

COMPARISON OF POLYELECTROLYTE THEORIES OF THE BINDING OF CATIONS TO DNA

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ABSTRACT Predictions of the binding of counterions to DNA made using the counterion condensation theory developed by Manning are compared with those made using the Poisson-Boltzmann equation, solved numerically by the Runge-Kutta procedure. Ions are defined as territorially or atmospherically bound if they fall within a given distance, defined by counterion condensation theory, from the DNA surface. Two types of experimental situations are considered. The first is the delocalized binding of a single type of counterion to DNA. In this case the Poisson-Boltzmann treatment predicts somewhat lower extents of binding to DNA, modeled as a 10-Å radius cylinder, than does Manning theory. The two theories converge as the radius decreases. The second type of experiment is the competition of ions of different valence for binding to DNA. The theories are compared with literature values of binding constants of divalent ions in the presence of monovalent ions, and of spermidine³⁺ in the presence of Na⁺ or Mg²⁺. Both predict with fair accuracy the salt dependence of the equilibrium constants.

INTRODUCTION

The polyelectrolyte nature of DNA has long been recognized as contributing to its behavior in solution. Recently a theory based on the concept of counterion condensation has been applied to DNA, with Manning (1) being largely responsible for its development and application to biological polyions. This theory has met with good success in predicting the extent of binding of charged ligands (2–4) and small ions (1, 5) to DNA.

Considerable theoretical work on polyelectrolytes is based on the Poisson-Boltzmann equation both in its full and linearized (Debye-Hückel) forms. Schellman and Stigter (6) have presented a rather detailed theoretical discussion of its application to DNA. The Poisson-Boltzmann approach differs from the counterion condensation theory in that the latter considers ions as either fully bound or free in solution, whereas the former results in a continuous distribution of the ions in solution.

We have recently applied both theories in considering the counterion-induced collapse of DNA (7, 8). In the process we naturally were concerned about the similarities and differences between the two approaches, especially since there is some disagreement as to the theoretical rigor of each (9, 10). Therefore, in this paper we present some detailed comparisons of binding calculations made using the full Poisson-Boltzmann equation and using Manning's theory, and compare both with experimental measurements.

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THEORY

The theory of counterion condensation is well described in a review article by Manning (1). Here we will reproduce some of the results which are important for this discussion. The theory is based on the concept that counterions will condense on a polyelectrolyte to lower its linear charge density to a limiting value. For a solution containing the polyelectrolyte and only one species of counterion with charge Z the charge on the polymer will be reduced by a factor of:

$$r = 1 - (Z\xi)^{-1} \text{ for } Z\xi > 1 \quad (1)$$

$$r = 0 \quad \text{for } Z\xi < 1, \quad (2)$$

where $\xi = q_p^2 / \epsilon k_B T b$ and q_p is the charge of a proton, ϵ is the bulk dielectric constant and b is the linear charge spacing of the polyelectrolyte in the absence of any associated ions. For DNA in aqueous solution $b = 1.7 \text{ \AA}$ and $\xi = 4.2$. Therefore, for example, the charge of DNA in solution with Na^+ ions is predicted to be reduced by 76%.

For a mixture of counterions with different valences the situation is more complicated because the counterions compete for association. Manning (1) has presented two treatments applicable to mixtures of two counterions. The first, or single variable theory, applies when one counterion species is in vast excess over the other. If the lower valent ion is assumed in excess an expression for the intrinsic binding constant of the higher valent ion is given by:

$$\log K_2 = \log (V_{p2}/1,000 \text{ e}) + \frac{Z_2}{Z_1} \log \left[\frac{1,000 \text{ e}}{V_{p1} c_1} (1 - (Z_1 \xi)^{-1}) \right], \quad (3)$$

where the subscripts 1 and 2 refer to the lower and higher valent counterions respectively, V_p is the volume per phosphate in which the ions are bound, defined by:

$$V_p = 4\pi e N_{AV} (1 + Z)(\xi - Z^{-1}) b^3, \quad (4)$$

c is the concentration of free ions, and e the base of natural logarithms. The equilibrium constant as defined above is the ordinate of a Scatchard plot and hence accessible to experiment.

