Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5227–5230, December 1977
Chemistry

"Virial" expansion of enzyme flux and use of quasi-chemical approximation for two-state enzymes with enzyme–enzyme interactions

(steady-state kinetics/enzyme lattice/Ising problem/phase transition/enzyme aggregation)

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, September 12, 1977

ABSTRACT **Two examples of enzyme systems with interactions, at steady state, are treated here. In both cases, the enzyme cycle has two states and quasi-equilibrium in spatial distributions obtains at steady state (because $f_\alpha + f_\beta = 1$). The first example is a dilute solution of enzyme molecules in a solvent. The flux (turnover) per molecule is expanded in powers of the enzyme concentration (a "virial" expansion). Aggregation of the enzyme molecules in solution is considered as a special case. In the second example, we treat an arbitrary lattice of enzyme molecules, with nearest-neighbor interactions, using the well-known quasi-chemical approximation. The flux per molecule is obtained. Critical behavior and hysteresis are illustrated.**

This paper is the third in a series (1, 2) concerned with the effect of enzyme–enzyme interactions on steady-state kinetic properties of enzymes. This subject can also be described by saying that we are beginning to extend some well-known molecular interaction problems in equilibrium statistical mechanics to steady-state conditions. For the present, at least, our primary concern is with the theoretical problem itself rather than with possible biological applications.

We have not treated transients yet except incidentally in our Monte Carlo work (see below).

This paper is devoted to the two topics mentioned in the title. In both of these cases, we can use the quasi-equilibrium approach explained in ref. 1: an isolated ("unperturbed") enzyme molecule has the states and rate constants shown in Fig. 1; and $f_\alpha + f_\beta = 1$ in equations 11 of ref. 1.

Strictly steady-state systems (i.e., where quasi-equilibrium does not apply) are usually much more difficult to treat theoretically because there is no general method available corresponding to the partition function formalism that is applicable to equilibrium systems. Some simple special cases of this type will be discussed in a fourth paper (see also ref. 1). Also, Monte Carlo calculations on strictly steady-state lattice systems have been initiated in collaboration with Yi-der Chen, as well as Bragg–Williams calculations with Leonard Stein.

Dilute two-state enzyme in solution

An introductory account of this topic was included in the previous paper (2). We consider a dilute solution of two-state enzyme molecules (Fig. 1), with $f_\alpha + f_\beta = 1$. Hence the steady-state population distributions are those of an equilibrium system (1). There are pairwise-additive interactions w_{ij} ($i,j = 1,2$) that perturb the enzyme flux; these might depend on the rotational orientation of the two molecules as well as on their distance apart, r. Equations (40.1) through (40.12) of ref. 3 show how to include rotational coordinates without complicating the formalism. Our object is to express J/J_0, where J is the flux per molecule and J_0 the unperturbed flux per molecule (equation 10 of ref. 1), as a power series in the enzyme concentration or number density ρ ($= \overline{N}/V$). The method used is general. We carry the work as far as the third "virial" coefficient.

The two-dimensional version of this and the following section would apply to enzyme molecules moving in a "clean" membrane (e.g., black-lipid or liposome).

It is advantageous to regard the solution of volume V as open with respect to enzyme molecules (grand partition function method). Let J_N be the total flux when there are N molecules in V. Then, on averaging over N,

$$\overline{J} = \sum_N J_N Q_N \lambda^N / \sum_N Q_N \lambda^N, \qquad [1]$$

where Q_N is the canonical partition function (4) and λ the absolute activity. The flux per molecule J (above) is $\overline{J}/\overline{N}$ in this notation, where

$$\overline{N} = \sum_N N Q_N \lambda^N / \sum_N Q_N \lambda^N. \qquad [2]$$

The unperturbed flux J_0 (above) is the same quantity as J_1 in Eq. 1.

