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ADVANCES IN GRAPHIC METHODS OF ENZYME KINETICS

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A concise description is presented of the recent progress in the graphic methods of enzyme kinetics, in order to help users to master these kinds of methods without the need for spending too much time in following the process of development and the details of mathematics.

1. Introduction

With deeper investigation into enzyme-catalysed mechanisms, we are often confronted with some complex reaction systems of which the derivation of their rate equations seems formidable if a computer is not employed. Unfortunately, by means of a computer, we can only obtain numerical results, but no analytic solution is available, which, however, is very important in analysing and discussing the mechanism concerned at a deeper level. In order to acquire the analytic expression, there is no alternative but to resort to the analytic derivation method although this is both tedious and error-prone. Fortunately, considerable progress in graphic methods of enzyme kinetics has been made recently [1–7], which makes it become feasible to find the rate equations for very complicated enzyme-catalysed mechanisms. However, because this set of new graphic methods was gradually developed and only became matured very recently, the relevant contents published are rather disperse, and some steps and notations need to be further simplified and unified. Also, during such a process of development, some sophisticated mathematics are inevitably involved in order to make these novel methods convincing. This, however, makes those people who are only interested in the application of the new methods feel it is difficult to follow if these 'obstacles' are not removed properly. To satisfy this kind of request from many users, the author will in this paper summarize these new graphic methods in a way that is easy to follow and to master for most biologists.

2. Method

In steady-state enzyme kinetics, the following two physical quantities often need to be calculated.

2.1. The concentration of enzyme species

Assume an enzyme-catalysed reaction system, in which there are n enzyme species $E_1, E_2, \ldots, E_n$. The concentration of any, say the mth, enzyme species can always be written in the form of [1,3]

$$[E_m] = \frac{N_m}{\sum_{i=1}^{n} N_i} e_0 \qquad (1)$$

where $[E_m]$ is the concentration of the mth enzyme species E_m, $e_0 = \sum_{m=1}^{n} [E_m]$ the total enzyme concentration, and N_i $(i = 1, 2, \ldots, n)$ a quantity whose

magnitude is apparently directly proportional to the concentration of the ith enzyme species E_i, and will be further discussed later.

2.2. The rate of product formation

Suppose P is a product in the enzyme-catalysed system. The rate of its formation can always be written as [3,5]

$$v = \frac{d[P]}{dt} = \frac{\vec{P} - \tilde{P}}{\sum_{i=1}^{n} N_i} e_0 \qquad (2)$$

where $\vec{P}$ and $\tilde{P}$ are the quantities related to the release and combination of the product, respectively, and will be further discussed as well.

From eqs. 1 and 2 we see that in order to obtain the above two important physical quantities, the key point is to find an efficacious method to derive N_i ($i = 1, 2, \ldots, n$), and $\vec{P}$ and $\tilde{P}$.

2.3. Rule 1. The calculation method for N_i

(a) Draw a graph $\mathfrak{P}$ to express the enzyme-catalysed reaction mechanism. This graph consists of points and arcs: each point denotes an enzyme species, and each arc reflects the relationship between two enzymes, viz., if there is a direct chemical conversion between some two enzyme species, then there will be an arc to link the two corresponding points. The arc bears an arrow to indicate the conversion direction and is weighted by a rate constant to show the conversion rate. For instance, for the following enzyme-catalysed mechanism

$$\begin{array}{ll} E + A \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} EA & EA + B \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} EAB \\ EAB \underset{k_{-3}}{\overset{k_{+3}}{\rightleftarrows}} EQ + P & EQ \underset{k_{-4}}{\overset{k_{+4}}{\rightleftarrows}} E + Q \end{array} \qquad (3)$$

the corresponding graph $\mathfrak{P}$ is depicted in fig. 1. In this example there are four enzyme species. When using eq. 1, we may assign

$$E_1 = E,\ E_2 = EA,\ E_3 = EAB,\ E_4 = EQ \qquad (4)$$

(b) To each point in $\mathfrak{P}$ add a loop with a weight equal to the sum of the weights of the arcs that depart from this point. The graph thus obtained is denoted by $\mathfrak{P}^\circ$. For instance, the $\mathfrak{P}$ in fig. 1 is accordingly transformed to $\mathfrak{P}^\circ$ of fig. 2.

