ANALYSIS OF EXCHANGE RATES FOR THE PING PONG BI BI MECHANISM AND THE CONCEPT OF SUBSTRATE SYNERGISM

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1. Introduction

Bireactant enzyme mechanisms may be divided into two classes, sequential and Ping Pong [1] and they may be readily separated by initial rate experiments [2]. The cardinal feature of the Ping Pong mechanism is that the enzyme reacts with the substrate to form a product which dissociates before the second substrate adds to the active site of the enzyme. In the case of the sequential mechanism both substrates must be present on the enzyme simultaneously before product formation may occur.

One characteristic of the Ping Pong mechanism is the requirement for partial exchange reactions; however, these exchanges are often slow relative to the initial reaction velocity. Bridger et al. [3] have attempted to account for these slow partial exchange reactions in Ping Pong mechanisms by suggesting that if net reaction rates are rapid compared to partial reactions, synergism would appear probable. We wish to suggest here that before the concept of substrate synergism is invoked an important expression which relates the partial exchange rates and initial velocities must be satisfied.

2. Theory and discussion

Scheme 1 illustrates the well known Ping Pong Bi Bi mechanism along with the initial rate equation used to describe this pathway of enzyme and substrate interaction in Dalziel form [4].

$$E + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EA \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E' + P$$

$$E' + B \underset{k_6}{\overset{k_5}{\rightleftharpoons}} EB \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E + Q$$

Scheme 1.

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

E_0, v, ϕ_0, ϕ_1, ϕ_2, A, and B represent total enzyme concentration, initial velocity $k_3k_7/(k_3+k_7)$, $(k_2+k_3)/k_1k_3$, $(k_6+k_7)/k_5k_7$, substrate A and substrate B, respectively.

Some rather troublesome problems have become apparent in recent years in the case of Ping Pong mechanisms. One of these is that sequential mechanisms may appear to be Ping Pong if the $\phi_{12}/(A)(B)$ term is small relative to other terms in eq. 1. Indeed, this situation has occurred with at least three enzymes: brain hexokinase [5], phosphofructokinase [6], and D-amino acid oxidase [7]. That the Ping Pong mechanism for these enzymes may be an artifact was recognized using alternative substrates.

One would expect that an enzyme which exhibits a Ping Pong mechanism should catalyze so-called half reactions. In scheme 1, the enzyme should catalyze an A ⇌ P exchange in the absence of B and Q and a B ⇌ Q exchange in the absence of A and P. If these independent exchanges are slow relative to the initial reaction rate, investigators have come to the following conclusions: (a) The enzyme preparation contains contaminating activities which are responsible for the partial exchange(s) and the mechanism is not really Ping Pong; (b) Either the enzyme or a particular substrate–product pair is contaminated by one or both of the substrates and products; (c) The Ping Pong pathway is not a feature of the primary kinetic mechanism and (d) Substrate synergism is occurring.

When considering the rationale behind slow partial exchanges the first three explanations are obvious and do not warrant additional explanation. Substrate synergism, however, is a concept which requires further discussion in this context. This idea was first proposed by Bridger et al. [3] to explain certain slow partial exchange reactions obtained with succinyl coenzyme A synthetase. Similar slow exchanges were observed with other Ping Pong enzymes and the reader is referred to [3] for additional information on this point.

Bridger et al. [3] suggest that if net reaction rates are rapid compared with partial reactions, synergism would appear probable. These authors go on to suggest that net reaction rates (initial velocities) are measured in the absence of product required for the partial reaction and thus a comparison between the two different rates is difficult to make unambiguously. Intuitively, one would expect in viewing scheme 1 that the $A \rightleftharpoons P$ and $B \rightleftharpoons Q$ exchanges should be equal to or greater than the initial velocity of the reaction. Intuition in this setting appears to be misleading and no *direct* relationship exists between the maximal exchange rates and the maximal initial reaction velocity. These points are readily demonstrated as follows:

The rate equation for the $A \rightleftharpoons P$ exchange is:

$$\frac{1}{R_{A \rightleftharpoons P}} = \frac{(k_2+k_3)}{k_1 k_3 (E_0)}\left[\frac{k_1}{k_2} + \frac{k_1 k_3}{k_2 k_4 (P)} + \frac{1}{A}\right] \tag{2}$$

Plots of $1/R_{A \rightleftharpoons P}$ against $1/A$ at different fixed concentrations of P give a family of parallel lines with intercept $[(k_2+k_3)/k_2 k_3(E_0) + (k_2+k_3)/k_2 k_4(E_0)(P)]$ and slope ϕ_1/E_0. A secondary plot of intercepts versus $1/P$ will yield a straight line with intercept $(k_2+k_3)/k_2 k_3(E_0)$. This term is equal to $1/R_{(\max,\, A \rightleftharpoons P)}$. By analogy, one may determine $1/R_{(\max,\, B \rightleftharpoons Q)}$, which is equal to $(k_6+k_7)/k_6 k_7(E_0)$.

These equations reveal immediately that no direct relationship exists between the rate of the partial exchange reactions and maximal velocity in either direction, i.e., $V_f = k_3 k_7 E_0/(k_3+k_7)$ and $V_r = k_2 k_6 E_0/(k_2+k_6)$, where V_f and V_r represent the maximal initial rates for the forward and reverse reactions, respectively.

In considering the forward reaction and the maximal $A \rightleftharpoons P$ exchange, if $k_2 \gg k_3$ and $k_7 \gg k_3$, $V_f = R_{\max,\, A \rightleftharpoons P}$. On the other hand, V_f may be greater or less than $R_{\max,\, A \rightleftharpoons P}$ depending upon the relationship between k_2, k_3 and k_7. Similar arguments can be made with reference to the V_f and $R_{\max,\, B \rightleftharpoons Q}$ exchange.

Although no direct relationship exists between the partial exchange rates and the initial reaction velocities, the following equation pertains,

$$\frac{1}{R_{\max,\, A \rightleftharpoons P}} + \frac{1}{R_{\max,\, B \rightleftharpoons Q}} = \frac{1}{V_f} + \frac{1}{V_r} \tag{3}$$

From these considerations it is clear that one must evaluate all four parameters shown in eq. 3 before any definitive conclusions can be drawn regarding the importance of, and explanation for, "slow" partial exchange reactions, relative to initial rates.

Another criterion that has been used to evaluate substrate synergism involves comparison of the partial exchange reactions in the presence and absence of the substrate–product pair not involved in the isotopic exchange reaction [3]. Here it would be expected that the presence of B and Q would serve to decrease the $A \rightleftharpoons P$ exchange of scheme 1. If the $A \rightleftharpoons P$ exchange increases, the concept of synergism may be invoked. It can be seen that in the case of the Ping Pong Bi Bi mechanism the overall exchange, $A \rightleftharpoons Q$, is related to the partial exchanges in the presence of both substrates and products as follows:

$$\frac{1}{R_{A \rightleftharpoons Q}} = \frac{1}{R_{A \rightleftharpoons P}} + \frac{1}{R_{B \rightleftharpoons Q}} \tag{4}$$

Eq. 4 suggests that the partial exchange rates must be equal to or greater than the overall exchange rate.

3. Conclusion

The purpose of this report is to point out those criteria which may be used to invoke substrate synergism in order to account for "slow" partial exchange reactions. It is quite valid to compare partial exchange reactions in the presence of the other substrate and product components of the Ping Pong system as was done by Bridger et al. [3]. On the other hand, it is not possible to relate partial exchanges to initial reaction velocities in an attempt to explain so-called slow exchange reactions. Unfortunately, this correlation is too often made.

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