This is formally the same problem as in equations (12–14), (12–17), (15–78), and (15–81) of ref. 4 for the electric polarization of an imperfect gas (J_N replaces the electric moment M_N). As in equation (15–78), we use Eq. 1 to write $\overline{J}$ as a single power series in λ. In this series we then replace λ by the concentration activity $z = Q_1\lambda/V$ and replace Q_N by the configuration integral Z_N, from equation (15–5) of ref. 4. Finally, we replace z by ρ, using equation (15–11), and divide by $J_1\rho V$. The result is

$$\frac{J}{J_0} = 1 + \left[a_2 + \left(\frac{J_2 Z_2}{2J_1V^2} - 1\right)V\right]\rho + \left[a_3 + 2\left(\frac{J_2Z_2}{2J_1V^2} - 1\right)a_2 V + \left(\frac{J_3Z_3}{6J_1V^3} - \frac{J_2Z_2}{2J_1V^2} - \frac{Z_2}{2V^2} + 1\right)V^2\right]\rho^2 + \cdots, \qquad [3]$$

where (4)

$$a_2 = -2b_2, \quad a_3 = -3b_3 + 8b_2^2$$
$$2Vb_2 = Z_2 - v^2, \quad 6Vb_3 = Z_3 - 3VZ_2 + 2V^3.$$

The term in ρ is the same as in equation 50 of ref. 2.

The explicit expression for Z_2 is

$$Z_2 = \int[(1-\theta_0)^2 y_{11} + 2\theta_0(1-\theta_0)y_{12} + \theta_0^2 y_{22}]d\tau_a d\tau_b, \qquad [4]$$

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.

Abbreviations: BW, Bragg–Williams; QC, quasi-chemical.

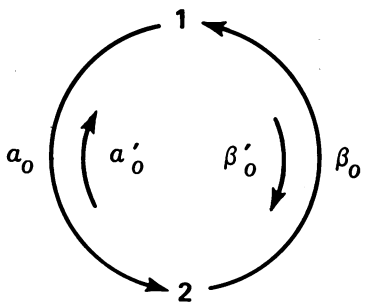

FIG. 1. Two-state cycle with unperturbed rate constant notation. The counterclockwise direction is supposed dominant.

where $y_{ij} = e^{-w_{ij}/kT}$, θ_0 is the unperturbed probability of state 2 (equation 9b, ref. 1), and τ_a represents the translational and rotational coordinates of molecule a [equations (40.11) and (40.90), ref. 3] of the pair a,b. The zero of w_{ij} is infinite separation ($r = \infty$). Similarly, for Z_3 we have

$$Z_3 = \int[(1-\theta_0)^3 y_{11}(ab)y_{11}(ac)y_{11}(bc) + 3(1-\theta_0)^2\theta_0 y_{11}(ab)y_{12}(ac)y_{12}(bc) + 3(1-\theta_0)\theta_0^2 y_{12}(ab)y_{12}(ac)y_{22}(bc) + \theta_0^3 y_{22}(ab)y_{22}(ac)y_{22}(bc)]d\tau_a d\tau_b d\tau_c. \quad [5]$$

The notation $y_{12}(ac)$, for example, means that molecule a is in state 1 and molecule c is in state 2.

We turn now to J_2 and use the α,α' transitions to evaluate this quantity (the β,β' transitions give the same result, on using $f_\alpha + f_\beta = 1$). From Eq. 4, the probability that both a and b are in state 1 and are located in $d\tau_a d\tau_b$ is $(1-\theta_0)^2 y_{11} d\tau_a d\tau_b / Z_2$. In this case the probability of a $11 \rightarrow 12$ or 21 transition, per unit time, is $2\alpha_0 y_{12}^{f_\alpha}/y_{11}^{f_\alpha}$ (equation 11, ref. 1). These two probabilities are multiplied together. We must add a similar term for 12 or $21 \rightarrow 22$ (α) and subtract terms for $22 \rightarrow 12$ or 21 (α') and 12 or $21 \rightarrow 11$ (α'). Finally, we factor out $J_1 \equiv J_0 = \alpha_0(1-\theta_0) - \alpha'_0\theta_0$ and integrate, to obtain

$$J_2 = (2J_1/Z_2)\int[(1-\theta_0)y_{11}^{1-f_\alpha}y_{12}^{f_\alpha} + \theta_0 y_{12}^{1-f_\alpha}y_{22}^{f_\alpha}]d\tau_a d\tau_b. \quad [6]$$

