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A Lattice Theory for Counterion Binding on Polyelectrolytes

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ABSTRACT: A lattice theory is developed to treat the problem of counterion binding on polyelectrolytes and to assess the effects of both short-range site-specific interactions and long-range electrostatic interactions between the counterion and the polyion. In the special case of no short-range term or in the limit of nearly zero ionic strength, the present theory gives results identical to those of counterion condensation theory. But in general cases the short-range terms introduce new modifications into the theoretical expressions. The counterion binding fraction is found to deviate from the limiting law at finite ionic strengths if there exist short-range effects. The magnitudes of short-range interactions are estimated by comparing the theory with experimental results. It is found that, for monovalent species, the short-range effect can be neglected. For divalent counterions Mg^{2+} and Mn^{2+} binding on DNA in excess 1:1 salt, a short-range interaction energy of about 1.3 kcal/mol is required to fit experimental binding constants. For Eu^{3+} binding on DNA in excess NaCl, the fitted short-range factor is 2.7 kcal/mol. The theory is further applied to the problem of the binding isotherm of a higher valence counterion on polyions in solutions with excess 1:1 salt. For these cases, the theoretical results are found to be in agreement with experiments.

Introduction

Many important biopolymers are polyelectrolytes. Aqueous polyelectrolyte solutions always contain small counterions to the polyion, and they usually contain excess simple electrolytes. There has been great interest in studying the distribution of small ions around the polyion,[1–4] as this information provides a key to the understanding of the various effects of simple electrolytes on the properties of polyions. A well-established result has been that an extra amount of counterions will condense or bind on the polyion at almost all bulk ionic concentrations.[1–4] The physical picture of counterion binding on the polyion, however, is somehow dependent on the interaction modes between the two species. Under purely long-range electrostatic interactions, counterions tend to be nonspecifically associated with the polyion, resulting in the territorial binding model.[1] On the other hand, if the short-range site-specific interactions are dominant, the counterion may bind on specific sites of the polyion, leading to the site binding model.[5]

The distribution of counterions around a polyion has been studied by a number of theoretical approaches. These include counterion condensation (CC) theory,[1,6–9] analysis of the cylindrical Poisson–Boltzmann equation,[10–13] hypernetted chain (HNC) theory,[14,15] and Monte Carlo computer simulations.[16,17] Most of these theoretical approaches have reached qualitatively similar conclusions and found reasonable agreement with experiments. These theories, however, have been primarily concerned with the long-range electrostatic interactions between the counterion and the polyion. Short-range site-specific interactions are given much less consideration. In most cases, short-range effects are only treated as repulsive interactions, such as excluded-volume and ion size effects.[18,19] In some theories,[1] the short-range terms are neglected. While the major attractions between counterions and polyions are probably the long-range electrostatic forces, experiments have indicated the influence of short-range attractive forces.[20–22] For example, the calculated binding constant for Eu^{3+} binding on DNA[22] based on purely long-range electrostatic interaction was found to be nearly 2 orders of magnitude smaller than the experimental value. It is also impossible to explain, without invoking short-range effects, the species selectivity among similar valence counterions in binding on the polyion.[7] Further investigations on counterion theory, particularly the short-range effects, are therefore desirable.

The present study is concerned with three problems in counterion binding on polyions: (1) how can short-range interactions be incorporated into the counterion theory? (2) What is the magnitude of the short-range interaction energy? (3) What are the effects of short-range interactions? We have developed a lattice theory to treat these problems systematically. Our approach is based on the one-dimensional lattice-gas model. We explicitly introduce two energy terms in our model: the free energy of a counterion bound on a single binding site and the long-range electrostatic interactions of the counterion with all other binding sites of the polyion. This approach is an extension of the standard lattice model where only short-range interactions are treated. It also generalizes earlier theories on counterion binding, in which only long-range electrostatic interactions are considered.

The lattice model is admitted to be oversimplified in describing the association of counterions with the polyion, but the model has the advantage of analytical solubility. A simple partition function can be constructed for the polyion–counterion system, and analytical expressions for the equilibrium binding fraction of the counterion on the polyion can be obtained. It is also easily extended to systems containing multiple counterions. The model is found to be equivalent to the two-state counterion condensation theory in the special case of no short-range term, but it introduces new modifications to the theory when both short-range and long-range interactions exist. The present theory provides a simple model by which the effects of various interactions on counterion binding can be studied in some detail and compared with experiments.

Theory

A polyion is modeled here as a linear array of N binding sites, each of which has two possible states, bound or unbound by counterions. For simplicity, it is assumed that N is sufficiently large so that end effects can be neglected. It is also assumed that the concentrations of

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the counterions in the solution are in far excess over the equivalent concentration of polyion charges, so that the binding of the counterions to the polyion does not appreciably change the counterion concentration in the solution.

Single Counterion Species Systems. Let c be the concentration of the counterion in the solution. We introduce a binding constant K for a single site of the polyion. Then, the binding free energy of a counterion on the site can be calculated as $-k_BT \ln (Kc)$, where k_B is Boltzmann's constant and T the absolute temperature. The bound counterions can be freely exchanged with those in the bulk solution, so the grand partition function of a polyion–counterion system can be written as[23]

$$\Gamma = \sum_{m=0}^{N} \Omega_m (Kc)^m \exp[-\Delta g(m)/k_BT] \tag{1}$$

where Ω_m is a combinatorial factor and $\Delta g(m)$ is the free energy of m bound counterions, resulting from neighboring interactions. The exact expression of the partition function is dependent on the neighboring interactions. We assume that the only interaction of a bound ion with its neighbors is electrostatic. As the electrostatic interaction is a long-range effect, it may involve all the charges on the polyion.

The long-range electrostatic energy is calculated based on the theory developed by Manning.[1,6] This treatment involves two approximations. First, the interactions between charges on the polyion are calculated according to Debye–Hückel theory for charges in dilute ionic solution. Second, the counterions bound on the polyion are assumed to distribute randomly over the whole lattice. The latter approximation is equivalent to the Bragg–Williams mean-field approximation in lattice theory.[24] Thus, when m counterions are bound on N lattice sites, each site on average is occupied by m/N ions. Defining b as the average distance between two neighboring sites of the linear lattice, the electrostatic energy of a bound ion from neighboring interactions is expressed as

$$\Delta g = -2\frac{zq_e^{\,2}}{\epsilon b}\sum_{i=1}^{\infty}\frac{e^{-i\kappa b}}{i} + \frac{zq_e^{\,2}}{\epsilon b}\left(\frac{zm}{N}\right)\sum_{i=1}^{\infty}\frac{e^{-i\kappa b}}{i} \tag{2}$$

where $z(>0)$ is the valence of the counterion, q_e the electronic charge, ϵ the dielectric constant of water, and κ the Debye–Hückel parameter calculated from the ionic strength of the solution. The first term on the right-hand side of eq 2 is the electrostatic interaction energy of the bound ion with the polyion charges (charges both above and below the site where the ion is bound); and the second term is the electrostatic interaction energy of the ion with m bound counterions (where there is no factor of 2 to avoid calculating each interaction twice). Defining the linear charge density of the polyion by a dimensionless parameter ξ,[1] $\xi = q_e^2/(\epsilon k_BTb)$, the sum of eq 2 is calculated to be

$$\Delta g = z\xi(2 - zm/N)k_BT \ln (1 - e^{-\kappa b}) \tag{3}$$

Inserting eq 3 into eq 1, the grand partition function is now expressed as

$$\Gamma = \sum_{m=0}^{N} \frac{N!}{m!(N-m)!}(Kc)^m (1 - e^{-\kappa b})^{-mz\xi(2-zm/N)} \tag{4}$$

