Cooperative Ligand Binding on Multidimensional Lattices: Bethe Approximation

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Synopsis

A binding isotherm for ligands on multidimensional lattices has been calculated in the Bethe approximation. In this lattice model, a single bound ligand is assumed to occupy a contiguous sequence of lattice sites in all possible configurations; interactions between neighboring bound ligands are included. This lattice model is equivalent to one used for treating polymer solutions; a configurational entropy of athermal polymer solutions has been utilized directly to represent bound ligands. Binding isotherms are examined for their dependences on lattice coordination numbers and the number of sites occupied by a single bound ligand. Critical conditions for phase transitions in single-ligand-species systems are also presented. The binding isotherm reported here can be applied to a wide variety of binding phenomena; it is exact for one-dimensional cases, i.e., it reduces to the binding isotherm of McGhee and von Hippel [(1974) *J. Mol. Biol.* **86,** 469–489] and should serve as a good approximation for higher-dimensional binding phenomena.

INTRODUCTION

Ligand-site binding phenomena widely observed in a variety of biological systems have stimulated the development of theories to analyze binding data in order to study underlying mechanisms. Since Scatchard[1] initially tried to analyze binding data by a graphic method subsequently termed Scatchard plots, there have been many attempts to extend theories and to apply them to particular phenomena. The simplest case in which a ligand occupies a single site with no interaction between bound ligands is well known as Langmuir adsorption theory. Generally, systems of molecules bound on an array of sites represented by regular arrangements of molecules of different species are often referred to as lattice gases. Lattice gases are equivalent to spin systems in magnetic fields, the so-called Ising model, with well-known extensions to helix–coil transitions of macromolecules.[2] Consequently, one-to-one binding cases have been examined in detail for any dimensional lattice. However, the range of ligand binding situations encompassed in biological systems is extremely broad. Ligands can range in size from atomic ions to protein molecules; general cases of single-ligand to multiple-site binding should be treated. Most theories that treat single-ligand binding to multiple sites are limited to ligand binding on a one-dimensional lattice such as ligand binding on linear polymers.[3–8] However, there are many ligand-binding biological systems whose proper representation should not be one-dimensional lattices but

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Biopolymers, Vol. 22, 2253–2271 (1983)

CCC 0006-3525/83/102253-19$02.90

rather two- or three-dimensional lattices. For two-dimensional lattices, special cases such as adsorption of monovalent and bivalent cations by membranes[9] and the binding of flexible ligands to the surface of proteins and other macromolecules[10–12] have been discussed; interligand interactions were not taken into account in either case. Another typical example of high-dimensional systems would be ligand binding to cell surface receptors[13,14]; although in this case receptors might be mobile rather than rigidly fixed on the cell surface, lattice models should be a good approximation even for such fluidlike systems, as exemplified by the success of lattice models of solutions.[15] Here we develop a lattice theory applicable to binding phenomena manifested by biological systems of arbitrary dimensionality.

The theories of polymer solution lattice models[15] already developed can be modified easily to calculate a binding isotherm for single ligand to multiple site binding on multidimensional lattices. In the lattice model treated here for ligand binding, a single bound ligand is assumed to be represented by a flexible chain that occupies a contiguous sequence of sites on a multidimensional lattice. Also, interactions between nearest-neighbor sites are taken into account; the interaction energies depend on whether those sites are bound to ligands or not. With these assumptions, the lattice model for ligand binding becomes equivalent to lattice models for polymer solutions; solvent molecules and linear polymers will correspond to unoccupied sites and ligands bound to multiple sites, respectively. We report a binding isotherm calculated by using a partition function[16] for polymer solutions based on the Bethe approximation. The effects of the coordination number of lattices and the number of sites occupied by a bound ligand on the binding isotherm are examined, as well as the critical condition for phase transitions in multidimensional lattices. The binding isotherm reported here is general and should be widely applicable to a variety of binding phenomena.

The Bethe approximation is well known as a higher-order representation of the Bragg-Williams or Weiss mean-field approximation. This approximation is exact for Bethe lattices that have no loop of lattice points and would be a good approximation for multidimensional binding phenomena. Here it should be noted that a one-dimensional lattice is a special case of Bethe lattices for which the Bethe approximation gives an exact solution.

BASIC THEORY

The grand canonical partition function for bound ligands is

$$\Xi = \sum_{\{N_\zeta\}} \left(\prod_{i=1} \lambda_i^{N_i} \right) Z(N;\{N_\zeta\}) \tag{1}$$

for multiligand species systems, where λ_i is the absolute activity of the ith ligand species and $Z(N;\{N_\zeta\})$ is a canonical partition function for a particular set of N_ζ, which specifies the number of bound ligands of the ζth species. N is the total number of sites. In the product over i, the index i takes values from 1 to the number of ligand species. The most probable number of bound ligands, N_j^*, which corresponds to the dominant term in the summation of the above equation, satisfies the familiar thermodynamic relation:

$$\ln \lambda_j + \frac{\partial \ln Z(N;\{N_\zeta^*\})}{\partial N_j^*} = 0 \tag{2}$$

These N_j^* are thermodynamic quantities observed as the number of bound ligands for a particular set of absolute activities. In a large system, i.e., for large N, N_j^* approaches the average of N_j. Subsequently, the superscript $*$ will be omitted.

From the equalities of chemical potentials between free and bound ligands, an intrinsic binding constant, K_j^0, for jth ligand species is related to λ_j as

$$K_j^0 N_j^f = \lambda_j Z(N;\{N_\zeta | N_j = 1, N_{\zeta \neq j} = 0\})/N \tag{3}$$

where N_j^f is the concentration of free ligands of the jth species. Then, a binding isotherm can be represented by eliminating λ_j from Eqs. (2) and (3):

$$\ln K_j^0 N_j^f = -\frac{\partial \ln Z(N;\{N_\zeta\})}{\partial N_j} + \ln Z(N;\{N_\zeta | N_j = 1, N_{\zeta \neq j} = 0\}) - \ln N \tag{4}$$

Partition Function, *Z*, in the Bethe Approximation

Lattice models can be used to represent more or less regular arrangements of sites, each of which can bind a ligand, and to take account of site exclusion by bound ligands and interactions between sites; each lattice point is identified as one site. The site exclusion of bound ligands has been simplified while retaining the characteristics of ligand binding in order to get the analytical forms of binding isotherms rather than taking account of complicated configurations of bound ligands.[10–12] A single bound ligand of the ith species is simply assumed to occupy a contiguous sequence of n_i sites or lattice points in all possible configurations. In other words, a bound ligand is assumed to be like a flexible linear chain consisting of n_i units each of which occupies a site. Also, interactions are assumed to occur only between nearest-neighbor sites. These assumptions are appropriate for many situations and have the advantage of making this binding representation completely equivalent to lattice models[15] for polymer solutions; the analogy is that solvent molecules and linear polymers correspond to unoccupied sites and ligands bound to multiple sites on a lattice, respectively. Thus, the partition function available for lattice models of polymer solutions can be utilized directly to treat ligand binding.

