ENZYME KINETICS

SYSTEMATIC GENERATION OF VALID KING-ALTMAN PATTERNS

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ABSTRACT One of the most generally applicable algorithms for the derivation of steady-state rate equations for complex enzyme reaction mechanisms is that of King and Altman. Several modifications of this algorithm have been suggested; however, each requires the generation of numerous valid and invalid patterns and the subsequent elimination of those that are invalid. A method is presented, employing topological theory of linear graphs, for the systematic generation of only those patterns which are valid. This method is readily adaptable to use on a digital computer. An independent method for the calculation of the number of valid patterns is also presented. This calculation can be used to substantiate the accuracy of the patterns obtained. This calculation is also adaptable to computerization. Examples are included to demonstrate both the generation of patterns and the calculation of their number for specific enzyme mechanisms.

INTRODUCTION

In order to use effectively steady-state kinetic techniques to study enzyme mechanisms, it is necessary to have available a method for testing the agreement between the initial velocities predicted by a proposed reaction mechanism and those obtained experimentally. More precisely, the steady-state kinetic approach can only be used to eliminate those mechanisms that are inconsistent with experimentally measured parameters. It is thus necessary that a sufficiently general set of reaction mechanisms be considered if maximum benefit is to be gained from this technique. The major deterrent to the consideration of general two- and three-substrate reaction mechanisms has been the forbiddingly tedious nature of the derivation of rate equations therefrom. This difficulty, however, in no way invalidates the possibility that such mechanisms do exist.

King and Altman (1) have made a major contribution to the simplification of the derivation of rate equations. Their schematic method has greatly simplified the solution of the set of simultaneous equations describing steady-state concentrations of enzyme intermediates. To apply their method it is necessary to draw a series of

patterns, each containing one less intermediate than is present for the entire mechanism. For random mechanisms closed loops are obtained which are invalid and must be deleted. This procedure becomes more difficult as mechanisms become more complex. They have further provided a formula for the calculation of the total number of patterns that can be generated for any mechanism. This calculation, however, includes patterns containing closed loops which must subsequently be discarded. These inherent difficulties in the application of the King-Altman approach have stimulated the development of other methods for the derivation of steady-state rate equations for enzymic mechanisms.

Fisher and Hoagland (2) have applied a fractional velocity concept to the ordered sequence approach of Hearon et al. (3) and obtained a systematic method for the derivation of rate equations for many two-substrate reaction mechanisms. Volkenstein and Goldstein (4) have used an approach involving signal flow graphs of electrical networks by applying Mason's rule. These methods, however, are difficult to program for a digital computer and computerization is highly desirable in dealing with the complex system of algebraic equations inherent in steady-state rate equations.

Fromm (5) has applied a modification of the theory of signal graphs to the King-Altman schematic method in an attempt to make these derivations more systematic. This method nevertheless requires the examination of patterns to eliminate those that are invalid because they contain closed loops or redundant terms. Fisher and Schulz (6) have computerized the method of King and Altman through the incorporation of a connection matrix; however, their method generates all patterns, including those containing loops, which must be eliminated by a time-consuming testing procedure. In addition, the only method available for testing the accuracy of their computer-derived equations is hand calculation, since it has not been proven that their method will generate all of the necessary valid patterns. A method is reported here which will systematically generate only the necessary valid patterns, and an independent means is provided for calculating the number of these valid patterns.

RESULTS AND DISCUSSION

Systematic Generation of King-Altman Patterns

Several investigators (4, 5) have pointed out the applicability of the theory of graphs to the solution of enzyme kinetic problems. Such approaches have taken into account the similarity between electrical networks and complex enzymatic mechanisms. The various postulated enzyme species are treated as nodes and the reactions incorporating substrates or products giving rise to these enzyme species are treated as branches. The intent is invariably to obtain an algorithm which will allow a rapid calculation of the steady-state concentration of enzyme species present and thereby obtain an expression for the rate of product accumulation.