The combinatorial factor has been taken as the binomial coefficient because the ions are assumed to be randomly distributed on the lattice. The exact evaluation of the partition function is difficult due to the m^2 term in the exponents. However, the equilibrium distribution of the counterions is determined by the largest term of the partition function when m is sufficiently large.[24] By setting the derivative of the logarithm of the mth term in eq 4 to zero, the equilibrium binding fraction of the counterions on the polyion is obtained as

$$\theta/(1-\theta) = Kc(1 - e^{-\kappa b})^{-2z\xi(1-z\theta)} \tag{5}$$

where θ is the binding fraction. The neutralization fraction of the polyion charges is $r = z\theta$.

It is noted that the binding fraction derived above is different from Manning's theory only on the left-hand side of eq 5, where the term $\theta/(1-\theta)$ corresponds to a single θ in the earlier theory.[1] In fact, Manning's expression can be exactly recovered with the lattice model if we use a combinational factor $(N/m)^m$ instead of the binomial coefficient. (The derivative of $\ln ((N/m)^m)$ with respect to m is $1 - \ln (\theta)$. But the binomial coefficient is consistent with the assumption that the counterions are randomly distributed on the lattice. The grand partition function defined in eq 1 is formally identical to the binding polynomial in the ligand binding model developed by Dewey.[25] However, in Dewey's treatment, the electrostatic interaction energy of a counterion with the polyion is treated as a constant, independent of the actual neutralization of the polyion charge in a given binding state, and the combinatorial factors in the binding polynomial are taken as unity. Even though these two assumptions in Dewey's work compensate each other somewhat, the result is not equal to the exact binding polynomial.

Dilute Salt Solutions. The effects of various interactions on counterion binding on the polyion can be further clarified in the case of dilute salt solutions. When the ionic strength is sufficiently low, the exponential term on the right-hand side of eq 5 can be linearized as $1 - e^{-\kappa b} \rightarrow \kappa b$. From the definition of the Debye–Hückel parameter κ, we have the relation

$$b^2\kappa^2 = 4\pi(10^{-3})N_A(e^2/\epsilon k_BT)b^2(z^2\nu + z'^2\nu')c \tag{6}$$

where c is the counterion concentration in solution, ν, z and ν', z' are the stoichiometric numbers and valences of the counterion and co-ion, respectively, and N_A is Avogadro's number. For convenience, we represent the coefficient in front of the variable c in eq 6 by a single factor j, so that $b^2\kappa^2 = jc$. Equation 5 then can be expressed as

$$\frac{\theta}{1-\theta} = \frac{K}{j}(jc)^{[1-z\xi(1-z\theta)]} \tag{7}$$

By making the variable change, $y = 1 - z\xi(1 - z\theta)$, eq 7 can be transformed into the following form:

$$\frac{1}{\ln (jc)} = \frac{y}{\ln\left[\dfrac{j(z\xi + y - 1)}{K(z^2\xi - z\xi - y + 1)}\right]} \tag{8}$$

The above expression has the standard form $\omega = y/f(y)$, where $\omega = [\ln (jc)]^{-1}$ and $f(y)$ represents the function of the denominator on the right-hand side of eq 8. When the condition $jc \ll 1$ is satisfied, the variable $|[\ln (jc)^{-1}|$ has an upper bound. The function y therefore can be expanded as a series in $[\ln (jc)]^{-1}$ according to the Lagrange inversion formula:[25,26]

$$y = \sum_{k=1}\frac{1}{k!}\left[\left(\frac{\mathrm{d}}{\mathrm{d}y}\right)^{k-1}(f(y))^k\right]_{y=0}[\ln c]^{-k} \tag{9}$$

Substituting the variable θ back into y, we obtain an expression for the binding fraction:

$$\theta = \frac{1}{z}\left(1 - \frac{1}{z\xi}\right) + \ln\left[\frac{j(z\xi - 1)}{K(z^2\xi - z\xi + 1)}\right] \times \left[\frac{\ln (jc)^{-1}}{z^2\xi} + \frac{\ln (jc)^{-2}}{(z\xi - 1)(z^2\xi - z\xi + 1)}\right] + O[\ln (jc)^{-3}] \quad (10)$$

which is valid when $jc \ll 1$. In the limit $c \to 0$, $\ln c \to -\infty$, the limit value of θ is given as

$$\lim_{c \to 0} \theta = \frac{1}{z}\left(1 - \frac{1}{z\xi}\right) \quad (11)$$

The result is indicated to the limiting law of CC theory.[1]

Equation 10 can be simplified by a decomposition of the single-site binding constant K. To this end, we substitute the limiting value of θ in eq 11 for the actual θ in eq 7. This yields a constant in place of K:

$$K_v = \frac{z\xi - 1}{(z^2\xi - z\xi + 1)}j \quad (12)$$

where j is defined from eq 6 and has the unit mol^{-1}. The parameter K_v in eq 12 is, however, not equal to the single-site binding constant K. The reason is that in the above substitution the limiting value of θ is used, not its actual value. Nevertheless, we can retain the full value of the constant K by representing it as the product of the parameter K_v and a dimensionless parameter f, such as

$$K = K_v f \quad (13)$$

The value of f can be either larger or smaller than unity. Now by substituting eqs 12 and 13 into eq 10, we obtain the simplified expression for θ:

$$\theta = \frac{1}{z}\left(1 - \frac{1}{z\xi}\right) - \ln (f)\left[\frac{\ln (jc)^{-1}}{z^2\xi} + \frac{\ln (jc)^{-2}}{(z\xi - 1)(z^2\xi - z\xi + 1)}\right] + O[\ln (jc)^{-3}] \quad (14)$$

We note that the factor $\ln (f)$ is a common factor to all the remaining terms in eq 14, even though the series is only explicitly written up to the third term.

The physical meaning of eq 14 can be interpreted as follows. In a polyelectrolyte solution with dilute salt, the binding fraction of the counterions on the polyion consists of two components. The first is a limiting value at infinitely low salt concentration, which is completely determined by the long-range electrostatic interactions between the polyion and the counterions. The short-range effects are related only to the parameter f, which we therefore call the short-range factor; if $f = 1$, all the short-range corrections vanish. The factor K_v is irrelevant to the short-range corrections because it is absent from eq 14.

