A SIMPLIFIED SCHEMATIC METHOD FOR DERIVING STEADY-STATE RATE EQUATIONS USING A MODIFICATION OF THE "THEORY OF GRAPHS" PROCEDURE*

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Summary

A simple schematic procedure for the derivation of steady-state enzyme rate equations is presented. The method is a modification of an approach described by Volkenstein and Goldstein (1966) based upon the "Theory of Graphs" procedure. The advantage of this method for rate equation derivations is that it requires little algebra and avoids problems of the type encountered in other schematic methods.

A number of attempts have been made in recent years to simplify the derivation of steady-state rate equations for enzyme mechanisms (Volkenstein and Goldstein, 1966; King and Altman, 1956; Hurst, 1967). The schematic method of King and Altman (1956) has been in use since 1956 and has proven to be a valuable tool to enzymologists interested in the solution of steady-state kinetic problems. As has been pointed out by others, this procedure becomes extremely tedious and complicated as the number of enzyme-substrate forms in a mechanism increases (Volkenstein and Goldstein, 1966; Melchior, 1965; Cleland, 1967). Volkenstein and Goldstein (1966) have employed an elegant schematic method based upon suggestions of Mason and Zimmerman (1960) for

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deriving steady-state rate equations which appears to be less complicated than the method of King and Altman (1956).

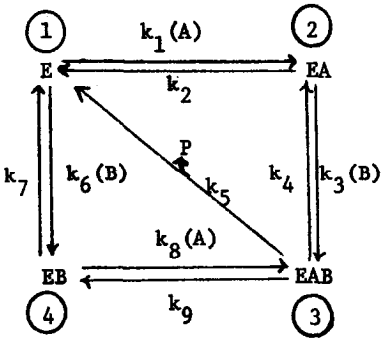
Our attempt to use the Volkenstein-Goldstein (1966) approach proved to be quite satisfactory when dealing with the mechanisms illustrated by these authors; however, certain complications arose when attempts were made to derive rate equations such as the two substrate system ordered sequential mechanism with two ternary complex intermediates.

In this report a modification of the "Theory of the Graphs" method is presented which in this author's opinion is the simplest schematic approach to the derivation of steady-state rate equations yet encountered. Like other schematic methods, its great advantage is that it requires only a rudimentary understanding of algebra.

Procedure

The following steps are used in the derivation of the steady-state rate equations. The Bi Bi random mechanism (King, 1956) is presented for the purpose of illustration.

1. The kinetic mechanism is set up in geometric form as suggested by King and Altman (1956), and the enzyme terms numbered as shown in Scheme 1.



Scheme 1

2. (a) Each enzyme form in the diagram is referred to as a node.

(b) To obtain the determinant for node 1, the shortest one-step paths to node 1 from other nodes are chosen. In Scheme 1 there are three routes leading to node 1 in a single step: 2 → 1, 3 → 1, and 4 → 1.

(c) Each step is characterized by a particular rate constant, i.e., $2 \rightarrow 1$, k_2; $3 \rightarrow 1$, k_5; $4 \rightarrow 1$, k_7.

3. (a) Next to each branch are written the products of the nodes which do not appear in the particular one-step process, i.e., $2 \rightarrow 1$ (3)(4), $3 \rightarrow 1$ (2)(4), and $4 \rightarrow 1$ (2)(3).

(b) Each number within the parentheses in 3(a) is the summation of two or more rate constants which lead away from the node. In the case of node 3 (EAB) this summation is $(k_4 + k_5 + k_9)$. For nodes 1, 2, and 4 the terms are, $(k_1(A) + k_6(B))$, $(k_2 + k_3(B))$, and $(k_7 + k_8(A))$, respectively[1].

4. The determinant for node 1 is:

$$E = 2 \rightarrow 1\ (3)(4) + 3 \rightarrow 1\ (2)(4) + 4 \rightarrow 1\ (2)(3), \text{ or}$$

$$E = k_2(k_4+k_5+k_9)(k_7+k_8(A)) + k_5(k_2+k_3(B))(k_7+k_8(A)) + k_7(k_2+k_3(B))(k_4+k_5+k_9)$$

5. Certain terms are eliminated from the determinant upon expansion by inspection.

(a) In the case of redundant terms, only one such term is included in the determinant.

(b) All forbidden terms are eliminated from the determinant. These are combinations of rate constants which contain products of the constants for a reversible step, i.e., for $EB \underset{k_8(A)}{\overset{k_9}{\rightleftharpoons}} EAB$, terms containing the product $k_8k_9(A)$, such as $k_2k_8k_9(A)$ are omitted from the determinant.

6. Expansion of E gives:

(a) $E = k_2k_4k_7 + k_2k_4k_8(A) + k_2k_5k_7 + k_2k_5k_8(A) + k_2k_7k_9 + \cancel{k_2k_8k_9(A)} +$
$\cancel{k_2k_5k_7} + \cancel{k_2k_5k_8(A)} + k_3k_5k_7(B) + k_3k_5k_8(A)(B) + \cancel{k_2k_7k_7} +$
$\cancel{k_2k_5k_7} + \cancel{k_2k_7k_9} + \cancel{k_3k_4k_7(B)} + \cancel{k_3k_5k_7(B)} + k_3k_7k_9(B).$

[1] Parallel path mechanisms are treated as suggested elsewhere (Volkenstein and Goldstein, 1966). Thus in Scheme 1, if two paths characterized by rate constants k_2 and k_x are available for going from (EA) to (E), then $2 \rightarrow 1$ would be $(k_2 + k_x)$ and (2) would be $(k_2 + k_x + k_3(B))$.