The two variable theory is more generally applicable but requires that numerical methods be used to solve the coupled equations:

$$1 + \ln \left(\frac{1,000 \theta_1}{c_1 V_{p1}} \right) = -2Z_1 \xi (1 - Z_1 \theta_1 - Z_2 \theta_2) \ln(1 - e^{-\kappa b}) \quad (5)$$

and

$$\ln \left(\frac{\theta_2}{c_2} \right) = \ln \left(\frac{V_{p2}}{1,000 \text{ e}} \right) + \frac{Z_2}{Z_1} \ln \left(\frac{1,000 \theta_1 \text{ e}}{c_1 V_{p1}} \right) \quad (6)$$

where θ_i is the number of associated counterions of type i per charge on the polyion, and κ is the Debye-Hückel shielding parameter.

A detailed description of our use of the Poisson-Boltzmann approach to DNA in solution with added simple electrolyte is presented elsewhere (8). It basically involves modeling DNA as a charged rod with a uniform surface charge density and solving the Poisson-Boltzmann

equation with appropriate boundary conditions using the Runge-Kutta method. The solution is in the form of a set of electrostatic potentials corresponding to various distances from the surface of the rod. These potentials can then be used to calculate the distribution of ions about the rod. It is this distribution that we use to compute the extent of counterion binding.

RESULTS AND DISCUSSION

In discussing the binding of ions to a polyelectrolyte we are required to make some comment on what is meant by a "bound" ion. Current practice is to divide the ions associated with a polyelectrolyte into two categories (11, 12) those that are site bound and those that are atmospherically or territorially bound. Site bound ions are restricted to the vicinity of a charged group on the polyelectrolyte with no intervening water. The more loosely bound territorial ions are retained in the neighborhood of the polyion by its electrostatic field, but retain freedom of mobility within a bound domain around the polymer and suffer little or no change in hydration relative to ions free in solution. A similar distinction is made by Schellman and Stigter (6) who in their discussion of the binding of counterions to DNA distinguish between the ions in close association with the charges on the chain (Stern layer) and those to be treated by the Poisson-Boltzmann theory (Gouy double layer). It is with the territorially bound ions that the counterion condensation and Poisson-Boltzmann theories are concerned so we shall limit our discussion to this mode of binding.

It is somewhat difficult to apply this definition of territorial binding to experimental data. Different experiments measure different properties of the ions or polymer, so that binding

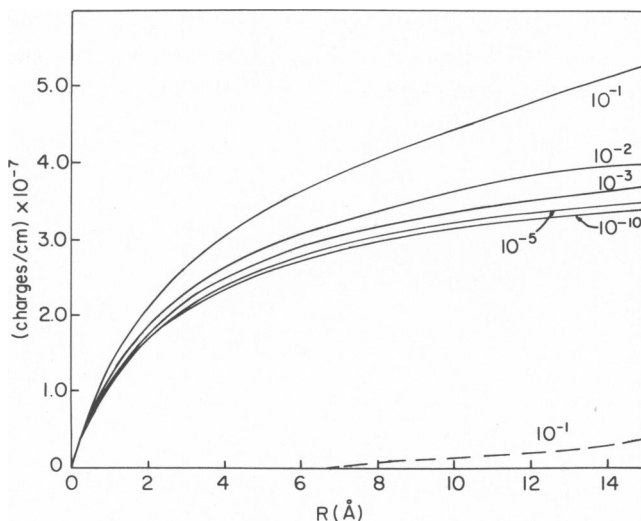


FIGURE 1 A plot of the calculated number of charges from univalent counterions (—) and univalent coions (----) within distance R of the surface of a charged cylinder as a function of R and the indicated bulk molar concentration of ions. The cylinder was selected to model DNA with a radius of 10 \AA and a linear charge density $Z_p = 5.9 \times 10^7$ charges/cm. In this and the next two figures, the fraction of DNA charges neutralized by mobile ions is given by the difference in total charge contributed by counterions and coions within R divided by Z_p .

