

(i) If you want to obtain the steady-state concentration of one or more of the enzyme species then type .Species = followed by the notation used for the corresponding species in the reaction scheme separated by one or more blank spaces. If you want the steady-state concentration of all of the enzyme species, then you can either write all notations or an asterisk
on the right-hand side of the equal symbol. For example:

\[ \text{.Species} = \text{E EM} \]
\[ \text{.Species} = * \]

(ii) If you want to obtain the steady-state rate of one or more of the ligand species then type .Ligands = followed by the notation used for the corresponding ligand species in the reaction scheme separated by one or more blank spaces. If you want the steady-state rate of all of the ligand species, then you can either write all notations or an asterisk on the right-hand side of the equal symbol. For example:

\[ \text{.Ligands} = \text{S P} \]
\[ \text{.Ligands} = * \]

(iii) In those cases in which one or more pairs of enzyme species are in rapid equilibrium, there are two possible forms to give the results: as a function of only the individual rate constants or including the corresponding equilibrium constants. If you choose the second form, type:

\[ \text{.Equilibrium Constants} \]

or its abbreviation:

\[ \text{.EC} \]

at the beginning of any line. By default, i.e. if you omit this line, the expressions are printed using the individual rate constants. If you enter this command when there are no steps at rapid equilibrium, then this line is ignored by the program.

(iv) By default, the program sets the width of the output file to 80 columns. If you want another width, you can use the option line .Width = followed by a number (>49) indicating the number of columns you want. If you write a number <50, the program changes it to 50. In complex mechanisms, some terms may be too long to fit it in the width chosen. In these cases, the program automatically uses the width necessary for the term and the highest width used is given at the end of the output file.

The .Species, .Ligands and .Width parameters in the option lines may be replaced by .S, .L and .W, respectively.

Starting the execution of the program

When the input file has been written, as described above, then save it and exit to the operating system. Then type referass followed by the name of that file. Suppose your input file is named foo.in, then you must type:

\[ \text{referass foo.in} \]

This will be valid if the program referass.exe and the input file foo.in are located in the same subdirectory and this is the current subdirectory. You can also execute the program from any other directory if you type the full path for the program and/or the input file:

\[ \text{drive:|path|referass drive:|path|foo.in} \]

After that, you will see a presentation screen while the program is running. When the execution of the program has finished, the time elapsed in the process is displayed and then you must press any key to return to the operating system.

Output of the results

After the execution, the program automatically creates an ASCII file with the same name as you chose for the input file with the extension .lis, e.g., if your input file is named foo.in, then the output file is named by the program as foo.lis. We select an ASCII file to present the results, because of the facility for display and printing, compatibility with word processors, etc.

This file contains the expressions of the steady-state concentrations of the enzyme species selected and/or those of the steady-state rate of the ligand species selected in the most simplified possible form. In those cases in which one or more reversible steps are in rapid equilibrium, the results are printed in the form you selected, i.e. containing or not equilibrium rate constants.

To unify the form of the steady-state equations derived by the program and contained in the file created, these equations are printed as:

\[ [X_1] = \frac{N(X_i)[E]_o}{\text{Den}} \quad (13) \]
\[ v(Y_i) = \frac{M(Y_i)[E]_o}{\text{Den}} \quad (14) \]

and then expressions of N(Xi), M(Yi) and Den are given.

Obviously, the quotient N(Xi)/Den coincides with the corresponding quotient G_i/G, H_i/H or L_i/L after making all possible simplifications. Owing to equations (5), (8) and (12), no repeated ligand species concentration appears in the terms of the denominator Den. Likewise, quotient M(Yi)/Den coincides with one of the quotients G_i/G, H_i/H and L_i/L in the most simplified form.

In most cases, as indicated in equations (13) and (14), the denominator of the corresponding equation (13) coincides with the denominator of equation (14), Den. Nevertheless, the program foresees the possibility that in some reaction schemes the denominator, resulting after simplification of the corresponding quotient H_i/H and L_i/L, does not coincide with Den. In this case, the denominator Den is not modified, but the numerator of the expression is divided by the appropriate factor given by the program.

Obviously, the concentrations of an enzyme species or/and the rate of a ligand species can be zero at the steady state, these cases also being foreseen by the program, which yields 0 for the corresponding concentration or rate.

Expressions such as [S]^2, [I]^3, etc. in the resulting equations must be interpreted as [S]2, [I]3, etc.
Error messages

During the execution of the program, any error in the input data is detected and the corresponding error message is displayed on the screen for its correction and then the program stops its execution. Once the error in the input file has been corrected, you must execute the program again.

Examples

To show the range and complexity of the mechanisms that the program is able to handle, the steady-state equations of different well-known reaction mechanisms under certain assumptions have been derived. Input and output files for these cases are provided with the program on the floppy disk that is available from the authors. The equations steady-state equations derived for each mechanism (e.g. concentrations of one or more of the enzyme species or/and rates of one or more of the ligand species), the conditions under which these equations were derived (e.g. strict steady-state or rapid equilibrium conditions of one or more of the reversible reaction steps) and the format wanted for the printout of the results are given in the corresponding input files. The equations derived are shown in the corresponding output files.