Maxwell and Cline (7) have provided proof of a method for the generation of

trees from linear graphs which is adaptable to the treatment of complex enzymatic reaction mechanisms. The technique they have used incorporates the principles of Wang algebra (8), whereby the sum or product of two or more identical constants is equal to zero, or

$$\sum_{i=1}^{n} c = 0, \text{ for } n > 1, \tag{1}$$

$$\prod_{i=1}^{n} c = 0, \text{ for } n > 1. \tag{2}$$

Application of this method to the systematic generation of King-Altman type patterns can be obtained through the use of the following stepwise procedure. (*a*) Describe the mechanism as a set of branches (reactions) that connect nodes (enzyme forms). (*b*) Inscribe circles about any $n - 1$ nodes, where n is the total number of nodes. (*c*) List separately the branches cut by the $n - 1$ circles. (*d*) Using the principles of Wang algebra stated above, multiply "alphanumerically" the listing obtained in step (*c*) above.[1]

Application of the procedure outlined above is facilitated by consideration of a specific enzyme mechanism. The random substrate addition, ordered product release mechanism shown in Fig. 1 has been selected for this purpose because it has been considered by others (2, 9), thus allowing direct comparison. Fig. 1 A shows all of the enzyme species, substrates, products, and rate constants necessary to describe the mechanism. Fig. 1 B is a graphic representation of this mechanism in which the numbered nodes represent enzyme forms and the numbered branches individual reactions. Dashed circles have been inscribed around $n - 1$ nodes. The result is independent of the node which is ignored. Branches 1, 2, and 6 are cut by the circle around node 1. Branches 1 and 4 are cut at node 3 and so on. The listings for each of the four nodes are shown below, with the product of their alphanumeric multiplication employing the Wang algebra principles.

$$(1,2,6)\ (1,4)\ (3,4,5)\ (5,6) \Rightarrow \begin{matrix} 1345 & 1346 & 1235 \\ 1236 & 2345 & 2346 \\ 1245 & 1246 & 1256 \\ 2456 & 1356 & 3456 \end{matrix}$$

It should be noted that no invalid or redundant terms have been generated. This

[1] The alphanumeric multiplication of integers (or any other symbols) is here defined to be a listing rather than a numerical value, e.g. the alphanumeric multiplication of 2 and 4 is equal to 24 rather than 8.

It is important to note that application of the Wang algebra principles should be made at all steps in the multiplication process. For example, the alphanumeric multiplication of (C_1, C_2) (C_1, C_2, C_3) yields $C_1 C_1 + C_1 C_2 + C_1 C_3 + C_2 C_1 + C_2 C_2 + C_2 C_3$. The terms $C_1 C_1$ and $C_2 C_2$ are discarded on the basis of the product principle of Wang algebra (equation 2) and the terms $C_1 C_2 + C_2 C_1$ are discarded on the addition principle (equation 1). In practice the above operations can be performed simultaneously.

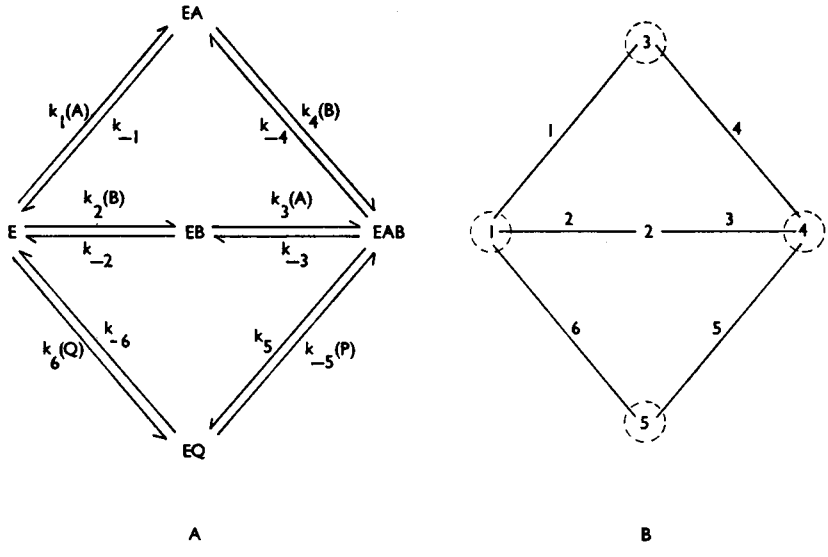

FIGURE 1 A, random substrate addition-ordered product release enzyme mechanism. B, linear graph representation of mechanism A.

procedure does not require reference to any pattern after the original listing obtained in step *c* above. The results are identical with those of other workers.