(b) The eliminated terms are either redundant or forbidden.

(c) $E = k_2k_4k_7 + k_2k_4k_8(A) + k_2k_5k_7 + k_2k_5k_8(A) + k_2k_7k_9 + k_3k_5k_7(B) + k_3k_5k_8(A)(B) + k_3k_7k_9(B)$.

7. The rate equation for this mechanism as shown by King and Altman (1956) is,

$$v = \frac{E_o[k_5(EAB)]}{E + EA + EB + EAB} \qquad (1)$$

where v, E_o, E, EA, EB, and EAB represent velocity, total enzyme, determinant for E, determinant for EA, determinant for EB, and determinant for EAB, respectively.

8. Determinants for the other enzyme forms are as follows:

$EA = k_1k_4k_7(A) + k_1k_4k_8(A)^2 + k_1k_5k_7(A) + k_1k_5k_8(A)^2 + k_1k_7k_9(A) + k_4k_6k_8(A)(B)$

$EAB = k_1k_3k_7(A)(B) + k_1k_3k_8(A)^2(B) + k_3k_6k_8(A)(B)^2 + k_2k_6k_8(A)(B)$

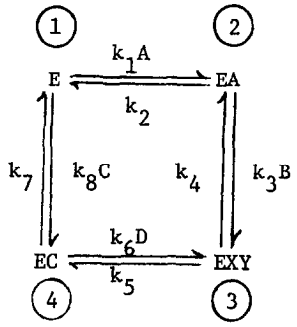
$EB = k_2k_4k_6(B) + k_2k_5k_6(B) + k_2k_6k_9(B) + k_3k_5k_6(B)^2 + k_3k_6k_9(B)^2 + k_1k_3k_9(A)(B)$

9. The values for the different enzyme forms are finally substituted into Equation (1) to yield the final rate equation.

Discussion

When the "Theory of Graphs" procedure is modified as indicated above, it appears to be simpler than either the King and Altman (1956) or the Volkenstein and Goldstein (1966) method. The reason for this involves the fact that the new procedure is basically algebraic. If one considers the usual sequential ordered mechanism shown in Scheme 2 and the analogous case with two ternary complexes, the limitations of the original "Theory of Graphs" method can be appreciated.

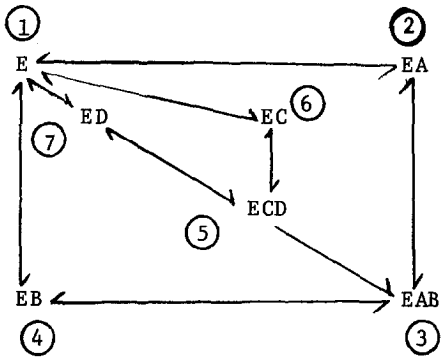
The determinant for E using the Volkenstein and Goldstein (1966) approach is $E = 3\rightarrow4\rightarrow1(2) + 3\rightarrow2\rightarrow1(4)$ or $E = k_2k_4k_7 + k_2k_5k_7 + k_3k_5k_7(B) + k_2k_4k_6(D)$. In order to obtain the correct determinant for the case involving two ternary



Scheme 2

complexes, the outlined procedure proves inadequate and "node compression", a relatively complicated manipulation, is required.

The advantageous nature and simplicity of the present procedure is best illustrated when considering highly complex mechanisms such as the Bi Bi random pathway illustrated in Scheme 3.



Scheme 3

Using the rules already outlined, the determinant for E is as follows:

$E = 2 \rightarrow 1\ (3)(4)(5)(6)(7) + 4 \rightarrow 1\ (2)(3)(5)(6)(7) + 6 \rightarrow 1\ (2)(3)(4)(5)(7)$

$+ 7 \rightarrow 1\ (2)(3)(4)(5)(6)$

For highly complex mechanisms of the type shown in Scheme 3, "closed loops' (King and Altman, 1956) will be generated, and they are eliminated from the determinant by inspection.

The modification of the "Theory of Graphs" procedure of Volkenstein and Goldstein (1966) as presented in this report has been tested on a large number of kinetic mechanisms involving one, two, and three substrates. In all cases, the resulting rate equations agree with expressions either in the literature or with equations derived by other means by this author.

References

Cleland, W. W., Ann. Rev. Biochem., 36, 77 (1967).
Hurst, R. O., Can. J. Biochem., 45, 2015 (1967).
King, E. L., J. Phys. Chem., 60, 1378 (1956).
King, E. L., and Altman, C., J. Phys. Chem., 60, 1375 (1956).
Mason, S., and Zimmerman, G., Electronic Circuits, Signals and Systems, John Wiley and Sons, Inc., New York, 1960.
Melchior, J. B., Biochemistry, 4, 1518 (1965).
Volkenstein, M. V., and Goldstein, B. N., Biochim. Biophys. Acta, 115, 471 (1966)