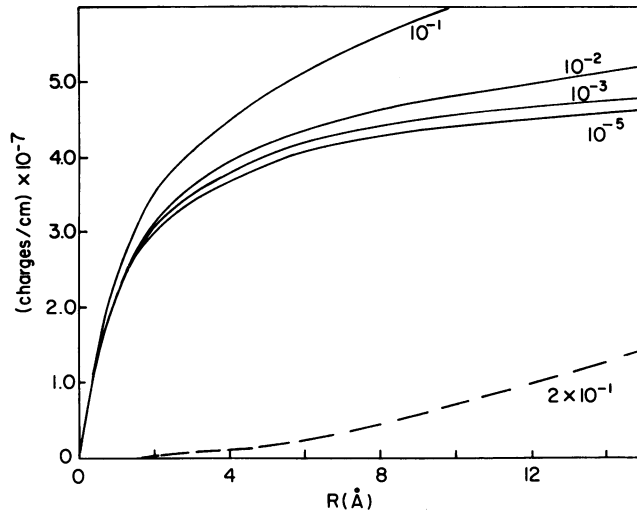


FIGURE 2 A plot of the calculated number of charges from divalent counterions (—) and univalent coions (----) within distance R of the surface of a charged cylinder as a function of R and the indicated bulk molar concentration of ions. Cylinder modeled as in Fig. 1.

may be defined differently for different experimental situations. In most cases, however, it should be possible to define some distance from the polymer such that ions within this distance are considered bound and those outside, free.

The counterion condensation theory supplies such a distance through the bound volume V_p . For example, for DNA and monovalent counterions (see Eq. 4) $V_{p1} = 646 \text{ cm}^3/\text{mol}$. If DNA is modeled as a rod with a 10-Å Diam this corresponds to a radius for binding of $\sim 7 \text{ Å}$ from the surface of the rod. Some attempts have been made to define a binding distance consistent

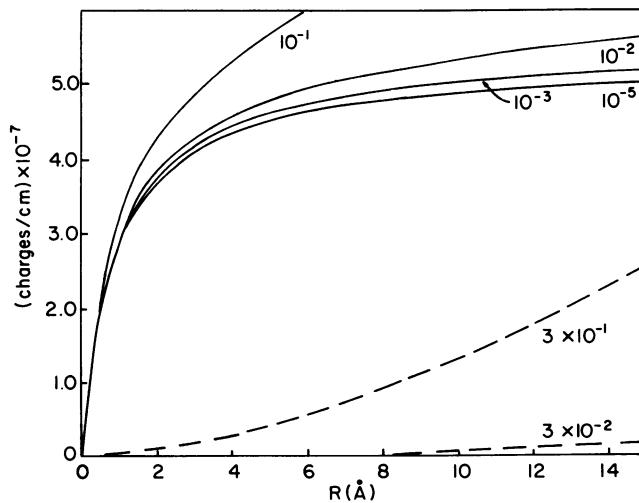


FIGURE 3 A plot of the integrated number of charges from trivalent counterions (—) and monovalent coions (----) within distance R of the surface of a charged cylinder as a function of R and the indicated bulk molar concentration of ions. Cylinder modeled as in Fig. 1.

with the Poisson-Boltzmann approach (13, 14). The assignment of such a distance within this theory seems somewhat arbitrary, especially in view of the variety of experimental procedures which have been applied to the problem. For comparison between the counterion condensation and Poisson-Boltzmann theories we therefore elect to use the same binding radius in both cases.

The extent of binding of Na^+ ions to DNA has been shown to be largely independent of their concentration (5). This nonmass action law behavior is a common property of the binding of counterions to linear polyelectrolytes (15) and gave much of the impetus to the development of condensation theories. In some forms, Manning's theory allows for a change in the fraction of DNA charges neutralized by counterions as a function of their concentration, but in its most often used form this charge fraction is determined only by the valence of the counterions (see Eqs. 1 and 2). The invariance of the charge fraction to concentration is also evidenced to a certain extent by Poisson-Boltzmann theory. In Figs. 1-3 are plotted the number of counterion charges within a radius R of the surface of a 10-Å radius cylindrical model for DNA, as a function of R . Note that over a wide range of counterion concentrations there is only a slight dependence of the curves on the bulk concentration. Large variance only occurs for concentrations of 0.1 M or greater. Part of this variance can be accounted for by the need to neutralize coions, whose concentrations in the vicinity of the rod begin to become important at these higher bulk concentrations (see dashed curves in Figs. 1-3).