Calculation of Number of Valid Patterns

An independent method for the calculation of the correct *number* of valid patterns substantiates their accuracy. The method described here again follows the theory of linear graphs. Matrix A, which we will call a cut matrix, is described by using the $n - 1$ nodes of a mechanism as the row numbers and the directed branches as column numbers. The determinant of the product of A and A transpose yields the correct number of valid patterns for any mechanism (8).

$$\text{Number of valid patterns} = | A A' | \tag{3}$$

where

$$A = \begin{bmatrix} a_{11} & a_{12} & a_{13} & \cdots & a_{1n} \\ a_{21} & \cdot & \cdot & \cdot & \\ a_{31} & \cdot & \cdot & \cdot & \\ \vdots & & & & \\ a_{m1} & \cdot & \cdot & \cdot & a_{mn} \end{bmatrix},$$

$$A' = \begin{bmatrix} a_{11} & a_{21} & a_{31} & \cdots & a_{m1} \\ a_{12} & \cdot & \cdot & \cdot & \\ a_{13} & \cdot & \cdot & \cdot & \\ \vdots & & & & \\ a_{1n} & \cdot & \cdot & \cdot & a_{mn} \end{bmatrix},$$

a_{ij} = 1 if branch j enters node i,

= -1 if branch j leaves node i,

= 0 otherwise,

and

m = total number of nodes,

n = total number of branches.

The use of this formula may be demonstrated by reference to Fig. 2. The mechanism is the same as that shown in Fig. 1 except direction has been assigned to the branches in this graphic representation. Any other assignment of direction could have been selected for any or all of the branches; however, a direction is necessary to set up the desired matrix. Circles are inscribed around any $n - 1$ nodes cutting the branches shown. The cut matrix for the graph in Fig. 2 is shown below.

$$A = \begin{array}{c} \\ \text{Node number} \\ 1 \\ 2 \\ 3 \\ 4 \end{array} \begin{array}{c} \text{Branch number} \\ \begin{bmatrix} 1 & 2 & 3 & 4 & 5 & 6 \\ -1 & -1 & 0 & 0 & 0 & +1 \\ 0 & +1 & -1 & 0 & 0 & 0 \\ +1 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & +1 & +1 & -1 & 0 \end{bmatrix} \end{array}.$$

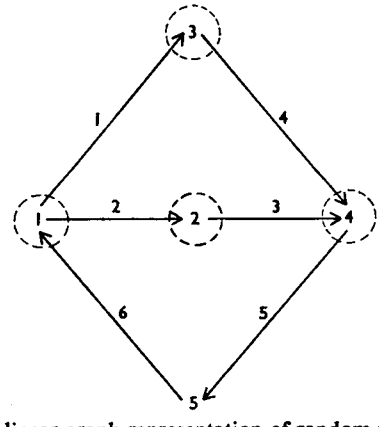

FIGURE 2 Directed linear graph representation of random mechanism of Fig. 1.

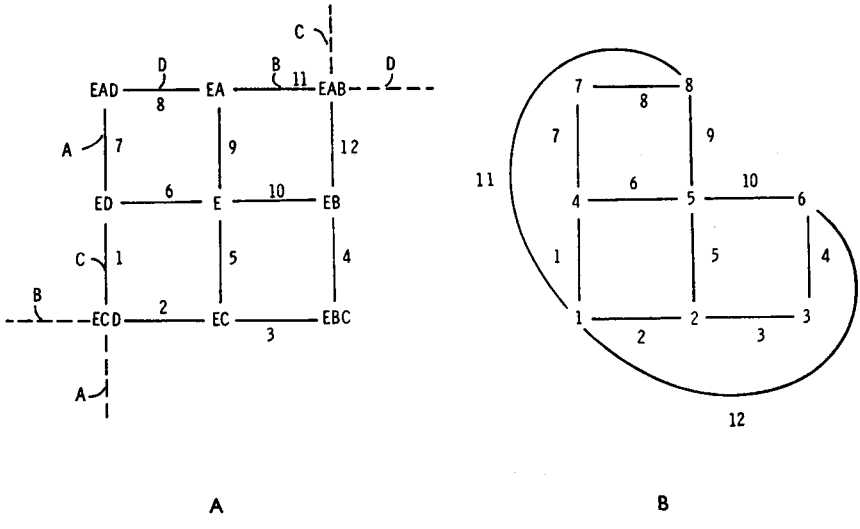

FIGURE 3 A, general two-substrate–two-product enzyme mechanism. B, graphic representation of A.