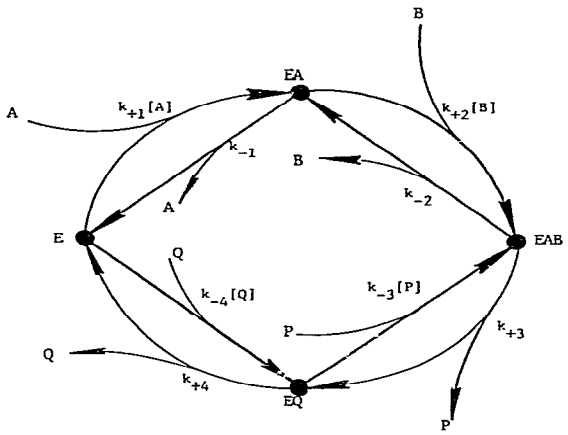


Fig. 1. The graph $\mathfrak{P}$ for mechanism 3.

(c) Select any point in $\mathfrak{P}^\circ$, say EAB, as a starting reference point. Then, for any specified point, say E, find all subgraphs each of which possesses a path from EAB to E, and all cycles and loops that intersect with neither each other nor the path. For instance, we find the following

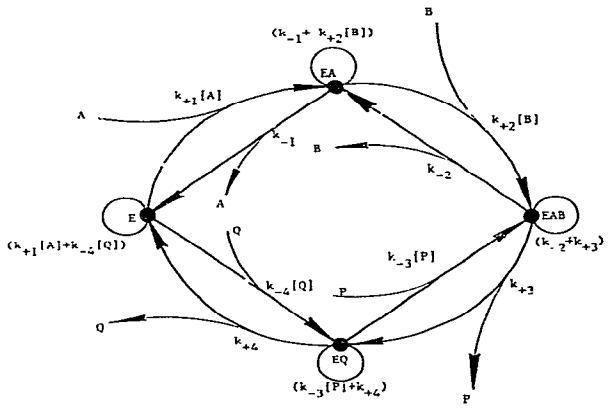
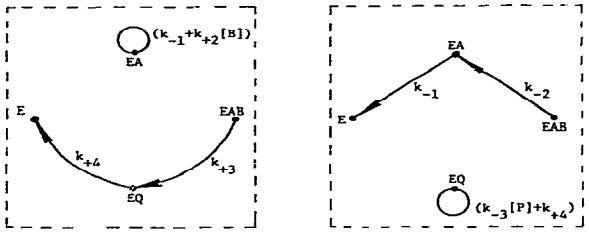


Fig. 2. The transformed graph $\mathfrak{P}^\circ$ obtained from $\mathfrak{P}$ in fig. 1.

two subgraphs from fig. 2 which satisfy the above condition:



Then for each such subgraph, take the product of all its weights and multiply with a sign factor given by

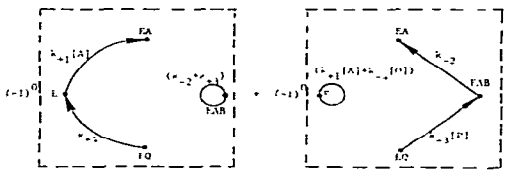
$$(-1)^{c_y} \tag{5}$$

where c_y is the number of the cycles (not including loops) in the corresponding subgraph. For instance, for the above two subgraphs, we have $(-1)^0 k_{+3}k_{+4}(k_{-1}+k_{+2}[B])$ and $(-1)^0 k_{-1}k_{-2}(k_{-3}[P]+k_{+4})$, respectively. Taking the sum of these results, we immediately obtain N_E; i.e.

