Further study of the effect of enzyme–enzyme interactions on steady-state enzyme kinetics

(linear enzyme chain/Ising problem/Bragg–Williams approximation/phase transition/dilute enzyme solution)

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Contributed by Terrell L. Hill, June 15, 1977

ABSTRACT   This paper continues an earlier one [Hill, T. L. (1977) *Proc. Natl. Acad. Sci. USA* 74, 3632–3636] and presents further introductory examples. Most attention is devoted to a closed linear chain of two-state enzyme molecules with nearest-neighbor interactions. The one-dimensional Ising theory can be used here. The Bragg–Williams (mean field) approximation is introduced to deal with a one-, two-, or three-dimensional lattice of enzyme molecules, at steady state, with an arbitrary kinetic diagram. The behavior of the flux in a phase transition is noted. Finally, a treatment is given for the first effect (second "virial" coefficient) of interactions on the flux in a dilute solution of two-state enzyme molecules.

This subject was introduced in a previous publication (1). The present paper is a continuation of the earlier one, using the same notation, in which we examine more complicated systems. We continue to use the non-unique rate constant convention in equation 4 of ref. 1. This is appropriate for present purposes, but is too restrictive for some biochemical applications.

Closed One-Dimensional Two-State Chain. As a theoretical prototype, we study a closed linear chain (ring) of M identical enzyme molecules, each of which has a two-state cycle with unperturbed (1) rate constants as shown in Fig. 1. There are interaction free energies w_{11}, w_{12}, w_{22} between nearest-neighbor pairs of molecules in the chain in states 11, 12 (or 21), 22, respectively. These interactions alter the unperturbed rate constants: the instantaneous rate constant for any transition of a given molecule will depend, in general, on the instantaneous states (1 or 2) of its two nearest neighbors. We shall make the symmetrical choice (1) for the "split" of inverse constants: $f_\alpha = f_\beta = 1/2$. Explicitly, for the three kinds of nearest-neighbor pairs, the central molecule of a triplet of molecules is assumed to have rate constants as follows:

$$111 \rightleftarrows 121 \quad \alpha = \alpha_0 y_{12}/y_{11}, \ \alpha' = \alpha_0' y_{11}/y_{12}$$
$$\beta = \beta_0 y_{11}/y_{12}, \ \beta' = \beta_0' y_{12}/y_{11} \qquad [1]$$

$$211 \rightleftarrows 221 \quad \alpha = \alpha_0 y_{22}^{1/2}/y_{11}^{1/2}, \ \alpha' = \alpha_0' y_{11}^{1/2}/y_{22}^{1/2}$$
$$\beta = \beta_0 y_{11}^{1/2}/y_{22}^{1/2}, \ \beta' = \beta_0' y_{22}^{1/2}/y_{11}^{1/2} \qquad [2]$$

$$212 \rightleftarrows 222 \quad \alpha = \alpha_0 y_{22}/y_{12}, \ \alpha' = \alpha_0' y_{12}/y_{22}$$
$$\beta = \beta_0 y_{12}/y_{22}, \ \beta' = \beta_0' y_{22}/y_{12}, \qquad [3]$$

where $y_{ij} \equiv e^{-w_{ij}/kT}$. As already explained (1), the "population" properties of this particular system at steady state will be those of a quasi-equilibrium system. Hence we can obtain these properties, as needed, from the well-known one-dimensional equilibrium Ising problem (2, 3), which can be solved exactly and easily by the matrix method (3–5).
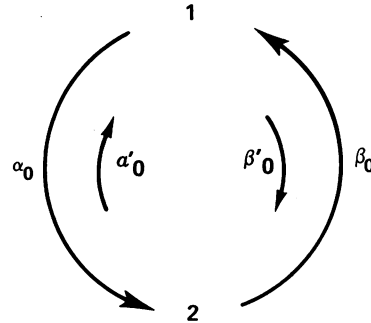
FIG. 1.   Two-state cycle with unperturbed rate constant notation. The counterclockwise direction is supposed dominant.

The flux per molecule, J, around the cycle in Fig. 1 but including the effect of interactions, can be expressed in terms of the mean numbers of triplets of various kinds, $\overline{N}_{ijk}$. The total number of triplets (also pairs) in the chain is M. Using the α, α' reactions,

$$MJ = \overline{N}_{111}\alpha_0(y_{12}/y_{11}) - \overline{N}_{121}\alpha_0'(y_{11}/y_{12})$$
$$+ 2[\overline{N}_{211}\alpha_0(y_{22}/y_{11})^{1/2} - \overline{N}_{221}\alpha_0'(y_{11}/y_{22})^{1/2}]$$
$$+ \overline{N}_{212}\alpha_0(y_{22}/y_{12}) - \overline{N}_{222}\alpha_0'(y_{12}/y_{22}). \qquad [4]$$

An equivalent expression can be written using the β, β' reactions. To proceed further, we need the $\overline{N}_{ijk}$ from the Ising theory.

