Biophysical Chemistry 7 (1977) 141–145
© North-Holland Publishing Company

THEORY OF THE DELOCALIZED BINDING OF Mg(II) TO DNA: PRELIMINARY ANALYSIS FOR LOW BINDING LEVELS

Gerald S. MANNING
Wright and Rieman Chemistry Laboratories, Rutgers, The State University, Piscataway, New Jersey 08854, USA

Received 4 January 1977

A simple theoretical equation for the binding of Mg^{2+} to DNA in the presence of excess 1 : 1 salt is derived from a model that does not specify discrete binding sites but rather allows the associated metal ions to move freely near the surface of the DNA polyion. Use of a numerical value for the free volume, determined uniquely, in a separate communication, by a free energy minimization, leads to predicted values for the Mg^{2+} binding constant that are in essential agreement with measured values taken from the literature.

1. Introduction

With no exceptions of which I am aware, data for the binding of metal ions to polynucleotides have been interpreted by mass-action or extended mass-action formulas that assume, at least phenomenologically, the existence of discrete binding sites on the polynucleotide molecule. Yet for strongly hydrated closed-shell ions like Na^+, Mg^{2+}, and Ca^{2+} there is no evidence in the range of physiological concentrations to indicate localized binding. In fact, the diffusion-limited association rate [1] and very small dilatometric effects [2] are consistent with the view that a "bound" Mg^{2+} ion is free to move in a nearly fully hydrated state near the polynucleotide surface. Evidence for delocalized binding will be reviewed in detail elsewhere [3]; here I am concerned only to show that existing binding data for Mg^{2+} are consistent with the delocalized mode of association. In previous papers it has been demonstrated that Mg^{2+} effects on DNA conformational changes also do not require postulation of specific binding sites [4–7].

2. Theory

The task at hand is to derive the following binding relation:

$$\theta/c_{2d} = Ac_s^{-2}(1 - \xi^{-1} - 2\theta)^2 . \tag{1}$$

In this equation θ is the ratio of moles of associated divalent cation n_{2a} to moles of nucleotide phosphate n_p

$$\theta = n_{2a}/n_p . \tag{2}$$

c_{2d} is the molarity of dissociated divalent cation, c_s is the molarity of 1 : 1 salt in the system, and ξ is the charge density parameter for DNA defined by

$$\xi = q^2/\epsilon kTb , \tag{3}$$

where b is the average axial charge spacing for DNA, equal to 1.7 Å because the step height in the B form is 3.4 Å and takes in two phosphate groups, and the factor that multiplies b^{-1} is the square of the electronic charge divided by the product of the dielectric constant of pure solvent, Boltzmann's constant k, and the absolute temperature T. Then $\xi_{DNA} = 4.2$ at 25°C. The constant A in eq. (1) is given by

$$A = 1000\, e\, V_p^{-1} , \tag{4}$$

where e is the base of natural logarithms, and V_p is the free volume in cm^3 per mole nucleotide for associated univalent and divalent cations. That is, in accord with the discussion in section 1, the associated cations are visualized as moving freely in a fully hydrated state within cylindrical shells axially centered on each DNA molecule and possessing total volume $n_p V_p$. For the sake of simplicity, the free volume V_p will be regarded as the same for both univalent and divalent cations. Another important assumption underlying eq. (1) is that univalent cation condensation is complete [8] so that the net value of ξ is unity; in other words, if the net value of ξ, including the charge of associated divalent, but not univalent cations, is greater than unity, then the extent of univalent cation association is such as to reduce further the net value of ξ to unity [4,5]. A final assumption is that the 1 : 1 salt concentration c_s is in excess over both the nucleotide phosphate concentration c_p and the total alkaline-earth cation concentration c_{2t},

$$c_p/c_s \ll 1\ , \quad c_{2t}/c_s \ll 1\ . \tag{5}$$

The concentration c_s is thus negligibly different from the ionic strength of the solution.