The determinant of the product of this matrix times its transpose, $| A A' | = 12$, may be solved either by hand or by computer. As more complex mechanisms are considered, computer methods become more desirable. A program[2] has been written to form the cut matrix and calculate the number of valid patterns for any mechanism using equation 3.

Treatment of a General Two-Substrate–Two-Product Mechanism

Wong and Hanes (9) among others have pointed out the desirability of considering general mechanisms and reducing them systematically for particular systems. Several general two-substrate–two-product mechanisms have been proposed. One of those suggested by Fisher et al. (J. R. Fisher, D. G. Priest, and J. S. Barton, unpublished results) lends itself well to the demonstration of the versatility of the method presented in this report. Their first-level, two-substrate–two-product mechanism has been reproduced in Fig. 3 A and the graphic representation in Fig. 3 B. All of the valid King-Altman patterns for this general mechanism have been obtained using the methods described in the previous sections by means of a digital computer.[2] The 288 valid patterns have been identified in the Appendix. Equation 3 independently predicts an identical number of patterns, thus enhancing the validity of those shown. It is of interest that equation 1 of King and Altman (1) gives rise to 792 total patterns. Thus a large number of patterns, 504, would have to be examined and eliminated by previous methods.

[2] The computer programs used in this paper to obtain patterns and number of patterns will be made available by the authors upon request.

APPENDIX

King-Altman patterns for the general two-substrate–two-product mechanism shown in Fig. 3.

(145)	2	3	4	7	8	10	11
(148)	2	3	4	7	8	9	11
(151)	2	3	4	6	7	8	11
(154)	2	3	4	5	7	8	11
(157)	2	3	4	6	8	10	11
(160)	2	3	6	7	9	11	12
(163)	2	3	4	6	8	9	11
(166)	2	3	5	6	7	11	12
(169)	2	3	4	5	6	8	11
(172)	1	2	3	7	10	11	12
(175)	1	2	3	8	9	11	12
(178)	1	2	3	7	9	10	11
(181)	1	2	3	6	8	11	12
(184)	1	2	3	6	7	10	11
(187)	1	2	3	5	8	11	12
(190)	1	2	3	5	7	10	11
(193)	2	4	5	7	8	9	12
(196)	2	4	5	6	7	8	10
(199)	2	4	5	6	8	9	10
(202)	1	2	4	5	7	9	12
(205)	2	3	5	7	8	9	12
(208)	2	3	5	6	7	8	12
(211)	2	3	5	6	8	9	12
(214)	2	3	5	6	7	9	10
(217)	1	2	3	5	8	9	12
(220)	1	2	3	5	7	9	10
(223)	2	3	4	7	8	9	10
(226)	2	3	4	6	7	9	10
(229)	1	4	5	7	8	10	12
(232)	1	4	5	6	7	8	12
(235)	1	4	5	6	7	9	12
(238)	1	3	5	7	8	10	12
(241)	1	3	4	5	7	8	9
(244)	1	3	4	5	6	7	8
(247)	1	3	5	6	8	9	10
(250)	1	3	4	5	6	7	9
(253)	1	3	4	7	8	9	10
(256)	1	3	4	5	7	8	12
(259)	1	3	4	6	8	9	10
(262)	1	2	4	7	8	9	12
(265)	1	2	4	6	7	8	10
(268)	1	2	4	6	8	9	12
(271)	1	2	4	6	7	9	10
(274)	1	2	3	7	8	9	12
(277)	1	2	3	6	7	8	12
(280)	1	2	3	5	7	8	12
(283)	1	2	3	6	8	9	12
(286)	1	2	3	6	7	9	10