$$N_E = N_1 = k_{+3}k_{+4}(k_{-1}+k_{+2}[B]) + k_{-1}k_{-2}(k_{-3}[P]+k_{+4}) \tag{6}$$

Likewise, for EA, if we take EQ as the starting reference point, it follows

$N_{EA} = N_2 =$



$$= k_{+1}k_{+4}[A](k_{-2}+k_{+3}) + k_{-2}k_{-3}[P](k_{+1}[A]+k_{-4}[Q]) \tag{7}$$

In a similar way, we obtain

$$N_{EAB} = N_3 = k_{+1}[A]k_{+2}[B](k_{-3}[P]+k_{+4}) + k_{-4}[Q]k_{-3}[P](k_{-1}+k_{+2}[B]) \tag{8}$$

$$N_{EQ} = N_4 = k_{-1}k_{-4}[Q](k_{-2}+k_{+3}) + k_{+2}[B]k_{+3}(k_{+1}[A]+k_{-4}[Q]) \tag{9}$$

Substituting eqs. 6–9 into eq. 1, we immediately obtain $[E_m]$ $(m = 1, 2, 3, 4)$, as follows:

$$[E_1] = [E] = [(k_{-1}k_{+3}k_{+4} + k_{-1}k_{-2}k_{+4}) + (k_{+2}k_{+3}k_{+4})[B] + (k_{-1}k_{-2}k_{-3})[P]]e_0/D \tag{10}$$

$$[E_2] = [EA] = [(k_{+1}k_{-2}k_{+4} + k_{+1}k_{+3}k_{+4})[A] + (k_{+1}k_{-2}k_{-3})[A][P] + (k_{-2}k_{-3}k_{-4})[P][Q]]e_0/D \tag{11}$$

$$[E_3] = [EAB] = [(k_{+1}k_{+2}k_{+4})[A][B] + (k_{-1}k_{-3}k_{-4})[P][Q] + (k_{+1}k_{+2}k_{-3})[A][B][P] + (k_{+2}k_{-3}k_{-4})[B][P][Q]]e_0/D \tag{12}$$

$$[E_4] = [EQ] = [(k_{-1}k_{-2}k_{-4} + k_{-1}k_{+3}k_{-4})[Q] + (k_{+1}k_{+2}k_{+3})[A][B] + (k_{+2}k_{+3}k_{-4})[B][Q]]e_0/D \tag{13}$$

where

$$\begin{aligned} D = \sum_{i=1}^{4} N_i = {} & (k_{-1}k_{+3}k_{+4} + k_{-1}k_{-2}k_{+4}) \\ & + (k_{+1}k_{-2}k_{+4} + k_{-1}k_{+3}k_{+4})[A] \\ & + (k_{+2}k_{+3}k_{+4})[B] + (k_{-1}k_{-2}k_{-3})[P] \\ & + (k_{-1}k_{-2}k_{-4} + k_{-1}k_{+3}k_{-4})[Q] \\ & + (k_{+1}k_{+2}k_{+3} + k_{+1}k_{+2}k_{+4})[A][B] \\ & + (k_{+1}k_{-2}k_{-3})[A][P] + (k_{+2}k_{+3}k_{-4})[B][Q] \\ & + (k_{-2}k_{-3}k_{-4} + k_{-1}k_{-3}k_{-4})[P][Q] \\ & + (k_{+1}k_{+2}k_{-3})[A][B][P] \\ & + (k_{+2}k_{-3}k_{-4})[B][P][Q] \end{aligned} \tag{14}$$

It should be emphasized that the starting reference point can be chosen arbitrarily, and the results thus obtained are all the same. However, there will be fewer subgraphs to count if the starting point is selected properly. Generally speaking, we prefer to choose the starting reference point as far as possible from the specified point.