The derivation of eq. (1) is remarkably simple. The total Gibbs free energy G_t may be decomposed into eight obvious components,

$$G_t = G_{1d} + G_{1a} + G_{2d} + G_{2a} + G_- + G_{H_2O} + G_t^{el} + G_t^0\ . \tag{6}$$

The term G_{1d} is the contribution of dissociated (free) univalent cations.

$$G_{1d}/RT = (n_s + \xi^{-1} n_p + 2n_{2a}) \times \ln[10^{-3}(c_s + \xi^{-1} c_p + 2c_{2a})]\ . \tag{7}$$

The first factor on the right-hand side of eq. (7) is the total number of moles of dissociated univalent cation in the system, which includes n_s moles from the 1 : 1 salt, $\xi^{-1} n_p$ moles that were originally counterions to the phosphate groups in the solid state but which dissociate in aqueous solution (the remaining $(1-\xi^{-1})n_p$ moles of univalent cation remain associated, or condensed, in aqueous solution in the absence of divalent cations [8]), and $2n_{2a}$ moles that dissociate upon association of n_{2a} moles of divalent cation in order to maintain the net value of ξ at unity. The argument of the logarithm is the concentration of dissociated univalent cation in units of mole cm^{-3}, c designating molarity. Omitted from eq. (7) is the standard state free energy for the dissociated univalent cations, since it is included in the last term G_t^0 of eq. (6) as discussed below; for theoretical simplicity, the activity coefficient is also omitted. Eq. (7) may be simplified by first generally defining

$$\bar g_i = G_i/n_p RT\ , \tag{8}$$

and then expanding the logarithmic factor in powers of the small quantities c_p/c_s and c_{2a}/c_s, with final retention only of the leading, zeroth-order term,

$$\bar g_{1d} = (c_s/c_p + \xi^{-1} + 2\theta)\ln(10^{-3} c_s) + \xi^{-1} + 2\theta\ . \tag{9}$$

The contribution G_{1a} in eq. (6) is from associated univalent cations,

$$G_{1a}/RT = (n_p - \xi^{-1} n_p - 2n_{2a}) \times \ln[(n_p - \xi^{-1} n_p - 2n_{2a})/n_p V_p]\ , \tag{10}$$

where the first factor is the number of moles of associated univalent cation (expressed as the number of moles of counterions in the solid state n_p minus the number of moles that dissociate upon dissolution in water and addition of divalent cation), and the argument of the logarithm is the local concentration in mole cm^{-3} of associated univalent cation, $n_p V_p$ being the total free volume in cm^3 accessible to the associated cations. Again, the standard state term is omitted, for it will be taken into account in the term G_t^0 of eq. (6). With eq. (8) one may then write

$$\bar g_{1a} = (1 - \xi^{-1} - 2\theta)\ln[V_p^{-1}(1 - \xi^{-1} - 2\theta)]\ . \tag{11}$$

G_{2d} is the free energy component due to dissociated divalent cations,

$$G_{2d}/RT = n_{2d}\ln(10^{-3} c_{2d})\ , \tag{12}$$

where n_{2d} and c_{2d} are the mole number and molarity, respectively, of dissociated divalent cation. Let n_{2t} be the total mole number of divalent cations,

$$n_{2t} = n_{2a} + n_{2d}\ , \tag{13}$$

and define

$$\theta_t = n_{2t}/n_p\ . \tag{14}$$

Then, with eq. (8),

$$\bar g_{2d} = (\theta_t - \theta)\ln[10^{-3} c_p(\theta_t - \theta)]\ . \tag{15}$$

G_{2a} is the contribution of associated divalent cations to the total free energy,

$$G_{2a}/RT = n_{2a}\ln(n_{2a}/n_p V_p)\ , \tag{16}$$

or,

$$\bar{g}_{2a} = \theta \ln(V_p^{-1}\theta) . \tag{17}$$

G_- is the contribution of the coions, assumed univalent,

$$G_-/RT = (n_s + 2n_{2t}) \ln\{10^{-3}(c_s + 2c_{2t})\} , \tag{18}$$

or,

$$\bar{g}_- = (n_s/n_p + 2\theta_t) \ln[10^{-3} c_p(c_s/c_p + 2\theta_t)] . \tag{19}$$