The same type of argument can be used for J_3. There are six terms rather than four, as above. For example, for $111 \rightarrow 211$, 121, or 112 (α) we have

$$\frac{(1-\theta_0)^3 y_{11}(ab)y_{11}(ac)y_{11}(bc)d\tau_a d\tau_b d\tau_c}{Z_3} \times \frac{3\alpha_0 y_{12}^{f_\alpha}(ba)y_{12}^{f_\alpha}(ca)}{y_{11}^{f_\alpha}(ab)y_{11}^{f_\alpha}(bc)}.$$

We combine the six terms, factor out J_1, and integrate:

$$J_3 = (3J_1/Z_3)\int[(1-\theta_0)^2 y_{11}^{1-f_\alpha}(ab)y_{11}^{1-f_\alpha}(ac)y_{12}^{f_\alpha}(ba) \times y_{12}^{f_\alpha}(ca)y_{11}(bc) + 2\theta_0(1-\theta_0)y_{11}^{1-f_\alpha}(ab)y_{12}^{1-f_\alpha}(ac) \times y_{12}^{f_\alpha}(ba)y_{22}^{f_\alpha}(ac)y_{12}(bc) + \theta_0^2 y_{12}^{1-f_\alpha}(ab)y_{12}^{1-f_\alpha}(ac) \times y_{22}^{f_\alpha}(ab)y_{22}^{f_\alpha}(ac)y_{22}(bc)]d\tau_a d\tau_b d\tau_c. \quad [7]$$

Eqs. 4 through 7 may now be substituted into Eq. 3. The coefficient of ρ in Eq. 3 (the second "virial" coefficient in the flux) becomes

$$\frac{1}{V}\int\{[(1-\theta_0)y_{11}^{1-f_\alpha}y_{12}^{f_\alpha} + \theta_0 y_{12}^{1-f_\alpha}y_{22}^{f_\alpha}] - [(1-\theta_0)^2 y_{11} + 2\theta_0(1-\theta_0)y_{12} + \theta_0^2 y_{22}]\}\, d\tau_a d\tau_b. \quad [8]$$

The integrand is zero except when molecules a and b are close enough to each other to interact. If the state change ($1 \rightleftarrows 2$) has no effect on the interaction free energy (i.e., if $y_{11} \equiv y_{12} \equiv y_{22}$), the integrand is zero everywhere and $J = J_0$. If the w_{ij} depend on intermolecular distance r only, then Eq. 8 becomes $\int_0^\infty\{\}4\pi r^2 dr$, as in equation 53, ref. 2. If in this case we also have $w_{11} = w_{12}$ and $w_{22} = w_{11} + W$, where $W/kT \ll 1$ (i.e., the 22 interaction differs slightly from the 11 and 12 interactions), then Eq. 8 simplifies to

$$\theta_0(\theta_0 - f_\alpha)\int_0^\infty y_{11}(W/kT)4\pi r^2 dr. \quad [9]$$

This is similar to equation 20 of ref. 1 and to equation 48 of ref. 2. The radial distribution function of the enzyme molecules is y_{11}.

The necessary ingredients for the third "virial" coefficient in Eq. 3 are given above. But we leave further details to the interested reader (see also Eq. 12, below).