(d) In order to facilitate checking, and avoid missing any subgraph during calculations, we can provide a method to calculate the number of the involved subgraphs beforehand. The method is as follows. According to the transformed graph $\mathfrak{T}^\circ$, construct a matrix $A = [a_{ij}]$, wherein

$$a_{ij} = \begin{cases} 1, & \text{if there is a connecting line from } E_i \text{ to } E_j \text{ in } \mathfrak{T}^\circ \\ 0, & \text{if there is no connecting line from } E_i \text{ to } E_j \text{ in } \mathfrak{T}^\circ \end{cases} \tag{15}$$

Then for some specified point E_m, if we take E_q as the starting reference point, the number of subgraphs to be counted will be

$$n^{q \to m} = \text{per}\, A_{m,q} \tag{16}$$

where $A_{m,q}$ denotes the submatrix obtained by eliminating the mth row and the qth column from the matrix A and 'per' denotes taking the permanent value of a matrix. The so-called permanent value of a matrix is the sum of all terms obtained by expanding the determinant corresponding to the matrix, but discarding the sign $\text{sgn}\sigma$ that appears in each term of the determinant expansion. So, it is very easy to calculate the permanent value of a matrix defined by eq. 15, where the elements are either 1 or 0. For instance, for $\mathfrak{D}^0$ in fig. 2, according to eq. 15 we have

$$A = \begin{bmatrix} 1 & 1 & 0 & 1 \\ 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 \end{bmatrix} \tag{17}$$

I. we take $m = 1$ (i.e., we wish to calculate N_E) and $q = 3$ (i.e., take EAB as the starting reference point), then from eqs. '6 and 17 we immediately have

$$n^{3 \to 1} = \text{per}\begin{bmatrix} 1 & 1 & 0 \\ 0 & 1 & 1 \\ 1 & 0 & 1 \end{bmatrix} = \text{per}\begin{bmatrix} 1 & 1 \\ 0 & 1 \end{bmatrix} + \text{per}\begin{bmatrix} 0 & 1 \\ 1 & 1 \end{bmatrix} = 1 + 1 = 2$$

Likewise, we have

$$n^{4 \to 2} = \text{per}\begin{bmatrix} 1 & 1 & 0 \\ 0 & 1 & 1 \\ 1 & 0 & 1 \end{bmatrix} = 2$$

$$n^{1 \to 3} = n^{2 \to 4} = 2$$

These results mean that no subgraph was missed in the above calculations.

Furthermore, we can also predict the number of terms in N_m by the following method: From the matrix $A = [a_{ij}]$, reconstruct a matrix $B = [b_{ij}]$, where

$$b_{ij} = \begin{cases} \sum_{i \neq j} a_{ij}, & \text{if } i = j \\ -a_{ij}, & \text{if } i \neq j \end{cases} \tag{18}$$

then the number of terms in N_m can be expressed as

$$P_m = \det B_{m,m} \tag{19}$$

where $B_{m,m}$ denotes the submatrix obtained by removing the mth row and the mth column from B, and the symbol det means taking the determinant value for the matrix next to it. For example, from the matrix A of eq. 17, we have

$$B = \begin{bmatrix} 2 & -1 & 0 & -1 \\ -1 & 2 & -1 & 0 \\ 0 & -1 & 2 & -1 \\ -1 & 0 & -1 & 2 \end{bmatrix} \tag{20}$$

Then it follows that

$$P_1 = \begin{vmatrix} 2 & -1 & 0 \\ -1 & 2 & -1 \\ 0 & -1 & 2 \end{vmatrix} = 4, \qquad P_2 = \begin{vmatrix} 2 & 0 & -1 \\ 0 & 2 & -1 \\ -1 & -1 & 2 \end{vmatrix} = 4$$

$P_3 = P_4 = 4$ *

The above results indicate that the numbers of terms given in eqs. 6–9 are correct. The checking formulae, eqs. 16 and 19, are very helpful when the treated systems are more complex.

The proof of Rule 1 is not given here, since it will involve considerable mathematical knowledge and terminology. Interested readers can find the proof in ref. 3.