The parameter K_v defined in eq 12 is equivalent to the binding volume v in the CC theory.[1] In the earlier theory, a binding free energy $\delta\mu$ for short-range interactions has also been introduced to account for short-range effects appearing in the problem of species selectivity.[7] The equivalence of K_v and v means that it is the product $ve^{-\delta\mu/k_BT}$ in the earlier theory that is equivalent to the single-site binding constant in the present lattice theory. However, in the earlier treatment,[7] an approximation was made such that the entire product $ve^{-\delta\mu/k_BT}$ is determined by a substitution procedure similar to that used in obtaining eq 12. This procedure cannot be used here to determine the constant K. A similar case occurs with the simple function $y(x) = 1/(b - x)$, where one cannot use the limiting value $y_{\lim(x\to\infty)} = 0$ to determine the constant b. Through the above derivations we see that the direct substitution of the limiting value of θ into eq 7 only recovers the parameter K_v; the factor f is not included. It is interesting to point out that, in many applications of CC theory, the parameter v (equivalent to K_v in the present model) has been directly used in place of the single-site binding constant K. Fortunately, the factor f is often close to unity for many systems, so that the earlier theory has been found to be quite consistent with experiments.

Two Counterion Species Systems. The two counterion species will be denoted as type 1 and type 2 and their valences as z_1 and z_2, respectively. For simplicity, we assume that the polyion is a polyanion, while both counterion species are cations. Let m_1 and m_2 be the number of bound counterions of types 1 and 2, respectively, and their corresponding binding fractions be defined as $\theta_1 = m_1/N$ and $\theta_2 = m_2/N$. The grand partition function for two counterion species systems can be expressed as

$$\Gamma = \sum_{m_1=0}^{N} \sum_{m_2=0}^{N} \frac{N!(K_1c_1)^{m_1}(K_2c_2)^{m_2}}{m_1!m_2!(N - m_1 - m_2)!} \exp[(-\Delta g_1(m_1,m_2) - \Delta g_2(m_1,m_2))/k_BT] \quad (15)$$

where K_1 and K_2 are the single-site binding constants for type 1 and type 2 counterions, respectively, c_1 is the bulk concentration of type 1, and c_2 is that of type 2. Δg_1 and Δg_2 are respectively the long-range electrostatic interaction energies of the two types of counterions with the polyion. They are calculated to be

$$\Delta g_1(m_1,m_2) = m_1\xi k_BTz_1(2 - m_1z_1/N - m_2z_2/N) \ln (1 - e^{-\kappa b}) \quad (16a)$$

$$\Delta g_2(m_1,m_2) = m_2\xi k_BTz_2(2 - m_1z_1/N - m_2z_2/N) \ln (1 - e^{-\kappa b}) \quad (16b)$$

If the individual terms in the double summation of eq 15 are represented by the functions $y(m_1,m_2)$, the equilibrium distribution can be determined by the maximum term method:[24]

$$\mathrm{d}\{\ln [y(m_1,m_2)]\}/\mathrm{d}m = 0 \quad (17a)$$

$$\mathrm{d}\{\ln [y(m_1,m_2)]\}/\mathrm{d}m_2 = 0 \quad (17b)$$

These relations lead to the equilibrium binding fractions of the two counterion species:

$$\theta_1/(1 - \theta_1 - \theta_2) = K_1c_1(1 - e^{-\kappa b})^{-2z_1\xi(1-z_1\theta_1-z_2\theta_2)} \quad (18a)$$

$$\theta_2/(1 - \theta_1 - \theta_2) = K_2c_2(1 - e^{-\kappa b})^{-2z_2\xi(1-z_1\theta_1-z_2\theta_2)} \quad (18b)$$

The decomposition of the single-site binding constant may now be introduced to both ion species, such that $K_1 = K_{v1}f_1$ and $K_2 = K_{v2}f_2$, where f_1 and f_2 are the short-range factors for species 1 and 2, respectively, and K_{vi} are defined according to eq 12:

$$K_{vi} = \frac{z_i\xi - 1}{(z_i^2\xi - z_i\xi + 1)}j; \quad i = 1 \text{ and } 2 \quad (19)$$

Since the factor j originates from the Debye–Hückel parameter of ionic solutions, it should be identical for both types of ions in the two-species system. The differences between K_1 and K_2 are the valence z_i and the factor f_i. The relative strength of the short-range interactions between the two counterion species is determined by the ratio f_1/f_2.

If the valences of the two counterion species in the system are identical, the long-range electrostatic components in the binding fraction expression are exactly the same for both species. The ratio of their binding fractions is then given as

$$\theta_1/\theta_2 = (f_1/f_2)(c_1/c_2) \tag{20}$$

Defining the selectivity coefficient of the polyion for the two counterion species as $\alpha = (\theta_1/c_1)/(\theta_2/c_2)$, one obtains $\alpha = f_1/f_2$. It is seen that experimentally measured selectivity coefficients are a direct measurement for the relative short-range interactions of the polyion with two counterion species of identical valence.

When the valences of the two counterion species are different, the binding fraction on the polyion will be dominated by the higher valence species if the bulk concentrations of the two species are comparable. The more important case is where higher valence counterions bind on polyions in solutions with excess 1:1 salt. Our attention below is focused on the latter kind of system. Assign c_1 and z_1 as the concentration and valence of the monovalent counterion, respectively, and c_2 and z_2 as those of the higher valence species. It is assumed that $c_1 \gg c_2$. According to eqs 18a and 18b, the ratio of the binding fractions of the two counterion species is expressed as

$$\frac{\theta_2}{\theta_1} = \frac{K_2c_2}{K_1c_1}(1 - e^{-\kappa b})^{-2(z_2-1)\xi(1-\theta_1-z_2\theta_2)} \tag{21}$$

An approximate expression for the binding isotherm of the higher valence counterion species can be obtained as follows. In the limit $c_2 \rightarrow 0$, θ_1 can be reliably approximated as $1 - \xi^{-1}$. At higher c_2, the higher valence counterion will effectively compete with the univalent counterion in binding on the polyion. We may write

$$\theta_1 = 1 - 1/\xi - x\theta_2 \tag{22}$$

where x is a parameter whose exact value is smaller than z_2. As an approximation, we set $x = z_2$. Then applying eq 22 to eq 21, and after rearrangement, we obtain

$$\theta_2 = \left(1 - \frac{1}{\xi}\right)\frac{K'c_2}{1 + z_2K'c_2} \tag{23}$$

where K' is expressed as

$$K' = \frac{1}{c_1}\frac{K_{v2}f_2}{K_{v1}f_1}(1 - e^{-\kappa b})^{-2(z_2-1)} \tag{24}$$

Equation 23 is a good approximation when c_2 is small so that $1 - (z_2 - x)\theta_2 \simeq 1$, but the expression leads to an incorrect limiting result as $K'c_2 \rightarrow \infty$. It is possible, however, to modify the expression to give the correct limiting value for θ_2 by changing the parameter z_2 in eq 23 to $z_2^2\xi/(z_2\xi - 1)$. Another approximate expression may be obtained by the Lagrange inversion formula, but the resulting expression is much more complicated than eq 23. The approximate expression of eq 23 provides a qualitatively correct description about the binding isotherms of the higher valence counterion species. Each counterion of valence z_2 occupies approximately z_2 sites when bound on the polyion.