One-Dimensional Ising Theory (3–5). The grand partition function for the ring of M molecules (regarding the system as "open" with respect to the number N_2 of molecules in state 2) can be written as the sum of a product of contributions L from each molecular pair in the ring:

$$\Xi = \sum_{\sigma_m = 1,2} \prod_{m=1}^{M} L(\sigma_m, \sigma_{m+1}), \qquad [5]$$

where σ_m is the state index for the mth molecule in the chain ($M + 1 \equiv 1$). The sum is over the 2^M possible states of the whole chain ($\sigma_m = 1,2; m = 1,\cdots,M$). L is a 2×2 matrix:

$$L = \begin{pmatrix} y_{11} & y_{12}x^{1/2} \\ y_{12}x^{1/2} & y_{22}x \end{pmatrix}, \qquad [6]$$

where (1) $x \equiv (\alpha_0 + \beta_0')/(\beta_0 + \alpha_0')$. In the notation of section 14-1 of ref. 2, $x = q\lambda$. Eq. 6 gives proper weight to each kind of pair and, in Ξ, to each of the 2^M system-states. If we perform the sum in Eq. 5 in the order $\sigma_M, \sigma_{M-1}, \cdots, \sigma_2$, we get

$$\Xi = \sum_{\sigma_1} L^M(\sigma_1, \sigma_1) = L^M(1,1) + L^M(2,2) = \lambda_1^M + \lambda_2^M, \qquad [7]$$

Abbreviation: BW, Bragg–Williams.

where L^M represents a matrix product and λ_1, λ_2 are the two eigenvalues of L,

$$\lambda_1,\lambda_2 = (y_{11} + y_{22}x \pm \sqrt{\ })/2 \qquad [8]$$

with

$$\sqrt{\ } = [(y_{11} - y_{22}x)^2 + 4y_{12}^2 x]^{1/2}. \qquad [9]$$

We take λ_1 as the larger eigenvalue (+ sign).

If we define $\theta = \overline{N}_2/M$, then because each of the 2^M states in Ξ is weighted properly,

$$\theta = L^M(2,2)/\Xi. \qquad [10]$$

If we sum over $\sigma_M, \cdots, \sigma_3$ in Eq. 5,

$$\Xi = \sum_{\sigma_1} \sum_{\sigma_2} L(\sigma_1,\sigma_2) L^{M-1}(\sigma_2,\sigma_1). \qquad [11]$$

Therefore, as in Eq. 10,

$$\overline{N}_{\sigma_1\sigma_2}/M = L(\sigma_1,\sigma_2) L^{M-1}(\sigma_2,\sigma_1)/\Xi. \qquad [12]$$

Similarly, for triplets,

$$\overline{N}_{\sigma_1\sigma_2\sigma_3}/M = L(\sigma_1,\sigma_2)L(\sigma_2,\sigma_3)L^{M-2}(\sigma_3,\sigma_1)/\Xi. \qquad [13]$$

To use Eqs. 10, 12, and 13, we need also (ref. 5, equation 2.80)

$$L^s(\sigma,\sigma') = \lambda_1^s \phi_1(\sigma)\phi_1(\sigma') + \lambda_2^s \phi_2(\sigma)\phi_2(\sigma'), \qquad [14]$$

where ϕ_1 and ϕ_2 are the eigenvectors belonging to λ_1 and λ_2, respectively. For example,

$$\phi_1(1) = (y_{11} - y_{22}x + \sqrt{\ })A_1$$
$$\phi_1(2) = 2y_{12}x^{1/2}A_1, \qquad [15]$$

where the normalization constant is given by

$$A_1^{-2} = 2[(y_{11} - y_{22}x)^2 + 4y_{12}^2 x + (y_{11} - y_{22}x)\sqrt{\ }]. \qquad [16]$$

Eqs. 4 and 13 provide the flux J for any finite value of $M \geq 3$. However, the algebra is quite involved except in simple special cases (e.g., equations 25 of ref. 1 with $x = 1$).

