Derivation of Rate Equations for Multisite Ping-Pong Mechanisms with Ping-Pong Reactions at One or More Sites

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SUMMARY

Rate equations are derived for three-site ping-pong mechanisms in which one or two of the individual sites have a ping-pong rather than random sequential mechanism, and the expected kinetic patterns are discussed.

It has become clear as the result of the pioneering studies of Northrop on transcarboxylase (1), and the later studies in Lardy’s (2) and Utter’s (3) laboratories on pyruvate carboxylase that biotin-containing enzymes have two-site ping-pong mechanisms in which biotin is carboxylated at one site, and in turn carboxylates a substrate at the second site. The rate equations for such mechanisms have been derived by assuming that the reaction at each site between biotin or carboxybiotin and the other reactants was random sequential. Thus, the conversion of biotin to carboxybiotin at a site was considered proportional to the fractional occupancy of that site by the correct substrates, and to the proportion of the enzyme existing in the form with free biotin at that site. Since the fractional occupancy is given by a simple expression such as:

\[ \frac{OAA}{K_i \cdot OAA} \cdot \frac{1}{1 + \frac{OAA}{K_i \cdot OAA} + \frac{Pyr}{K_i \cdot Pyr}} \]  

for a site where pyruvate and oxalacetate combine, the rate equation is easily derived by classical means by multiplying the appropriate rate constants for the steps where biotin is carboxylated or decarboxylated by the corresponding fractional occupancy factor. The resulting equations predict the observed initial velocity and product and dead end inhibition patterns, and thus this approach seems valid in these cases.

Pyruvic dehydrogenase contains a bound lipoic acid and might be expected to behave similarly to the biotin enzymes and show a three-site ping-pong mechanism. The kinetics when only substrates are present are indeed ping-pong regardless of which pair of substrates are varied, as shown in the accompanying paper (4). However, reaction at all three sites in the complex is not random sequential, and good evidence is available to suggest that the reactions at two of the sites are ping-pong:

\[
\begin{align*}
\text{Pyruvate} & \quad \text{CO}_2 & \quad \text{Ox Lipoic} & \quad \text{Ac-Lipoic} \\
\text{TPP} & \quad ( ) & \quad \text{HetPP} & \quad ( ) & \quad \text{TPP} \\
\text{Red Lipoic} & \quad \text{Ox Lipoic} & \quad \text{DPN} & \quad \text{DPNH} \\
\text{E}_{\text{ox}} & \quad ( ) & \quad \text{E}_{\text{red}} & \quad ( ) & \quad \text{E}_{\text{ox}} \\
\end{align*}
\]

Reaction at such a site cannot be considered as proportional to the fractional occupancy by substrate and the proportion of the lipoic acid that is in the proper form and at the site, since half of the reaction can take place while the lipoate is elsewhere and even in one of its other two forms. A new approach is thus needed to enable derivation of the expected rate equations, and the purpose of this paper is to present such an approach.

THEORY

Three-site Ping-Pong Mechanism with One Ping-Pong and Two Random Sequential Sites—Let us consider first a case where a bound carrier has three possible forms which we will call S, X, and L (corresponding to oxidized, acetylated, and reduced lipoate in pyruvic dehydrogenase), and where conversion of S to X is a ping-pong reaction, but conversion of X to L and L to S are random sequential reactions. We can then diagram such a mechanism as:

\[ \text{T} \quad \text{H} \quad \text{K} \]

\[ k_1 \quad k_2 \quad k_3 \quad k_4 \]

1 The notation and nomenclature used are those of Cleland (5).

2 We are assuming only one site reaction to be ping-pong to illustrate the method; see the following section for derivation of the equation for the actual pyruvic dehydrogenase mechanism which assumes both first and third site reactions to be ping pong.