The experimentally measured binding constant of higher valence counterions on the polyion in solutions with excess 1:1 salt can be defined as

$$K_0 = \lim_{c_2 \rightarrow 0} (\theta_2/c_2) = (1 - \xi^{-1})K' \tag{25}$$

where K' is defined in eq 24. From the salt dependence of K_0, the ratio of the short-range factors, f_2/f_1, between the higher valence and the univalent counterions can be obtained. For convenience of comparison with experimental results, we may take the logarithm of both sides of eq 25. Denote the constant and variable components on the right-hand side of eq 25 as C and $h(c_1)$, respectively, so we have the relation

$$\ln (K_0) = \ln C - \ln h(c_1) \tag{26}$$

where

$$C = \frac{(z_1\xi - 1)}{(z_2^2\xi - z_2\xi + 1)\xi}\left(\frac{f_2}{f_1}\right) \tag{27}$$

and

$$h(c_1) = c_1(1 - e^{-\kappa b})^{2(z_2-1)} \tag{28}$$

Then if one plots $\ln K_0$ with respect to $\ln h(c_1)$, the result is a straight line. The slope of the line is -1, and the intercept is equal to $\ln C$. From the known valence of the counterion and the linear charge density of the polyion, the ratio f_2/f_1 can be obtained.

The explicit dependence of K_0 on c_1 can be obtained by taking the derivative of $\ln K_0$ with respect to $\ln c_1$. We obtain

$$\frac{\mathrm{d} \ln K_0}{\mathrm{d} \ln c_1} = -\left[1 + (z_2 - 1)\frac{\kappa b}{\exp(\kappa b) - 1}\right] \tag{29}$$

In dilute solutions, $\kappa b \ll 1$, so the above expression reduces to $\mathrm{d}[\ln K_0]/\mathrm{d}[\ln c_1] \sim -z_2$. This result is in agreement with earlier theories.[1]

Results and Discussion

(a) One Counterion Species System. A primary objective of this study is to evaluate how the various kinds of interactions affect the binding behaviors of counterions on polyions beyond the limiting law of the CC theory.[1] Essential information can be obtained from eq 14. First, the dominant term in the series of eq 14 is $-\ln(f)/\ln(jc)$, so the dependence of the binding fraction on bulk concentration c is basically controlled by the parameter f. Assuming that $jc \ll 1$, the higher order terms can be neglected. This condition also implies that $\ln jc < 0$. Then, if $f > 1$ and $\ln f > 0$, the binding fraction will monotonously increase with the bulk ionic concentration; on the other hand, if $f < 1$ and $\ln f < 0$, the binding fraction will monotonously decrease with the concentration. The larger the magnitude of $\ln f$, the stronger the dependence of the binding fraction on the ionic concentration. In the special case $f = 1$, the binding fraction is independent of the concentration, because all correction terms that are dependent on the concentration vanish. Second, the leading term of the series is proportional to $z^{-2}\xi^{-1}$, so the influence of short-range interactions on the binding fraction is modulated by the valence of the counterions and the linear charge density of the polyion. The higher the counterion valence and/or the linear charge density of the polyion, the weaker the influence of the short-range factor f on the binding fraction. Finally, the binding fraction is dependent on the logarithm of the bulk ion concentration, and the signs of the $\ln(jc)^{-k}$ terms in the series vary alternately between positive and negative for even and odd k. Thus, the variation of the binding fraction with concentration c is very low at low concentrations. This explains why the counterion binding fraction varies little when the bulk counterion concentration increases many orders of magnitude.

Equation 14 is valid only for dilute salt solutions. It is therefore relevant to consider the general cases where eq

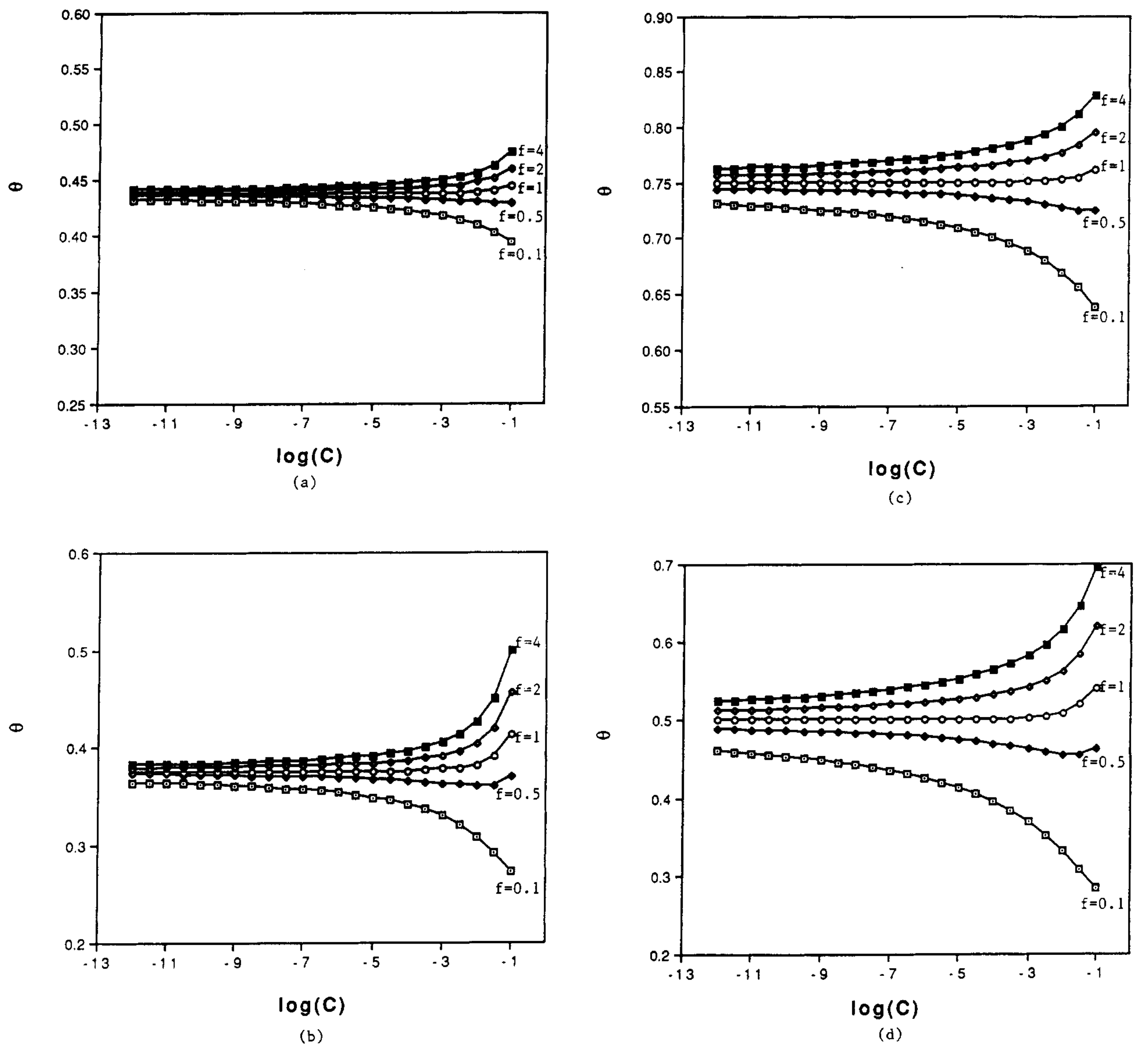

Figure 1. Effects of short-range interactions on the counterion binding fraction. θ is the binding fraction and c the bulk concentration of counterion. The short-range factor f for different binding curves is shown in the figure: (a) $\xi = 4$; $z = 2$; (b) $\xi = 4$, $z = 1$; (c) $\xi = 2$, $z = 2$; and (d) $\xi = 2$, $z = 1$.

