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# A SIMPLE METHOD FOR THE DERIVATION OF THE STEADY-STATE RATE EQUATION FOR AN ENZYME MECHANISM

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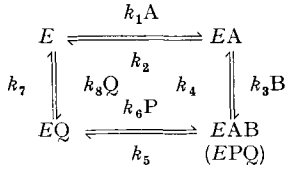
SUMMARY

A general method, based on the King–Altman scheme, for the derivation of the complete steady-state rate equation for an enzyme-catalysed reaction is illustrated. This method is simple and appears to be less liable to errors than other methods. Its use in the derivation of isotope exchange equations is also illustrated.

The derivation of the steady-state rate equation for an enzyme-catalysed reaction is based on the solution of a set of linear non-homogeneous simultaneous equations. The normal process for such a solution involves the use of Cramer's rule with determinants[1-3]. However, this procedure becomes very laborious if the number of equations is greater than three. In order to get over this difficulty KING AND ALTMAN[4] developed a schematic method for obtaining the equations and their method has been used by most practising kineticists ever since[5,6]. The basis of the method is to write down in diagrammatic form all possible combinations of reaction steps that lead to the formation of a particular enzyme-containing species, certain of these combinations being eliminated if they form loops or cycles. This is repeated for each enzyme-containing species in turn. In complex mechanisms the process of obtaining all permissible combinations can be very tedious and liable to errors of omission. VOLKENSTEIN AND GOLDSTEIN[7] have applied graph theory from electrical networks to this problem in an attempt to simplify the King–Altman method. HURST[8,9] proposed a coding system for the solution of the determinants in an attempt to simplify the calculations for a complex enzyme system[8] and wrote a Fortran IV program to facilitate the solution of the problem[9]. A somewhat more complex but systematic approach has been devised by FISHER AND HOAGLAND[10].

The method described here is based on a suggestion made by WONG AND HANES[11]. It is simple although, as with all other methods, it gets laborious with a large number of intermediates. This method, as with the King–Altman scheme, can be used without a complete understanding of how it works.

The method is best described by illustrating its use with an example. If an Ordered BiBi mechanism[6] is selected then the first step is to write the mechanism as a cycle in terms of enzyme-containing intermediates including free enzyme:




Associated with each forward and reverse step is a rate constant $k_1$, $k_2$ . . . . . and possibly a concentration term [A], [B] . . . . . .

For each enzyme-containing species write the sum of the rate constants (and any associated concentration terms) of all the steps that lead away from that enzyme species.

For $E$ : $(k_1[\mathrm{A}] + k_8[\mathrm{Q}])$
$EA$ : $(k_2 + k_3[\mathrm{B}])$
$EAB$: $(k_4 + k_5)$
$EQ$ : $(k_6[\mathrm{P}] + k_7)$

These sums can be called Reaction Terms and it is of interest that they are found on the main diagonal of the numerator determinant of KING AND ALTMAN[4].

Using these Reaction Terms it is now possible to calculate the distribution equation for each enzyme containing species. These distribution equations show how the total amount of enzyme ($[E_0]$) is distributed amongst the different forms. The equations are represented by $[E]/[E_0]$, $[EA]/[E_0]$, . . . . . where $[E]$, $[EA]$, . . . . . are the steady-state concentrations of the enzyme forms.

In order to simplify the derivation the distribution equations will be written as:

$$[E]/[E_0] = \frac{(E)}{D}\ ;\ [EA]/[E_0] = \frac{(EA)}{D}\ ;\ \ldots\ldots\ldots\ldots$$

where $D = (E) + (EA) + (EAB) + (EQ)$

The terms $(E)$, $(EA)$ . . . . . can be called Distribution Terms and once they have been obtained the problem is essentially solved.

