Proc. Natl. Acad. Sci. USA
Vol. 75, No. 3, pp. 1101–1105, March 1978
Biochemistry

Unsymmetrical and concerted examples of the effect of enzyme–enzyme interactions on steady-state enzyme kinetics

(heterologous dimer/excluded transitions/conformational changes/isologous dimer/half-the-sites reactivity)

TERRELL L. HILL

Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

Contributed by Terrell L. Hill, November 28, 1977

ABSTRACT **In previous papers of this series, emphasis has been placed on the steady-state phase transition and critical properties of large lattices of interacting, symmetrical, and identical enzyme molecules. The present paper is concerned with a number of examples of enzyme–enzyme interactions that do not belong to the class of models of the earlier papers. These are more biochemically oriented and include heterologous dimers, a linear chain with unsymmetrical interactions, and concerted isologous dimers (half-the-sites reactivity).**

This is the fifth in a series (1–4) of theoretical papers on the effect of enzyme–enzyme interactions on steady-state enzyme kinetics. In all of these papers we treat the steady state as an explicit kinetic problem and do not use an equilibrium approximation to the steady-state population of states. The reader is assumed to be familiar with the notation and terminology being employed (1–4). Although small (oligomeric) aggregates of identical enzyme molecules (subunits) have received some attention (1, 4), emphasis so far has been placed on the statistical physics problem of large lattices of interacting, symmetrical, identical enzyme molecules. For this latter purpose, primarily, we have been using the relatively simple rate constant convention introduced in equations 1, 4, and 5 of ref. 1. As mentioned at the outset (1), this convention is not unique. (Equation 2 of ref. 1, on the other hand, is a general requirement.) The convention treats equivalently the interactions of a given molecule with all of its nearest neighbors. This helps simplify the already very difficult large lattice problem, but the degree of molecular symmetry implied would not be realistic for most actual enzyme oligomers.

The purpose of this paper is to present a few illustrative examples that are *not* based on the above-mentioned convention. It is hoped, in future work, to extend these considerations to some models of real oligomeric enzyme systems. Thus, this paper is meant to serve as a bridge between the earlier models of more physical interest and future biochemical models.

Other work to be reported later includes Monte Carlo (with Y. Chen) and Bragg–Williams (with L. Stein) approaches to critical behavior of enzyme lattices at steady state and an analytical study of a special case (1, 4) of the lattice-diffusion problem (by R. J. Rubin).

Heterologous dimer

The first example is presented in Fig. 1. Two identical enzyme molecules associate as shown. Each separated molecule (monomer) has the (unperturbed) rate constants, for a two-state cycle, given in Fig. 2*A*. The enzymatic process we have in mind is the usual E(1) → ES(2) → (EP) → E(1), where EP is a transient intermediate. The rate constants for subunit I (Fig. 1) are the same in the dimer as in the monomer (Fig. 2*B*). State 2 (ES) has a bound substrate molecule (Fig. 1). Binding and release of both substrate and product are sterically hindered in subunit II of the dimer, as suggested schematically in Fig. 1 and as is reflected by the rate constants in Fig. 2*B*. That is, $0 \le a_\alpha \le 1$ and $0 \le a_\beta \le 1$ in Fig. 2*B*. These are steric *kinetic* factors. There are no interaction free energy effects in this idealized model. That is, $w_{11} = w_{12} = w_{21} = w_{22}$. But there *are* rate constant effects (a_α, a_β). In other words, the interactions appear here in transition states but not in initial and final states. Equations 4 and 5 of ref. 1 are clearly inapplicable (except as an asymptotic limit). In equation 2 of ref. 1, $w_{ie} = w_{je}$; hence α_0 and α_0' have the *same* factor a_α in Fig. 2*B*, and similarly for the factor a_β multiplying β_0 and β_0'.