Fortunately, the case of most interest, $M \to \infty$, is easy to handle because the smaller eigenvalue λ_2 can be ignored. We find from Eqs. 10, 12, and 13,

$$\theta = 2y_{12}^2 x/\sqrt{\ } (y_{11} - y_{22}x + \sqrt{\ }) \qquad [17]$$

$$\overline{N}_{11}/M = y_{11}(y_{11} - y_{22}x + \sqrt{\ })/\sqrt{\ }( ) \qquad [18]$$
$$\overline{N}_{12}M = 2y_{12}^2 x/\sqrt{\ }( )$$

$$\overline{N}_{22}/M = y_{22}x(y_{22}x - y_{11} + \sqrt{\ })/\sqrt{\ }( )$$

$$\overline{N}_{111}/M = y_{11}^2(y_{11} - y_{22}x + \sqrt{\ })/\sqrt{\ }[ ]$$

$$\overline{N}_{211}/M = 2y_{11}y_{12}^2 x/\sqrt{\ }[ ]$$

$$\overline{N}_{121}/M = y_{12}^2 x(y_{11} - y_{22}x + \sqrt{\ })/\sqrt{\ }[ ] \qquad [19]$$

$$\overline{N}_{212}/M = y_{12}^2 x(y_{22}x - y_{11} + \sqrt{\ })/\sqrt{\ }[ ]$$

$$\overline{N}_{221}/M = 2y_{22}y_{12}^2 x^2/\sqrt{\ }[ ]$$

$$\overline{N}_{222}/M = y_{22}^2 x^2(y_{22}x - y_{11} + \sqrt{\ })/\sqrt{\ }[ ],$$

where

$$( ) = y_{11} + y_{22}x + \sqrt{\ }$$
$$[ ] = y_{11}^2 + 2y_{12}^2 x + y_{22}^2 x^2 + (y_{11} + y_{22}x)\sqrt{\ }.$$

Symmetrical pairs and triplets are omitted above ($\overline{N}_{21}$, etc.).

The Steady-State Flux. Though exact, the $\overline{N}_{ij}$ and $\overline{N}_{ijk}$ obey simple "quasi-chemical" relations (see the *Appendix*). These

permit Eq. 4 for J to be rewritten as

$$MJ = [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')][\overline{N}_{111}(y_{12}/y_{11}) + 2\overline{N}_{211}(y_{22}/y_{11})^{1/2} + \overline{N}_{212}(y_{22}/y_{12})]. \qquad [20]$$

Using Eqs. 19, this becomes, finally,

$$J = [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')][(y_{11} - y_{22}x)^2 + 4(y_{11}y_{22})^{1/2}y_{12}x + (y_{11} + y_{22}x)\sqrt{\ }]y_{12}/\sqrt{\ }[ ]. \qquad [21]$$

An expression for J/J_0, where J_0 is the unperturbed flux (equation 10 of ref. 1), is obtained by replacing the α_0,β_0 bracket [ ] (in the numerator) by $1 + x$.

Special Cases. There are many special cases of Eq. 21. We consider a few. Suppose, for example, that $y_{12}^2 = y_{11}y_{22}$, which might be expected for nonspecific van der Waals forces between the molecules. Then we find

$$J/J_0 = (y_{11}y_{22})^{1/2}(1 + x)/(y_{11} + y_{22}x). \qquad [22]$$

The distribution of states 1 and 2 along the chain is random, with $\theta = y_{22}x/(y_{11} + y_{22}x)$.

Suppose $w_{11} = w_{22} = -w_{12}$ (e.g., state 1 has a charge -1 and state 2 a charge $+1$). Then $y_{11} = y_{22}$ and $y_{12} = 1/y_{22}$. If y_{22} is small as well ($w_{22}/kT \to \infty$),

$$J/J_0 \to y_{22}^2(1 + x^{1/2})^2 (1 + x)/2x \to 0. \qquad [23]$$

In this case the molecules tend to alternate their states: 1212⋯.

The main special case we consider (1) is $w_{11} = w_{12} = 0$ and $w_{22} \neq 0$. That is, $y_{11} = y_{12} = 1$. Incidentally, this could mean physically (as in a real aggregate of enzymes), that w_{11}, say, is chosen as the *arbitrary zero* of interaction free energy and that $w_{12} = w_{11}$ while $w_{22} \neq w_{11}$. Actually, relative to infinite separation, w_{11} would presumably be negative. For simplicity of notation, we write $y \equiv y_{22}$. Then

$$J = \left(\frac{\alpha_0\beta_0 - \alpha_0'\beta_0'}{\beta_0 + \alpha_0'}\right) \frac{[(1 - yx)^2 + 4y^{1/2}x + (1 + yx)\sqrt{\ }]}{\sqrt{\ }[1 + 2x + y^2x^2 + (1 + yx)\sqrt{\ }]},$$
$$[24]$$

where

$$\sqrt{\ } = [(1 - yx)^2 + 4x]^{1/2}.$$

Eq. 24 will be illustrated numerically below. When $y \to 0$ (22 pairs excluded),

$$J = \left(\frac{\alpha_0\beta_0 - \alpha_0'\beta_0'}{\beta_0 + \alpha_0'}\right) \frac{[1 + (1 + 4x)^{1/2}]}{(1 + 4x)^{1/2}[1 + 2x + (1 + 4x)^{1/2}]}. \qquad [25]$$

In this case, from Eq. 17, an alternative expression is

$$J = (\alpha_0\beta_0 - \alpha_0'\beta_0')\theta/(\alpha_0 + \beta_0'), \qquad [26]$$

which is the same as equation 26 of ref. 1 (for small M).

