Biochem. J. (1987) 243, 159–164 (Printed in Great Britain)

A residence-time analysis of enzyme kinetics

Jacqueline J. SINES and David D. HACKNEY
Department of Biological Sciences, Carnegie–Mellon University, Pittsburgh, PA 15213, U.S.A.

A 'first-passage-time' analysis is applied to enzyme kinetics. It is shown that the residence times determined in this way are directly related to the steady-state parameters and are particularly useful in analysis of isotopic exchange. A simple linear means is used for the calculation of these residence times that makes this method easily applicable to the numerical evaluation of complex models. This stochastic type of approach provides an alternative that avoids the classical steady-state approximation that the concentrations of enzyme intermediates are constant. Instead, steady state is defined as the randomization of the states of the enzyme following initial mixing due to completion of the turnovers of individual enzyme molecules at different times.

INTRODUCTION

Many reaction schemes can be formulated as a set of first-order or pseudo-first-order interconversions. These schemes can be analysed by a first-passage-time treatment (Weiss, 1967), in which the average lifetime or residence time of a species before first passage through a selected step is determined by integration of the time course of the reaction. The present paper reports the application of this approach to initial-rate enzyme kinetics and isotopic exchange. The residence times for the enzyme intermediates are evaluated during an isolated turnover starting from a single intermediate. Certain of these residence times are shown to be proportional to the steady-state concentrations. Furthermore, ratios of residence times can provide information about the partitioning of intermediates, and this is directly useful in analysis of isotopic-exchange reactions. This stochastic analysis underlies approaches based on net rate constants and transit times (Yagil & Hoberman, 1969; Cleland, 1975; Fersht, 1985). The evaluation of residence times follows directly and unambiguously from the definition of the kinetic scheme and can be simply determined numerically by matrix inversion for even highly complex models.

Steady-state and isotopic-exchange parameters are classically derived by direct solution of the set of differential equations describing the kinetic scheme (see Huang, 1979). The stochastic approach now presented here represents an alternative type of analysis that is useful both for the insights obtained from its different perspective and for the unique set of residence times that it can provide.

THEORY AND DISCUSSION

The mechanism of eqn. (1) serves as a minimal model to illustrate the application of first time of passage analysis to enzymic kinetic schemes:

$$S+E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} EX \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E+P \qquad (1)$$

Pseudo-first-order conditions will be assumed, with the concentrations of S and P unchanging during the initial-rate phase. The differential equations describing this complete system are:

$$d[E]/dt = -k[E]+k'[EX]$$
$$d[EX]/dt = k[E]-k'[EX]$$

where $k = k_{+1}[S]+k_{-2}[P]$ and $k' = k_{-1}+k_{+2}$ are pseudo-first-order rate constants. On mixing E with S and P, a transient phase occurs as the concentrations of E and EX adjust to their steady-state values. This is illustrated in Fig. 1(*a*) and described by:

$$[E] = 1-\frac{k}{k+k'}(1-e^{-(k+k')t})$$

$$[EX] = \frac{k}{k+k'}(1-e^{-(k+k')t})$$

for the sum of concentrations of all enzyme species normalized to 1. (Here and throughout, concentrations will be normalized to 1, yielding a dimensionless parameter that is equivalent to probability.)

Consider now a hypothetical 'isolated turnover' of this system, defined as a turnover initiating with free E and terminating with the first passage through a ligand-release step, which for the scheme of eqn. (1) is step 2 or the reverse of step 1. This is a simple system of:

$$E \overset{k}{\rightarrow} EX \overset{k'}{\rightarrow}$$

with

$$d[E]/dt = -k[E]$$
$$d[EX]/dt = k[E]-k'[EX]$$

and

$$[E] = e^{-kt}$$

$$[EX] = \frac{k}{k'-k}(e^{-kt}-e^{-k't})$$

The time course of such an isolated turnover is illustrated for comparison in Fig. 1(*b*). Concentrations of all enzyme species will decay to zero in the limit of infinite time because the term for regeneration of E from EX is absent whereas the term for loss of EX is still present. Integration of these expressions for [E] and [EX] from time zero to infinity yields the residence times r_E