As is evident from Fig. 2*B*, the rate constants of I are independent of the state of II, and vice versa. Hence, we have immediately for the two fluxes (5)

$$J_{\mathrm{I}} = (\alpha_0\beta_0 - \alpha_0'\beta_0')/(\alpha_0 + \alpha_0' + \beta_0 + \beta_0') = J_0$$

$$J_{\mathrm{II}} = (\alpha_0\beta_0 - \alpha_0'\beta_0')a_\alpha a_\beta/[(\alpha_0 + \alpha_0')a_\alpha + (\beta_0 + \beta_0')a_\beta], \qquad [1]$$

where J_{I} is also the unperturbed flux J_0 for the monomer (Fig. 2*A*). Clearly, $J_{\mathrm{II}} < J_{\mathrm{I}}$. If either $a_\alpha = 0$ or $a_\beta = 0$, then $J_{\mathrm{II}} = 0$: only one of the two monomers in the dimer operates as an enzyme (a simple example of half-the-sites reactivity).

Consider next a more complicated version of the above model (Fig. 3). Here, binding of substrate induces a conformational change. Hence state 2 is shown (schematically, in Fig. 3) to have a different shape than state 1. We imagine that access of substrate and product to the site on II is less restricted when I is in state 2 than when it is in state 1. Hence there are two pairs of rate constant factors (Fig. 3), with $0 \le a_\alpha \le b_\alpha \le 1$ and $0 \le a_\beta \le b_\beta \le 1$. Also, we assume that the two subunits fit together less well (dispersion forces, etc.) when I is in state 2 than when it is in state 1. Hence we take $w_{11} = w_{12} = 0$ (this is a reference value) and $w_{21} = w_{22} \equiv w \ge 0$. We define $y = e^{-w/kT} \le 1$; hence $y^{-1} \ge 1$ (below).

Fig. 4 shows the new set of rate constants. One further assumption has been included in Fig. 4: the effect of w on the transitions in I has been put entirely in the release steps (α_0', β_0) rather than in the binding steps (α_0, β_0') as if, for example, binding on I is diffusion controlled. In the notation of ref. 1, $f_\alpha = 0$ and $f_\beta = 1$.

We note (Fig. 4) that the rate constants for I are independent of the state of II. Hence (5)

$$J_{\mathrm{I}} = (\alpha_0\beta_0 - \alpha_0'\beta_0')y^{-1}/[(\alpha_0 + \beta_0') + (\alpha_0' + \beta_0)y^{-1}]. \qquad [2]$$

The expression for J_{II} is rather more complicated so we turn to the special case of one-way cycles ($\alpha_0' = \beta_0' = 0$ in Fig. 4). From pp. 173–175 of ref. 5, we find

$$J_{\mathrm{II}} = \alpha_0\beta_0\{\quad\}/(\alpha_0 + \beta_0 y^{-1})[\quad], \qquad [3]$$

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

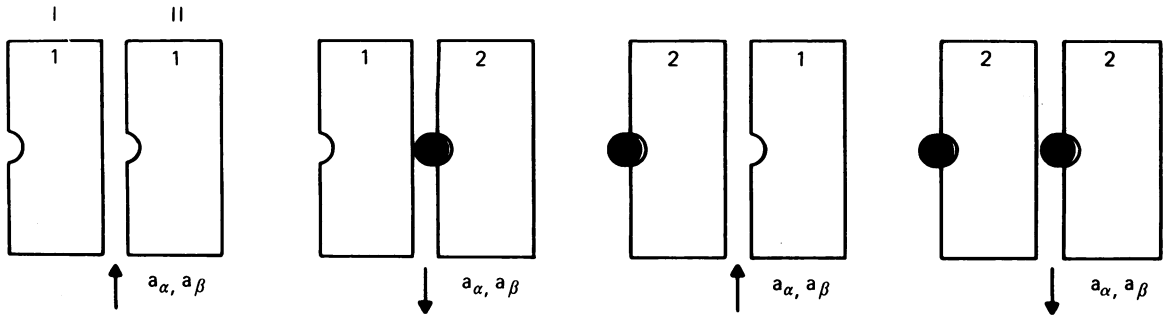

FIG. 1. Four states of two subunits (I,II) in a heterologous dimer. Solid ball represents bound substrate. Kinetic factors (see Fig. 2*B*) are a_α and a_β for subunit II.