Enzyme aggregation in solution

This section is concerned with the most important special case of the above quite general approach. We suppose here that enzyme–enzyme attractive interactions are strong enough to lead to the formation of aggregates of sizes two, three, four, etc. For example, we might have nonspecific (no distinction between states 1 and 2) associating forces supplemented by quite specific forces that influence the enzyme flux in an aggregate. We suppose further that the nonassociating forces (e.g., "hard" interactions) between monomers, dimers, etc. have no effect on enzyme flux. This is probably a very realistic assumption. The effects of interactions on the flux of the enzyme molecules of an aggregate are handled as for the small systems ($M = 2,3,\cdots$) already discussed in refs. 1 and 2, and discussed further in parts four and five (to be published). We give only general relations here and do not consider explicit models for the aggregates.

Fortunately, the theory of aggregation in solution (5) is already available, so very little new work is required here. One way to proceed is to introduce the necessary notation (5) for an equilibrium solution of aggregates into Eq. 3. We have done this, but it is much simpler to use equations (34)–(37) of ref. 5 directly. These equations give the number density ρ_s of aggregates of size s ($s = 1,2,\cdots$) as power series in ρ. We denote the total enzyme flux of a dimer by J_{01}, of a trimer by J_{001}, etc. Then the mean flux per enzyme molecule is simply

$$J = (\rho_1 J_1 + \rho_2 J_{01} + \rho_3 J_{001} + \cdots)/\rho \quad [10]$$

since

$$\rho = \rho_1 + 2\rho_2 + 3\rho_3 + \cdots. \quad [11]$$

On substituting equations (34)–(36) of ref. 5 into Eq. 10, and dividing by $J_1 \equiv J_0$, we obtain the special case of Eq. 3,

$$\frac{J}{J_0} = 1 + \left[K_2\left(\frac{J_{01}}{J_1} - 2\right)\right]\rho + \left[\left(\frac{J_{01}}{J_1} - 2\right)(-4K_2 + b_{110} - 4b_{20})K_2 + \left(\frac{J_{001}}{J_1} - 3\right)K_3\right]\rho^2 + \cdots, \quad [12]$$

where K_s is the equilibrium constant for s-mer formation from monomers, and b_{110} and b_{20} are nonassociative cluster integrals (4, 5) for monomer–dimer and monomer–monomer, respectively. The "equilibrium" between states 1 and 2 can be taken care of explicitly, if necessary, essentially as in ref. 6 (where a binding equilibrium is treated). The quantities in square

brackets are the second and third flux "virial" coefficients. If there are no interaction effects on the flux within the aggregates, then $J_{01} = 2J_1$, $J_{001} = 3J_1$, etc., and we obtain $J/J_0 = 1$. As already mentioned, particular models are needed (e.g., equations 25 of ref. 1) to evaluate $(J_{01}/J_1) - 2$, etc. The interested reader can easily write out the term in ρ^3 in Eq. **12**, using the additional terms given in equations (34)–(37) of ref. 5.

Incidentally, in the first method mentioned above (direct use of Eq. 3), we encounter also J_{20}, J_{30}, J_{110}, etc. In accordance with our assumptions, these are set equal to J_1^2, J_1^3, $J_1 + J_{01}$, etc., respectively.

Finally, we note that Eqs. **10–12** do not show explicitly that they refer to two-state enzymes with $f_\alpha + f_\beta = 1$. They might therefore provide the basis for an approximate treatment of flux and aggregation in much more complicated cases.

Quasi-chemical approximation of enzyme lattice

In the previous paper (2) we used the Bragg–Williams (BW) approximation for a steady-state lattice (z nearest neighbors) of interacting two-state enzyme molecules (Fig. 1), with $f_\alpha + f_\beta = 1$ (1). In the same paper, we gave an exact treatment of the one-dimensional system ($z = 2$), with $f_\alpha = f_\beta = 1/2$. In both cases we could use a quasi-equilibrium approach (1, 2). The objective of the present section is to introduce the quasi-chemical (QC) approximation (with $f_\alpha + f_\beta = 1$) as a refinement of the BW approximation. It is well-known (4) that QC is a significant improvement on BW, and also that QC is exact in one dimension. Hence, an incidental by-product of the present section is the extension of the exact one-dimensional treatment (2) from $f_\alpha = f_\beta = 1/2$ to $f_\alpha + f_\beta = 1$.