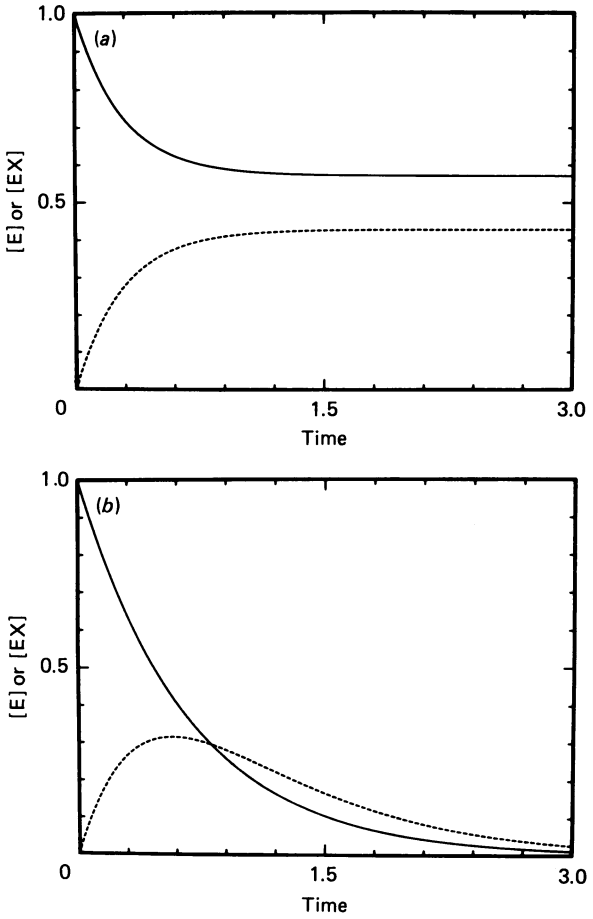


Fig. 1. Time course of first-order schemes, calculated for the scheme of eqn. (1) (*a*) under steady-state conditions or (*b*) as an isolated turnover

Rate constants were 1.5 and 2 for k and k' respectively in arbitrary units. ——, [E]; ------, [EX].

and r_{EX}, which are the total times spent as E or EX during a single isolated turnover and are given by:

$$r_E = \int_0^\infty [E]\cdot dt = \frac{1}{k}$$

$$r_{EX} = \int_0^\infty [EX]\cdot dt = \frac{1}{k'}$$

In a real experimental situation under pseudo-first-order conditions, the free E released in the steps with rate constants k_{+2} and k_{-1} of each isolated turnover will recycle through the scheme repeatedly, with E and EX reaching their steady-state concentrations as different enzyme molecules complete their cycles at different times. The relative times spent as E and EX at steady state will be equal to the relative times spent as E and EX during a single isolated turnover (r_E and r_{EX}), and the average turnover time at steady state will be equal to the total time spent as E and EX during a single isolated turnover ($r_{total} = r_E + r_{EX}$). Thus at steady state:

$$[E]_{ss} = \frac{r_E}{r_{total}}\cdot[E]_t = \frac{k'}{k+k'}\cdot[E]_t$$

and

$$[EX]_{ss} = \frac{r_{EX}}{r_{total}}\cdot[E]_t = \frac{k}{k+k'}\cdot[E]_t$$

The net velocity in the forward direction will be the difference between the rate of production of P and the rate of loss of P or:

$$v_f^{net} = k_{+2}[EX]_{ss} - k_{-2}[P][E]_{ss} \quad (2)$$

$$= \frac{k_{+2}k - k_{-2}[P]k'}{k+k'}\cdot[E]_t$$

which for the special case of [P] = 0 reduces to the well-known Briggs–Haldane equation. The reverse reaction can be analysed in an analogous manner. This approach is applicable, within the limits of the initial-rate approximation, to both the initial-rate period following first mixing of free E with S and P and to the instantaneous rate at each set of [S] and [P] values during the progress of the reaction.

