THE POINT OF CONVERGENCE OF SEQUENTIAL DOUBLE RECIPROCAL PLOTS AS A CRITERION OF BIREACTANT ENZYME KINETIC MECHANISMS. EVALUATION OF THE YEAST HEXOKINASE REACTION

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1. Introduction

It has long been recognized by kineticists that information on the kinetic mechanism of enzyme action for bireactant systems can be obtained by inspection of initial rate data. For example, in 1952 Segal et al. [1] suggested that a choice could be made between certain steady-state and equilibrium mechanisms by evaluation of double reciprocal plots. Alberty [2]* was able to demonstrate in 1953 that ping–pong and sequential mechanisms give different Lineweaver–Burk [4] type graphs. Finally, Rudolph and Fromm [5] in 1969 were able to show how a choice of mechanism could be made from among a number of terreactant mechanisms from slope and intercept replots of primary double reciprocal graphs. In this report, it will be shown that the point of intersection of sequential double reciprocal plots may be used as a criterion to distinguish among bireactant enzyme kinetic mechanisms.

2. Theory and discussion

Frieden [6] reported in 1957 that sequential mechanisms that conform to the rate expression described by eq. 1 give families of straight lines which converge at a common point on, above, or below the abscissa.

$$\frac{E_0}{v} = \phi_0 + \frac{\phi_1}{A} + \frac{\phi_2}{B} + \frac{\phi_{12}}{(A)(B)} \qquad (1)$$

In fig. 1 is shown the type of plot in double reciprocal form to be expected for sequential mechanisms. The graph also indicates the coordinates of the intersection point of the extrapolated initial velocity lines. In the analogous E_0/v verus $1/B$ plot, the ordinate or E_0/v coordinate would be the same as indicated in fig. 1.

Some rather interesting relationships, which have a direct bearing on the kinetic mechanism of yeast hexokinase action, can be obtained by evaluation of

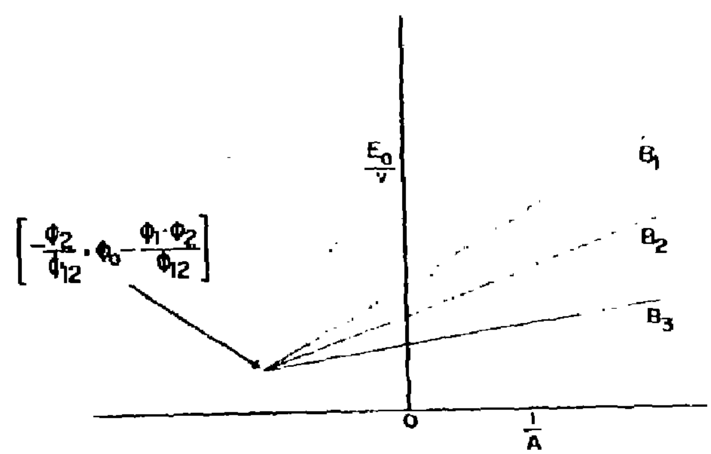

Fig. 1. Plot of E_0/v versus $1/A$ at different fixed levels of substrate B. The coordinates of the point of intersection of the family of straight lines indicated on the graph was obtained from eq. 1 of the text.

* The nomenclature is that of Cleland [3].

the E_0/v coordinate in both the forward and reverse reaction. The point of intersection of the converging lines is somewhat mechanism-dependent when considered in both directions. These relations and how they relate to hexokinase is illustrated as follows:

2.1. *Theorell–Chance mechanism* [7]

This kinetic pathway is illustrated in scheme 1:

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EQ + P$$

$$EQ \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E + Q$$

Scheme 1.

The ϕ† values for this mechanism are:

$$\phi_0 = \frac{1}{k_5} \qquad \phi_{0'} = \frac{1}{k_2}$$

$$\phi_1 = \frac{1}{k_1} \qquad \phi_{1'} = \frac{1}{k_6}$$

$$\phi_2 = \frac{1}{k_3} \qquad \phi_{2'} = \frac{1}{k_4}$$

$$\phi_{12} = \frac{k_2}{k_1 k_3} \qquad \phi_{12'} = \frac{k_5}{k_4 k_6}$$

It is clear that the E_0/v coordinates for this mechanism are,

$$E_0/v_f = \frac{1}{k_5} - \frac{1}{k_2} \text{ and } E_0/v_r = \frac{1}{k_2} - \frac{1}{k_5} \qquad (2)$$

where v_f and v_r represent velocity in the forward and reverse reaction, respectively.

From eq. 2 it can be seen that, if $E_0/v_f = 0$, i.e., if the curves intersect on the abscissa in the forward direction, they *must* also intersect on this axis in the reverse direction in the case of this mechanism. If on the other hand, intersection is above the abscissa in the forward direction, i.e., $(1/k_5) > (1/k_2)$, then intersection must be *below* the abscissa in the reverse reaction. Under no circumstances will this mechanism yield data in which plots in both directions are both above or both below the abscissa. Similarly, it is not possible to see intersection on the axis in one direction and convergence either above or below the abscissa in the other direction in the case of the Theorell–Chance mechanism.

2.2. *Random Bi Bi mechanism* [8]

Scheme 2 depicts the random Bi Bi kinetic mechanism. In this mechanism, it is assumed that all steps equilibrate rapidly relative to interconversion of the ternary complexes. The initial rate equation, which describes the kinetic behavior of the mechanism, is illustrated in eq. 1.

$$E + A = EA,\ K_1 \qquad EPQ = EP + Q,\ K_5$$

$$E + B = EB,\ K_2 \qquad EPQ = EQ + P,\ K_6$$

$$EA + B = EAB,\ K_3 \qquad EP = E + P,\ K_7$$

$$EB + A = EAB,\ K_4 \qquad EQ = E + Q,\ K_8$$

$$EAB \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EPQ$$

Scheme 2.