G_{H_2O} is the free energy of the solvent. Apart from the standard state of the solvent, which is lumped into G_t^0, the chemical potential of the solvent in units of RT is $-n_{\text{solute}}/n_{H_2O}$, where the numerator is the total mole number of solute particles, and the denominator is the number of moles of solvent, the osmotic coefficient being taken as unity. Since G_{H_2O} equals n_{H_2O} times the solvent's chemical potential, and the total number of moles of solute particles equals the sum of the mole numbers of dissociated uni- and divalent cations, coions, and macroions (i.e., DNA molecules together with associated cations), and since, further, the mole number of macroions is negligible in comparison with those of the small ions,

$$\bar{g}_{H_2O} = -\xi^{-1} - 2n_s/n_p - 3\theta_t - \theta . \tag{20}$$

The total electrostatic free energy G_t^{el} accounts for the free energy of charging the DNA polyions (considered as including the associated cations) and the free energy of interaction between dissociated ions and DNA molecules. The former contribution is assumed to depend only on the net charge density, which is maintained at a constant value corresponding to $\xi_{net} = 1$ [8]; expressed in the Born approximation with the units of $\bar{g}$, it is simply equal to $-\xi_{net} \ln a$ [9], where a is the radius of the equivalent cylinder that represents a DNA molecule. The interaction free energy in the same units is given by polyelectrolyte theory [8] as $-\xi_{net} \ln \kappa$, where κ is the Debye screening constant, proportional to $\Sigma n_i z_i^2$, where the sum is over all cations and anions and z_i is the ionic valence. It is easy to verify that, but for negligible terms of order c_p/c_s, κ may be replaced by κ_s, the Debye screening constant for an aqueous 1 : 1 salt solution of concentration c_s in the absence of DNA and divalent cations. Hence,

$$\bar{g}_t^{el} = -\xi_{net} \ln(\kappa_s a) , \quad \xi_{net} = 1 . \tag{21}$$

The final free energy component is G_t^0, the sum over the standard state free energies of all species. Since the standard states of dissociated and associated species are, by assumption, the same (an associated cation is fully hydrated and translates freely), all that need be noted is

$$g_t^0 = \bar{g}_t^0(n_p, n_s, n_{2t}, n_{H_2O}) , \tag{22}$$

and, in particular, that $\bar{g}_t^0$ does not depend on θ, the parameter that specifies the distribution of cations among associated and dissociated states.

It remains now merely to minimize the free energy with respect to n_{2a} (or θ), subject to the constraint that n_{2t} (or θ_t) is constant. Therefore, all the $\bar{g}$ terms are added, and the result is differentiated with respect to θ, the only variable. When the derivative is set equal to zero, simple rearrangement leads to the binding equation (1). Note particularly that of the eight components of the free energy, only five depend on θ and hence influence the equilibrium distribution of associated and dissociated ions; the coion free energy, the standard state free energy, and the electrostatic free energy play no role in determining the binding relation. Also, and especially, to be noted is that discrete binding sites on the DNA molecule have not been introduced into the model. The binding eq. (1) does not describe a multiple ligand-site equilibrium and therefore is not expressed in terms of fictitious binding constants or numbers of binding sites.

It is clear from the derivation that eq. (1) is restricted to the range $\theta < \frac{1}{2}(1 - \xi^{-1})$, since the net value of ξ computed from the phosphate charge and the charge of associated divalent, but not univalent, cations must remain greater than unity. For DNA this range is $\theta < 0.38$. The singularity at $\theta = \frac{1}{2}(1 - \xi^{-1})$ is a theoretical artifact due to the assumption of complete univalent cation condensation, i.e., that association of univalent cations maintains the net value of ξ at unity regardless of the value of θ. In fact, the maximum value of θ is $\frac{1}{2}(1 - \frac{1}{2}\xi^{-1})$, or 0.44 for DNA [5,10]. A more elaborate calculation in progress may clarify this point.

One of the most important conclusions from eq. (1) concerns the limiting behavior. In line with the limiting laws of polyelectrolyte theory [4,10], Mg^{2+} association becomes complete as the ionic strength, equal to c_s, tends to zero.