14 may not be applicable. We have calculated the binding fraction at higher ionic concentration regions by a numerical solution of the exact equation of eq 5. Figure 1 shows the results for uni- and divalent counterions binding on polyions as a function of bulk ionic concentration. To illustrate the effects of f, the following values have been selected: $f = 0.1, 0.5, 1.0, 2.0$, and 4.0. The systems outside the above f region may be deduced from results shown here. Parts a and b of Figure 1 show respectively the binding fractions of divalent and univalent counterions on a polyion with $\xi = 4$, and parts c and d of Figure 1 are respectively the binding fractions of divalent and univalent counterions on a polyion with $\xi = 2$. It is observed that all the essential features revealed from the approximate expression are retained in these numerical results. The major differences occur at ionic concentrations above 0.1 M, where the increase of the binding fraction with ionic concentration is much faster than the approximate expression predicted. This is expected, since at this concentration region, the approximate expression is no longer valid.

An important question is how the short-range factor f is related to experimental observations. It has been experimentally observed that the binding fractions of most uni- and divalent counterions on polyions are generally close to the limiting value: $z\theta \simeq 1 - 1/z\xi$.[1-4] In particular, the binding fractions are observed almost independent of bulk ionic strength over a wide concentration region (for a recent review, see ref 4). The limiting value obtained theoretically is found consistent with experiments.[1,2] The constancy of the binding fraction then has to be fitted by a proper short-range factor. The theory predicts that if $f > 1$, the binding fraction will increase with bulk ionic strength; if $f < 1$, the binding fraction will decrease with ionic strength. It is easy to see that only for those systems with f close to unity will the binding fraction remain constant. Our numerical calculations indicate that, for those systems with $0.25 < f < 2$, the counterion binding fractions are quite constant over a wide range of bulk ionic concentration. Particularly, it is observed that the binding fraction of the system with $f = 0.5$ is even less variable at higher ionic strengths than the system with $f = 1$. Comparing the theoretical results with experiments, we suggest that the short-range interactions between polyions and most univalent and divalent counterions should be quite small, so that f is near unity. The possible value of f may range from 0.25 to 2. In this region, the counterion binding fraction on the polyion is not significantly dependent on the bulk ionic strength. The short-range interaction energy can be estimated with the relationship $\delta\mu = -RT \ln f$.

(b) Two Counterion Species Systems. This section is devoted to a qualitative estimation of the relative magnitude of the short-range factor for a number of counterion species.

Body and Wilson measured the selectivity coefficient of synthetic poly(ethylene sulfonate) for alkali-metal ions: Na^+, K^+, and Cs^+.[27] They found that the selectivity coefficient among these species is almost independent of the bulk ionic composition over the entire experimental region. The selectivity coefficients α for K^+ and Cs^+ with respect to Na^+ are 1.33 and 1.47, respectively. Extensive measurements on the competitive binding of monovalent counterions on DNA by a ^{23}Na NMR technique have been reported from Record's group.[4,28,29] The selectivity coefficients of Li^+, K^+, and Cs^+ with respect to Na^+ when binding on DNA are 1.12, 1.59, and 1.56, respectively. Since the selectivity coefficient is directly related to the ratio of the short-range factors between two species of similar valence, the above experimental results indicate that the short-range effects among these monovalent ions are quite similar and the differences in the binding free energies are less than 0.3 kcal/mol. The organic cations appear different from the metal ions. For example, Paulsen et al. report that the selectivity coefficients of DNA for the organic cations Bu_4N^+ and Et_4N^+ are about 10–20 fold smaller than that of Na^+.[4,29] These results perhaps indicate the importance of short-range interactions for organic counterions.

Experimentally measured binding constants of Mg^{2+}, Mn^{2+}, and Eu^{3+} on double-stranded DNA in a solution with excess NaCl have been analyzed according to eq 26. The data for Mn^{2+} and Eu^{3+} are from single sources,[21,22] and the data for Mg^2 are taken from a number of published works.[30-32] Na^+ is taken as the common reference in all these systems. Plots of the logarithm of the binding constants of Mg^{2+}, Mn^{2+}, and Eu^{3+} as a function of ln $h(c_1)$ defined in eq 28 are shown in Figure 2. The straight lines that pass through the experimental data points are obtained by a least-squares fitting procedure. Two observations can be made from these analyses. (a) Despite the diversity of the experimental data, all these plots are excellently linear. The slopes of the plots for Mg^{2+}, Mn^{2+}, and Eu^{3+} data are −0.97, −0.97, and −1.09, respectively, all very close to −1. These results are in agreement with the relationship of eq 26. (b) The intercepts of the straight lines for Mg^{2+}, Mn^{2+}, and Eu^{3+} are 0.2, 0.36, and 1.92, respectively. If the linear charge density of DNA is taken as $\xi = 4.2$, the ratios of f_M/f_{Na^+} for $M = Mg^{2+}$, Mn^{2+}, and Eu^{3+} are calculated, according to Eq 27, to be 6.49, 7.61, and 64.71, respectively. The relative strength of Mn^{2+} binding on DNA is found to be greater than Mg^{2+}. The difference, however, is somewhat smaller than that reported by Granot and Kearns,[21] who found that Mn^{2+} is about 3 times stronger than Mg^{2+} in binding to DNA. The experimental data of Mn^{2+} used in the above analyses are from Granot and Kearns,[21] and the data for Mg^{2+} are taken from a number of different sources.[30-32] The difference is not quite clear.