(146)	2	3	7	8	9	11	12
(149)	2	3	6	7	8	11	12
(152)	2	3	5	7	8	11	12
(155)	2	3	6	8	10	11	12
(158)	2	3	4	6	7	10	11
(161)	2	3	6	8	9	10	11
(164)	2	3	4	6	7	9	11
(167)	2	3	5	6	8	10	11
(170)	2	3	4	5	6	7	11
(173)	1	2	3	4	8	10	11
(176)	1	2	3	7	9	11	12
(179)	1	2	3	4	8	9	11
(182)	1	2	3	6	7	11	12
(185)	1	2	3	4	6	8	11
(188)	1	2	3	5	7	11	12
(191)	1	2	3	4	5	8	11
(194)	2	4	5	7	8	9	10
(197)	2	4	5	6	8	9	12
(200)	2	4	5	6	7	9	10
(203)	1	2	4	5	8	9	10
(206)	2	3	5	7	8	9	10
(209)	2	3	5	6	7	8	10
(212)	2	3	5	6	7	9	12
(215)	2	3	4	5	6	8	9
(218)	1	2	3	5	7	9	12
(221)	1	2	3	4	5	8	9
(224)	2	3	4	6	7	8	10
(227)	1	2	3	4	8	9	10
(230)	1	4	5	7	8	9	12
(233)	1	4	5	6	7	8	10
(236)	1	4	5	6	8	9	10
(239)	1	3	5	7	8	9	12
(242)	1	3	5	6	7	8	12
(245)	1	3	5	6	8	9	12
(248)	1	3	5	6	7	9	10
(251)	1	3	4	7	8	10	12
(254)	1	3	4	6	7	8	12
(257)	1	3	4	6	8	9	12
(260)	1	3	4	6	7	9	10
(263)	1	2	4	7	8	9	10
(266)	1	2	4	5	7	8	12
(269)	1	2	4	6	7	9	12
(272)	1	2	3	7	8	10	12
(275)	1	2	3	7	8	9	10
(278)	1	2	3	6	7	8	10
(281)	1	2	3	5	7	8	10
(284)	1	2	3	6	7	9	12
(287)	1	2	3	4	6	8	9

(147)	2	3	7	8	9	10	11
(150)	2	3	6	7	8	10	11
(153)	2	3	5	7	8	10	11
(156)	2	3	6	7	10	11	12
(159)	2	3	6	8	9	11	12
(162)	2	3	6	7	9	10	11
(165)	2	3	5	6	8	11	12
(168)	2	3	5	6	7	10	11
(171)	1	2	3	8	10	11	12
(174)	1	2	3	4	7	10	11
(177)	1	2	3	8	9	10	11
(180)	1	2	3	4	7	9	11
(183)	1	2	3	6	8	10	11
(186)	1	2	3	4	6	7	11
(189)	1	2	3	5	8	10	11
(192)	1	2	3	4	5	7	11
(195)	2	4	5	6	7	8	12
(198)	2	4	5	6	7	9	12
(201)	1	2	4	5	8	9	12
(204)	1	2	4	5	7	9	10
(207)	2	3	4	5	7	8	9
(210)	2	3	4	5	6	7	8
(213)	2	3	5	6	8	9	10
(216)	2	3	4	5	6	7	9
(219)	1	2	3	5	8	9	10
(222)	1	2	3	4	5	7	9
(225)	2	3	4	6	8	9	10
(228)	1	2	3	4	7	9	10
(231)	1	4	5	7	8	9	10
(234)	1	4	5	6	8	9	12
(237)	1	4	5	6	7	9	10
(240)	1	3	5	7	8	9	10
(243)	1	3	5	6	7	8	10
(246)	1	3	5	6	7	9	12
(249)	1	3	4	5	6	8	9
(252)	1	3	4	7	8	9	12
(255)	1	3	4	6	7	8	10
(258)	1	3	4	6	7	9	12
(261)	1	2	4	7	8	10	12
(264)	1	2	4	6	7	8	12
(267)	1	2	4	5	7	8	10
(270)	1	2	4	6	8	9	10
(273)	1	2	3	4	7	8	10
(276)	1	2	3	4	7	8	9
(279)	1	2	3	4	6	7	8
(282)	1	2	3	4	5	7	8
(285)	1	2	3	6	8	9	10
(288)	1	2	3	4	6	7	9

The complete rate equation can be obtained from these patterns using existing methods (6, 10). Note that direction must be assigned to the patterns for each of the eight individual enzyme species. A total of 2304 (8×288) terms is obtained for this mechanism.

The method described here may be applied not only to this general multi-substrate mechanism but also to new general mechanisms as they arise, modifier or allosteric mechanisms, and isotope exchange studies.

SUMMARY

A method has been described and demonstrated that facilitates the rapid identification of King-Altman patterns directly and systematically without the necessity of testing numerous diagrams for their validity. An equation has also been reported which will independently predict the required number of patterns necessary to obtain a valid rate equation for any mechanism that can be described by a linear graph. These methods have been used, by hand and on a digital computer, to determine both the number of patterns and the patterns yielded by a general two-substrate–two-product enzyme reaction mechanism.

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