Linear solution for residence times

Evaluation of residence times by explicit integration of the time course of an isolated turnover was trivial for the minimal model of eqn. (1), but becomes highly involved for schemes even of only modest complexity. These integrations, however, reduce to a simple form for first-order reactions (see the Appendix) that is easily applied to complex mechanisms. For the scheme of eqn. (1), the differential equations describing an isolated turnover with E as the recycling species can be expressed in matrix notation as

$$\begin{vmatrix} d[E]/dt \\ d[EX]/dt \end{vmatrix} = \begin{vmatrix} -k & 0 \\ k & -k' \end{vmatrix} \begin{vmatrix} [E] \\ [EX] \end{vmatrix}$$

or

$$\begin{vmatrix} d[E]/dt \\ d[EX]/dt \end{vmatrix} = B\cdot\hat{X} \quad (3)$$

where B is the 2×2 matrix of differential coefficients and $\hat{X}$ is the column vector of the amounts of E and EX. The vector of residence times, $\hat{T}_r$, is defined as:

$$\hat{T}_r = \begin{vmatrix} r_E \\ r_{EX} \end{vmatrix}$$

and can be evaluated by matrix inversion (see the Appendix) by using:

$$\hat{T}_r = -B^{-1}\cdot\hat{X}_0 = R\cdot\hat{X}_0 \quad (4)$$

where R is a matrix of residence times calculated as the inverse of matrix $-B$ and $\hat{X}_0$ is the distribution of enzyme species at the start of the isolated turnover. For an isolated turnover of the scheme of eqn. (1) starting with all of the enzyme as free E and with E and EX designated intermediates 1 and 2 respectively:

$$\hat{T}_r = \begin{vmatrix} r_E \\ r_{EX} \end{vmatrix} = -\begin{vmatrix} -k & 0 \\ k & -k' \end{vmatrix}^{-1} \begin{vmatrix} 1 \\ 0 \end{vmatrix} = \begin{vmatrix} 1/k & 0 \\ 1/k' & 1/k' \end{vmatrix} \begin{vmatrix} 1 \\ 0 \end{vmatrix} = \begin{vmatrix} 1/k \\ 1/k' \end{vmatrix}$$

The first column of matrix R contains the residence times for an isolated turnover starting from free E. It can also be seen that the second column of R contains the residence times for E and EX starting from EX in this isolated turnover, with free E as the recycling species (i.e. multiply R by |0, 1|). Steady-state parameters for the scheme of eqn. 1 are taken from the column of R corresponding to free enzyme because free enzyme is the species that links successive isolated turnovers. We define $r_{i,j}$ to be the entries of the matrix R, and $\hat{T}_r$ is defined to be the vector of net residence times r_i from which

steady-state parameters are derived. $\hat{T}_r$ is obtained by multiplying R by the value of $\hat{X}_0$ that has all of the enzyme present as the recycling species (i.e. a vector of zeros except for a value of 1 for the recycling species).

It should be emphasized that free E was selected as the recycling species in the above treatment because this is the conventional perspective. It is equally valid, however, for any intermediate to be considered as the species that initiates the isolated turnover and recycles. Thus an isolated turnover can also be defined for EX as the recycling species as:

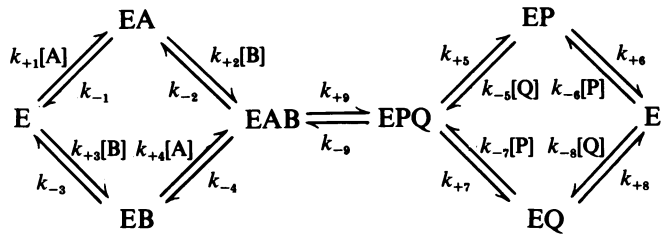
$$EX \xrightarrow{k'} E \xrightarrow{k}$$

with r_E and r_{EX} unchanged from above.

For branched schemes, the absolute values of the net residence times for isolated turnovers with different recycling intermediates will not in general be identical, but the relative values of the net residence times for any one recycling intermediate will still be proportional to the steady-state concentrations.

Complex schemes

The random binding scheme of eqn. (5) with two substrates and two products serves to illustrate the application to more complex mechanisms:



The kinetics for the isolated turnover, with free E as the recycling species, are given by:

$$\begin{vmatrix} d[E]/dt \\ d[EA]/dt \\ d[EB/dt \\ d[EAB]/dt \\ d[EPQ]/dt \\ d[EP]/dt \\ d[EQ]/dt \end{vmatrix} = \begin{vmatrix} -\sigma_1 & 0 & 0 \\ k_{+1}[A] & -\sigma_2-k_{-1} & 0 \\ k_{+3}[B] & 0 & -\sigma_3-k_{-3} \\ 0 & k_{+2}[B] & k_{+4}[A] \\ 0 & 0 & 0 \\ k_{-6}[P] & 0 & 0 \\ k_{-8}[Q] & 0 & 0 \end{vmatrix}$$

$$\begin{vmatrix} 0 & 0 & 0 & 0 \\ k_{-2} & 0 & 0 & 0 \\ k_{-4} & 0 & 0 & 0 \\ -\sigma_4 & k_{-9} & 0 & 0 \\ k_{+9} & -\sigma_5 & k_{-5}[Q] & k_{-7}[P] \\ 0 & k_{+5} & -\sigma_6-k_{+6} & 0 \\ 0 & k_{+7} & 0 & -\sigma_7-k_{+8} \end{vmatrix} \begin{vmatrix} [E] \\ [EA] \\ [EB] \\ [EAB] \\ [EPQ] \\ [EP] \\ [EQ] \end{vmatrix}$$

where σ_i is the sum of all the other rate constants in the ith column. Note that, in defining this matrix, steps 2, 4, 5, 7 and 9 are considered to be reversible, as they do not result in release of the recycling species, free E, whereas regeneration of free E in step 1, 3, 6 or 8 terminates the isolated turnover. Thus the k_{-1}, k_{-3}, k_{+6} and k_{+8} terms are included as negative entries on the diagonal, but are not included as corresponding positive entries in the off-diagonal positions. Solution of this system by eqn. (4), with the only non-zero entry of $\hat{X}_0$ corresponding to free E, yields the set of net residence times that are proportional to the steady-state concentrations.

This treatment is easily generalized to any first-order scheme with n species. The terms of the $n \times n$ matrix B are defined by the coefficients of the differential equations describing the isolated turnover for any designated recycling species. The $b_{i,j}$ terms for $i \neq j$ are equal to the rate constants $k_{j,i}$ for conversion of species j into species i (note inversion of subscript order between b and k terms), and the $b_{i,i}$ terms are the negative sums of all rate constants leading away from species i. This set of equations will be identical with the usual equations describing steady state ($d[E_i]/dt = 0$) except for the absence of the off-diagonal terms for the regeneration of the recycling species. Inversion of matrix $-B$ yields R, the matrix of residence times, in which the column corresponding to the designated recycling species contains the net residence times that are proportional to steady-state values. Each other $r_{i,j}$ term is the time spent as species i during a cascade from species j in the isolated turnover specific for the designated recycling intermediate.

If only the net residence times at steady state are desired, the calculation of the complete inverse can be avoided. Thus eqn. (4) can be multiplied on both sides by $-B$ to yield:

$$-B \cdot \hat{T}_r = \hat{X}_0 \qquad (6)$$

or for the scheme of eqn. (1):

$$\begin{vmatrix} k & 0 \\ -k & k' \end{vmatrix} \begin{vmatrix} r_E \\ r_{EX} \end{vmatrix} = \begin{vmatrix} 1 \\ 0 \end{vmatrix}$$

In the general case this will be a simple linear system of n simultaneous equations in n unknowns that can be solved by any of the standard methods for such systems.

These methods are useful conceptually for derivation of exact solutions to specific kinetic schemes with rate constants treated as variables, but such solutions would contain a very large number of terms for any but the simplest schemes. In practice with a complex kinetic scheme, numerical solutions can be readily obtained for each set of numerical values of the rate constants without explicit derivation of the exact solution.

Computer programs are available from the authors for performing such numerical evaluations.

Isotopic partitioning and exchange

In isotopic-exchange studies, it is useful to know the total flux of free S into free P, such as would be measured from the rate of appearance of radioactive label in P starting from radioactively labelled S. For the minimal scheme of eqn. (1), this forward flux, designated R_f, is given by:

$$R_f = k_{+1}[S][E]_{ss} F_{EX,P}$$

where $F_{EX,P}$ is the fraction of EX that releases ligand as P and is equal in this case to $k_{+2}/(k_{-1}+k_{+2})$ or:

$$R_f = \frac{k_{+1}[S]k_{+2}}{k+k'} \cdot [E]_t = \frac{[S]}{[S]+K'} \cdot k_{+2}[E]_t$$