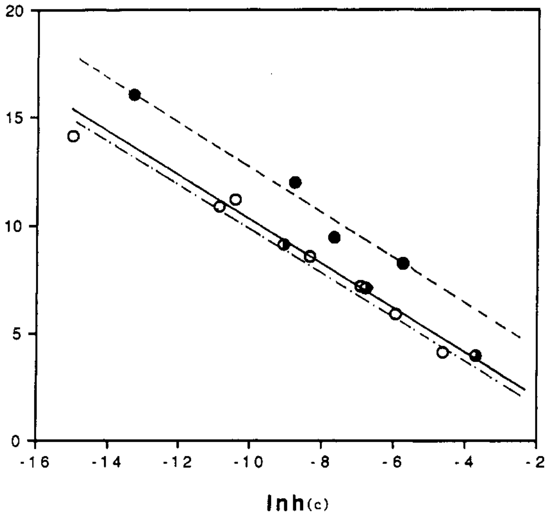

Figure 2. Dependence of counterion binding constants on the concentrations of NaCl. K_0 and $h(c)$ are defined in the text. The filled circles are the data points for Eu^{3+}, the half-filled circles are data points for Mn^{2+}, and the open circles are the data points for Mg^{2+}. The data of Eu^{3+} are from Draper,[22] the data of Mn^{2+} are from Granot and Kearns.[21] The data of Mg^{2+} are taken from Clement et al.[32] ($c_1 = 10^{-3}$, $K_0 = 0.33 \times 10^6$; $c_1 = 8.1 \times 10^{-3}$, $K_0 = 5.1 \times 10^{-6}$), from Archer et al.[31] ($c_1 = 10^{-2}$, $K_0 = 7.2 \times 10^4$; $c_1 = 2.9 \times 10^{-2}$, $K_0 = 5.0 \times 10^3$; $c_1 = 6.0 \times 10^{-2}$, $K_0 = 1.27 \times 10^3$; $c_1 = 10^{-1}$, $K_0 = 3.44 \times 10^2$), and from Zubay and Doty[30] ($c_1 = 2 \times 10^{-1}$, $K_0 = 5.8 \times 10^1$).

Table I
Short-Range Factors of Different Counterions Compared to That of Na^+

cations (M^{z+})	polyions	$f_{M^{z+}}/f_{Na^+}$	$\Delta\mu_{loc}$,[d] kcal/mol
K^+	PES[a]	1.33	−0.19
Cs^+	PES[a]	1.47	−0.25
Li^+	DNA[b]	1.12	−0.07
K^+	DNA[b]	1.19	−0.11
Cs^+	DNA[b]	1.56	−0.29
Mg^{2+}	DNA[c]	6.49	−1.28
Mn^{2+}	DNA[c]	7.61	−1.34
Eu^{3+}	DNA[c]	64.71	−2.75

[a] PES represents poly(ethylene sulfonate). Data from Body and Wilson.[27] [b] Data from Bleam, Anderson, and Record.[28] [c] Raw data are shown in Figure 2; the values $f_{M^{z+}}/f_{Na^+}$ are derived according to eq 26 by a least-squares fit of the data points shown in Figure 2. [d] $\Delta\mu_{loc}$ is the short-range interaction energy, calculated as $\Delta\mu_{loc} = -RT \ln (f_{M^{z+}}/f_{Na^+})$ with $T = 300$ K.

The short-range interaction factors for the various counterion species are summarized in Table I. These data provide quantitative measurements on the relative short-range interactions of the polyions (particularly DNA) with various counterion species. Inspection of the data in the table reveal several interesting points. First, the short-range factor f of the monovalent species can be considered to be small; they are all very close to unity. If the f factor of Na^+ is near unity, all the other monovalent cations will also be so. According to the results of the previous section, if f is near unity, the binding fraction of the counterion will be close to the limiting value and nearly independent of the bulk ionic strength. These results are consistent with the binding behaviors of the monovalent counterions observed experimentally.[1-4] Second, the data show the increasing importance of short-range interactions in higher valence counterion binding on polyions. The short-range factor of Mg^{2+} is about 6 times bigger than that of Na^+, and the short-range factor of Eu^{3+} is 65 times that of Na^+. Clearly, the long-range electrostatic interaction alone would not be adequate to explain the behavior of Eu^{3+} binding on DNA. Finally, counterion binding on polyions such as DNA is primarily controlled by the long-range electrostatic interaction. The binding of monovalent counterions is seen to be completely determined by long-range electrostatic interactions since their f factors are near unity. The modifications of the short-range interactions for divalent counterion binding on DNA are also not large. Even for the trivalent cation Eu^{3+} [22] in 27

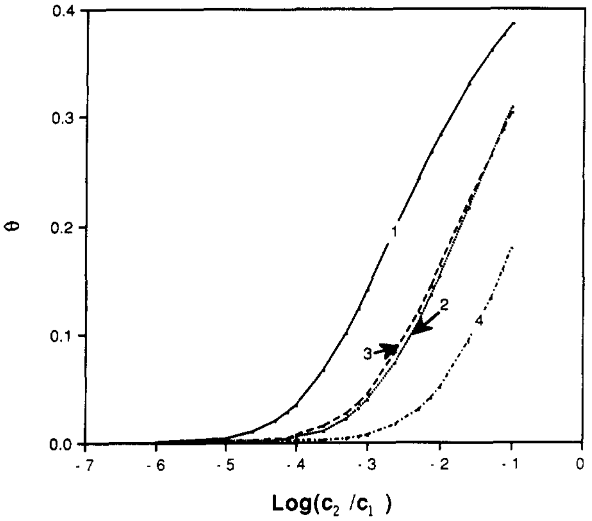

Figure 3. Calculated binding curves of divalent counterion on DNA in different salt concentrations and with different short-range factors. Curve 1: 0.01 M NaCl, $f = 7$. Curve 2: 0.1 M NaCl, $f = 7$. Curve 3: 0.01 M NaCl, $f = 1$. Curve 4: 0.1 M NaCl, $f = 1$. The short-range factor of Na^+ is taken as unity.

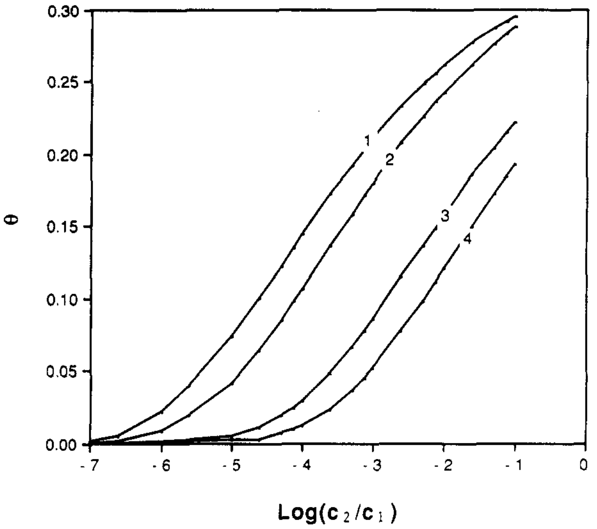

Figure 4. Binding curves of a trivalent counterion on DNA in different salt concentrations and with different short-range factors. Curve 1: 0.05 M NaCl, $f = 64.7$. Curve 2: 0.1 M NaCl, $f = 64.7$. Curve 3: 0.05 M NaCl, $f = 1$. Curve 4: 0.1 M NaCl, $f = 1$. The f factor of Na^+ is unity.

mM NaCl solution, the apparent binding constant of Eu^{3+} on DNA is 10^7 M^{-1}, to which the short-range interactions only contribute a factor of 65. At 400 mM NaCl concentration, the apparent binding constant is still much larger than that of the short-range factor. Considering the relative magnitude of the long-range electrostatic interactions and short-range interactions, it appears that counterion binding on DNA is primarily dominated by long-range electrostatic forces.