The ϕ values are as follows:

$$\phi_0 = \frac{1}{k_1} \qquad \phi_{0'} = \frac{1}{k_2}$$

$$\phi_1 = \frac{K_4}{k_1} \qquad \phi_{1'} = \frac{K_5}{k_2}$$

$$\phi_2 = \frac{K_3}{k_1} \qquad \phi_{2'} = \frac{K_6}{k_2}$$

$$\phi_{12} = \frac{K_1 K_3}{k_1} \qquad \phi_{12'} = \frac{K_6 K_8}{k_2}$$

The E_0/v coordinates of intersection for the random Bi Bi mechanism can be obtained from these ϕ values and the equation of fig. 1. These coordinates are,

$$E_0/v_f = 1/k_1\,(1 - K_4/K_1) \text{ and } E_0/v_r = 1/k_2(1 - K_5/K_8) \qquad (3)$$

† ϕ and ϕ' are taken to mean ϕ values in the forward and reverse directions, respectively.

Table 1
Types of intersections of double reciprocal plots to be expected for sequential bireactant mechanisms.

Mechanisms	Intersection of lines relative to abscissa: Forward direction		Reverse direction: Above	On	Below
Theorell–Chance	Above	+	F*	F	+
and	On	+	F	+	F
iso Theorell–Chance	Below	+	+	F	F
Random Bi Bi	Above	+	+	+	+
	On	+	+	+	+
	Below	+	+	+	+
Ordered Bi Bi	Above	+	+	+	+
and	On	+	+	F	F
iso ordered Bi Bi	Below	+	+	F	F

* F means that the type of intersection is forbidden
+ Indicates that the type of intersection is permissable.

Two points are obvious from eq. 3. First, the curves of the random Bi Bi mechanism may intersect above, below, or on the 1/substrate axis. Second, the point of convergence of the data in one direction is unrelated to that in the other direction.

2.3. *Ordered Bi Bi mechanism* [1]

The ordered Bi Bi mechanism with a single productive ternary complex is depicted in scheme 3:

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA + B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EXY \underset{k_6}{\overset{k_5}{\rightleftharpoons}} EQ + P$$

$$EQ \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E + Q$$

Scheme 3

The eight applicable ϕ values based upon this kinetic mechanism which also is described by equation 1, are:

$$\phi_0 = \frac{1}{k_5} + \frac{1}{k_7} \qquad \phi_{0'} = \frac{1}{k_2} + \frac{1}{k_4}$$

$$\phi_1 = \frac{1}{k_1} \qquad \phi_{1'} = \frac{1}{k_8}$$

$$\phi_2 = \frac{(k_4+k_5)}{k_3k_5} \qquad \phi_{2'} = \frac{(k_4+k_5)}{k_4k_6}$$

$$\phi_{12} = \frac{k_2(k_4+k_5)}{k_1k_3k_5} \qquad \phi_{12'} = \frac{k_7(k_4+k_5)}{k_4k_6k_8}$$

The E_0/v coordinates of the points of intersection of the lines obtained from double reciprocal plots for the ordered Bi Bi mechanism are

$$E_0/v_f = \left(\frac{1}{k_5}+\frac{1}{k_7}\right) - \frac{1}{k_2} \text{ and } E_0/v_r = \left(\frac{1}{k_2}+\frac{1}{k_4}\right) - \frac{1}{k_7}$$

It is possible from a knowledge of these coordinate points to predict whether certain experimentally obtained data are consistent with this mechanism. For example, if, in one direction (designated forward), the double reciprocal plots intersect on the abscissa, what constraints are placed on the point of convergence of the plots in the opposite direction with this mechanism? If $(1/k_2) = (1/k_5) + (1/k_7)(E_0/v_f = 0)$, then in order for convergence to occur on the 1/substrate axis in the reverse reaction, i.e., $E_0/v_r = 0$, $(1/k_4)$ must equal $(-1/k_5)$, a condition which is kinetically impossible. Thus, in the case of the ordered Bi Bi mechanism, it is theoretically not possible for double reciprocal plots to converge on the abscissa in both directions.

Table 1 summarizes the possible and forbidden points of intersection of double reciprocal plots for the three mechanisms that conform to eq. 1.

2.4. *Applicability to the iso Theorell–Chance and iso ordered Bi Bi mechanism* [3]

The rules listed in this report also apply if EA alone, or EA and EQ isomerize in either the Theorell–Chance or ordered Bi Bi mechanism.

2.5. *Application to the mechanism of yeast hexokinase*

The kinetic mechanism of hexokinase action appears not to have been resolved at this late date. Ricard and his co-workers [9] and others [10] have presented evidence in support of the ordered Bi Bi mechanism with glucose as the obligatory initial substrate. Rudolph and Fromm [11] have recently summarized what they consider to be conclusive evidence in support of hexokinase's exhibiting a random Bi Bi mechanism. A large body of evidence has been presented from Ricard's [9], Sols' [10], and Fromm's [12–14] laboratories which indicates that plots of $1/v$ versus 1/substrate, at different fixed levels of second substrate, intersect on the 1/substrate axis. Furthermore, studies of the back hexokinase reaction from our laboratory [14] and from Sols' [10] indicate convergence of the double reciprocal plots on the abscissa. If nothing else, these data serve to exclude the ordered Bi Bi mechanism with a single ternary complex as being a viable possibility for yeast hexokinase.

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