In the Bethe approximation, only the occurrence probabilities of specific site pairs and no longer site clusters are taken into account; of course, long-range correlations in the site-exclusion configurations of bound ligands are completely neglected. In other words, this approximation is exact only for Bethe lattices that have no loops of lattice points [Fig. 1(a)]. As a result, lattices are represented only by their coordination numbers, z, i.e., the number of nearest-neighbor points around a lattice point; $z = 6$ could represent either a cubic lattice or close-packed lattice in two dimensions. First, the Bethe approximation formalism is summarized briefly for ligand binding problems.

A partition function, Z, is represented by

$$Z(N;\{N_\zeta\}) = \exp\left(-\sum_{i=1} \frac{N_i(E_i + zq_iE_{ii}/2)}{kT}\right) \times \sum_{\{X_{\zeta\eta}\}} \exp\left(-\sum_{l>m=0} \sum \frac{zX_{lm}\epsilon_{lm}}{kT}\right) \omega(N;\{N_\zeta\}\{X_{\zeta\eta}\}) \quad (5)$$

where

$$q_i = [n_i - (2/z)(n_i - 1)] \quad (6)$$

$$\epsilon_{lm} = E_{lm} - (E_{ll} + E_{mm})/2 \quad (7)$$

k is the Boltzmann constant and T is temperature. E_i is the binding energy for the ith ligand species and E_{lm} ($= E_{ml}$) is the interaction energy between

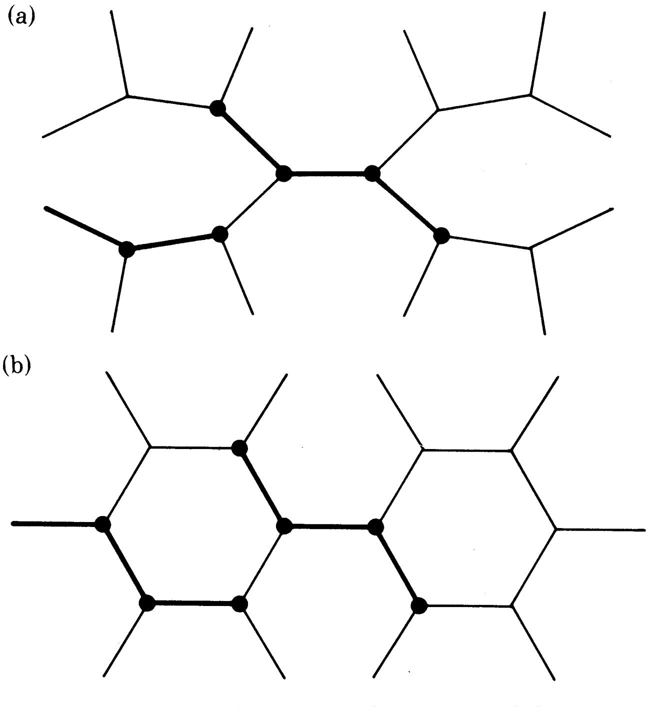


Fig. 1. (a) Bethe lattice with coordination number, $z = 3$, and (b) usual triangular lattice. In Bethe lattices, loops of lattice points are not included. Bold lines represent bound ligands.

nearest-neighbor sites that are bound to the lth and mth species; a subscript 0 represents unoccupied sites and E_{00} is defined as zero. zX_{lm} is the number of such nearest-neighbor site pairs. zq_i is the number of site pairs between a bound ligand of the ith species and neighboring sites. In Bethe lattices, these neighboring sites are either unoccupied or occupied by other ligands, because no loops are included in the lattice structures. Therefore, in the Bethe approximation that is exact for Bethe lattices, the interactions between nearest-neighbor sites, E_{lm}, are regarded as interligand interactions. In the present model, intraligand interactions for bound ligands are not taken into account beyond the assumption of random site exclusion. This basic assumption is employed to estimate the combinatory factor, $\omega(N;\{N_\zeta\}\{X_{\zeta\eta}\})$, which is the number of arrangements for placing N_ζ ligands of each ligand species on the N lattice points under the restriction that the number of neighboring site pairs bound to ζ and ηth ligand species is equal to $zX_{\zeta\eta}$. The combinatory factor, $\omega(N;\{N_\zeta\}\,\{X_{\zeta\eta}\})$, for Bethe lattices was previously calculated for lattice models of polymer solutions by Miller[17] and Kurata et al.[18] Therefore, the sum in Eq. (5) can be evaluated by a maximum term approximation. However, we take an alternative way, the quasi-chemical approximation, which was shown by Guggenheim[19,20] to be equivalent.

The sum in Eq. (5) is replaced by

$$\exp\left(-\sum_{l>m=0}\sum\frac{z\overline{\overline{X}}_{lm}\epsilon_{lm}}{kT}\right)\omega(N;\{N_\zeta\}) \equiv \sum_{\{X_{\zeta\eta}\}}\exp\left(-\sum_{l>m=0}\sum\frac{zX_{lm}\epsilon_{lm}}{kT}\right)\omega(N;\{N_\zeta\}\{X_{\zeta\eta}\}) \tag{8}$$

where

$$\omega(N;\{N_\zeta\}) \equiv \sum_{\{X_{\zeta\eta}\}}\omega(N;\{N_\zeta\}\{X_{\zeta\eta}\})$$

This $\overline{\overline{X}}_{lm}$ is related to a statistical average $\overline{X}_{lm}$ of X_{lm} as follows:

$$\overline{\overline{X}}_{lm}\epsilon_{lm} = \int_0^{\epsilon_{lm}}\overline{X}_{lm}\,d\epsilon_{lm} \tag{9}$$

Then, a binding isotherm of Eq. (4) is

$$\ln K_j^0 N_j^f = \frac{\partial}{\partial N_j}\sum_{l>m=0}\sum\frac{z\overline{\overline{X}}_{lm}\epsilon_{lm}}{kT} - \left(\sum_{l>m=0}\sum\frac{z\overline{\overline{X}}_{lm}\epsilon_{lm}}{kT}\right)_{N_j=1,\,N_{\zeta\neq j}=0} - \frac{\partial}{\partial N_j}\ln\omega(N;\{N_\zeta\}) + \ln\omega(N;\{N_\zeta|N_j=1,N_{\zeta\neq j}=0\}) - \ln N \tag{10}$$

If there are no interactions between nearest-neighbor sites, then the first and second terms reduce to zero. A combinatory factor, $\omega(N;\{N_\zeta\})$, which corresponds to a configurational entropy of athermal systems, will be taken from other earlier work on polymer solutions. The statistical average $\overline{X}$

is evaluated for a simple case in the quasi-chemical approximation and $\overline{\overline{X}}$ is calculated according to Eq. (9).