where

$$\{\ \} = \alpha_0^2(a_\alpha + 1)b_\alpha b_\beta + \beta_0^2(b_\beta + y^{-1})a_\alpha a_\beta y^{-1} + \alpha_0\beta_0[(a_\alpha y^{-1} + b_\beta)a_\beta b_\alpha + (a_\alpha b_\beta + a_\beta b_\alpha)y^{-1}], \text{ and}$$

$$[\ ] = \alpha_0^2(a_\alpha + 1)b_\alpha + \beta_0^2(b_\beta + y^{-1})a_\beta + \alpha_0\beta_0(a_\alpha y^{-1} + b_\beta + a_\alpha b_\beta + a_\beta b_\alpha).$$

If $a_\alpha = a_\beta = 0$ (no transitions between 11 and 12),

$$J_{II} = \alpha_0^2\beta_0 b_\alpha b_\beta/(\alpha_0 + \beta_0 y^{-1})(b_\alpha\alpha_0 + b_\beta\beta_0). \quad [4]$$

In earlier examples in this series, some states were eliminated from the kinetic diagram because of assumed interactions. Here ($a_\alpha = a_\beta = 0$) *transitions* are eliminated from the diagram, but not states. In the present case ($a_\alpha = a_\beta = 0$), states 11, 21, 22, and 12 are now connected linearly, in the order given. Hence a "detailed balance" solution (4) for the state probabilities at steady state is easy, even with two-way cycles.

If $a_\alpha = a_\beta \equiv a$ and $b_\alpha = b_\beta \equiv b$ (which is rather plausible), we have a quasi-equilibrium system (1, 4). Hence we find easily, for two-way cycles,

$$p_{11} = 1/(1 + x)(1 + xy), \qquad p_{12} = xp_{11}$$
$$p_{21} = xyp_{11}, \qquad p_{22} = x^2yp_{11}, \quad [5]$$

where $x = (\alpha_0 + \beta_0')/(\beta_0 + \alpha_0')$ and the p_{ij} are state probabilities. Then J_I is given by Eq. 2 and

$$J_{II} = \alpha_0(ap_{11} + bp_{21}) - \alpha_0'(ap_{12} + bp_{22}) = J_0(a + bxy)/(1 + xy). \quad [6]$$

If $a_\alpha = b_\alpha$ and $a_\beta = b_\beta$, II is independent of the state of I, and J_{II} is given again by Eq. 1.

It is easy to extend the above model to the case (ref. 5, pp. 173–175) in which I and II are *different* enzymes—for example, I is a regulatory subunit and II is a catalytic subunit (6).

Excluded transitions in a linear chain

Consider an "open" linear chain of M enzyme molecules of the type shown in Figs. 2A, 3, and 4 ($M = 2$ in Figs. 3 and 4), but in the simple case $y = 1$, $a_\alpha = a_\beta = 0$, and $b_\alpha = b_\beta = 1$. Thus, the only interaction between the enzyme molecules of the chain is the property that a given molecule is inoperative as an enzyme if its left-hand neighbor is in state 1 (compare p. 175 of ref. 5 for $M = 2$). The state of the right-hand neighbor is immaterial. This is illustrated in Fig. 5 for $M = 4$. All $2^M = 16$ states are shown. A line represents both a transition to the right with rate constant (see Figure 1C of ref. 1) $\alpha_0 + \beta_0'$ and to the left with rate constant $\beta_0 + \alpha_0'$. The dotted lines indicate those transitions excluded because of the above-mentioned type of interaction.

This is a quasi-equilibrium system (1, 4) with steady-state probabilities of the states proportional to $1, x, \cdots, x^4$ (Fig. 5). All states are accessible despite the excluded transitions, which therefore have no effect on state probabilities. Hence, for *any* M, the probability that any molecule of the chain is in state 2 is $x/(1 + x)$. Thus, the flux in the left-hand molecule is J_0 (Eq. 1) and in all of the other $M - 1$ molecules it is $J_0x/(1 + x)$.

The above is an "open" (1) chain. In the "closed" chain case, the last molecule is a nearest-neighbor of the first. In this case the transitions (lines) marked with a small circle (Fig. 5) are also excluded. Hence, state 1111 is inaccessible, but the other states are all accessible and have relative steady-state probabilities $x, \cdots, x^4$ as before. For arbitrary M it is easy to see, then, that the probability that any particular molecule is in state 2 is

$$x(1 + x)^{M-1}/[(1 + x)^M - 1] \xrightarrow{M\to\infty} x/(1 + x)$$

and that the mean flux, for any molecule, is this probability multiplied by J_0.