where

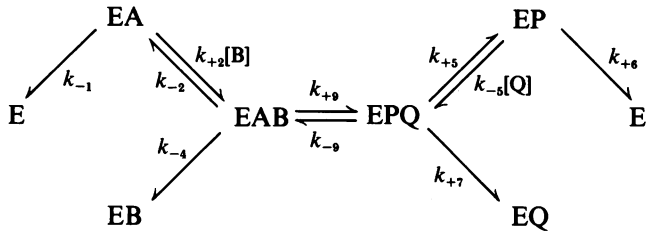
$$K' = K_m + \frac{k_{-2}[P]}{k_{+1}}$$

and K_m equals the Michaelis constant for the forward reaction as $(k_{-1}+k_{+2})/k_{+1}$. This expression is equivalent to eqns. (7) and (9) of Boyer (1959) and applies both to steady-state and equilibrium situations.

Analysis of the partitioning of enzyme intermediates was trivial for the case of eqn. (1), but is more complex for the scheme of eqn. (5). For example, if labelled A is converted into labelled P, then the initial rate of transfer of label from the A pool to the P pool, $R_{A,P}$, can be determined as:

$$R_{A,P} = k_{+1}[A][E]_{ss}F_{EA,P} + k_{+4}[A][EB]_{ss}F_{EAB,P}$$

where $F_{i,P}$ is the fraction of intermediate i that first releases ligand as P rather than as A. The relevant residence times for determination of these partitioning factors are those of an isolated turnover for a reduced scheme that begins with all intermediates containing A or P and terminates with the first step releasing A or P. Such a reduced scheme is given by:



and

$$\begin{vmatrix} d[EA]/dt \\ d[EAB]/dt \\ d[EPQ]/dt \\ d[EP]/dt \end{vmatrix} = \begin{vmatrix} -\sigma_1 - k_{+1} & k_{-2} & 0 & 0 \\ k_{+2}[B] & -\sigma_2 - k_{-4} & k_{-9} & 0 \\ 0 & k_{+9} & -\sigma_3 - k_{+7} & k_{-5}[Q] \\ 0 & 0 & k_{+5} & -\sigma_4 - k_{+6} \end{vmatrix} \begin{vmatrix} [EA] \\ [EAB] \\ [EPQ] \\ [EP] \end{vmatrix}$$

The residence times for this reduced scheme can be obtained by application of eqn. (4) and are designated $r'_{i,j}$. $F_{EA,P}$ can be determined from these $r'_{i,EA}$ values as:

$$F_{EA,P} = \frac{k_{+6}r'_{EP,EA} + k_{+7}r'_{EPQ,EA}}{\sigma_{EA}}$$

where σ_{EA} is the sum of the fluxes through all of the terminal steps in this reduced isolated turnover initiating with EA, which in this case will be:

$$\sigma_{EA} = k_{-1}r'_{EA,EA} + k_{-4}r'_{EAB,EA} + k_{+7}r'_{EPQ,EA} + k_{+6}r'_{EP,EA}$$

Similarly $F_{EAB,P}$ is given by:

$$F_{EAB,P} = \frac{k_{+6}r'_{EP,EAB} + k_{+7}r'_{EPQ,EAB}}{\sigma_{EAB}}$$

where

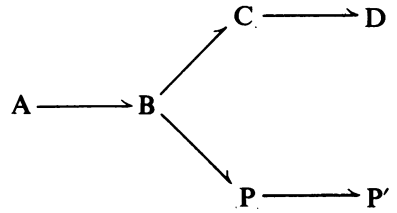
$$\sigma_{EAB} = k_{-1}r'_{EA,EAB} + k_{-4}r'_{EAB,EAB} + k_{+7}r'_{EPQ,EAB} + k_{+6}r'_{EP,EAB}$$

These partitioning factors are also directly applicable to analysis of isotope-trapping experiments (see Rose, 1980) in which, for example, a low concentration of labelled A is incubated with enzyme, chased with an excess of unlabelled A, B and P, and the fraction of the label resulting in A compared with P is determined. The residence-time approach is formally analogous to that employed by Rose *et al.* (1974) in that the partitioning of intermediates is determined by integration of a single turnover. The specific procedure presented here has the advantage that the algorithm is simple and unambiguous and can be easily applied to complex schemes. More importantly, however, is the fact that matrix inversion provides a simple and direct way to evaluate the residence times. Even the simple scheme considered by Rose *et al.* (1974) was difficult to integrate analytically, and such an approach could not be feasibly applied to a complex scheme. Matrix inversion, in contrast, can provide numerical evaluations of residence times even with large complex schemes, and programs for inversion are readily available.