3. Numerical results

To compare eq. (1) with published binding data, it is convenient to rewrite it in the form

$$\theta / c_{2d} = K_0 g(\theta) , \tag{23}$$

where

$$K_0 = \lim_{\theta \to 0} (\theta / c_{2d}) \tag{24}$$

and $g(\theta)$ is a polynomial in θ with the limiting property,

$$\lim_{\theta \to 0} g(\theta) = 1 . \tag{25}$$

In this form, and with the value $\xi = 4.2$ characteristic of DNA, eq. (1) reads,

$$\theta / c_{2d} = 1580 \, V_p^{-1} c_s^{-2} (1 - 5.3\theta + 6.9\theta^2) . \tag{26}$$

Tables 1 and 2 provide comparison of theory with data from five sources. Table 1 compares the theoretical values for K_0 with measured values. From eqs. (24) and (26), the theoretical expression for K_0 is $1580 \, V_p^{-1} c_s^{-2}$. The value of V_p is taken as 647 cm^3, the result of a free energy minimization for a polyelectrolyte in an environment of excess 1 : 1 salt with no divalent counterions present. The analysis, which will be published separately in this journal, establishes an expression for V_p that depends only on ξ (a slight dependence on ionic strength is insignificant for present purposes); the value 647 cm^3 corresponds to $\xi = 4.2$. Measured values of K_0 correspond to its definition according to eq. (24), not necessarily to the definition in the papers cited.

Table 1
Comparison of theoretical and experimental values of K_0

Source	c_s(M)	K_0 (theory)	K_0(exp)
SS [2]	0.2	61	156
ZD [11]	0.2	61	59
ACK [12]	0.1	244	359
ACK [12]	0.01	2.44×10^4	7.17×10^4
CSD [13]	0.001	2.44×10^6	7×10^5
SB [14]	0.002	6.1×10^5	6×10^4

The ACK data are unique in covering a range of ionic strengths (emphasis on the ionic strength dependence is the focus of data presently being collected in Record's laboratory); the measured coefficient of c_s was reported as −2.3, in reasonable agreement with the present theoretical value −2.0. In general, the good agreement with the ZD, ACK and CSD data should be noted. A factor of 3 in K_0 corresponds to the small free energy 0.65 kcal/mole base at 25°C, which, while of certain importance for some purposes, is within what can reasonably be demanded of a completely molecular theory with a priori predictive power (and also may be within experimental error). The discrepancy with the SS data can possibly be assigned to the use by SS of the tetramethylammonium cation, which is known not to condense completely and hence does not compete with Mg^{2+} as well as the Na^+ ion used by ZD. The only discrepancy that may be of concern is for the SB data. The data of Sander and Ts'o [15] were not used here because their experimental conditions did not satisfy the theoretical restriction (5).

The value 647 cm^3 for V_p corresponds to the volume of a cylindrical shell of thickness 7 Å centered about a cylinder (DNA) of diameter 20 Å. Agreement with data using this value supports the picture of associated Na^+ and Mg^{2+} ions, which possess diameters of about 7 Å when fully hydrated, being held by the DNA in a loose monolayer.

Table 2 compares the theoretical expression for $g(\theta)$ with data from the same sources as in table 1. Three of the five sets of data indicate curvature of $g(\theta)$; for the SS data the curvature agrees closely with

Table 2
Comparison of theoretical and experimental expressions for $g(\theta)$ a)

Source	$g(\theta)$	Range	B_f
theory, eq. (26)	$1 - 5.25\theta + 6.89\theta^2$	$\theta < 0.38$	0.19
SS	$1 - 6.1\theta + 10.7\theta^2$	$\theta < 0.28$	0.16
ZD	$1 - 4.8\theta$	$\theta \leq 0.12$	0.21
ACK b)	$1 - 7.1\theta + 24.7\theta^2$	$\theta < 0.1$	0.14
CSD	$1 - 7.5\theta + 26.1\theta^2$	$\theta < 0.1$	0.13
SB	$1 - 3.3\theta$	$\theta < 0.25$	0.30

a) See table 1 for references.
b) In this case the coefficients of θ and θ^2 are average values for the range of ionic strangths 10^{-2} M − 10^{-1} M; see text.

the theoretical result (compare the ratio of the second-order to the first-order coefficient), while the ACK and CSD data exhibit greater curvature than predicted but still of the same order of magnitude. The experimental linear coefficients are in reasonably good agreement with theory. The standard linear Scatchard representation is

$$\theta/c_{2d} = K_f(B_f - \theta) \qquad (27)$$

and can be used here to describe the ZD and SB data and the initial linear regions of the theoretical curve and the other three sets of data. Values of B_f are listed in table 2; their interpretation as numbers of sites per nucleotide is without meaning in the present theoretical context, as is that of K_f as a mass-action site-binding constant, and the subscript "f" has been used to indicate "fictitious".