Long linear chain with interactions

Here we consider a long linear chain (M large) of enzyme molecules with properties closely related to those used in Figs. 3 and 4. Here, however, we employ arbitrary $y_{ij} = e^{-w_{ij}/kT}$ (this is more general) but take $a_\alpha = a_\beta \equiv a$ and $b_\beta = b_\beta \equiv b$ (less general). We use $f_\alpha = 0$ and $f_\beta = 1$ as in Fig. 4. These last two choices ($a, b; f$) produce a quasi-equilibrium system; hence, we can take over the one-dimensional Ising results in ref. 2 (except that y_{12}^2 becomes here $y_{12}y_{21}$).

We shall express the flux as in equation 4 of ref. 2. The explicit α rate constants for the central molecule of triplets in the

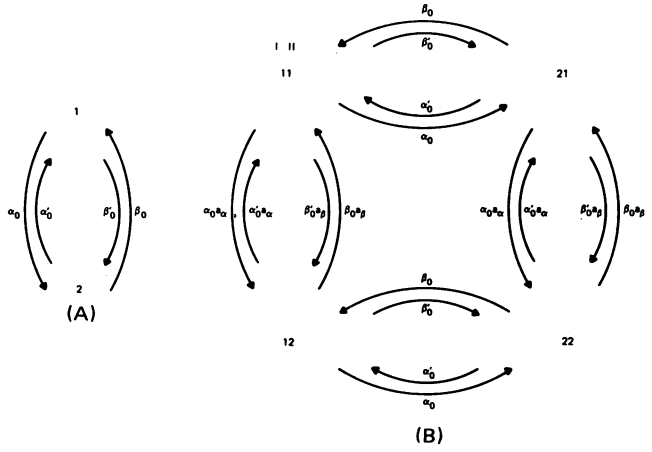

FIG. 2. (*A*) Rate constants for monomer. (*B*) Rate constants for dimer. See text and Fig. 1.

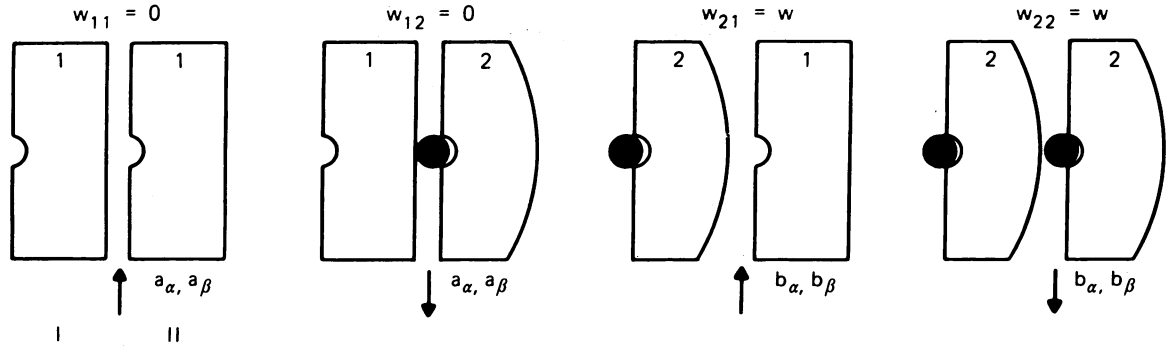

FIG. 3. Dimer with conformational change (two shapes) and interactions between subunits (w_{ij}). See text.

chain are:

$$\begin{aligned} 111 &\rightleftarrows 121 \quad \alpha = \alpha_0 a, \ \alpha' = \alpha_0' a y_{11}^2/y_{12}y_{21} \\ 112 &\rightleftarrows 122 \quad \alpha = \alpha_0 a, \ \alpha' = \alpha_0' a y_{11}/y_{22} \\ 211 &\rightleftarrows 221 \quad \alpha = \alpha_0 b, \ \alpha' = \alpha_0' b y_{11}/y_{22} \\ 212 &\rightleftarrows 222 \quad \alpha = \alpha_0 b, \ \alpha' = \alpha_0' b y_{12}y_{21}/y_{22}^2. \end{aligned} \quad [7]$$