(c) Binding Isotherms. A large number of experiments have been done on divalent and trivalent counterion binding on DNA in solutions with excess NaCl.[20-22,30-33] Our discussions below are only concerned with this kind of system. In particular, we are interested in how the short-range interactions and salt effects influence the binding isotherms of higher valence counterions on polyions. Qualitative descriptions can be obtained from eq 23. Higher valence counterion binding on the polyion in solutions with excess monovalent counterions has the characteristics of anticooperative binding. The binding curves have the typically sigmoidal shape, which is characterized by the position and slope of the curve in the θ_2–c_2 frame. The position of the binding curve may be defined by the point where θ_2 reaches half of its maximum value. According to eq 23, the half-maximum point of the binding curve is at $c_2 = 1/(z_2K')$, where K' is defined by eq 24. It is seen that the larger the binding constant and/or the higher the counterion valence, the more the binding curve is moved to the low concentration region. In graphical representations, it is usually more convenient to plot θ_2 with respect to $\ln c_2$. The slope of the binding curve in such a frame is, according to eq 23

$$d\theta_2/d \ln c_2 = (1 - \xi^{-1})K'c_2/(1 + z_2K'c_2)^2 \qquad (30)$$

At the half-maximum binding point, the slope of the curve is $(1 - \xi^{-1})/(4z_2)$. It is seen that the higher the counterion valence, the flatter the binding curve in the θ_2–$\ln c_2$ frame.

The above general features of the binding curve are consistent with the numerical solution of the exact relations of eqs 18a and 18b. Figure 3 shows the binding fractions of a divalent counterion as a function of its bulk concentration in 0.01 and 0.1 M NaCl solutions. The polyion is DNA with $\xi = 4.2$. The short-range factor of Na^+ is taken as unity. To illustrate the effects of the short-range factor, f_2 is chosen respectively as 1 (no short-range interaction) and 7, which is the average value for Mg^{2+} and Mn^{2+} determined with respect to Na^+. Figure 4 shows similar binding curves of Eu^{3+} on DNA in solution with NaCl concentrations of 0.05 and 0.1 M, respectively. The short-range of Eu^{3+} is taken as either 1 or 64.7 (the latter was determined previously). For the convenience of graphical presentation, the concentration of the higher valence counterion has been represented as the ratio $c_2/[Na^+]$, where $[Na^+]$ is the NaCl concentration.

Consider the binding curve of a divalent counterion as an example. Curves 1 and 2 in Figure 3 are both for the case of $f = 7$, but curve 1 is calculated in a 0.01 M NaCl solution and curve 2 in a 0.1 M NaCl solution. It is seen that curve 2 is shifted to a higher concentration position by about 2 orders of magnitude with respect to curve 1, corresponding to the ratio of their binding constants as 10^2. Comparing Figures 3 and 4 shows that the slope of the binding curves of a trivalent counterion is less than that of a divalent counterion. Furthermore, the slope of the binding curves of similar species at lower ionic strength is slightly less than that at higher ionic strength. These observations are in agreement with the qualitative features of eq 23. The short-range factor f only acts to shift the position of the binding curve but does not change the shape of the binding curves.

Comparison of the theoretical results with experimental data is made on two aspects: the position and the shape of the binding curves in the θ–$\ln c$ frame. As discussed above, the position of the binding curve is determined by the binding constant K_0. Clearly, the short-range factor is necessary in order for the theoretical binding curves to be in agreement with the experimental results. Consider Eu^{3+} binding on DNA as an example. From the experimental data of Draper,[22] the middle point of the binding curve in a 0.027 M NaCl solution is at about 10 μM Eu^{3+}. This can be fitted by the theoretical curve with a factor of $f = 64.7$. If the short-range factor is taken as unity, the middle point of the binding curve will be shifted to about 600 μM, which is nearly 2 orders of magnitude away from the experimental data. In the case of Mg^{2+} and Mn^{2+} binding on DNA, if the short-range factor is omitted, the position of the theoretical binding curve is about 1 order of magnitude away from the experimental results.

Table II
Comparison of Different Expressions for Divalent Counterion Binding on DNA

$\theta_2/c_2 = K_0(1 - A\theta + B\theta^2)$

source	ion	c_1, M	A	B
ref 33	Mg^{2+}	0.2	6.1	10.7
ref 32[a]	Mg^{2+}	0.1–0.01	7.1	24.7
ref 31[a]	Mg^{2+}	0.08	6	10
ref 21	Mn^{2+}	0.02	5.8	8.4
ref 21	Mn^{2+}	0.065	6.2	10.0
present work	M^{2+}	0.01	5.83	8.6
present work	M^{2+}	0.1	6.07	10.0
Manning's theory[34]	M^{2+}	no effect	5.25	6.89

[a] The experimental data are parametrized by Manning.[34]

Next, we compare the shapes of the binding curves between theoretical results and experiments for divalent counterion binding on DNA. To facilitate the comparison, the binding fraction θ_2 from the numerical solution of eqs 18a and 18b is parametrized according to the polynomial[35]

$$\theta/c_2 = K_0(1 - A\theta_2 + B\theta_2^{\,2}) \qquad (31)$$

where A and B are constants. This fitting procedure is found to be very accurate within the region $0 < \theta_2 < 0.38$, and the fitness R factor is larger than 0.997. The calculated coefficients of A and B at 0.1 and 0.01 M NaCl solutions are shown in Table II. Five different sets of experimental results are also collected in the table. The experimental data of Archer et al.[30] and Clement et al.[32] are presented in the form as parametrized by Manning.[34] The expression used for the data of Clement et al.[32] is taken in the range of $\theta_2 < 0.26$.[34] It is found that the theoretical results are in close agreement with four sets of experimental data. Moreover, the calculations indicate that as the NaCl concentration decreases from 0.1 to 0.01 M, the coefficients A and B of eq 31 decrease from 6.07 to 5.83 and from 10.0 to 8.61, respectively. The experimental data of Mn^{2+} binding on DNA show similar phenomenon.[21] The only different case is the data of Archer et al.,[31] which is parametrized based on the average value over the NaCl concentration region from 0.01 to 0.1 M.[34] The results of an earlier one-parameter theory for Mg^{2+} binding on DNA developed by Manning are also shown in the table.[34] It is found that the present theoretical results are closer to experimental data than the earlier theory. The agreement of the theoretical binding curve with that of Eu^{3+} binding on DNA determined experimentally is poor. This can be attributed to the experimental conditions. The theoretical expressions are derived under the assumption that the concentration of counterions is in far excess over that of equivalent polyion charges. The experiment, on the other hand, was performed under the condition that the equivalent concentration of the DNA phosphate charge is always higher than the concentration of Eu^{3+}.[34] For this reason, the theoretical results are not directly comparable with experiments except in the limit $\theta_2 \to 0$.