Combinatory Factor, $\omega(\mathrm{N};\{\mathrm{N}_\zeta\})$

The pioneering work in lattice models for polymer solution is the calculation of a combinary factor, $\omega(N;\{N_\zeta\})$, in the Bragg-Williams approximation by Flory[21]; $\omega(N;\{N_\zeta\})$ is the number of arrangements for placing N_ζ flexible chain molecules, each of which occupies a contiguous sequence of n_ζ sites, of each molecular species on the N lattice points. The higher-order combinatory factor in the Bethe approximation, in which account is taken of accommodation of the adjoining segment of the given chain on an adjacent site, was obtained by Huggins,[22,23] Miller,[17] Guggenheim,[24] and Kurata et al.[18] (See Refs. 2, 15, and 16 for reviews of their works.) The combinatory factor in the Bethe approximation is derived with a trivial extension to systems of multiligand species from equation (C-17) of Kurata et al.[18] Here, it should be noted that the assumption of random configurations for polymers that corresponds to random site exclusion has been employed to obtain this equation:

$$\ln \omega(N;\{N_\zeta\}) = \sum_{i=1} N_i \ln \frac{z(z-1)^{n_i-2}}{\sigma_i} - \sum_{m=0} N_m \ln\left(\frac{N_m}{N}\right) + \left(\frac{z}{2}\right)\left(N_0 + \sum_{i=1} q_i N_i\right) \ln \frac{N_0 + \sum_{i=1} q_i N_i}{N} \tag{11}$$

where N_0 is the number of unoccupied sites,

$$N_0 = N - \sum_{i=1} n_i N_i \tag{12}$$

The symmetry factor σ_i is equal to one for the case in which the head and tail of a ligand are distinguishable, otherwise two. This estimation of $\omega(N;\{N_\zeta\})$ is exact for Bethe lattices. The limiting case of $z \to \infty$ of Eq. (11) corresponds to the result for the Bragg-Williams approximation by Flory.[21] From the above equation, the third term in Eq. (10) is found to be

$$\frac{\partial \ln \omega(N;\{N_\zeta\})}{\partial N_j} = \ln \frac{z(z-1)^{n_j-2}}{\sigma_j} - \ln\left(\frac{N_j}{N}\right) + n_j \ln\left(\frac{N_0}{N}\right) - (n_j - 1) \ln \frac{(N_0 + \sum_{i=1} q_i N_i)}{N} \tag{13}$$

The fourth term in Eq. (10) is obvious for Bethe lattices:

$$\ln \omega(N;\{N_\zeta | N_j = 1, N_{\zeta \neq j} = 0\}) = \ln \frac{z(z-1)^{n_j-2}}{\sigma_j} + \ln N \tag{14}$$

Quasi-Chemical Approximation for the Statistical Average $\overline{X}$

As a simple case, let us consider nearest-neighbor interactions to be the same for all sites bound to different ligand species:

$$\begin{aligned} &\epsilon_{i0} = \epsilon_{0i} = \epsilon && \text{for all } i > 0 \\ &\epsilon_{ij} = \epsilon_{ji} = 0 && \text{for } i \neq j,\, i \neq 0, \text{ and } j \neq 0 \end{aligned} \tag{15}$$

There are $z(N_0 - X)/2$ free neighbor pairs, $z(\Sigma_{i=1} q_i N_i - X)/2$ neighboring site pairs occupied by ligands, and zX free-bound site pairs that are assumed to be in quasi-chemical equilibrium:

$$\frac{\overline{X}^2}{(N_0 - \overline{X})\,(\sum_{i=1} q_i N_i - \overline{X})} = \frac{1}{\gamma} \tag{16}$$

where

$$\begin{aligned} \overline{X} &\equiv \sum_{i=1} \overline{X}_{i0} \\ \gamma &\equiv \exp(2\epsilon/kT) \end{aligned} \tag{17}$$

Equation (16) is equivalent to the condition for maximizing the term on the right-hand side of Eq. (8), $\exp(-zX\epsilon/kT)\omega(N;\{N_\zeta\}X)$, with respect to X; $\omega(N;\{N_\zeta\}X)$ was given in Eq. (C-14b) of Kurata et al.[18] Then, $\overline{\overline{X}} \equiv \sum_{i=1} \overline{\overline{X}}_{i0}$ is from Eq. (9):

$$\frac{z\overline{\overline{X}}\epsilon}{kT} = \frac{z}{2}\left[N_0 \ln\left(1 + \frac{\sum_{i=1} q_i N_i(\beta - 1)}{N_0(\beta + 1)}\right) + \sum_{i=1} q_i N_i \ln\left(1 + \frac{N_0(\beta - 1)}{\sum_{i=1} q_i N_i(\beta + 1)}\right)\right] \tag{18}$$

where

$$\beta = \left[1 + \frac{4N_0 \sum_{i=1} q_i N_i}{(N_0 + \sum_{i=1} q_i N_i)^2}(\gamma - 1)\right]^{1/2} \tag{19}$$

This result is a trivial extension of that[16] for the single-component systems of polymer solutions. Then, the first term in Eq. (10) is found from Eq. (18):

$$\frac{\partial}{\partial N_j}\left(\frac{z\overline{\overline{X}}\epsilon}{kT}\right) = \frac{z}{2}\left[-n_j \ln\left(1 + \frac{\sum_{i=1} q_i N_i(\beta - 1)}{N_0(\beta + 1)}\right) + q_j \ln\left(1 + \frac{N_0(\beta - 1)}{\sum_{i=1} q_i N_i(\beta + 1)}\right)\right] \tag{20}$$

The second term for Bethe lattices is trivial:

$$\left[\frac{z\overline{\overline{X}}\epsilon}{kT}\right]_{N_j=1,\, N_{\zeta\neq j}=0} = \frac{zq_j\epsilon}{kT} \tag{21}$$