Comparison of residence times and transit times

The use of turnover analysis to derive steady-state parameters is also employed in methods that rely on net rate constants and transit times (Yagil & Hoberman, 1969; Cleland, 1975; Fersht, 1985). The reciprocal of the net rate constant is the time required for a given intermediate, once formed, to decay, and is often designated a transit time. A residence time is defined here as the actual time spent as a given intermediate during an isolated turnover. For a simple linear scheme, every intermediate is formed during each turnover and the reciprocals of the net rate constants are closely related to residence times starting from free enzyme. Specifically, the reciprocal of the net rate constant for an intermediate will be proportional to the sum of the residence times for itself and all species between it and the irreversible step in an isolated turnover starting from free E. The set of n transit times is in effect a subset of the complete $n \times n$ set of residence times with the transit times bering derived from only one column of the R matrix.

With branched kinetic schemes, however, this simple correspondence is no longer valid. For example with the scheme:



the relative residence times for C and D starting from A will be shorter than the total transit times for species C and D, as only part of the net flux will go through each branch.

Net rate constants are assigned by inspection in the method of Cleland (1975), which requires special rules for branched schemes and opens the possibility that errors can occur when complex assignments are required. In contrast, the linear method presented here for determination of residence times only requires

assignment of the rate constants to a simple square matrix by clear and unambigious rules. In effect the assignment of the terms of the matrix B is merely a restatement of the particular kinetic model that is desired. The calculation of residence times following this assignment only requires inversion of the matrix $-B$, which again is a simple and direct procedure. Thus residence times can be readily determined even for very large and complex kinetic schemes.

Comparison of stochastic and differential approaches

In usual practice (see Huang, 1979), steady-state parameters are obtained by solution of the set of linear differential equations that results from setting to zero the derivative with respect to time of the concentration of each enzyme species. This can be expressed in matrix notation for the minimal scheme of eqn. (1) as:

$$A \cdot \hat{X}_{ss} = \begin{vmatrix} 0 \\ 0 \end{vmatrix} \quad (8)$$

where matrix A is similar to matrix B, but contains the additional terms required for recycling of free E at steady state (compare with eqn. 3). This set of simultaneous equations in general has no unique solution, but solution is possible with the additional constraint of the conservation equation, either directly or by specialized algorithms such as the King–Altman method (King & Altman, 1956). In this classical differential approach the steady-state concentrations are determined as the solution of eqn. (8), whereas with the method presented here the residence times are obtained from solution of eqn. (4) or (6). The forms of the two solutions are similar and both require solution of a set of n simultaneous linear equations in order to obtain steady-state parameters. The residence-time analysis has the advantage, however, that the complete set of $n \times n$ residence times can be obtained by calculation of the inverse of the $-B$ matrix. The isotope-partitioning treatment presented here is an example of the usefulness of these $r_{i,j}$ terms beyond mere calculation of steady-state parameters.

The major conceptual difference between the two approaches lies in the way steady state is defined. In the differential approach, the net rate will initially be zero on mixing free E plus S and P, but will increase after a lag phase to its steady-state value. This initial transient phase is viewed as the time required for the intermediates to reach their steady-state concentrations and for the steady-state approximation of $d[E_i]/dt = 0$ to become valid. This assumption is more properly stated as $d[E_i]/dt \ll d[P]/dt$ and can be a source of confusion, but is avoided in the stochastic approach. From the stochastic view, all the enzyme molecules are initially in phase and begin their first isolated turnover at the same time as free E, but this uniformity is lost as different enzyme molecules complete their first and subsequent turnovers at different times. Thus steady state in this context is defined as the stage of the reaction that results when this randomization process is complete and no phase uniformity exists.

This work was supported by Grant AM25980 from the U.S. Public Health Service. D.D.H. was supported by an Established Investigatorship from the American Heart Association and J.J.S. was supported by N.I.H. Predoctoral Training Grant GM08067.