When $y \to \infty$ in Eq. 24 (strong attraction between state 2 neighbors),

$$J \to (\alpha_0\beta_0 - \alpha_0'\beta_0')/(\alpha_0 + \beta_0')y$$
$$J/J_0 \to (1 + x)/y. \qquad [27]$$

Almost all molecules are in state 2 ($\theta \to 1$); the flux is small.

Excluded 22 Pairs ($y = 0$). There is an alternative way to obtain Eq. 26, which we digress to consider. This is based on the finite M approach in ref. 1. $\Xi(M)$ is the grand partition function for a closed chain (Eq. 5); we denote the same function for an open chain by $\Xi_0(M)$ (we used subscripts *cl* and *op*, respectively, in ref. 1). For finite M the flux in Eq. 25 becomes (1)

$$J = [(\alpha_0\beta_0 - \alpha_0'\beta_0')/(\beta_0 + \alpha_0')]\Xi_0(M - 3)/\Xi(M). \qquad [28]$$
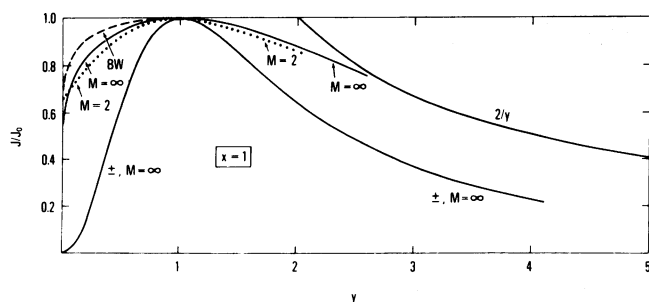
FIG. 2. Effect of interaction parameter y on relative flux J/J_0 at constant x ($x = 1$). The $M = \infty$ curve is for a one-dimensional system with $w_{11} = w_{12} = 0$. The $M = 2$ curve (dotted) is for a pair of interacting enzyme molecules with $w_{11} = w_{12} = 0$. The BW curve (broken) is for the Bragg–Williams approximation (see Fig. 4). BW follows $M = \infty$ closely for $y > 1$. All three of these curves approach $2/y$ asymptotically as $y \to \infty$. The lowest curve ($\pm$, $M = \infty$) is for the noncomparable case $w_{11} = w_{22} = -w_{12}$ (Eq. 38).

The Ξ quotient here can be seen directly (1) to be equal to θ/x. But we follow a more devious path. Using the relation (that can be observed in equations 25 of ref. 1; see also the *Appendix*)

$$\Xi_0(M) = \Xi(M) + x^2 \Xi_0(M - 4),\qquad [29]$$

we have for the Ξ quotient in Eq. 28:

$$\Xi \text{ quotient} = \frac{\Xi_0(M - 3)}{\Xi_0(M) - x^2\, \Xi_0(M - 4)}.\qquad [30]$$

In the limit $M \to \infty$, the subscript can be dropped because open and closed chains have the same properties. Thus,

$$\Xi \text{ quotient} \to \frac{[\Xi(M - 3)/\Xi(M)]}{1 - x^2[\Xi(M - 4)/\Xi(M)]}.\qquad [31]$$

Now, from Eqs. 8 and 17,

$$(\ln \Xi)/M = \ln \lambda_1 = \ln\{[1 + (1 + 4x)^{1/2}]/2\}$$
$$= \ln[(1 - \theta)/(1 - 2\theta)].\qquad [32]$$

Also, we need the Taylor expansion

$$\Xi(M - m) = \Xi(M) + \frac{\partial \Xi}{\partial M}(-m) + \frac{1}{2!}\frac{\partial^2 \Xi}{\partial M^2}(-m)^2 + \cdots,$$
$$[33]$$

where x and T are constant in the derivatives. Because $\ln \Xi$ is an extensive property,

$$\frac{\partial^n \Xi}{\partial M^n} = \Xi\left(\frac{\partial \ln \Xi}{\partial M}\right)^n = \Xi\left(\frac{\ln \Xi}{M}\right)^n.\qquad [34]$$

Thus, it follows from Eq. 33 that

$$\Xi(M - m)/\Xi(M) = \exp\{-m[(\ln \Xi)/M]\}$$
$$= [(1 - 2\theta)/(1 - \theta)]^m.\qquad [35]$$

We substitute this result (for $m = 3$ and 4) into Eq. 31 and use

$$x = \theta(1 - \theta)/(1 - 2\theta)^2\qquad [36]$$

from Eq. 32, to obtain, finally,

$$\Xi \text{ quotient} = (1 - 2\theta)^2/(1 - \theta) = \theta/x.\qquad [37]$$

Some further properties of Ξ and Ξ_0 are given in the *Appendix*.