Finally, from Eqs. (13), (14), (20), and (21), the binding isotherm Eq. (10) is represented in an appropriate form for Scatchard plots by

$$\frac{N_j}{K_j^0 N_j^f N} = \left(\frac{N_0}{N}\right)^{n_j} \left(\frac{N_0 + \sum_{i=1} q_i N_i}{N}\right)^{(1-n_j)}$$
$$\times \left[1 + \frac{\sum_{i=1} q_i N_i (\beta - 1)}{N_0(\beta + 1)}\right]^{z n_j/2} \left[1 + \frac{N_0(\beta - 1)}{\sum_{i=1} q_i N_i (\beta + 1)}\right]^{-z q_j/2} \gamma^{z q_j/2} \qquad (22)$$

In the sums over i, the index i takes values from 1 to the number of ligand species. Equation (22) is general and includes a number of cases previously treated; it should be noted that Eq. (22) for a case of $z = 2$ is just the same as the binding isotherms of Eq. (15) of McGhee and von Hippel[6] or Eq. (25) of Schwarz[25] for one-dimensional lattices, as expected from the fact that a one-dimensional lattice is a special case of Bethe lattices for which Eq. (22) is exact. The limit of Eq. (22) as $z \to \infty$ with a fixed value of $z\epsilon$ corresponds to a binding isotherm in the Bragg-Williams approximation:

$$\lim_{\substack{z \to \infty \\ z\epsilon = \text{const}}} \left(\frac{N_j}{K_j^0 N_j^f N}\right) = \left(\frac{N_0}{N}\right)^{n_j} \exp\left(\frac{2\epsilon z n_j \sum_{i=1} n_i N_i}{kTN}\right) \qquad (23)$$

In the following, we will discuss the dependences of the binding isotherm on lattice structure and multiple site exclusion by a bound ligand.

Phase Transitions in Two- or Three-Dimensional Bethe Lattices

Phase transitions can occur in two- and three-dimensional lattices. First, let us calculate the equation of state, in order to derive critical conditions for phase transitions. A thermodynamic variable, Φ, conjugate to the number of sites, N, is

$$\frac{\Phi N}{kT} = -\sum_{i=1} N_i \frac{\partial \ln Z}{\partial N_i} + \ln Z \qquad (24)$$

Φ and N are analogous to the usual pressure and volume, respectively. Then, from Eqs. (5), (8), (11), (13), (18), and (20), the equation of state becomes

$$\frac{\Phi}{kT} = \ln \frac{N}{N_0} + \frac{z}{2} \ln \left(\frac{(N_0 + \sum_{i=1} q_i N_i) N_0(\beta + 1)}{N[N_0(\beta + 1) + \sum_{i=1} q_i N_i (\beta - 1)]}\right) \qquad (25)$$

Conditions for phase transitions are the equalities of Φ and λ_i among different phases; λ_i can be calculated from Eqs. (2), (5), (8), (13), and (20). These equations are just the same as those for mixing chemical potentials of solvent and polymers among different phases; $-\Phi$ will be the mixing chemical potential of a solvent, if unoccupied sites in ligand binding are interpreted as solvent molecules. The lattice model treated here for ligand binding is equivalent to that for polymer solutions. Thus, discussions[15,21,26] with respect to phase transitions in lattice models of polymer solutions are applicable to the present problems of ligand binding. Critical temperatures and phase diagrams have been examined in detail for polymer solutions that include multicomponent systems, although the Bragg-Williams approximations rather than the Bethe approximation have commonly been employed.

For simplicity, let us consider a system of one ligand species. The critical point is the point where the first and second partial derivatives of Φ with respect to N are both zero. For a simple case of $n_1 = 1$, critical values of γ, n_1N_1/N, and $\ln(K_1^0N_1^f\gamma^{zq_1/2})$ are found at $[z/(z-2)]^2$, 1/2, and 0, respectively. In the limit as $z \to \infty$ with $z\epsilon$ fixed, which corresponds to the Bragg-Williams approximation, the critical point[15,21] becomes

$$z \ln \gamma_c = \frac{1}{n_1}(1 + n_1^{1/2})^2 \tag{26a}$$

$$\left(\frac{n_1N_1}{N}\right)_c = (1 + n_1^{1/2})^{-1} \quad \text{for } z \to \infty \tag{26b}$$

These two limits suggest that γ_c is a decreasing function of both z and n_1; calculations in the Bethe approximation confirm this dependence to be more general. Critical values of γ, n_1N_1/N, and $\ln(K_1^0N_1^f\gamma^{zq_1/2})$ have been calculated numerically in the Bethe approximation by using Eq. (25) for various values of z and n_1. Figure 2 shows that these calculated γ_c exhibit the expected behavior. The exact values of γ_c calculated from the equation,[27] $\cosh[(\ln \gamma_c)/2] = \sec(\pi/z)$, for cases of triangular, square, and hexagonal two-dimensional lattices with $n_1 = 1$ are also shown as crosses in Fig. 2. Although the values of γ_c for the Bethe approximation are incorrect in the limit of $n_1 = 1$, this approximation is useful to demonstrate the qualitative effects of z and n_1; γ_c decreases as either n_1 or z increases.

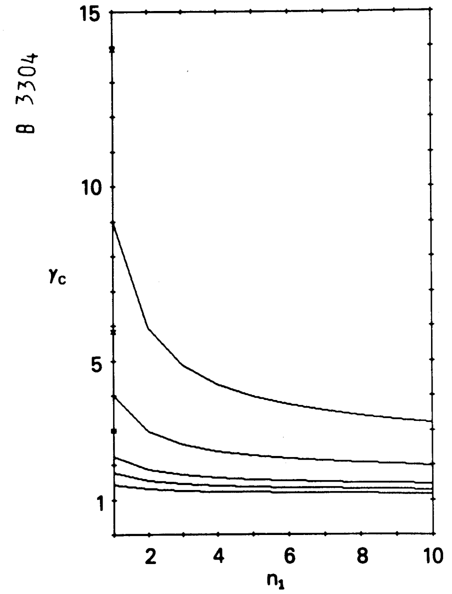


Fig. 2. Critical values of γ in the Bethe approximation for single ligand species. Curves from top to bottom are for z = 3, 4, 6, 8, and 12. X's show exact values of γ_c for $n_1 = 1$ from top to bottom for triangular, square, and hexagonal two-dimensional lattices. These exact values of γ_c are the solutions of the equation (Ref. 27), $\cosh[(\ln \gamma_c)/2] = \sec(\pi/z)$.