The flux per molecule, calculated from the α transitions, is then

$$\begin{aligned} J = {} & \alpha_0 a(\bar{N}_{111}/M) - \alpha_0' a(y_{11}^2/y_{12}y_{21})(\bar{N}_{121}/M) \\ & + \alpha_0(a+b)(\bar{N}_{112}/M) - \alpha_0'(a+b)(y_{11}/y_{22})(\bar{N}_{122}/M) \\ & + \alpha_0 b(\bar{N}_{212}/M) - \alpha_0' b(y_{12}y_{21}/y_{22}^2)(\bar{N}_{222}/M), \end{aligned} \quad [8]$$

in which we have made use of the chain symmetry. From equations 19 of ref. 2 we then obtain

$$\begin{aligned} J = {} & [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')][a y_{11}^2(y_{11} - y_{22}x + \sqrt{\ }) \\ & + 2(a+b)y_{11}y_{12}y_{21}x \\ & + b y_{12}y_{21}x(y_{22}x - y_{11} + \sqrt{\ })]/\sqrt{\ }[\ ], \end{aligned} \quad [9]$$

where, as in ref. 2,

$$\begin{aligned} \sqrt{\ } &= [(y_{11} - y_{22}x)^2 + 4y_{12}y_{21}x]^{1/2} \\ [\ ] &= (y_{11} + y_{22}x + \sqrt{\ })^2/2. \end{aligned} \quad [10]$$

In the special case $a = 0$ (an enzyme molecule does not operate if its left-hand neighbor is in state 1),

$$\begin{aligned} J &= [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')]2bxy_{12}y_{21}/\sqrt{\ }(\ ) \\ &= [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')]b(\bar{N}_{12}/M), \end{aligned} \quad [11]$$

where ( ) is the quantity in parentheses in Eq. 10.

Another special case of interest is $y_{12}y_{21} = y_{11}y_{22}$. In this case we find

$$\begin{aligned} \theta &= \bar{N}_2/M = y_{22}x/(y_{11} + y_{22}x) \\ J &= [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')]y_{11} \\ &\quad \times (a y_{11} + bxy_{22})/(y_{11} + y_{22}x)^2 \\ &= [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')](1-\theta)[a(1-\theta) + b\theta]. \end{aligned} \quad [12]$$

Actually, these results hold for any closed linear chain with $M \geq 2$. The particular w_{ij} in Fig. 3 are a special case: $y_{11} = y_{12} = 1$, $y_{21} = y_{22} \equiv y$. In this case,

$$J = [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')](a + bxy)/(1 + yx)^2. \quad [13]$$

Heterologous dimer of three-state enzymes

In this example we consider a heterologous dimer of the sort shown in Fig. 3. However, here we have a one-way, three-state enzyme with the unperturbed rate constants and states shown in Fig. 6A. We suppose that the enzyme has the two conformational shapes included in the figure. As a consequence, we assume that, when molecule I is in state 1, S and P do not have access to the binding site on II. Hence we take $a = 0$ in Fig. 6B, which shows the rate constants for the dimer. That is, the transitions 11 → 12 and 13 → 11 are excluded. Furthermore, we assume that, when I is in either state 2 or state 3, the substrate binding rate constant for II is $\alpha_0 b$ and the product release rate constant for II is $\alpha_0 c$ (see Fig. 6B), where $0 \leq b, c \leq 1$. These are the only interactions in the model.

With $a = 0$ in Fig. 6B, we have the straightforward algebraic problem of finding the nine steady-state probabilities. These turn out to be

$$\begin{aligned} p_{11} &= p_{31} = c(14 + 7b + 7c + 2bc)/\Sigma, \\ p_{21} &= c(14 + 7b + 7c + 3bc)/\Sigma \\ p_{12} &= bc(6 + 3b + 3c + bc)/\Sigma, \\ p_{22} &= bc(10 + 5b + 5c + 2bc)/\Sigma \\ p_{32} &= bc(12 + 6b + 6c + 2bc)/\Sigma, \\ p_{13} &= b(14 + 7b + 12c + 5bc + 3c^2 + bc^2)/\Sigma \\ p_{23} &= b(14 + 7b + 8c + 3bc)/\Sigma, \\ p_{33} &= b(14 + 7b + 6c + 2bc)/\Sigma \end{aligned} \quad [14]$$

where

$$\begin{aligned} \Sigma = {} & 3(14b + 14c + 7b^2 + 25bc \\ & + 7c^2 + 8b^2c + 8bc^2 + 2b^2c^2). \end{aligned}$$