To obtain the Distribution Term for a particular enzyme form the product of all the Reaction Terms, but excluding the Reaction Term belonging to the enzyme form under consideration, must be evaluated.

$$\begin{aligned}(E) \quad &= (k_2 + k_3[\mathrm{B}])\,(k_4 + k_5)\,(k_6[\mathrm{P}] + k_7) = k_2k_4k_6[\mathrm{P}] \\ &+ k_2k_4k_7 + \underline{k_2k_5k_6[\mathrm{P}]} + k_2k_5k_7 + \underline{k_3k_4k_6[\mathrm{B}]\,[\mathrm{P}]} \\ &+ \underline{k_3k_4k_7[\mathrm{B}]} + \underline{k_3k_5k_6[\mathrm{B}]\,[\mathrm{P}]} + k_3k_5k_7[\mathrm{B}]\end{aligned}$$

$$\begin{aligned}(EA) \quad &= (k_1[\mathrm{A}] + k_8[\mathrm{Q}])\,(k_4 + k_5)\,(k_6[\mathrm{P}] + k_7) = k_1k_4k_6[\mathrm{A}]\,[\mathrm{P}] \\ &+ k_1k_4k_7[\mathrm{A}] + \underline{k_1k_5k_6[\mathrm{A}]\,[\mathrm{P}]} + k_1k_5k_7[\mathrm{A}] + k_4k_6k_8[\mathrm{P}]\,[\mathrm{Q}] \\ &+ \underline{k_4k_7k_8[\mathrm{Q}]} + \underline{k_5k_6k_8[\mathrm{P}]\,[\mathrm{Q}]} + \underline{k_5k_7k_8[\mathrm{Q}]}\end{aligned}$$

$$\begin{aligned}(EAB) &= (k_1[\mathrm{A}] + k_8[\mathrm{Q}])\,(k_2 + k_3[\mathrm{B}])\,(k_6[\mathrm{P}] + k_7) \\ &= \underline{k_1k_2k_6[\mathrm{A}]\,[\mathrm{P}]} + \underline{k_1k_2k_7[\mathrm{A}]} + k_1k_3k_6[\mathrm{A}]\,[\mathrm{B}]\,[\mathrm{P}]\end{aligned}$$

$$+ k_1k_3k_7[A]\,[B] + k_2k_6k_8[P]\,[Q] + \underline{k_2k_7k_8[Q]}$$

$$+ k_3k_6k_8[B]\,[P]\,[Q] + \underline{k_3k_7k_8[B]\,[Q]}$$

$$(EQ) = (k_1[A] + k_8[Q])\,(k_2 + k_3[B])\,(k_4 + k_5)$$

$$= \underline{k_1k_2k_4[A]} + \underline{k_1k_2k_5[A]} + \underline{k_1k_3k_4[A]\,[B]}$$

$$+ k_1k_3k_5[A]\,[B] + k_2k_4k_8[Q] + k_2k_5k_8[Q]$$

$$+ \underline{k_3k_4k_8[B]\,[Q]} + k_3k_5k_8[B]\,[Q]$$

Some of these terms are redundant (underlined) because they contain the forward and reverse constants of the same reaction (one-step cycles). Terms that contain rate constants that give multistep cycles (not possible with this mechanism but common in more complex mechanisms) must also be discarded. Each of these Distribution Terms consists of the same number (four in this mechanism) of rate constant terms. Each of the individual rate constant terms consist of $n-1$ rate constants and associated concentration terms where n is equal to the number of enzyme containing species. It is now possible to evaluate the steady-state concentration of each enzyme form.

$$D = (E) + (EA) + (EAB) + (EQ) = k_2k_7(k_4 + k_5)$$

$$+ k_1k_7(k_4 + k_5)\,[A] + k_3k_5k_7[B] + k_1k_3(k_5 + k_7)\,[A]\,[B]$$

$$+ k_1k_4k_6[A]\,[P] + k_3k_5k_8[B]\,[Q] + k_2k_8(k_4 + k_5)\,[Q]$$

$$+ k_2k_4k_6[P] + k_6k_8(k_2 + k_4)\,[P]\,[Q] + k_1k_3k_6[A]\,[B]\,[P]$$

$$+ k_3k_6k_8[B]\,[P]\,[Q]$$

$$\text{and } [E] = \frac{(E)\,[E_0]}{D} = \frac{(k_2k_7(k_4 + k_5) + k_3k_5k_7[B] + k_2k_4k_6[P])\,[E_0]}{D}$$

In a similar manner the steady-state values for $[EA]$, $[EAB]$ and $[EQ]$ may be obtained. The rate of reaction in the forward direction is then given by:

$$v = \frac{dQ}{dt} = [EQ]k_7 - [E]\,[Q]k_8$$

Substituting the values for $[EQ]$ and $[E]$ and simplifying yields the final steady-state rate equation in rate constant form.

$$v = \frac{(k_1k_3k_5k_7[A]\,[B] - k_2k_4k_6k_8[P]\,[Q])\,[E_0]}{D}$$

Before use this equation should be converted into the kinetic constant form involving only maximum velocities, Michaelis constants, inhibition constants, and the equilibrium constant[6].