A rather similar enzyme kinetics problem was discussed in 1952 (7). We use the same method here. A characteristic of the QC approximation is that the nearest-neighbor pairs of the lattice can be treated as statistically independent of each other, except for QC and conservation relations (4). It is this feature that we exploit below.

There are M enzymes in the lattice. The fraction of these in state 2 is $\theta = \bar{N}_2/M$. The numbers of nearest-neighbor pairs of the different types, in this approximation, are (4)

$$\bar{N}_{11}/M = (z/2)(1-\theta)(\beta + 1 - 2\theta)/(\beta + 1)$$
$$\bar{N}_{12}/M = \bar{N}_{21}/M = (z/2)2\theta(1-\theta)/(\beta + 1)$$
$$\bar{N}_{22}/M = (z/2)\theta(\beta - 1 + 2\theta)/(\beta + 1), \quad [13]$$

where

$$\beta^2 = 1 - 4\theta(1-\theta)[1 - (y_{11}y_{22}/y_{12}^2)]. \quad [14]$$

If an enzyme is in state 1, the probability r that any particular one of its z nearest neighbors is in state 1 is determined by $r/(1-r) = \bar{N}_{11}/\bar{N}_{12}$ (here we use the assumed statistical independence of pairs, mentioned above). This gives

$$r = (\beta + 1 - 2\theta)/(\beta + 1). \quad [15]$$

Similarly, if an enzyme is in state 2, the probability p that a particular nearest neighbor is in state 1 is determined by $p/(1-p) = \bar{N}_{21}/\bar{N}_{22}$. Hence,

$$p = 2(1-\theta)/(\beta + 1). \quad [16]$$

If an enzyme is in state 1, the probability that j of its z nearest neighbors are in state 2 is then

$$R_j = z!r^{z-j}(1-r)^j/j!(z-j)!. \quad [17]$$

If the "central" enzyme is in state 2, replace r by p and R_j by P_j. These are the probabilities of different environments for the central molecule; they are needed for the corresponding instantaneous rate constants (which we designate by $\alpha_{(j)}$, $\alpha'_{(j)}$, etc.). Explicitly, from equation 11 of ref. 1 and $f_\alpha + f_\beta = 1$, we have

$$\alpha_{(j)}/\alpha_0 = \beta'_{(j)}/\beta'_0 = s_{12}^{z-j}s_{22}^j/s_{11}^{z-j}s_{12}^j$$
$$\alpha'_{(j)}/\alpha'_0 = \beta_{(j)}/\beta_0 = (s_{12}/y_{12})^{z-j} \times (s_{22}/y_{22})^j/(s_{11}/y_{11})^{z-j}(s_{12}/y_{12})^j, \quad [18]$$

where $s_{ik} \equiv y_{ik}^{f_\alpha}$.

Using the α,α' transitions, the flux per enzyme molecule is

$$J = \sum_{j=0}^{z}[(1-\theta)R_j\alpha_{(j)} - \theta P_j\alpha'_{(j)}]$$
$$= \alpha_0(1-\theta)\left[\frac{rs_{12}}{s_{11}} + \frac{(1-r)s_{22}}{s_{12}}\right]^z - \alpha'_0\theta\left[\frac{ps_{12}y_{11}}{s_{11}y_{12}} + \frac{(1-p)s_{22}y_{12}}{s_{12}y_{22}}\right]^z. \quad [19]$$