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where T and H are the two stable enzyme forms at the first site (TPP and hydroxyethyl TPP for pyruvate dehydrogenase). A + P are the substrate and product reacting at this site (pyruvate and CO2), and f6, f7, f5, f8 are the fractional occupancy factors for the other two sites given by the following equations:

\[ f_6 = \frac{E}{K_i \beta} \frac{x}{(1 + \frac{E}{K_i \beta} + \frac{x}{K_i \alpha})} \]

\[ f_7 = \frac{x^2}{K_i \eta \gamma} \frac{y}{1 + \frac{x}{K_i \eta \gamma}} \]

where B, C, Q, and R are the substrates and products at the other two sites (CoA, DPN, AcCoA, DPNH), and the K_i values are their dissociation constants. Note that Steps 3 and 4 are common to the first two diagrams, and that the action of reactants A and P is related to the main scheme only through their common to the first two diagrams, and that the action of reactants A and P is related to the main scheme only through their influence on the relative levels of T and H.

If standard methods (such as King's method (6)) are now used to derive the rate equation from mechanism 5, we obtain:

\[ v = (k_2 + k_4 + k_2 f_6 + k_4 f_7 + k_6 f_8 + k_8 f_8) \]

\[ v = \frac{k_2 k_4 k_6 k_8 f_6 f_7 f_8}{k_2 k_4} \]

Clearly, we can substitute for the f's, but we must also figure out how to eliminate H and T in order to have a rate equation that is in terms only of rate constants and reactant concentrations. To do this we write for the reaction at the first site:

\[ V = (k_1 A T - k_2 P H) E_1 \]

or, since: H + T = 1 if the concentrations of H and T are considered as fractions of their potential values,

\[ \frac{v}{v} = k_1 A T - k_2 P (1 - T) = (k_1 A + k_2 P) T - k_2 P \]

Solving for T:

\[ T = (\frac{v}{v}) \frac{1}{k_1 A + k_2 P} \]

\[ H = 1 - T = \frac{k_1 A - (\frac{v}{v})}{k_1 A + k_2 P} \]

Since in the steady state v in these equations must be the same as v for the over-all reaction given by Equation 8, we can substitute these values of T and H into Equation 8 and then solve for v. The resulting rate equation is a quadratic one in (v/v):

\[ (\frac{v}{v})^2 \left( k_1 k_2 A + k_2 k_4 P + k_2 k_4 P \right) - k_2 k_4 P (1 - T) = (k_1 A + k_2 P) T - k_2 P \]

The model described in detail above illustrates the approach required when reaction at one of the sites in a multisite ping-pong mechanism is itself ping-pong. However, for pyruvic dehydrogenase there is good evidence that the lipoyl dehydrogenase site, as well as the pyruvate decarboxylating site, has a ping-pong mechanism. Thus, in this shorthand notation, 1 = k_1, 2 = k_2, etc., for the rate constants, and each B is really B/K_i, each C is C/K_i, and each R is R/K_i.

Three-site Ping-Pong Mechanism with Two Ping-Pong Sites—The model described in detail above illustrates the approach required when reaction at one of the sites in a multisite ping-pong mechanism is itself ping-pong. However, for pyruvic dehydrogenase there is good evidence that the lipoyl dehydrogenase site, as well as the pyruvate decarboxylating site, has a ping-pong mechanism. In this case, Equation 5 must be changed to:

\[ V = (k_1 k_2 k_4 k_6 k_8) \frac{ABC}{K_i \alpha \beta \gamma} + \frac{k_2 k_4 k_6 k_8}{K_i \beta \gamma} \]

In this shorthand notation, 1 = k_1, 2 = k_2, etc., for the rate constants, and each B is really B/K_i, each C is C/K_i, and each R is R/K_i.

and we must also add:

\[ k_4 S \]

\[ k_4 T \]

\[ k_4 P \]

\[ k_1 Q \]

\[ k_1 R \]

\[ k_1 C \]
By the same reasoning used in the above model, we can derive equations for Ox and Red analogous to Equation 11:

\[
\begin{align*}
\text{Red} &= \left( \frac{v}{E_t} \right) + k_{10} R \\
\text{Ox} &= k_9 C - \left( \frac{v}{E_t} \right)
\end{align*}
\]  

(17)