From these curves it is possible to extract the fraction of DNA charges neutralized by associated counterions if it is assumed that ions within 7 Å for univalent, 11 Å for divalent, and 14 Å for trivalent ions are bound. These radii are calculated for a cylinder with a 10-Å radius using values of $V_p = 646, 1,121, \text{ and } 1,563 \text{ cm}^3/\text{mol PO}_4$, respectively. The results are contained in Table I. The larger values correspond to assumed concentrations of 0.1 M and the smaller to 10^{-10} M. A feature of Manning's counterion condensation theory is the independence of the fraction of polymer charges neutralized on the bulk counterion concentration. Poisson-Boltzmann theory, we find here, predicts a strict independence of the fraction of charges neutralized only in the limit of very low bulk counterion concentrations. In each case

TABLE I
EFFECT OF THE ASSUMED RADIUS, a , ON THE FRACTIONAL CHARGE REDUCTION IN THE PRESENCE OF COUNTERIONS FOR CYLINDRICAL MODELS OF DNA

Radius	Counterion valence		
	1	2	3
Å			
0.01	0.75-0.93	0.88-1.00*	0.93-1.00*
1.0	0.69-0.88	0.86-1.00*	0.92-1.00*
5.0	0.60-0.79	0.81-0.97	0.88-1.00*
10.0	0.51-0.67	0.74-0.93	0.84-0.98
12.5	0.44-0.62	0.72-0.91	0.83-0.98
Manning	0.76	0.88	0.92

Values were calculated as described in the text using the Poisson-Boltzmann equation and surface charge density $Z_p q_p / 2\pi a$, where Z_p , the linear charge density, equals 5.9×10^7 charges/cm. In each pair the lower value corresponds to a counterion concentration of 10^{-10} M, the higher to 10^{-1} M. Values given are the net total charge for counter and coions within distance R divided by Z_p .

*The net charge remaining outside the cylinder of radius $R + a$ is $<0.5\%$ of the total DNA charge.

these values of $r = 0.51, 0.74,$ and 0.84 are lower than the $r = 0.76, 0.88,$ and 0.92 predicted by Manning theory for DNA. We feel that this is largely the result of modeling differences between the two calculations. In counterion condensation theory, the polymer is modeled as a line of charges each separated by 1.7 \AA . In the other case, a 10-\AA radius cylinder with a uniform surface charge density is assumed. As can be seen in Table I, much closer agreement between the two approaches is obtained if, for the Poisson-Boltzmann calculation, a very small radius of 0.01 \AA is assumed. This latter model is more like that used by Manning.

We are aware of only one attempt to determine the charge fraction of DNA in solution containing only one kind of counterion. From their ^{23}Na nuclear magnetic resonance experiments, Anderson et al. (5) determined a value of $r = 0.75 \pm 0.10$ over a wide range of Na^+ concentrations. On the basis of this determination it seems that for electrostatic calculations DNA is better represented by a cylinder with a radius much smaller than its actual radius. This agrees with a major assumption of counterion condensation theory: the replacement of the actual polyion charge distribution by a line charge. We wish to caution that this discrimination is based on an interpretation of NMR data using a rather primitive two state, bound/free model of the Na^+ ions. This model may not be appropriate for spatially distributed ions such as are predicted by Poisson-Boltzmann theory.

More data are available concerning the competition of ions for binding to DNA. These data can be compared with the predictions of the counterion condensation theory using Eq. 3 and with the results calculated using the Poisson-Boltzmann approach. In this case calculations were performed with the higher valent cation assumed in extreme dilution, such that the ratio of the fraction of bound to free higher valent ions was independent of their concentration. Results for divalent ions in the presence of monovalent ions are shown in Fig. 4 along with a

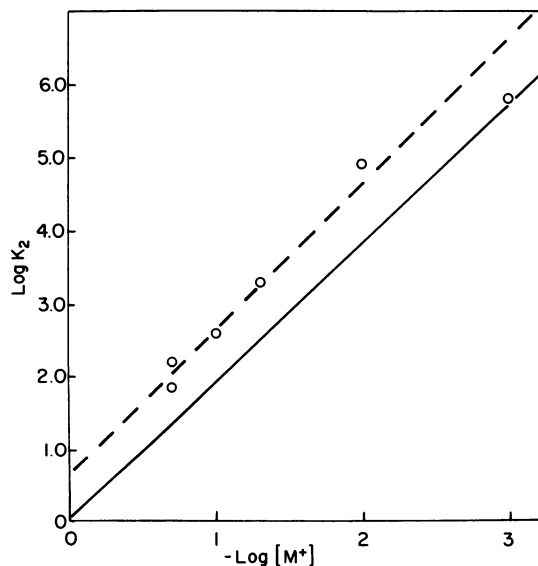


FIGURE 4 Experimental and theoretical binding constants of divalent ions to DNA in the presence of monovalent ions. Data from reference 1. Dashed line is the theoretical prediction from Eq. 3, solid line the theoretical prediction from the Poisson-Boltzmann theory.

