Graphic analysis of relaxation times of enzyme-catalysed reactions

An extension of the graphic method of King and Altman

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An extension of the graphic method of King & Altman (1956) (J. Phys. Chem. **60**, 1375–1378) is applied to the analysis of relaxation times of enzyme-catalysed reactions and a simple graphic method is presented. Clear-cut graphs, simple drawing, easy operation (without the need to perform the usual complex mathematical operations), and reliable results are the main characteristics of this kind of graphic method. A system of enzyme-catalysed reactions ($E \rightleftharpoons ES \rightleftharpoons EP$) is used as the actual example for illustrating the graphic method.

INTRODUCTION

At present, the relaxation times of enzyme reactions are usually derived by using the methods of differential equations, matrix algebra and Fourier analysis, etc. (Kustin & Shear, 1965; Hammes & Schimmel, 1966; Espenson, 1981; Bernasconi, 1986a; Schwarz, 1986). It is somewhat difficult for biologists to master these complex methods. The advantages of utilizing graphic methods to solve the problems of steady-state enzymic kinetics were demonstrated early on (King, 1956; King & Altman, 1956; Volkenstein & Goldstein, 1966; Fromm, 1970; Chou *et al.*, 1978; Huang, 1981). Now these graphic methods are widely used in the area of enzymic kinetics (Whitehead, 1970; Guan, 1989). This paper shows that the relaxation times of enzyme reactions can be derived by using an extension of the graphic method of King and Altman (King, 1956; King & Altman, 1956). The graphic method demonstrated in this paper is very simple and useful. As enzymologists are very familiar with the graphic method of King and Altman, they should also easily understand the present method which is based on that of King and Altman.

GENERAL THEORY

For the general case of a mechanism involving n enzyme-containing species, $E_1, E_2, \dots, E_n$, each of which can be converted into every other enzyme-containing species, the n kinetic differential equations can be written as follows:

$$\frac{dE_i}{dt} = \sum_{j \neq i}^n K_{ji} E_j - E_i \sum_{j \neq i}^n K_{ij} \quad (1)$$

where K_{ij} or K_{ji} represents the kappa, or the product of the rate constant and the associated concentration factors (not including concentrations of enzyme-containing species, however) for the reaction which converts E_i into E_j . We define

$$E_i = E_{ie} + \Delta E_i \quad (2)$$

E_{ie} is the equilibrium concentration and ΔE_i measures how far the respective concentration is from equilibrium. It is easy to show that eqns. (1) and (2) can then be rewritten as

$$\frac{d\Delta E_i}{dt} = \sum_{j \neq i}^n K_{ji} \Delta E_j - \Delta E_i \sum_{j \neq i}^n K_{ij} \quad (3)$$

Let $\Delta E_i = X_i = X_i(t)$ and define q by the Laplace transform X_i^* of X_i :

$$X_i^*(t) = \int_0^\infty e^{-qt} X_i(t) dt \quad (4)$$

The standard Laplace transform of dX_i/dt is $[qX_i^* - X_i(0)]$, where t represents time, $X_1(0), X_2(0), \dots, X_n(0)$ are initial values. Then we obtain

$$\begin{cases} qX_1^* - X_1(0) = -X_1^* \sum_{j \neq 1}^n K_{1j} + X_2^* K_{21} + \dots + X_n^* K_{n1} \\ qX_2^* - X_2(0) = X_1^* K_{12} - X_2^* \sum_{j \neq 2}^n K_{2j} + \dots + X_n^* K_{n2} \\ \vdots \\ qX_n^* - X_n(0) = X_1^* K_{1n} + X_2^* K_{2n} + \dots - X_n^* \sum_{j \neq n}^n K_{nj} \end{cases} \quad (5)$$

According to eqn. (5) we have

$$\begin{cases} -X_1(0) = -X_1^* \left(\sum_{j \neq 1}^n K_{1j} + q \right) + X_2^* K_{21} + \dots + X_n^* K_{n1} \\ -X_2(0) = X_1^* K_{12} - \left(\sum_{j \neq 2}^n K_{2j} + q \right) X_2^* + \dots + X_n^* K_{n2} \\ \vdots \\ -X_n(0) = X_1^* K_{1n} + X_2^* K_{2n} + \dots - X_n^* \left(\sum_{j \neq n}^n K_{nj} + q \right) \end{cases} \quad (6)$$

(As the rank of the system matrix of eqn. (6) is $n-1$, one of the n equations of eqn. (6) is useless and must be deleted.)

Since

$$X_1 + X_2 + \dots + X_n = 0 \quad (7)$$

We have

$$(X_1^* + X_2^* + \dots + X_n^*) q = 0 \quad (8)$$

So the first line of eqn. (6) can be rewritten as follows:

$$-X_1(0) = -X_1^* \sum_{j \neq 1}^n K_{1j} + X_2^* (K_{21} + q) + \dots + X_n^* (K_{n1} + q) \quad (9)$$

Let

$$K_{ij}^* = \begin{cases} K_{ij} & (i = 1, 2, \dots, n; j = 2, 3, \dots, n; i \neq j) \\ K_{ij} + q & (i = 2, 3, \dots, n; j = 1) \end{cases} \quad (10)$$

Combining eqns. (6), (7), (9) and (10) affords

$$\begin{cases} -X_1(0) = -X_1^* \sum_{j=1}^n K_{1j}^* + X_2^* K_{21}^* + \dots + X_n^* K_{n1}^* \\ -X_2(0) = X_1^* K_{12}^* - X_2^* \sum_{j=2}^n K_{2j}^* + \dots + X_n^* K_{n2}^* \\ \vdots \\ -X_n(0) = X_1^* K_{1n}^* + X_2^* K_{2n}^* + \dots - X_n^* \sum_{j=n}^n K_{nj}^* \\ 0 = X_1^* + X_2^* + \dots + X_n^* \end{cases} \quad (11)$$