Numerical Examples. The effect of the interaction free energy w_{22} on the flux is illustrated in Fig. 2. Here the solid curve $M = \infty$ is a plot of J/J_0, from Eq. 24, as a function of $y = e^{-w_{22}/kT}$ with x held constant at $x = 1$ (i.e., there is no bias
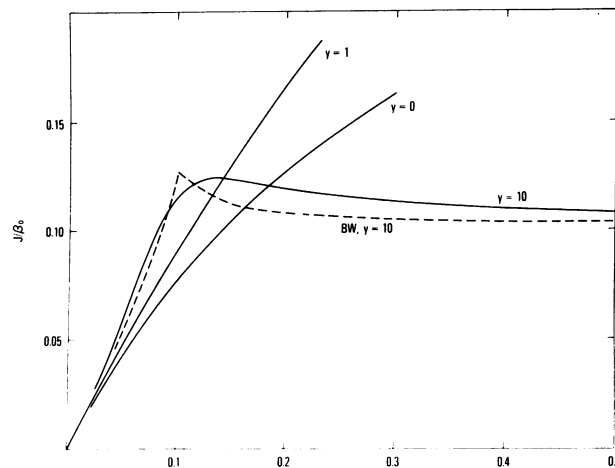

FIG. 3. One-dimensional $M = \infty$ system (Eq. 24) with one-way cycle: J/β_0 as a function of x (ligand concentration), with y constant. See *text* for further details. The BW curve (Fig. 4) is for comparison.

with respect to state 1 or 2). Either attraction or repulsion between state 2 molecules reduces the flux below J_0. When y is large, $J/J_0 \to 2/y$ (Eq. 27). When $y \to 0$, $J/J_0 \to 2\theta = 1 - 5^{-1/2} = 0.553$ (Eq. 26).

Also included in Fig. 2 is a solid curve labeled $\pm$, $M = \infty$. This is for the case $w_{11} = w_{22} = -w_{12}$ (compare Eq. 23) with $y \equiv y_{22}$ and $x = 1$. Here we find

$$J/J_0 = 4y^2/(1 + y^2)^2.\qquad [38]$$

This curve has symmetry with respect to y and y^{-1}.

The dotted curve in Fig. 2 is the $x = 1$ case for a pair of molecules only ($M = 2$), with $w_{11} = w_{12} = 0$ and $w_{22} \neq 0$ as in Eq. 24 for $M = \infty$. From equation 19 of ref. 1,

$$J/J_0 = 2(1 + y)/(3 + y^2),\qquad [39]$$

where here $y \equiv y_{22}^{1/2} = e^{-w_{22}/2kT}$. The general definition of a comparable variable for any system of this type (22 interactions) is $y \equiv e^{-zw_{22}/2kT}$, where z = number of nearest neighbors (this is used in the next section). When $M = 2$, $z = 1$. Eq. 39 also gives $J/J_0 \to 2/y$ for $y \to \infty$, but $J/J_0 \to 2/3$ for $y \to 0$. There is surprisingly little difference between the $M = 2$ and $M = \infty$ curves.

As a final example, we consider Eq. 24 as a function of x, with y constant. To attach an explicit physical significance to the x variation, one can suppose that (Michaelis–Menten): β_0' is negligible; β_0 and α_0' are held constant; and $\alpha_0 = (\beta_0 + \alpha_0')x$ is a pseudo-first-order rate constant that is proportional to the concentration of a ligand that is bound in the $\alpha_0 (1 \to 2)$ process. That is, x is proportional to ligand concentration.