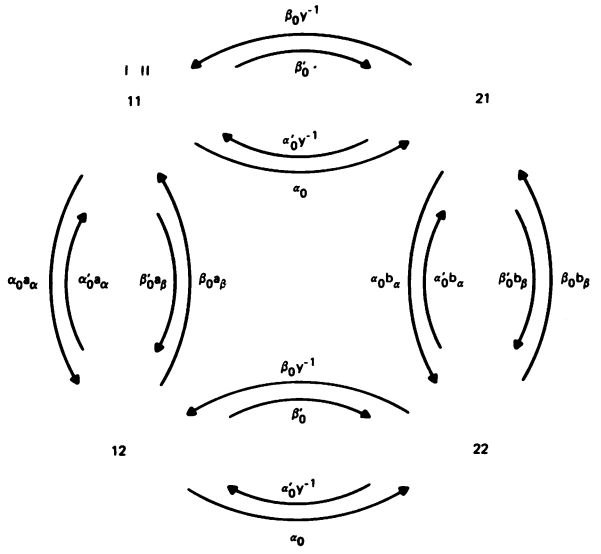

FIG. 4. Rate constants for model in Fig. 3. See text.

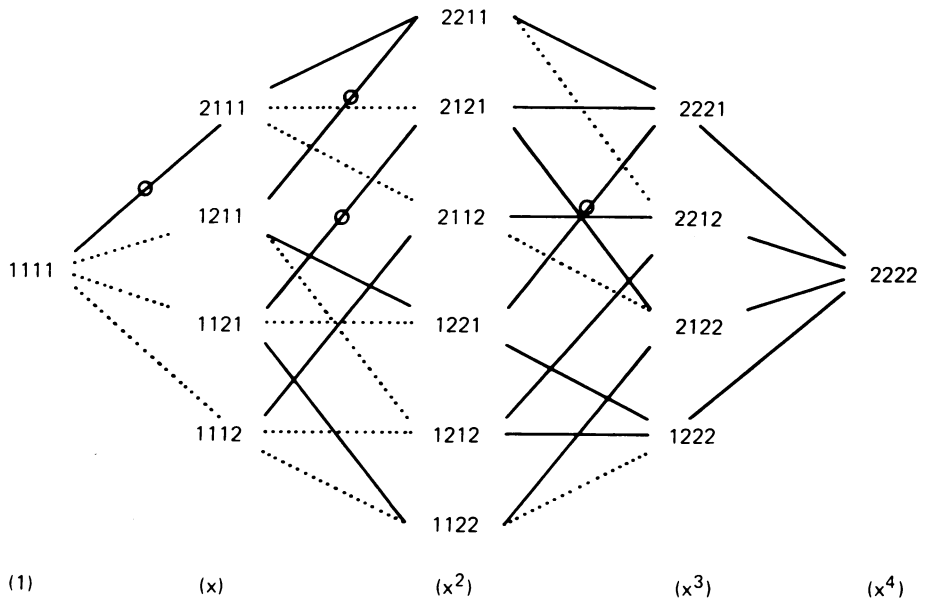

FIG. 5. States and transitions of "open" linear tetramer of two-state enzyme molecules. See text. A subunit cannot operate as an enzyme if subunit on left is in state 1. Dotted lines represent excluded transitions. Lines with small circles are excluded also if tetramer is "closed."

The two fluxes are then

$$J_I = \alpha_0/3 = J_0$$
$$J_{II} = \alpha_0 bc(28 + 14b + 14c + 5bc)/\Sigma. \quad [15]$$

When $b = c = 1$, $J_{II} = 61\alpha_0/255$ (exclusion effect only: $a = 0$). When $b = c \ll 1$, $J_{II} = \alpha_0 b/3$. States 12, 22, and 32 are practically unoccupied; the other six states have $p_{ij} \cong 1/6$.