REFERENCES

Boyer, P. D. (1959) Arch Biochem. Biophys. **82**, 387–410
Cleland, W. W. (1975) Biochemistry **14**, 3220–3224
Fersht, A. (1985) Enzyme Structure and Mechanism, 2nd edn., pp. 117–119, W. H. Freeman, New York
Huang, C. Y. (1979) Methods Enzymol. **63**, 54–84
King, E. L. & Altman, C. (1956) J. Phys. Chem. **60**, 1375–1378
Rose, I. A. (1980) Methods Enzymol. **64**, 47–59
Rose, I. A., O'Connell, E. L., Litwin, A. & Bar-Tana, J. (1974) J. Biol. Chem. **249**, 5163–5168
Weiss, G H. (1967) Adv. Chem. Phys. **13**, 1–18
Yagil, G. & Hoberman, H. D. (1969) Biochemistry **8**, 352–360

APPENDIX

Linear determination of residence times

A general solution for mean first-passage times is well known (see Weiss, 1967) and reduces to the matrix-inversion method for residence times in first-order kinetic schemes. The following is a simplified derivation specific to such cases that illustrates the validity of eqn. (4) of the main paper.

For the scheme of eqn. (1) of the main paper, the residence times in T_r are equal to the integrated areas under the curves for E and EX, which can be calculated numerically as:

$$\hat{T}_r = \int_0^\infty \hat{X}_t \cdot dt = \lim_{\Delta t \to 0} \sum_{i=0}^{\infty} \hat{X}_{i\Delta t} \cdot \Delta t \quad (A1)$$

Now each $\hat{X}_{t+\Delta t}$ is related to each $\hat{X}_t$ by:

$$\begin{vmatrix} [E] \\ [EX] \end{vmatrix}_{t+\Delta t} = \begin{vmatrix} 1-\Delta t \cdot k & 0 \\ \Delta t \cdot k & 1-\Delta t \cdot k' \end{vmatrix} \begin{vmatrix} [E] \\ [EX] \end{vmatrix}_t$$

or

$$\hat{X}_{t+\Delta t} = [I + \Delta t \cdot B] \cdot \hat{X}_t$$

where I is a 2×2 identity matrix. At any time point that is a multiple of Δt, $\hat{X}_{i\Delta t}$ can be defined in terms of the initial distribution $\hat{X}_0$ by:

$$\hat{X}_{\Delta t} = [I+\Delta t\cdot B]\cdot\hat{X}_0$$
$$\hat{X}_{2\Delta t} = [I+\Delta t\cdot B]\cdot\hat{X}_{\Delta t} = [\mathrm{I}+\Delta t\cdot B]^2\cdot\hat{X}_0$$
$$\vdots$$
$$\hat{X}_{i\Delta t} = [I+\Delta t\cdot B]^i\cdot\hat{X}_0 \qquad \text{(A2)}$$

Thus combination of eqns. (A1) and (A2) yields:

$$\hat{T}_r = \lim_{\Delta t\to 0}\Delta t\sum_{i=0}^{\infty}[I+\Delta t\cdot B]^i\cdot\hat{X}_0$$

It can be verified by expansion that:

$$[I+M+M^2+M^3+\ldots][I-M] = I$$

for any square matrix M for which the terms of M^n vanish as n approaches infinity. Multiplying both sides by the inverse of $[I-M]$ gives:

$$[I+M+M^2+M^3+\ldots] = [I-M]^{-1}$$

[This is the matrix equivalent of the result:

$$\sum_{i=1}^{\infty} a^i = (1-a)^{-1}$$

for a real number whose absolute value is less than 1.]

Now this relationship applies to the matrix $[I+\Delta t\cdot B]$, since the terms of $\hat{X}$ must approach zero at infinite time, and substitution of $[I+\Delta t\cdot B]$ for M yields:

$$\sum_{i=0}^{\infty}[I+\Delta t\cdot B]^i = (I-[I+\Delta t\cdot B])^{-1} = (-\Delta t\cdot B)^{-1}$$

and thus:

$$\hat{T}_r = \lim_{\Delta t\to 0}\Delta t(-\Delta t\cdot B)^{-1}\cdot\hat{X}_0 = -B^{-1}\cdot\hat{X}_0 = R\cdot\hat{X}_0$$

Reference

Weiss, G. H. (1967) Adv. Chem. Phys. **13**, 1–18

Received 5 August 1986/2 October 1986; accepted 1 December 1986