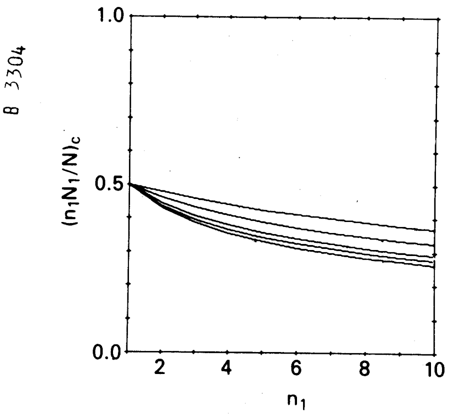


Fig. 3. Fractions of sites occupied by ligands at critical points in the Bethe approximation for single ligand species of various sizes n_1. Curves from top to bottom are for $z = 3, 4, 6, 8,$ and 12.

These effects would be due to the fact that the increase of z or n_1 yields more neighboring site pairs around a bound ligand. Another interesting feature of changing n_1 is that the critical values of (n_1N_1/N) shift from $\frac{1}{2}$ toward smaller values as n_1 increases, as suggested by Eq. (26b) for a limiting case (see Fig. 3). This is consistent with the fact that in binding curves of n_1N_1/N versus $\ln(K_1^0N_1^f\gamma^{zq_1/2})$, central symmetry around $(n_1N_1/N) = \frac{1}{2}$ is not present for cases with $n_1 > 1$. See Eq. (22) and Fig. 4.

SCATCHARD PLOTS AND BINDING CURVES

Scatchard plots[1] are often used for examining the cooperative character and binding modes of a system. For single-ligand-species systems, Scatchard plots exhibit a linear relationship for the case of $n_1 = 1$ and $\gamma = 1$, i.e., a one ligand–one site binding case without nearest-neighbor interactions. Deviations from linearity can yield information about the cooperativity and binding modes. Here, we will use normalized Scatchard plots; $N_1/(K_1^0N_1^fN)$ versus n_1N_1/N. To examine the effect of coordination

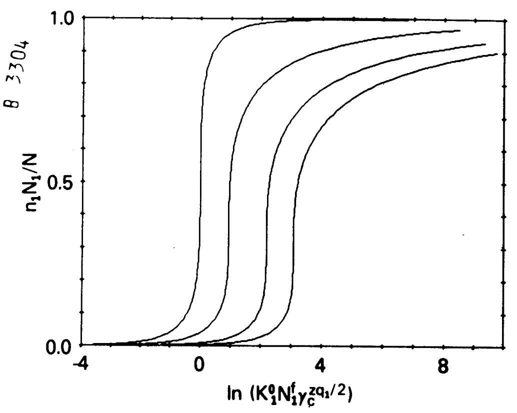


Fig. 4. Binding curves at critical points for single ligand species calculated with Eq. (22). Curves from left to right are for $n_1 = 1, 5, 8,$ and 10 with $z = 6$.

number, z, and the number of sites occupied by a bound ligand, n_i, on normalized Scatchard plots, we first consider binding at the two extreme limits where most of sites are either unoccupied or occupied.

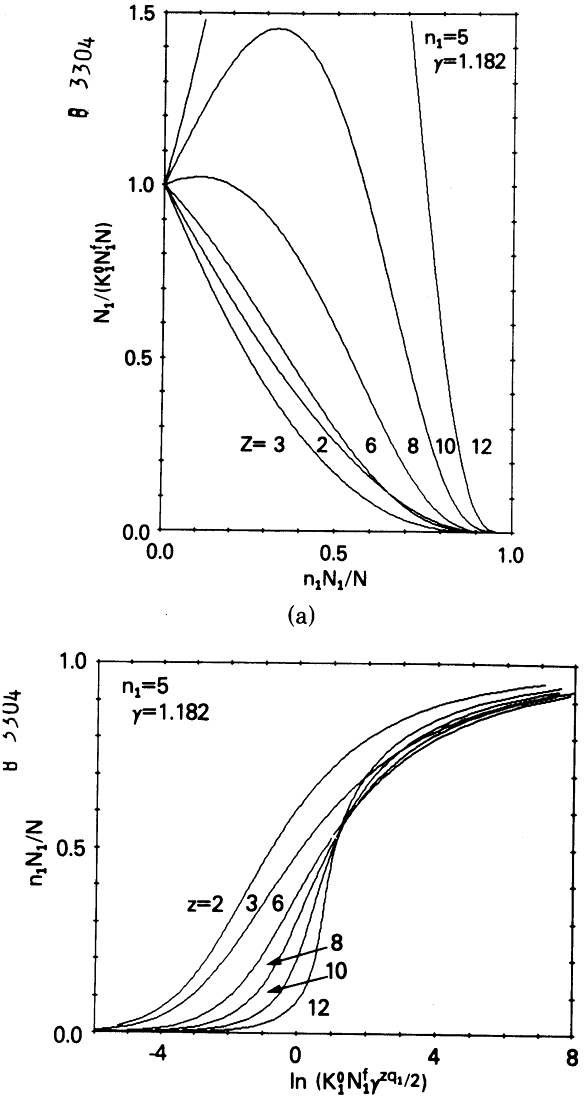
In the case of low occupancy, $\Sigma n_i N_i/N \ll 1$, Eq. (22) becomes

$$\frac{N_j}{K_j^0 N_j^f N} = 1 - \frac{\sum_{i=1} n_i N_i}{N} + [zq_j(\gamma - 1) - (n_j - 1)] \frac{\sum_{i=1} q_i N_i}{N} + o\left(\frac{\sum_{i=1} n_i N_i}{N}\right) \tag{27}$$

For single-ligand-species systems, the slope of Scatchard plots at small values of $n_1 N_1/N$ is $-1 + [zq_1(\gamma - 1) - (n_1 - 1)]q_1/n_1$, whose first and third terms represent contributions from site exclusion by a bound ligand and second term from the creation of sites with the interaction statistical weight γ; the contributions from the excluded-volume effects are always negative, but the second term can be positive or negative depending on whether $(\gamma - 1)$ is positive or negative. Here, it should be noted that $\gamma > 1$ or $\gamma < 1$ indicates positive (attractive) or negative (repulsive) interactions between bound ligands, respectively. Then, the initial slope takes values larger or smaller than -1, depending on whether the term, $zq_1(\gamma - 1) - (n_1 - 1)$, is positive or negative. The limiting value, γ_0, of γ for the initial slope of -1 as a function of z and n_1 is