After some manipulation, we find the alternate but equivalent forms

$$J = \left(\frac{\alpha_0\beta_0 - \alpha'_0\beta'_0}{\beta_0 + \alpha'_0}\right)(1-\theta) \times \left[\frac{(\beta + 1 - 2\theta)s_{12}}{(\beta+1)s_{11}} + \frac{2\theta s_{22}}{(\beta+1)s_{12}}\right]^z \quad [20]$$
$$= \left(\frac{\alpha_0\beta_0 - \alpha'_0\beta'_0}{\alpha_0 + \beta'_0}\right)\theta \times \left[\frac{2(1-\theta)s_{12}y_{11}}{(\beta+1)s_{11}y_{12}} + \frac{(\beta - 1 + 2\theta)s_{22}y_{12}}{(\beta+1)s_{12}y_{22}}\right]^z. \quad [21]$$

The variable $x \equiv (\alpha_0 + \beta'_0)/(\beta_0 + \alpha'_0)$ plays the role here of an "activity" (1, 2). Its relation to θ follows immediately from the simulated detailed balance (1) expression

$$[\alpha_{(j)} + \beta'_{(j)}](1-\theta)R_j = [\beta_{(j)} + \alpha'_{(j)}]\theta P_j \quad [22]$$

for $1 \rightleftarrows 2$ transitions with j nearest neighbors in state 2. We find (for any j)

$$x\left(\frac{y_{22}}{y_{11}}\right)^{z/2} = \frac{\theta}{1-\theta}\left[\frac{(\beta - 1 + 2\theta)(1-\theta)}{(\beta + 1 - 2\theta)\theta}\right]^{z/2}, \quad [23]$$

in agreement with equation (14–61) of ref. 4. In numerical calculations, one would assign θ a value and then calculate J and x from Eqs. **20** and **23**. One can show that these equations agree with one-dimensional and BW results in ref. 2 as special or limiting cases. As is well known (4), Eq. **23** can show a phase transition. The critical value of $y_{11}y_{22}/y_{12}^2$ is $z^2/(z-2)^2$.

Special Case. We turn now to the special case (2) $y_{11} = y_{12} = 1$, $f_\alpha = f_\beta = 1/2$, $\beta'_0 = 0$. This is a Michaelis–Menten enzyme with 22 interactions only. To be more explicit (2), w_{11} is arbitrarily chosen as zero, $w_{12} = w_{11}$, and $w_{22} \neq w_{11}$. Equation **20** becomes in this case

$$J/\beta_0 = x(1-\theta)[(\beta + 1 - 2\theta + 2\theta y_{22}^{1/2})/(\beta + 1)]^z. \quad [24]$$

The behavior of $J(x)/\beta_0$ and $\theta(x)$ (Fig. 2) is similar to that seen in figure 4, ref. 2. When $y_{22} = 1$, $J/\beta_0 = x/(1+x)$ (unperturbed); that is, $J = J_0$.

The critical point occurs at

$$y_{22} = z^2/(z-2)^2, \quad x = [(z-2)/z]^z$$
$$\theta = 1/2, \quad J/\beta_0 = (1/2)[(z-2)/(z-1)]^z. \quad [25]$$

For example, if $z = 6$ (hexagonal planar lattice), $y_{22} = 2.25$ ($w_{22} = -472$ cal mol^{-1} at 20°C), $x = 0.088$, and $J/\beta_0 = 0.131$.