(d) Origin of Short-Range Interactions. Since the formal expressions of the present lattice model are identical to counterion condensation theory[1] and the short-range factor has not been used in the earlier theory, there is the question as to whether it is necessary to introduce such a factor. The question may be alternatively asked, what is the origin of the short-range forces? The latter question can be considered from three aspects. First, an examination of the basic formalism of the lattice model shows that long-range interactions are calculated based on the concept of neighboring interactions. The electrostatic interactions between a counterion and its own binding site (therefore the polyion charge at the site) are excluded from the long-range terms. The electrostatic interaction between a counterion and its binding site will contribute to the short-range term. Second, the interactions between a bound ion and the binding site may involve several charged groups of the polyion near the site. The collective interactions of a number of charged groups with a bound ion may not be identical to the sum of individual pair interactions. For this reason, the interactions between the counterion and its binding site cannot be simply treated as the interactions between two individual charges. Third, when a counterion binds to a binding site of the polyion, short-range repulsive forces (such as exclusive volume and ion size effects) and the change in the hydration energy of the ion will also contribute to the short-range effect. The above three types of interactions constitute the origins of the short-range forces between the polyion and the counterions. The main difference between the long-range interactions and the short-range interactions is that the former are strongly dependent on the ionic strength of the solution, while the latter are basically independent of the ionic strength.

The present study demonstrates the necessity of including short-range attractive forces in the theory of counterion binding on polyions. The short-range effects influence the counterion binding behavior in both single and two counterion species systems. In the single species system, the short-range factor drives the counterion binding fraction away from the limiting law and becomes dependent on bulk ionic strength. For higher valence counterion binding on a polyion in solutions with excess 1:1 salt, the short-range factor shifts the position of the binding curve and changes the magnitude of the binding constant. The short-range factor, however, does not change the shape of the binding curves. This explains why in an earlier theory for Mg^{2+} binding on DNA,[34] the shape of the binding curve can be satisfactorily explained without the involvement of short-range interactions. The short-range interactions are not large for most uni- and divalent counterions, however. This may be the reason why, in many of the earlier theories on counterion binding on polyions, the short-range interactions are basically neglected but the theoretical results are still in reasonable agreement with experiment. The introduction of the short-range factor is required from theoretical considerations. Furthermore, in practical applications, the short-range factor can be quite large for higher valence counterions, so that without this factor the theory will not be able to satisfactorily explain experimental observations.

Conclusions

In this paper, we have investigated three problems concerning the short-range interactions in counterion binding on polyions based on a new lattice theory. First, we show that the short-range effect can be introduced into the counterion binding theory by a dimensionless factor. Second, by comparing theory with experiments, we have made quantitative estimates of the magnitude of the short-range factor for a number of counterion species. Third, the effects of the short-range interactions on counterion binding are examined for two types of systems. It is shown that the short-range factor drives counterion binding fractions away from the limiting law of CC theory, and such a factor is desirable in explaining experimentally measured binding constants, particularly for multivalent counterions. While the present approach is based on a site-binding model, it is found that the final results are comparable to those of counterion condensation theory based on the territorial binding model. This consistency implies that the overall description of counterion binding is not particularly dependent on the mathematical formalism but is instead dependent on the physical forces that dominate the system. The critical element is the long-range electrostatic interactions between the polyion and the counterions. With such a potential, the counterion condensation phenomenon can be obtained from a site-binding formalism as well.

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References and Notes

(1) Manning, G. S. *Quart. Rev. Biophys.* **1978**, *11*, 179.
(2) Manning, G. S. *Acc. Chem. Res.* **1979**, *12*, 443.
(3) Anderson, C. F.; Record, M. T., Jr. *Annu. Rev. Phys. Chem.* **1982**, *33*, 191.
(4) Anderson, C. F.; Record, M. T., Jr. *Annu. Rev. Biophys., Biophys. Chem.* **1990**, *19*, 423.
(5) Poland, D. *Cooperative Equilibria in Physical Biochemistry*; Clarendon Press: Oxford, U.K., 1978.
(6) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924.
(7) Manning, G. S. *J. Phys. Chem.* **1984**, *88*, 6654.
(8) Iwasa, K. *J. Chem. Phys.* **1975**, *62*, 2976.
(9) Fenley, M. O.; Manning, G. S.; Olson, W. K. *Biopolymers* **1990**, *30*, 1191.
(10) Hill, T. L. *Arch. Biochem. Biophys.* **1955**, *57*, 229.
(11) Dolar, D.; Peterlin, A. *J. Chem. Phys.* **1969**, *50*, 3011.
(12) Bratko, D.; Vlachy, V. *Chem. Phys. Lett.* **1982**, *90*, 434.
(13) Jayaram, B.; Beveridge, D. L. *J. Phys. Chem.* **1991**, *94*, 4666.
(14) Bacquet, R.; Rossky, P. J. *J. Phys. Chem.* **1984**, *88*, 2660.
(15) Bacquet, R.; Rossky, P. J. *J. Phys. Chem.* **1988**, *92*, 3604.
(16) Le Bret, M.; Zimm, B. H. *Biopolymers* **1984**, *23*, 271.
(17) Olmsted, M.; Anderson, C. F.; Record, M. T., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7766.
(18) Gregor, H. P.; Gregor, J. M. *J. Chem. Phys.* **1977**, *66*, 1934.
(19) Pack, G. R.; Klein, B. J. *Biopolymers* **1984**, *23*, 2801.
(20) Granot, J.; Feigon, J.; Kearns, D. R. *Biopolymers* **1982**, *21*, 181.
(21) Granot, J.; Kearns, D. R. *Biopolymers* **1982**, *21*, 203.
(22) Draper, D. E. *Biophys. Chem.* **1985**, *21*, 91.
(23) Hill, T. L. *Cooperativity Theory in Biochemistry*; Springer-Verlag: New York, 1985.
(24) Hill, T. L. *An Introduction to Statistical Thermodynamics*; Addison-Wesley: Reading, MA, 1960.
(25) Dewey, T. G. *Biopolymers* **1990**, *29*, 1793.
(26) de Bruijn, N. G. *Asymptotic Methods in Analysis*; Dover Publications: New York, 1981.
(27) Body, G. E.; Wilson, D. P. *Macromolecules* **1982**, *15*, 78.
(28) Bleam, M. L.; Anderson, C. F.; Record, M. T., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3085.
(29) Paulsen, M. D.; Anderson, C. F.; Record, M. T., Jr. *Biopolymers* **1987**, *27*, 1247.
(30) Zubay, G.; Doty, P. *Biochim. Biophys. Acta* **1958**, *29*, 47.
(31) Archer, B. G.; Craney, C. L.; Krakauer, H. *Biopolymers* **1972**, *11*, 781.
(32) Clement, R. M.; Sturm, J.; Daune, M. P. *Biopolymers* **1972**, *12*, 405.
(33) Skerjanc, J.; Strauss, U. P. *J. Am. Chem. Soc.* **1968**, *90*, 3081.
(34) Manning, G. S. *Biophys. Chem.* **1977**, *7*, 141.

Registry No. Mg^{2+}, 7439-95-4; Mn^{2+}, 7439-96-5; Eu^{3+}, 7440-53-1.