$$\gamma_0 = [(z - 1)n_1 + 1]/[(z - 2)n_1 + 2] \tag{28}$$

Thus, if γ is larger than γ_0, Scatchard plots will effectively exhibit positive cooperativity of the system. If a system does not show an effectively positive cooperativity, a phase transition will never occur. Therefore, critical values, γ_c, must be greater than γ_0. γ_0 is a monotonically increasing function of n_1 and a decreasing function of z; it is notable that the dependence of γ_0 on n_1 is opposite that of γ_c, while γ_0 and γ_c depend on z in a similar manner. On the other hand, the dependences of the initial slope of Scatchard plots on z and n_1 are not simple, although the initial slope is an increasing function of γ. As either z or n_1 increases, the contributions to the initial slope from excluded-volume effects, $-1 - (n_1 - 1)q_1/n_1$, become more negative, because the increase of excluded-volume effects strengthens negative cooperativities. On the other hand, the second term in the equation for the initial slope, $zq_1^2(\gamma - 1)/n_1$, becomes more positive or negative depending on the sign of $(\gamma - 1)$, as z increases. In other words, cooperativities are amplified whether positive or negative; this is due to the fact that the increase of lattice coordination numbers, z, yields more neighboring site pairs which could interact with a bound ligand. For higher-dimensional lattices, $z > 2$, the second term depends on n_1 in the same way except at small values of n_1 and z; for one-dimensional lattices with $z = 2$, the contribution of the second term becomes smaller as n_1 increases. Consequently, the dependences of Scatchard plots at low occupancy on z and n_1 are affected by these two counteracting effects in cases of $\gamma > 1$. The two effects correspond to the amplification of positive cooperativities and the increase of negative cooperativities due to excluded-volume effects. The derivative of the initial slope with respect to z is $[(\gamma - 1)zn_1 + 2(\gamma - \gamma_0)(n_1 - 1)]q_1/(zn_1)$. Thus, the initial slope is an increasing function of z in the range of $\gamma \geqq \gamma_0(z,n_1)$ and a decreasing function of z if $\gamma \leqq 1$, but it may decrease with increase of z in the range of $1 < \gamma < \gamma_0$. As an example, Fig. 5(a) shows that the initial slope decreases when z changes from 2 to 3 in the case of $n_1 = 5$ and a particular value of γ that is equal to γ_0 for $z = 6$ and $n_1 = 5$. This is in contrast to the case of $n_1 = 1$ where the initial slope will be an increasing function of z if

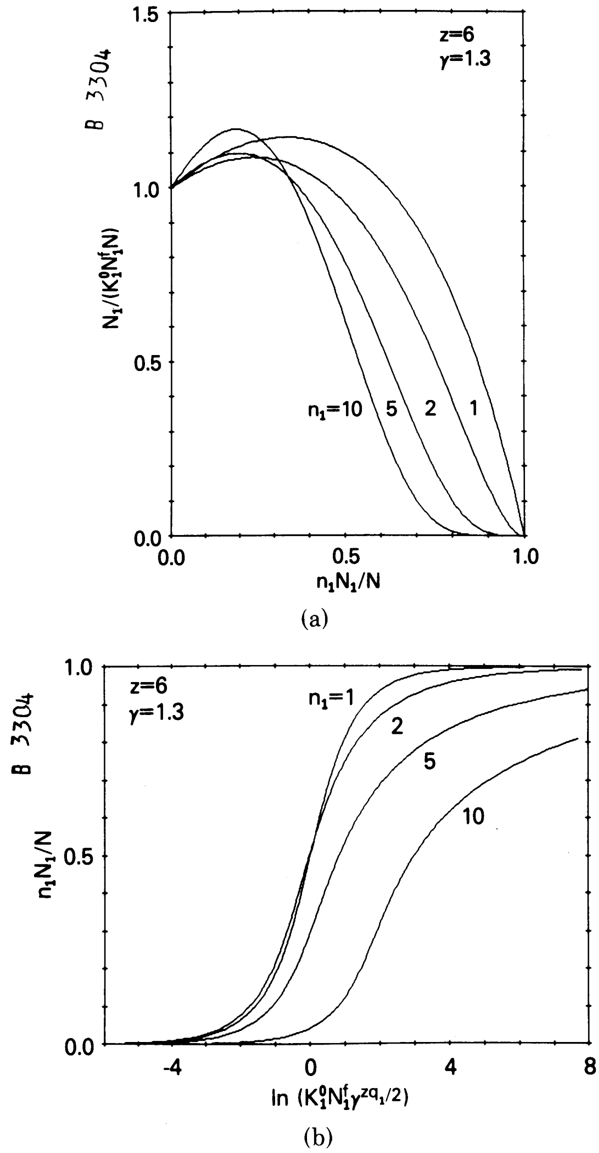


(a)

(b)

Fig. 5. (a) Normalized Scatchard plots and (b) binding curves for single-ligand-species systems for $\gamma = 1.18182$ and $n_1 = 5$. Curves in (a) are from top to bottom for $z = 12, 10, 8, 6, 2$, and 3. Curves in (b) are for $z = 2, 3, 6, 8, 10$, and 12 from left to right at small values of the ordinate. The value of γ is lower than the critical value for phase transition, γ_c, for all cases. The initial slope of Scatchard plots for $z = 6$ is -1 for these specific values of γ and n_1.

$\gamma - 1$ is positive. The derivative of the initial slope with respect to n_1 is $[n_1^2(z - 2)\{(z - 2)\gamma - (z - 1)\} - 4(\gamma - \frac{1}{2})]/(zn_1^2)$. For higher-dimensional lattices with $z > 2$, the slope will be a decreasing function of n_1 if $\gamma \leqq \gamma_0(z, n_1 = \infty) = (z - 1)/(z - 2)$; otherwise it may increase with the increase of n_1 except at small values of n_1. In contrast, the initial slope for one-dimensional lattices with $z = 2$ is a decreasing function of n_1 except for the cases of strongly negative interactions, $\gamma \leqq \frac{1}{2}$. This is one of the most important differences between one- and higher-dimensional lattices. Figure 6(a) gives a typical example of the dependence of Scatchard plots on n_1. For $z = 6$ and $\gamma = 1.3$ which is larger than $(z - 1)/(z - 2)$, the initial slope increases



(a)

(b)

Fig. 6. (a) Normalized Scatchard plots and (b) binding curves for single-ligand-species systems for $z = 6$ and $\gamma = 1.3$. Curves in (a) and (b) are for $n_1 = 1, 2, 5$, and 10 from top to bottom at large values of the abscissa. The value of γ is lower than the critical value for phase transition, γ_c, for all cases.

as n_1 increases beyond 2. The amplifications of positive cooperativities accompanied by the increases of lattice coordination numbers, z, and the numbers of sites occupied by a bound ligand, n_1, would be responsible for the dependences of the critical value of γ for phase transition on z and n_1; in Fig. 2, γ_c ($>\gamma_0$) decreases as either n_1 or z increases.