In using Cramer's method, the fractional concentration of the k th species can be expressed as the quotient of two determinants, Δ_k/Δ_0 , as shown in eqn. (12):

$$X_k^* = \frac{\Delta_k}{\Delta_0} = \frac{\begin{vmatrix} -\sum K_{1j}^* & K_{21}^* & \dots & -X_1(0) & \dots & K_{n1}^* \\ K_{12}^* & -\sum K_{2j}^* & \dots & -X_2(0) & \dots & K_{n2}^* \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ 1 & 1 & \dots & 0 & \dots & 1 \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ K_{1n}^* & K_{2n}^* & \dots & -X_n(0) & \dots & -\sum K_{nj}^* \end{vmatrix}}{\begin{vmatrix} -\sum K_{1j}^* & K_{21}^* & \dots & K_{k1}^* & \dots & K_{n1}^* \\ K_{12}^* & -\sum K_{2j}^* & \dots & K_{k2}^* & \dots & K_{n2}^* \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ 1 & 1 & \dots & 1 & \dots & 1 \\ \vdots & \vdots & \ddots & \vdots & \ddots & \vdots \\ K_{1n}^* & K_{2n}^* & \dots & K_{kn}^* & \dots & -\sum K_{nj}^* \end{vmatrix}} \quad (12)$$

In eqn. (12), the denominator determinant Δ_0 (not the numerator determinant Δ_k) can be calculated by using the schematic rule of King and Altman because the denominator determinant Δ_0 of eqn. (12) is similar to the denominator determinant Δ_0 of King & Altman's (1956) method. [Note: the numerator determinant Δ_k of eqn. (12) cannot be calculated by using the graphic method of King and Altman.]

On the other hand, let

$$X_k^* = \frac{b_0 q^m + b_1 q^{m-1} + \dots + b_{m-1} q + b_m}{q^{n-1} + a_1 q^{n-2} + \dots + a_{n-2} q + a_{n-1}} \quad (\text{where } k \neq 1, n-1 > m)$$

$$= \frac{Q_1}{q-q_1} + \frac{Q_2}{q-q_2} + \dots + \frac{Q_{n-1}}{q-q_{n-1}} \quad (13)$$

and

$$\Delta_0 = q^{n-1} + a_1 q^{n-2} + \dots + a_{n-2} q + a_{n-1} \quad (14)$$

Where $q_1, q_2, \dots, q_n$ are the roots of the equation $\Delta_0 = 0$. Then the inverse Laplace transformation of eqn. (13) is

$$X_k(t) = \sum_{i=1}^{n-1} Q_i \exp(q_i t) \quad (15)$$

From eqn. (15), we know that the reciprocal relaxation time $1/\tau_i$ is equal to $-q_i$.

So we can conclude that the denominator determinant Δ_0 of eqn. (12) can be calculated by using the schematic rule of King and Altman under limiting conditions

$$K_{ij}^* = \begin{cases} K_{ij} \cdot (i=1,2,\dots,n; j=2,3,\dots,n; i \neq j) \\ K_{ij}^* + q \cdot (i=2,3,\dots,n; j=1) \end{cases}$$

and that the reciprocal relaxation times of the enzyme-catalysed reactions are equal to the negative roots of the equation $\Delta_0 = 0$.

ILLUSTRATION OF THE GRAPHIC METHOD

Let us consider the reaction

Under the conditions of eqn. (10), eqn. (16) can be written in cyclical form (Scheme 1):

$$K_{12}^* = K_{12}S; \quad K_{23}^* = K_{23}; \quad K_{31}^* = K_{21} + q;$$

$$K_{32}^* = K_{32}; \quad K_{21}^* = 0 + q = q$$

On the basis of King and Altman's method, we can obtain the graphic solutions of E, ES, EP:

$$\Delta_e = q(K_{21} + q) + K_{23}q + K_{32}(K_{21} + q)$$

$$\Delta_{es} = K_{12}Sq + K_{32}K_{12}S$$

$$\Delta_{ep} = K_{12}K_{23}S$$

Then we have

$$\Delta_0 = \Delta_e + \Delta_{es} + \Delta_{ep} = q^2 + \beta q + \alpha \quad (17)$$

Where we let

$$\alpha = K_{21}K_{32} + K_{12}K_{32}S + K_{12}K_{23}S$$

$$\beta = K_{12}S + K_{21} + K_{23} + K_{32}$$

Here the roots of the equation $\Delta_0 = 0$ are

$$q_{1,2} = [-\beta \pm \sqrt{(\beta^2 + 4\alpha)}]/2 \quad (18)$$

So we have

$$\begin{cases} 1/\tau_1 = -q_1 = [\beta - \sqrt{(\beta^2 + 4\alpha)}]/2 \\ 1/\tau_2 = -q_2 = [\beta + \sqrt{(\beta^2 + 4\alpha)}]/2 \end{cases} \quad (19)$$

or

$$\begin{cases} \frac{1}{\tau_1} + \frac{1}{\tau_2} = \beta = K_{12}S + K_{21} + K_{23} + K_{32} \\ \frac{1}{\tau_1 \tau_2} = \alpha = K_{21}K_{32} + K_{12}S(K_{23} + K_{32}) \end{cases} \quad (20)$$

From eqns. (19) and (20), we find that the relaxation times of eqn. (16) derived by the graphic method of this paper agree with those derived using the conventional solution of differential equations in the literature (Bernasconi, 1986b).

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