It is convenient to plot J/β_0 as a function of x. For the unperturbed system ($y = 1$), $J_0/\beta_0 = x/(1 + x) = \theta$ (Michaelis–Menten kinetics). For arbitrary y, $J(x)/\beta_0$ is given by Eq. 24 if we replace the α_0, β_0 parentheses ( ) in that equation by x. Fig. 3 shows $J(x)/\beta_0$ for $y = 0$, $y = 1$, and $y = 10$. The $y = 0$ and $y = 1$ curves are also plots of $\theta(x)$ (above, and Eq. 26). The $y = 1$ curve saturates at a value 1, the $y = 0$ curve at 1/2 (Eq. 36). The $y = 10$ curve corresponds to fairly strong attraction. In the Bragg–Williams (BW) approximation (next section), there is a phase transition at this y (the broken BW curve in Fig. 3 is taken from Fig. 4). As y gets larger, the $J(x)/\beta_0$ curves become flatter and lower, $J/\beta_0 \to 1/y$ (Eq. 27), except for small x, where $J/\beta_0 \cong x \cong \theta$. Note the dominant qualitative fact that this flux ($1/y$) is very small compared to the maximum flux

$J_0/\beta_0 \to 1$ (as $x \to \infty$), because the β transition $2 \to 1$ occurs against the attraction of two neighbors in state 2.

The two $y = 10$ curves in Fig. 3 are fairly similar in magnitude, though the BW curve has a discontinuity in slope. As is well known, this one-dimensional system will not show a phase transition (discontinuity) at any y.

Qualitatively (solid $y = 10$ curve), attractive interactions increase the flux, compared to J_0/β_0, at small x but decrease it at larger x. Computer calculations show that small repulsive interactions decrease the flux for $x < 1$ and increase it for $x > 1$.

Bragg–Williams Approximation. This equilibrium approximation (2, 6, 7), also known as the mean field approximation, is extended here to steady states (8–12). It can be applied to one-, two-, and three-dimensional lattices (but may predict a nonexistent phase transition in one dimension). The basic idea is that the fluctuating instantaneous environment of a given molecule, emphasized in ref. 1, is here replaced—as an approximation—by a steady average environment. There is no need, in this approach, to enumerate or consider the system-states. It suffices to study a single (average) molecule in the system and its states i (1).

Let p_i (an unknown, to begin with) be the probability of any state i for the "central" molecule. The same p_i applies to the nearest neighbors. The interaction free energy between the central molecule in state i, and its z nearest neighbors, is then $w_{ie} = z\Sigma_k p_k w_{ik}$, in which the sum is over all possible states k. Similarly, if the central molecule is in state j, $w_{je} = z\Sigma_k p_k w_{jk}$. Thus, the altered rate constants for the arbitrary transition pair ij of the kinetic diagram are, from equation 4 of ref. 1,

$$\alpha_{ij} = \alpha_{ij}^0 \exp\left[zf_{ij}\sum_k p_k(w_{ik} - w_{jk})/kT\right]$$

$$\alpha_{ji} = \alpha_{ji}^0 \exp\left[z(1 - f_{ij})\sum_k p_k (w_{jk} - w_{ik})/kT\right]. \tag{40}$$

The steady-state probabilities p_i of the central molecule can now be expressed, in the same way as for an independent molecule (13), in terms of the rate constants α_{ij} of the kinetic diagram. But the α_{ij} values in the present problem themselves depend on the p_i (Eq. 40). Thus, we have a set of implicit equations in the p_i, together with $\Sigma_i p_i = 1$, from which a self-consistent set of p_i must be found (numerically). The flux is then calculated from the α_{ij} and the p_i as for independent molecules (13).

Two-State Cycle. As an explicit example, consider the two-state cycle in Fig. 1 (an advantage of the BW approximation is that arbitrarily more complicated kinetic diagrams than this can also be handled numerically). The rate constants, from equations 40 and 11 of ref. 1, are

$$\alpha = \alpha_0 Y^{f_\alpha}, \quad \alpha' = \alpha_0' Y^{f_\alpha - 1}$$

$$\beta = \beta_0 Y^{-f_\beta}, \quad \beta' = \beta_0' Y^{1-f_\beta}, \tag{41}$$

where

$$Y = (y_{12}/y_{11})^z (y_{11}y_{22}/y_{12}^2)^{z\theta} \tag{42}$$

and $\theta = p_2 = 1 - p_1$. Also, $p_2 = \theta$ as a function of rate constants is (13)

$$\theta = (\alpha + \beta')/(\alpha + \beta' + \beta + \alpha'). \tag{43}$$

Eq. 43 is the one implicit equation, here, in the one unknown θ. Having calculated θ from Eq. 43, the flux per molecule is given by (13)

$$J = (\alpha\beta - \alpha'\beta')/(\alpha + \beta' + \beta + \alpha'). \tag{44}$$

In the special case $f_\alpha + f_\beta = 1$, in this two-state system (1), Eq. 43 takes the *equilibrium* form

$$\theta = Yx/(1 + Yx)$$

or $\tag{45}$

$$Yx = \theta/(1 - \theta).$$

As is well known (2), this equation can produce a phase transition. Also, Eq. 44 becomes

$$J/J_0 = Y^{f_\alpha}(1 + x)/(1 + Yx), \tag{46}$$

where J_0 is given by equation 10 of ref. 1. We take $f_\alpha = 1/2$, as usual (1).