Next, let us consider the other limit where most sites are occupied. In the limit of high occupancy, $N_0/\Sigma q_i N_i \ll 1$, Eq. (22) is approximated by

$$\frac{N_j}{K_j^0 N_j^f N} = \left(\frac{N_0}{N}\right)^{n_j} \left(\frac{\sum_{i=1} q_i N_i}{N}\right)^{(1-n_j)} \gamma^{z(q_j+n_j)/2} \left[1 + O\left(\frac{N_0}{\sum_{i=1} q_i N_i}\right)\right] \qquad (29)$$

And if $n_j = 1$,

$$N_j/(K_j^0 N_j^f N) = (N_0/N)\gamma^z$$

Then, the slopes of Scatchard plots approach zero except for $n_1 = 1$ as most sites become occupied. As shown in Fig. 6(a), the larger n_1 is, the more quickly the slope approaches zero, because of the n_1th power dependence on (N_0/N). This is due to excluded-volume effects of bound ligands; the negative cooperativities originating from multiple site exclusion become strongest when most of sites are occupied by ligands and n_1 increases.

A slightly different representation of the binding isotherm in the limit of high occupancy might be useful when the number of sites occupied by a bound ligand, n_i, is estimated from experimental data. Equation (29) is transformed as follows:

$$\frac{\sum_{i=1} n_i N_i}{N} = 1 - \left[\left(\frac{\sum_{i=1} q_i N_i}{N}\right)^{-1+n_j} \gamma^{-z(q_j+n_j)/2} \frac{N_j}{K_j^0 N_j^f N}\right]^{1/n_j} \qquad (30)$$

For single-ligand-species systems, we get the following equation by approximating N_1/N in the right-hand side of Eq. (30) by $1/n_1$:

$$\frac{N_1}{N_1 + N_1^f} = \frac{1}{n_1}\left(\frac{N}{N_1 + N_1^f}\right) \times \left\{1 - \left[\left(\frac{q_1}{n_1}\right)^{-1+n_1} \gamma^{-z(q_1+n_1)/2} \left(\frac{1}{K_1^0\, n_1 N}\right)\left(\frac{N}{N_1 + N_1^f}\right)\right]^{1/n_1}\right\} \qquad (31)$$

This equation is appropriate only for the high-occupancy limit: $N/(N_1 + N_1^f) \rightarrow 0$. Then, as indicated by Schwarz,[3,28] the number of sites occupied by a bound ligand, n_1, might be estimated from the initial slope in the plots of the fraction of bound ligands, $N_1/(N_1 + N_1^f)$, versus the number of sites per ligand, $N/(N_1 + N_1^f)$; this limit should be taken carefully to avoid contributions of the second term in Eq. (31); otherwise n_1 will be overestimated.

Another representation of a binding isotherm is the binding curve, such as the ratio of bound sites plotted as a function of the logarithm of the concentration of free ligands. Figures 5(b) and 6(b) show binding curves for the same parameter sets as in the Scatchard plots of Figs. 5(a) and 6(a), respectively. In these binding curves, the effects of parameters, z and n_1, are not so apparent as in the Scatchard plots, but one should note the effect of z on the sharpness of the binding curves in Fig. 5(b). Also, an increase in n_1 produces more asymmetric binding curves as shown in Fig. 6(b). The asymmetry in binding curves results from the fact that the negative cooperativities due to excluded-volume effects become stronger as more sites are occupied and n_1 increases.

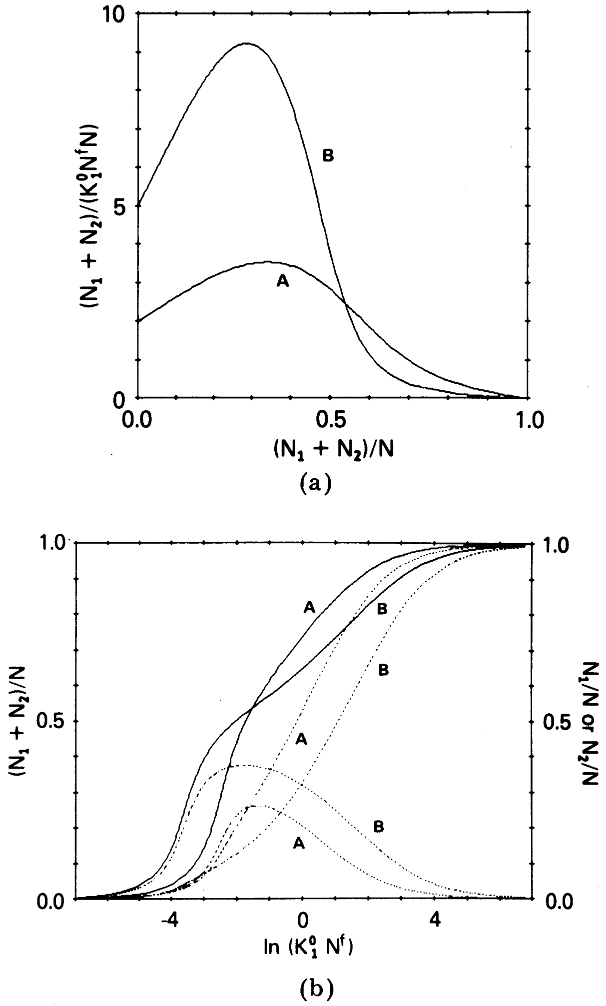
However, this type of binding curve is useful for analyzing systems in which the same species of ligands can bind in different modes, i.e., a ligand can exclude different numbers of sites with different intrinsic binding constants. Let us consider a typical case in which ligands can bind in both a monovalent and bivalent form such as antibodies, but the assumption of Eq. (15), for nearest-neighbor interactions between bound ligands is still in effect. The binding model for bivalent antibodies has been studied for a one-dimensional lattice.[29] Such one-dimensional treatments are of limited applicability for most phenomena observed in biological systems, because these phenomena usually occur on surfaces. One example is the binding of antibodies to membrane-bound antigens. The use of the binding isotherm, Eq. (22), is clearly more appropriate than previous theories for modeling of such systems. For the present case, the simultaneous equations of Eq. (22) must be solved with the condition $N^f \equiv N_1^f = N_2^f$, where the subscripts 1 and 2 represent the two binding modes. Figures 7(a,b) show Scatchard plots and binding curves for the particular case of $n_1 = 1$ and $n_2 = 2$. Although the Scatchard plots do not exhibit any remarkable qualitative differences from those for systems with only one binding mode, two-step saturation of binding curves can be observed for cases in which bivalent binding occurs with larger intrinsic binding constants than monovalent binding mode or positive nearest-neighbor interactions exist. At low free ligand concentrations, more ligands can bind in a bivalent mode than in a monovalent mode because of larger binding affinities originating in a larger intrinsic binding constant or positive nearest-neighbor interactions; from Eq. (27),