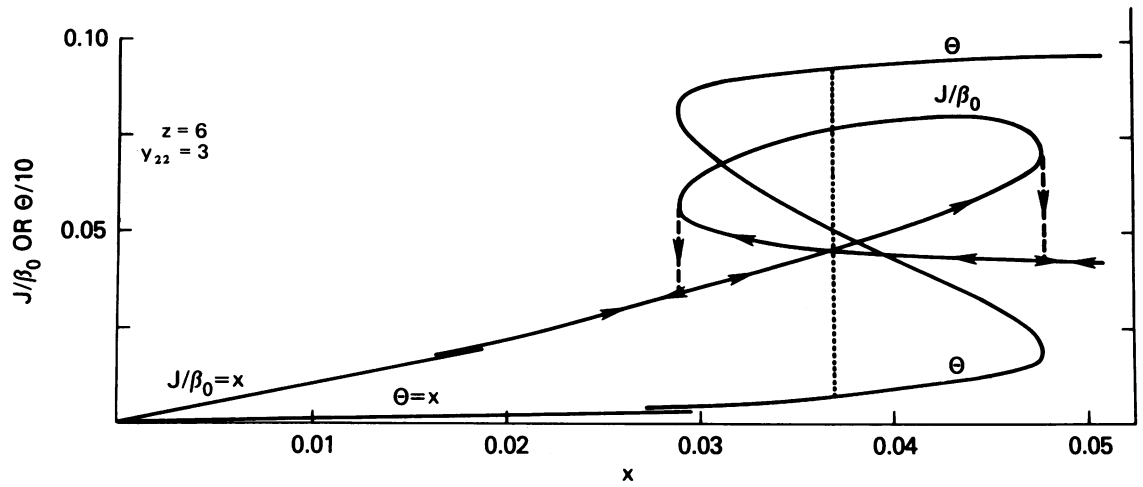

FIG. 2. Example of a phase transition, at steady state, in a lattice of enzyme molecules with $z = 6$ and $y_{22} = 3$, according to the quasi-chemical approximation. The flux per molecule (as J/β_0) and the fraction of molecules in state 2 (as $\theta/10$) are plotted against the "activity" x. The stable phase transition occurs at the dotted line. Metastable transitions (broken lines) lead to a "bow-tie" hysteresis loop (arrows) in the flux. Hysteresis in θ is not shown. The straight lines show asymptotic behavior at small x.

One can show analytically that, in a phase transition, J/β_0 has the same value in the two phases (2). See also Fig. 2. This is not the case in less symmetrical, strictly steady-state models (part four, to be published). The kind of "bow tie" hysteresis loop (2) that could occur in the flux is illustrated in Fig. 2, where $y_{22} = 3$ and $z = 6$.

It is well known (4) that θ is a symmetrical function of $\ln x$. The center of symmetry is at $\theta = 1/2$, $x = y_{22}^{-z/2}$. This is also the value of x at which the phase transition occurs, if y_{22} is larger than the critical value. For small x, $\theta \to x$ and $J/\beta_0 \to x$. For large x, $\theta \to 1 - y_{22}^{-z}x^{-1}$ and $J/\beta_0 \to y_{22}^{-z/2}$. This last result is in agreement with Eq. 18: $\beta_{(z)} = \beta_0 y_{22}^{-z/2}$ (since $\theta \to 1$). When y_{22} is large, $J/\beta_0 \cong x$ up to the point of phase transition, $x = y_{22}^{-z/2}$. Then $J/\beta_0 \cong y_{22}^{-z/2} =$ constant, beyond this value of x. The example in Fig. 2 comes fairly close to exhibiting these properties. Note especially that J/β_0 here is very small compared to the maximum possible flux, $J_0/\beta_0 \to 1$ (as $x \to \infty$). The same asymptotic behavior was found earlier (2) for the one-dimensional and BW systems.

In the limit $y_{22} \to 0$ (strong w_{22} repulsion), we obtain

$$x = [\theta/(1-\theta)][(1-\theta)/(1-2\theta)]^z, \quad J/\beta_0 = \theta. \qquad [26]$$

These should be compared with the exact results for $z = 2$ (1, 2).

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. (1956) *Statistical Mechanics* (McGraw-Hill, New York).
4. Hill, T. L. (1960) *Statistical Thermodynamics* (Addison-Wesley, Reading, MA).
5. Hill, T. L. & Chen, Y. (1973) *Biopolymers* **12**, 1285–1312.
6. Hill, T. L. (1966) *J. Chem. Phys.* **23**, 2270–2274.
7. Hill, T. L. (1952) *J. Am. Chem. Soc.* **74**, 4710–4711.