We turn now to the special case $w_{11} = w_{12} = 0$, for which $Y = y_{22}^{z\theta}$. We define $y = y_{22}^{z/2}$ (see the sentence following Eq. 39). Then $Y = y^{2\theta}$ and

$$y^{2\theta}x = \theta/(1 - \theta)$$

$$J/J_0 = y^\theta(1 + x)/(1 + y^{2\theta}x). \tag{47}$$

When w_{22}/kT is very small ($y \to 1$),

$$J/J_0 \to 1 + (zw_{22}/2kT)\theta_0(2\theta_0 - 1) + \cdots, \tag{48}$$

where $\theta_0 = x/(1 + x)$. The linear correction term here is zero in Fig. 2 because $\theta_0 = 1/2$ ($x = 1$).

Eq. 48 agrees with equation 20 of ref. 1 ($z = 1$) and with Eq. 24 ($z = 2$) in the limit $y \to 1$. It is well known (2) that BW is exact, in equilibrium systems, in the limit $y \to 1$. It appears that BW is also exact for any two-state, steady-state lattice system with $f_\alpha = f_\beta = 1/2$ (despite the additional kinetic instantaneous $\to$ average environment assumption).

Numerical Examples. The broken curve in Fig. 2 is a plot of Eq. 47 (J/J_0 as a function of y) with $x = 1$. At large y, again $J/J_0 \to 2/y$. The slope of $J(y)/J_0$ becomes infinite as $y \to 0$ (the BW approximation is especially unrealistic in this limit). Generally, the three top curves in Fig. 2 ($M = 2$, $M = \infty$, BW) are very similar.

We next consider J as a function of x, with y constant, as in Fig. 3. As in the preceding section, we take $\beta_0' = 0$ and $\alpha_0 = (\beta_0 + \alpha_0)x$. We have here

$$J(x)/\beta_0 = y^\theta x/(1 + y^{2\theta}x). \tag{49}$$

Fig. 4 shows $J(x)/\beta_0$ and $\theta(x)/10$ for a number of choices of y. The critical curve, with a cusp in the flux, is $y = e^2$. There is a phase transition for $y > e^2$, for example, for $y = 10$. At the phase transition, there is a discontinuity in $\theta(x)$ but only a discontinuity in the slope of $J(x)$ (the broken regions in the $y = 10$ curves are metastable or unstable). That is, the flux is the same in the two phases (as can be verified analytically). For large y, $J/\beta_0 \to 1/y$ (except at very small x, where $J/\beta_0 \cong x \cong \theta$). This is the same asymptotic behavior as in the one-dimensional chain (above).

This phase transition effect in J is interesting physically but rather unlikely biologically. Note that whereas $\theta(x)$ allows the usual single hysteresis loop (ref. 2, p. 251), $J(x)/\beta_0$ would exhibit a corresponding "double loop" (like a bow tie).

In less symmetrical, non-quasi-equilibrium BW cases, the flux is *not* the same in the two phases (part four, to be published).

Dilute Two-State Enzyme in Solution. This problem will be considered more extensively elsewhere. Here we state a few results, without a detailed proof. We consider the simplest possible case: we take $f_\alpha = f_\beta = 1/2$ so that the steady-state molecular distributions are as at equilibrium; we assume radially dependent distributions only; also, we find only the sec-
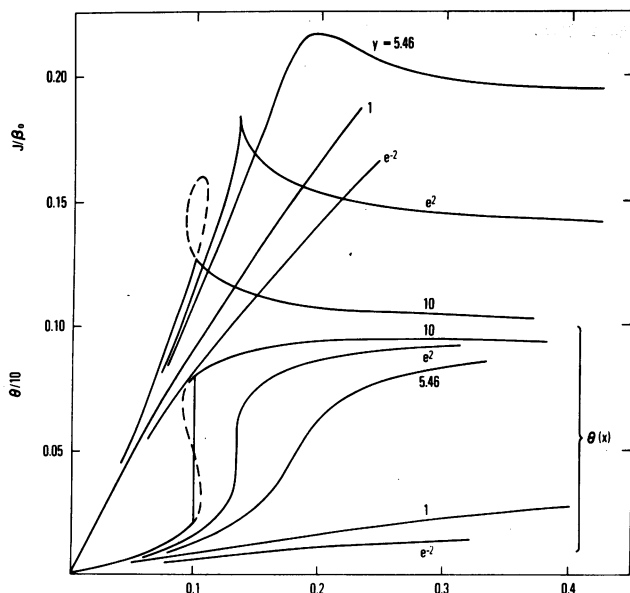
FIG. 4.   Plots of J/β_0 (upper set) and $\theta/10$ (lower set) as function of x in the BW approximation with a one-way cycle and $w_{11} = w_{12} = 0$. This corresponds to Fig. 3 for a one-dimensional system. There is a phase transition for $y > e^2$. See text for further details.

ond "virial" coefficient for the flux. Infinite separation ($r = \infty$) is chosen as the zero of interaction free energy for all pairs (compare Eq. 24).