Two-state concerted dimer

This model (Fig. 7) is suggested by the paper of Seydoux *et al.* (7). We have an isologous dimer of two identical subunits, each of which can be in one of two conformations (C = circle, R = rectangle). The two conformations in the dimer are always different (one C, one R); the subunits change conformation in concert. Otherwise the subunits do not interact. Each subunit is an enzyme for S → P (with one enzymatic site): E → ES → (EP) → E, where EP is a transient intermediate. The symbol X in Fig. 7 represents S bound on the site. The R site, let us say, is a better enzymatic site than the C site ($\alpha_1 > \alpha_2$, $\beta_1 > \beta_2$). In the extreme, $\alpha_2 = \beta_2 = 0$ (only the R site operates).

The κ transitions (concerted conformational changes) in Fig. 7 are inverses of each other. But α_i and β_i ($i = 1,2$) do *not* refer to inverse transitions. The inverse rate constants are designated α_i' and β_i', respectively (these are not shown in the figure, for simplicity). It is easy to show (5) that there are two restraints on choices of rate constants in this model:

$$\frac{\kappa_1}{\kappa_1'} = \frac{\beta_1'\beta_2}{\beta_2'\beta_1} = \frac{\alpha_1\alpha_2'}{\alpha_2\alpha_1'}. \quad [16]$$

From this point we limit ourselves to one-way cycles, as in Fig. 7 (i.e., we take $\alpha_1' = \beta_1' = \alpha_2' = \beta_2' = 0$). Because of symmetry, the steady-state probabilities satisfy

$$p_1 = p_5, \quad p_2 = p_7, \quad p_3 = p_6, \quad p_4 = p_8$$
$$p_1 + p_2 + p_3 + p_4 = 1/2. \quad [17]$$

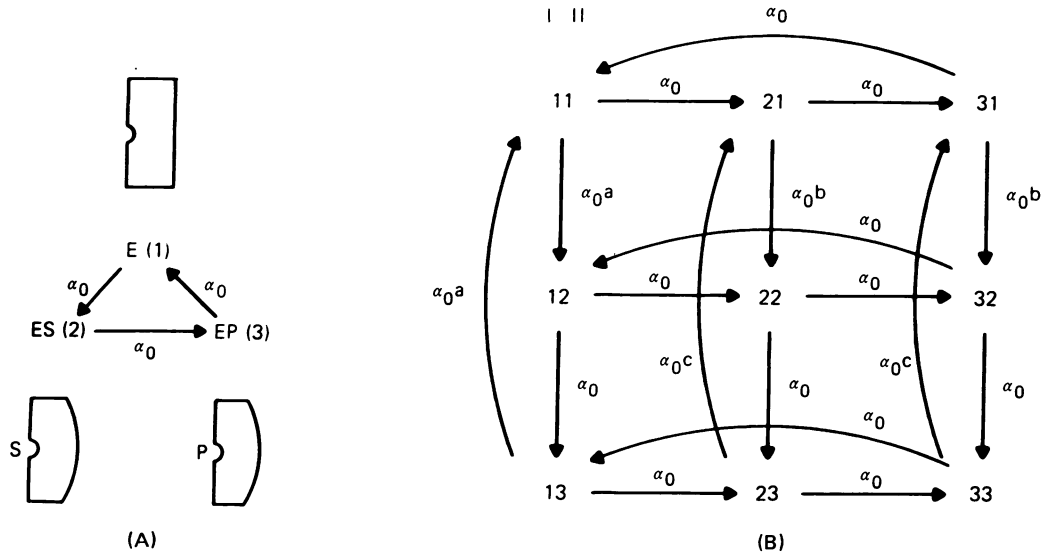

FIG. 6. (*A*) Rate constants and conformations for three-state monomer. (*B*) Rate constants for dimer. See text.