$$\frac{N_j}{N_k} = \left(\frac{K_j^0}{K_k^0}\right)\left[1 + [z(q_j - q_k)(\gamma - 1) - (n_j - n_k)]\left(\sum_{i=1} \frac{q_i N_i}{N}\right)\right]$$

at the low-occupancy limit. Here, it should be noted that in cases of $\gamma = 1$ and $K_j^0 = K_k^0$, ligands always bind more often in binding modes in which fewer sites are occupied; from Eq. (22), $N_j/N_k = [N_0/(N_0 + \sum_{i=1} q_i N_i)]^{n_j - n_k} > 1$ for $n_j < n_k$. As the free ligand concentration increases, ligands bound in a monovalent mode gradually increase in order to balance chemical potentials between free and bound ligands by increasing the total number of bound ligands; from Eq. (29),

$$\frac{N_j}{N_k} = \left(\frac{K_j^0}{K_k^0}\right)\left[\frac{N_0}{(\sum_{i=1} q_i N_i)}\right]^{n_j - n_k} \gamma^{z(q_j + n_j - q_k - n_k)/2}$$

at the high-occupancy limit. As a result, the number of ligands binding in a bivalent mode shows a maximum, and two-step binding curves are observed [see the solid and dotted curves in Fig. 7(b)]. The two-step



(a)

(b)

Fig. 7. (a) Scatchard plots and (b) binding curves for single-ligand-species binding in two modes for $z = 6$ and $\gamma = 1.5$. Ligands can bind in either a monovalent mode, $n_1 = 1$, or a bivalent mode, $n_2 = 2$. Simultaneous equations, Eq. (22), have been solved under the condition of $N^f \equiv N_1^f = N_2^f$. Curves A and B are for $K_2^0/K_1^0 = 1$ and 4, respectively. In (b), the ordinate shows the ratio of the total number of bound ligands to the number of sites for solid curves and the ratio of the number of monovalent or bivalent bound ligands to the total number of sites for the dotted curves. Binding in a bivalent mode exhibits a maximum.

binding curves will be amplified by increases in the coordination number, z, if $\gamma > 1$.

Interpretation of Experimental Data

Because Scatchard plots and binding curves can be similar in appearance for markedly different sets of parameter values, their extraction from experimental data can be a treacherous exercise. As pointed out previously,[6,25,30,31] careful estimates are required of all parameters, including intrinsic binding constants K_i^0, an interaction parameter γ, the number of sites occupied by a bound ligand n_i, the total number of sites N, and coordination number z. One possible procedure for a simple case of single-ligand-species system is outlined below. First, K_1^0 may be estimated from the initial intercept in Scatchard plots. Then, n_1 might be determined from the initial slope in the plots of the fraction of bound ligands versus the number of sites per ligand by using Eq. (31). If N is not explicitly known, determining n_1 will also require the analysis based on Eq. (29) of the power dependence of Scatchard plots on N_0/N at the high-occupancy limit, which might not be possible. The relationship between the values of z and γ may be obtained from the initial slope of Scatchard plots by using Eq. (27). Finally, the exact values of z and γ would be obtained by fitting Scatchard plots and binding curves over the whole region to experimental data. This procedure may be iterated to get consistent values for parameters. Because of the large number of parameters, it is highly desirable for some parameters to be determined by external means before analyzing binding data. Generally, both Scatchard plots and binding curves should be employed to fit experimental data, because of differing sensitivities to parameter values.

DISCUSSION

A binding isotherm has been calculated on the basis of the Bethe approximation. The extension of theory to multidimensional lattices would increase its applicability to analyses of binding data observed for a wide variety of biological systems. The effects of n_1, the numbers of sites occupied by single bound ligands, and z, coordination numbers of lattices, on binding isotherms in single-ligand-species systems are noteworthy. (1) Exclusion of multiple sites yields negative cooperativities of ligand binding, but in higher-dimensional lattices it can also amplify positive cooperativities originating in attractive interactions between bound ligands. This positive amplification can be sufficient to overcome the negative excluded-volume cooperativities at low occupancy. Multiple-site exclusions also yield asymmetric binding curves, because the negative cooperativities due to excluded-volume effects become stronger as more sites are occupied. (2) Larger coordination numbers amplify cooperativities of ligand binding. (3) As a result, increases of z and n_1 decrease the critical value, γ_c, of the interaction parameter between nearest-neighbor sites bound to ligands for phase transition as well as making the fraction of occupied sites at the critical point shift from $1/2$ toward smaller values. In applications to real systems, n_i and z should be regarded as effective numbers, because the numbers of occupied sites might vary and the array of sites could be fluidlike rather than rigid and regular.

In the derivation of the binding isotherm, Eq. (22), we have assumed that nearest-neighbor interactions are the same for all sites bound to different ligand species, i.e., Eq. (15). For multiligand species systems, this assumption might not be satisfied. If it is not satisfied, it will be difficult to evaluate $\overline{X}_{lm}$ with the quasi-chemical approximation. Instead, the Bragg-Williams approximation might be used in this case to estimate $\overline{X}_{lm}$ in the form $\overline{X}_{lm} = q_l N_l q_m N_m/(N_0 + \Sigma_{i=1} q_i N_i)$ with $q_0 \equiv 1$; however, it is appropriate only for cases with weak nearest-neighbor interactions. This yields

$$\frac{N_j}{K_j^0 N_j^f N} = \left(\frac{N_0}{N}\right)^{n_j} \left(\frac{N_0 + \sum_{i=1} q_i N_i}{N}\right)^{(1-n_j)} \times \exp\left[\frac{z}{kT} \sum_{l=1} \left\{(\epsilon_{l0} n_j - \epsilon_{lj} q_j + \epsilon_{0j} q_j) + \frac{(\epsilon_{l0} N_0 + \sum_{m>l} \epsilon_{lm} q_m N_m)}{(N_0 + \sum_{i=1} q_i N_i)} (q_j - n_j)\right\} \frac{q_l N_l}{(N_0 + \sum_{i=1} q_i N_i)}\right] \tag{32}$$

Here, q_i may be replaced by n_i by taking the limit of $z \rightarrow \infty$. Although the Bethe approximation is higher order than the Bragg-Williams approximation, the former does not necessarily improve agreement with experiments for polymer solutions.[32,33] This may be attributed to imperfections inherent in the lattice model itself for polymer solutions.[15] However, it is unknown whether this is true for ligand binding systems.

We thank R. L. Jernigan for critical reading of the manuscript and valuable comments and suggestions, and K. Iwasa for valuable comments.

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Received December 22, 1982
Accepted March 25, 1983