The interaction free energies are w_{ij} (r), with $i, j = 1, 2$. We use the grand partition function method employed in equations (15-78) and (15-79) of ref. 2 for the polarization of an imperfect gas in an electric field. For the second "virial" coefficient, we need consider only one molecule in the macroscopic volume V (with flux J_1, denoted by J_0 above) and two molecules in V (total flux J_2 for the two molecules, including interaction effects). From the above two equations (replacing the electric moment M_N by J_N), we find

$$J/J_0 = 1 + (Z_2/J_1 V)\left(\frac{1}{2}J_2 - J_1\right)\rho + \cdots, \qquad [50]$$

where ρ is the total enzyme number density (N/V) and Z_2 is the configuration integral (2) for two molecules in V (see below).

To find J_2, we have to take into account the probability the two molecules are in states ij, then the probability that they are a distance apart between r and $r + dr$, and finally the instantaneous transition probabilities (equations 11 of ref. 1). We obtain from these considerations

$$J_2/2 = (J_1 V/Z_2) \int_0^\infty [(1 - \theta_0)(y_{11}y_{12})^{1/2}$$
$$+ \theta_0(y_{22}y_{12})^{1/2}]4\pi r^2 dr. \qquad [51]$$

The explicit expression for Z_2/V (2) is

$$Z_2/V = \int_0^\infty [(1 - \theta_0)^2 y_{11} + 2\theta_0(1 - \theta_0)y_{12} + \theta_0^2 y_{22}]4\pi r^2 dr,$$
$$\qquad [52]$$

where $\theta_0 = x/(1 + x)$. Thus, finally, on substituting Eqs. 51 and 52 in Eq. 50, we have

$$J/J_0 = 1 + \rho \int_0^\infty \{[(1 - \theta_0)(y_{11}y_{12})^{1/2} + \theta_0(y_{22}y_{12})^{1/2}]$$
$$-[(1 - \theta_0)^2 y_{11} + 2\theta_0(1 - \theta_0)y_{12} + \theta_0^2 y_{22}]\}4\pi r^2 dr + \cdots.[53]$$

The integral is the second "virial" coefficient for the flux.

APPENDIX

Inspection of Eqs. 18 and 19 shows the following "quasi-chemical" relations (2), mentioned in the text:

$$\overline{N}_{11}\overline{N}_{22}/\overline{N}_{12}\overline{N}_{21} = y_{11}y_{22}/y_{12}^2 \qquad [54]$$

$$\overline{N}_{121}/\overline{N}_{111} = y_{12}^2 x/y_{11}^2, \ \overline{N}_{221}/\overline{N}_{211} = y_{22}x/y_{11}$$
$$\overline{N}_{222}/\overline{N}_{212} = y_{22}^2 x/y_{12}^2, \ \overline{N}_{212}\overline{N}_{111}/\overline{N}_{211}^2 = 1. \qquad [55]$$

Alternative combinations are possible.

Relations involving Ξ and Ξ_0 (Eqs. 28 and 29) are:

$$\theta = M^{-1} d\ln \Xi/d\ln x = x\ \Xi_0(M - 3)/\Xi(M) \qquad [56]$$

$$x\ \Xi(M - 2) + \Xi(M - 1) = \Xi(M) \text{ (or } \Xi_0 \text{ for } \Xi \text{ throughout)}$$
$$\qquad [57]$$

$$x\ \Xi_0(M - 3) + \Xi_0(M - 1) = \Xi(M) \qquad [58]$$

$$\Xi(M) = 1 + Mx + \frac{M(M - 3)}{2!}x^2 + \frac{M(M - 4)(M - 5)}{3!}x^3$$
$$+ \frac{M(M - 5)\cdots(M - 7)}{4!}x^4 + \cdots \qquad [59]$$

$$\Xi_0(M) = 1 + Mx + \frac{(M - 1)(M - 2)}{2!}x^2$$
$$+ \frac{(M - 2)\cdots(M - 4)}{3!}x^3 + \cdots. \qquad [60]$$

Eqs. 59 and 60 are polynomials that break off at the first zero term (1). Eq. 29 follows from Eqs. 57 and 58.

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