When the rate equation for mechanism 15 is derived by the usual techniques, it will contain T, H, Ox, and Red, as well as rate constants and \( f_k \). When T and H are substituted from Equation 11, and Ox and Red from Equation 17, the resulting equation analogous to Equation 12 will contain terms in \( (v/E_t)^2 \), as well as in \( (v/E_t) \) as in \( v/E_t \). Again, all higher power terms are dropped, and only the \( (v/E_t) \) term kept. The resulting equation can be written:

\[
v = \left( \frac{k_1 k_2 k_3 k_9 k_{10} \ ABC - k_2 k_4 k_8 k_{10} \ PQR}{K_{ib}} \right) \frac{v}{E_t}
\]

where \( \Delta = 1357 AB + 1379 AC + 3579 BC + I(35 + 37 + 57) 9 ABC + 2468 PQ + 24610 PR + 46810 QR + 2(4+5)79BCP + 2479 CP \)

The notation is similar to that used for Equation 14, except that only B is B/\( K_{ib} \) and Q is Q/\( K_{iq} \).

**Dead End Inhibition**—In multisite ping-pong mechanisms the action of dead end inhibitors is also often of interest, particularly if a product acts both as a product and dead end inhibitor. When a dead end inhibitor combines at a site with a random sequential mechanism, the only change is in the denominator of the \( f \)’s. Thus, for mechanism 5 or 15, the combination of a dead end inhibitor at the B, Q site changes the denominators in Equation 6 to \( (1 + B/\ K_{ib} + Q/\ K_{iq} + I/\ K_i) \), where \( K_i \) is the dissociation constant of the inhibitor. The CP, AC, and PR terms in Equation 19 are then multiplied by \( (1 + I/\ K_i) \).

If a dead end inhibitor combines at a site with a ping-pong mechanism, however, the rate equation for reaction at this site must be divided by \( (1 + 1/\ K_i) \). Thus, for mechanism 16 we would now have:

\[
v = \left( \frac{k_9 C \text{ Red} - k_{10} R \text{ Ox}}{1 + 1/\ K_i} \right) \frac{v}{E_t}
\]

The \( (v/E_t) \) terms in Equation 17 are now multiplied by \( (1 + 1/\ K_i) \) as are the AB and PQ terms in Equation 19. Similarly, a dead end inhibitor combining at the A, P site causes the BC and QR terms in Equation 19 to be multiplied by \( (1 + 1/\ K_i) \).

This formulation assumes that the inhibition is competitive versus C and R, which is usually reasonable.

**DISCUSSION**

Regardless of whether the individual site reactions are random sequential or ping-pong, rate equations for three-site ping-pong mechanisms will have numerators similar to those of Equations 13 and 18, and the denominators will all have AB, AC, BC, PQ, PR, QR, PQR, ABR, ACP, BCP, BPR, AQR, and CPQ terms. Note that the triple terms constitute all combinations of one reactant from each site. If all three site reactions are random sequential, the denominator includes in addition AQ, BR, and CP terms. If reaction at the A, P site is ping-pong, the BR term is missing, and if reaction at the C, R site is ping-pong the AQ term disappears. The CP term would also go if reaction at the B, Q site were ping-pong.

The initial velocity patterns called for by all of these mechanisms are parallel regardless of which substrate is varied at fixed levels of a second. This has been confirmed for pyruvate dehydrogenase (4). The product inhibition patterns call for each product to be competitive versus the substrate combining at that site, regardless of whether reaction at that site is random sequential or ping pong (unless central complexes are kinetically important, in which case the ping-pong site gives noncompetitive and the random competitive inhibition) and this, too, has been confirmed for acetyl-CoA and DPNH with pyruvic dehydrogenase. Each product will be uncompetitive against other substrates, except that it will be noncompetitive against the substrate that combines at the next site in the over-all sequence, if reaction at that site is random sequential. Combination as a dead end inhibitor at another site will also make a product noncompetitive versus the substrate combining there. With pyruvnic dehydrogenase acetyl-CoA and DPNH are both uncompetitive versus pyruvate, but acetyl-CoA is noncompetitive versus DPN, and DPNH noncompetitive versus CoA, suggesting that each may combine as a dead end inhibitor at the other site, or in some other way hinder reaction at that site. The data in the accompanying paper suggest that the latter is the correct explanation (4).

**REFERENCES**

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