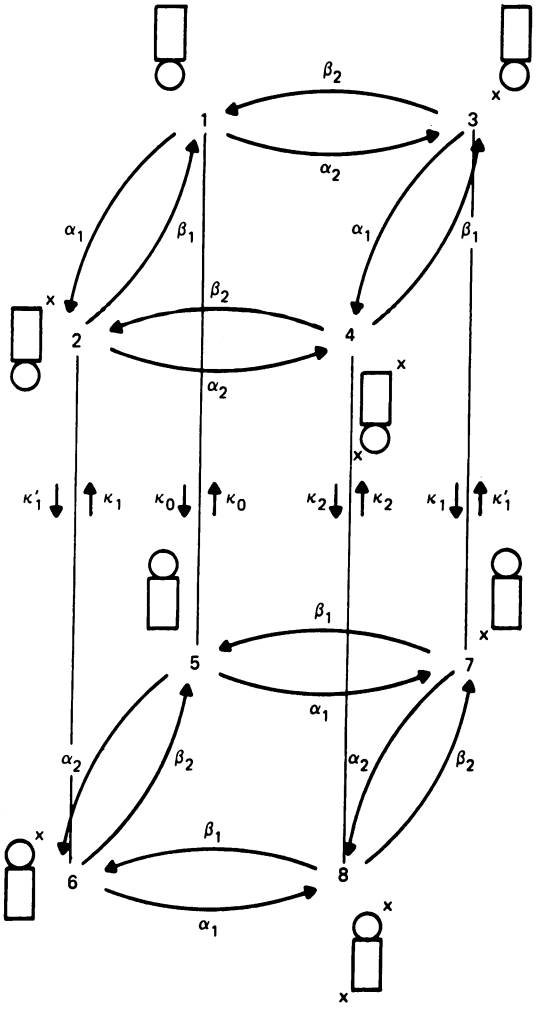

FIG. 7. Kinetic diagram for isologous dimer of two-state enzyme with concerted conformational changes. Rectangle (R) and circle (C) represent two conformations; X represents bound substrate. See text.

We find for these probabilities

$$\begin{aligned} p_1 &= (\beta_1 + \beta_2)(\beta_2 C + \beta_1 D)/E, \\ p_2 &= (\alpha_1 + \alpha_2)(\beta_1 + \beta_2)D/E \\ p_3 &= (\alpha_1 + \alpha_2)(\beta_1 + \beta_2)C/E, \\ p_4 &= (\alpha_1 + \alpha_2)(\alpha_1 C + \alpha_2 D)/E, \end{aligned} \qquad [18]$$

where

$$E = 2(AD + BC)$$

$$A = \beta_1(\beta_1 + \beta_2) + \alpha_2(\alpha_1 + \alpha_2) + (\alpha_1 + \alpha_2)(\beta_1 + \beta_2)$$

$$B = \beta_2(\beta_1 + \beta_2) + \alpha_1(\alpha_1 + \alpha_2) + (\alpha_1 + \alpha_2)(\beta_1 + \beta_2)$$

$$C = (\alpha_1 + \alpha_2)(\beta_1 + \beta_2)\kappa_1' + \alpha_2\beta_1(\alpha_1 + \alpha_2 + \beta_1 + \beta_2)$$

$$D = (\alpha_1 + \alpha_2)(\beta_1 + \beta_2)\kappa_1 + \alpha_1\beta_2(\alpha_1 + \alpha_2 + \beta_1 + \beta_2).$$

The two subunits have the same flux J (by symmetry). Using α_i transitions in the top subunit,

$$\begin{aligned} J &= \alpha_1(p_1 + p_3) + \alpha_2(p_5 + p_7) \\ &= (\alpha_1 + \alpha_2)(\beta_1 + \beta_2)[(\alpha_1 + \beta_2)C \\ &\qquad + (\alpha_2 + \beta_1)D]/E. \end{aligned} \qquad [19]$$

In the most important special case $\alpha_2 = \beta_2 = 0$ (half-the-sites reactivity):

$$p_1 \sim \beta_1^2\kappa_1, \quad p_2 \sim \alpha_1\beta_1\kappa_1, \quad p_3 \sim \alpha_1\beta_1\kappa_1', \quad p_4 \sim \alpha_1^2\kappa_1'$$

$$J = \alpha_1\beta_1/2(\alpha_1 + \beta_1). \qquad [20]$$

1. Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3632–3636.
2. Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4111–4115.
3. Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5227–5230.
4. Hill, T. L. (1978) *J. Theor. Biol.*, in press.
5. Hill, T. L. (1977) *Free Transduction in Biology* (Academic Press, New York).
6. Stryer, L. (1975) *Biochemistry* (W. H. Freeman and Co., San Francisco).
7. Seydoux, F., Malhotra, O. P. & Bernhard, S. A. (1974) *Crit. Rev. Biochem.* **2**, 227–257.