The reaction mechanisms solved, as well as the corresponding input and output files, are: general mechanism for two-substrate systems (Cha, 1968) (input file: examp1.in, output file: examp1.lis), Michaelis-Menten mechanism in which the enzyme species are unstable (Duggleby, 1986) (examp2.in, examp2.lis), general modifier mechanism of Botts and Morales (1953) (examp3.in, examp3.lis), reversible single substrate–single modifier mechanism (three different examples: examp4a.in, examp4a.lis, examp4b.lis, examp4c.lis), partial equilibrium ping-pong bi-bi mechanism (Cha, 1968) (examp5.in, examp5.lis), mechanism with enzyme species not included in rapid equilibrium segment (Cha, 1968) (examp6.in, examp6.lis) and the random ter-ter mechanism, a very complex scheme proposed by Fromm (1979a) (examp7.in, examp7.lis).

Discussion

When Cha (1968) published his important contribution, the derivation of strict steady-state solutions necessarily had to be manual, either using algebraic or graphic procedures, because there was no efficient computerized method to carry out this task. For this reason, the derivation of the solutions of partial or total equilibrium reactions from the corresponding strict steady-state solutions, although elegant, was time consuming and prone to human error, even for relatively simple reaction mechanisms. Cha’s procedure to derive solutions of partial or total equilibrium mechanisms avoided the derivation of the strict steady-state solutions. The method of Cha was developed not only to simplify the derivation of rate equations, but also to provide rate equations that are tractable to experimental verification.

Subsequently, several authors (Orsi, 1972; Cornish-Bowden, 1977; Fromm, 1979b; Lam, 1981; Canela, 1983; Herries, 1984; Ishikawa et al., 1988; Varon et al., 1990) published methods that allow the computerized derivation of the strict steady-state solutions and some of these can be applied to very complex reaction mechanisms (Ishikawa et al., 1988; Varon et al., 1990). Therefore, the main difficulty which justified Cha’s procedure is now circumvented. However, most contributions which offer the possibility of the computerized derivation of both the strict steady-state solutions and those corresponding to partial or total equilibrium mechanisms (Cornish-Bowden, 1977; Ishikawa et al., 1988) obtain the latter by incorporating Cha’s procedure into their computer program instead of taking advantage of the strict steady-state expressions to delete the terms which, according to the equilibrium assumptions made, are much smaller than the others.

The possibility of both the computerized derivation of the strict steady-state solutions and the subsequent computerized elimination of determined terms to furnish the corresponding solution, if in the reaction mechanism one or more of the reversible reaction steps are in rapid equilibrium, is a powerful alternative to Cha’s method, saving a lot of time and not prone to human error. As far as we know, the only contributions which offer this possibility are the computer program developed by Kinderlerer and Ainsworth (1976) and that of Varon et al. (1995), with the limitations already pointed out in the Introduction.

The computer program presented here allows the user to derive the equations for the concentration of the enzyme species and ligand rates for steady-state mechanisms either without any reversible step in rapid equilibrium (i.e. strict steady-state equations) or with one or more of these reaction steps in rapid equilibrium. This principle has already been described above. The present program handles enzyme-catalysed reactions in which up to 255 enzyme forms can be involved, with a limit of 255 non-null rate constants in the reaction mechanism. For this task, the program uses all free RAM memory of the computer (up to 128 Mbytes) and the free space of the hard disk (up to 128 Mbytes) when necessary. The memory requirement is a function of the complexity of the reaction mechanism. In those, probably few, cases in which more than 255 enzyme species and/or non-null rate constants could be involved, the source program can easily be adapted to handle up to 2^n – 1 enzyme forms and/or 2^n – 1 non-null K_{i,j} with n = 16 or 32, but this option will double (if n = 16) or quadruple (if n = 32) the amount of memory and CPU time used. Obviously, the execution time depends on the CPU of the computer, the access time of the hard disk used and the complexity of the reaction scheme.
This computer program offers as a printout option the strict steady-state equations of the reaction mechanism, i.e. those corresponding to the situation in which no reversible reaction step is in rapid equilibrium. The other options, which justify this contribution, are those corresponding to the existence of one or more reversible reaction steps in rapid equilibrium in the reaction mechanism. In this case, the results are delivered, according to the user’s choice, as a function of the rate constants or as a function of the equilibrium constants and those rate constants not involved in the reversible steps in rapid equilibrium.

Our program is valid for those reaction mechanisms which fit the general model described by reaction steps [1]–[6], i.e. those of most enzyme systems, irrespective of whether it is a branched or unbranched mechanism, irrespective of whether there are parallel steps, irreversible steps, repeated rate constants, closed loops, etc. It is not applicable to mechanisms of reactions for zymogen activation or for cyclic reversible enzyme cascades, because they do not fit the model. The program is also not applicable to mechanisms involving two or more enzymes acting simultaneously on one or more substrates.

The correct running of the program has been checked using many different cases, besides the examples given here. Nevertheless, in order to improve future versions, we would appreciate any suggestions or comments from readers.

Acknowledgement

This work was supported by grant ALI96-1111-C04 of the Comision Interministerial de Ciencia y Tecnologia (Spain).

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Received on August 19, 1996; revised on October 22, 1996; accepted on October 25, 1996