2.4. Rule 2. The calculation method for $\bar{N}$ and $\tilde{N}$

(a) Following the same steps a and b of Rule 1.

(b) Define cycles which satisfy the following condition as the product-releasing cycles: if a circuit is made along any of these cycles, the net number of the product P released must be greater than zero. Likewise, define the product-combining cycles as those along which the net number of P combined is greater than zero when a circuit is made. For instance, the product-releasing cycle and product combining cycle for the enzyme-catalysed system described in fig. 2 are illustrated in fig. 3a and b, respectively. Both these kinds of cycles could be regarded as the master cycles, which play a key role during calculations.

(c) Find all subgraphs in $\mathfrak{D}^0$, each of which possesses one product-releasing cycle (or product-combining cycle), and all the other cycles and

* In fact, it can be proved [1] that when the arcs in a graph form a 'two-way traffic' between any two connecting points, which means in the graph the arc from E_i to E_j and that from E_j to E_i always coexist as shown in figs. 1 and 2, we must have $P_1 = P_2 = \ldots = P_n$.

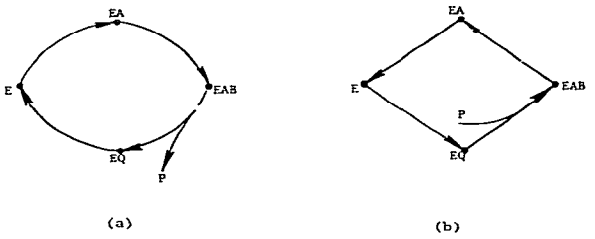


Fig. 3. The master cycles in the graph $\mathfrak{D}^0$ of fig. 2: (a) the product-releasing cycle, (b) the product-combining cycle.

loops that neither intersect with each other nor with the master cycle. Then for each of these subgraphs, take the product of all its weights and multiply with a sign factor given by

$$(-1)^{c'_y} g \tag{21}$$

where c'_y is the number of the cycles (not including the master cycle and the loops) in the respective subgraph, and g is the number of P released (or combined) when a circuit of the product-releasing cycle (or the product-combining cycle) is made. For instance, g is equal to 1 for fig. 3a (or fig. 3b). Taking a sum of these results, we immediately obtain $\vec{P}$ (or $\bar{P}$). For instance, for mechanism 3, following the above steps, we immediately obtain (see fig. 2):

$$\vec{P} = (-1)^0 k_{+1}[A]k_{+2}[B]k_{+3}k_{+4} \tag{22}$$

$$\bar{P} = (-1)^0 k_{-4}[Q]k_{-3}[P]k_{-2}k_{-1} \tag{23}$$

Substituting eqs. 22 and 23 as well as eqs. 6–9 into eq. 2, we obtain

$$v = \frac{d[P]}{dt} = [(k_{+1}k_{+2}k_{+3}k_{+4})[A][B] - (k_{-4}k_{-3}k_{-2}k_{-1})[P][Q]]e_0/D$$

where D has already been given in eq. 14.

The proof of Rule 2 can be obtained by combining the conclusions in refs. 4 and 7.

It is interesting to note the factor $(-1)^{c'_y} g$ as given in eq. 21. At first sight, there seems no point in introducing such a factor. But when we treat more complex mechanisms, e.g., those with branched pathways [5], its role will become quite significant. It is through g, the number of P released or the number of P combined, that the incorporation of the crossed terms is realized and wasted labor avoided automatically; while it is $(-1)^{c'_y}$, the sign for the weight product in each subgraph, that makes it possible to give a rational formulation by which the number of the counted subgraphs can be greatly reduced.

Especially, after some practice, there is no need to depict the decomposed subgraphs one by one in applying Rule 1 and Rule 2 as illustrated above; the desired results can be directly written down according to the graph $\mathfrak{D}^0$ even for very complicated mechanisms as shown in refs. 5 and 6.

The graphic method for nonsteady-state enzyme kinetics has also been developed recently; interested readers may refer to ref. 8.

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