Representation of the ACK and CSD data by quadratic polynomials is not without subtleties. In both cases the authors effectively used a linear polynomial times an exponential to represent $g(\theta)$ over a much wider range than indicated in table 2, wherein $g(\theta)$ is given only as the expansion of the authors' expression to second order. In other words, the quadratic polynomials in table 2 for these cases are strongly determined by larger values of θ for which the theoretical equation (26) may not be accurate. A good representation of the CSD data for $\theta \leqslant 0.26$ is, in fact, given by the formula

$$\theta/c_{2d} = (6 \times 10^5)(1 - 6\theta + 10\theta^2) , \quad \theta \leqslant 0.26 , \qquad (28)$$

that is, with a K_0 value close to that given by the authors (see table 1) but with a quadratic polynomial $g(\theta)$ very similar both to theory and to the SS data (see table 2). Of course, eq. (28) is not a good representation of the CSD data for larger θ values, which may require an improved theory. To recapitulate, the most direct comparison of theory with experiment is obtained by fitting the data with a quadratic polynomial in θ with emphasis on small values of θ, allowing, if necessary, appreciable deviation of data points from the curve at high θ.

A second interesting curve-fitting problem is illustrated by the ACK data, the only set in table 2 taken over a range of ionic strengths (see table 1). These authors found that the numerical coefficient required to fit the exponential (of which $g(\theta)$ in table 2 is the second-order expansion) was a function of ionic strength. Thus, a more faithful representation of their data would have had the form $g(\theta) = 1 + b_1(c_s)\theta + b_2(c_s)\theta^2$, and table 2 actually lists average values of b_1 and b_2 over the concentration range studied 10^{-2} M $< c_s < 10^{-1}$ M. For example the coefficient B_f in the Scatchard representation for small θ, eq. (27), has the reported values 0.09 at $c_s = 0.01$ M and 0.34 at $c_s = 0.1$ M. Note that at 0.2 M (table 1), the B_f values of SS and ZD are *less* then the ACK value at 0.1 M, while at 0.001 M and 0.002 M, respectively, the CSD and SB values of B_f are *greater* than the ACK value at 0.01 M. Published data therefore appear to be internally inconsistent on the question of the ionic strength dependence of $g(\theta)$, and, in fact, an average of the ACK data for $g(\theta)$ is in good agreement with the $g(\theta)$ of CSD at a much lower ionic strength (table 2). The theoretical equation (1), or (26), predicts that $g(\theta)$ is independent of ionic strength (with the prediction expected to be more accurate at small values of θ). The theoretical and experimental evidence that the equivalent of $g(\theta)$ for the binding of protons to polyacrylate and polymethacrylate is independent of 1 : 1 salt concentration over the low end of the θ range (high degrees of neutralization of the polymeric weak acids) should also be recalled [9].

References

[1] D. Porschke, Biophys. Chem. 4 (1976) 383.
[2] J. Skerjanc and U.P. Strauss, J. Am. Chem. Soc. 90 (1968) 3081.
[3] M.T. Record Jr. and G.S. Manning, Quant. Rev. Biophys., in preparation.
[4] G.S. Manning, Biopolymers 11 (1972) 951.
[5] N. de Marky and G.S. Manning, Biopolymers 14 (1975) 1407.
[6] N. de Marky and G.S. Manning, Biopolymers 15 (1976) 457.
[7] G.S. Manning, Biopolymers 15 (1976) 1333.
[8] G.S. Manning, J. Chem. Phys. 51 (1969) 924.
[9] G.S. Manning and A. Holtzer, J. Phys. Chem. 77 (1973) 2206.
[10] G.S. Manning, in: Polyelectrolytes, ed. E. Selegny (Reidel, Dordrecht, 1974).
[11] G. Zubay and P. Doty, Biochim. Biophys. Acta 29 (1958) 47.
[12] B.G. Archer, C.L. Craney and H. Krakauer, Biopolymers 11 (1972) 781.
[13] R.M. Clement, J. Sturm and M.P. Daune, Biopolymers 12 (1973) 405.
[14] J. Shack and B.S. Bynum, Nature 184 (1959) 635.
[15] C. Sander and P.O.P. Ts'o, J. Mol. Biol. 55 (1971) 1.