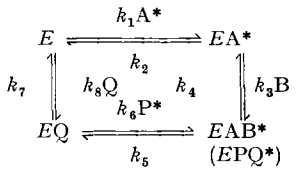
This method appears to be quite general and has been used successfully for a number of years with a variety of different mechanisms. Equations for mechanisms, wholly or partially in equilibrium are, however, more easily evaluated by the method of CHA[12]. Fully random mechanisms may be evaluated by the method described but with the further complication that additional terms will be reduntant. These terms

are ones that include single isolated rate constants or isolated groups of rate constants that do not lead to the particular enzyme form under consideration[4].

In the last few years it has become increasingly obvious that measurement of the rates of isotope exchange is a powerful and sensitive tool in the elucidation of an enzyme mechanism[13–15]. In 1967 CLELAND[16] developed a variation of the King–Altman schematic method in order to evaluate the isotope exchange equations for systems not necessarily at chemical equilibrium.

Evaluation of isotope exchange equations can also be done by the method described in this paper and again it can be illustrated by considering as an example the Ordered BiBi mechanism. There are four possible isotope exchanges for this mechanism, A↔P, A↔Q, B↔P, B↔Q, although not all these could take place in a given reaction.

Consider only the A*→P exchange. Write the mechanism as a cycle and mark in some way (with an asterisk) all compounds, including enzyme forms, that are labelled or become labelled during the reaction:




The initial rate of isotope exchange from A into P is given by:

$$v^* = \frac{dP^*}{dt} = k_5[EAB^*]$$

The problem is to find $[EAB^*]$ which is not necessarily identical to $[EAB]$. For convenience the following relationship will be used:

$$[EAB^*] = \frac{(EAB^*)\,[E]}{D^*}$$

$[E]$ is the steady-state concentration of free enzyme and has already been obtained. This and no other is used because it is with this enzyme form and no other that the initially labelled A* combines. If the B*→P exchange is being considered then:

$$[EAB^*] = \frac{(EAB^*)\,[EA]}{D^*}$$

$D^*$, the denominator of the exchange equation is obtained by again considering the Reaction Terms. However, only those Reaction Terms are considered that belong to the enzyme forms that become labelled. In the example only $EA$ and $EAB$ become labelled.

$$D^* = (k_2 + k_3[B])\,(k_4 + k_5) = k_2k_4 + k_2k_5 + k_3k_4[B] + k_3k_5[B]$$

Any cycles, either one step or multistep, are again eliminated.

$$D^* = k_2(k_4 + k_5) + k_3k_5[B]$$

For the B*→P exchange $D^* = k_4 + k_5$. Each term in this exchange denominator will be composed of m rate constants and associated concentration terms where m is the number of enzyme forms that become labelled.

The exchange Distribution Term, $(EAB^*)$, is very simply obtained as the product of rate constants and associated concentration terms that lead from the unlabelled enzyme form with which A* combines to the labelled form that releases P*.

$$(EAB^*) = k_1k_3[A^*]\,[B]$$

For the B*→P exchange $(EAB^*) = k_3[B^*]$.

It may be noticed that this term is the reverse of one of the terms found in $D^*$. The exchange equation may now be written:

$$v^* = k_5[EAB^*] = \frac{k_5(EAB^*)}{D^*}\,[E] = \frac{k_1k_3k_5[A^*]\,[B]\,[E]}{k_2(k_4 + k_5) + k_3k_5[B]}$$

For the B*→P exchange

$$v^* = \frac{k_3k_5[B^*]\,[EA]}{k_4 + k_5}$$

As with the overall rate equation this should be converted into kinetic constant form before use[6,16].

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