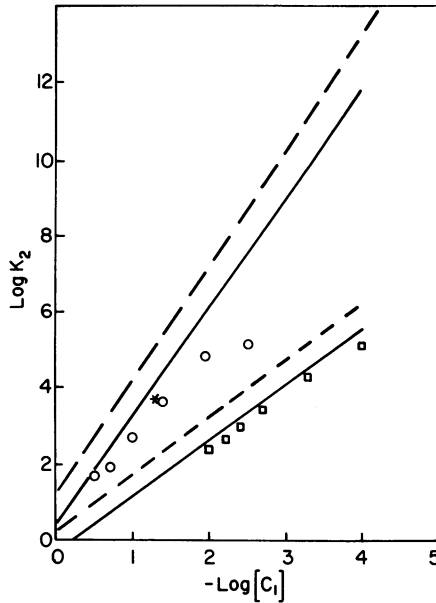


FIGURE 5 Experimental and theoretical binding constants of spermidine to DNA in the presence of Na^+ or Mg^{2+} . Data for Na^+ (\circ) from Rubin (16) and ($*$) from Ascoli et al. (17). Data for Mg^{2+} (\square) from Rubin. Dashed lines are the theoretical prediction from Eq. 3 and the solid line the prediction from the Poisson-Boltzmann theory. Upper curves refer to Na^+ data and lower to Mg^{2+} .

number of experimental points (see reference 1 and other references cited therein) and a plot of Eq. 3 which, with substitution of the appropriate numerical values, becomes:

$$\log K_2 = 0.63 - 2 \log c_1. \quad (7)$$

Both lines can be seen to be in good agreement with the experimental data, the Poisson-Boltzmann line having slightly too small an intercept. The intercept can be raised by using a thinner cylinder to represent the polymer.

In Fig. 5 are similar results for spermidine³⁺ in the presence of Na^+ and Mg^{2+} . The data come from the work of Rubin (16) with one point from Ascoli et al. (17). For the monovalent-trivalent counterion system, Eq. 3 becomes:

$$\log K_2 = 1.27 - 3 \log c_1. \quad (8)$$

A least squares fit to the data gives:

$$\log K_2 = 0.51 - 2.22 \log c_1, \quad (9)$$

and the Poisson-Boltzmann approach yields:

$$\log K_2 = 0.4 - 2.9 \log c_1. \quad (10)$$

For the divalent-trivalent counterion system, Eq. 3 becomes:

$$\log K_2 = 0.25 - 1.5 \log c_1, \quad (11)$$

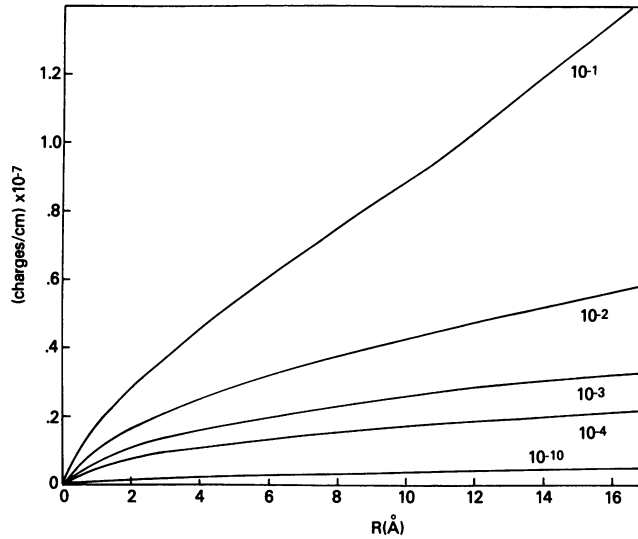


FIGURE 6 Plot of the calculated number of charges from univalent counterions within distance R of the surface of a charged cylinder as a function of R and the indicated bulk molar concentration of ions. The cylinder has a radius of 1 Å and a linear charge density $Z_p = 1.4 \times 10^7$ charges/cm, giving $\xi = 1.0$.

while the data are fit by:

$$\log K_2 = -0.39 - 1.39 \log c_1, \quad (12)$$

and the Poisson-Boltzmann results are:

$$\log K_2 = -0.3 - 1.46 \log c_1. \quad (13)$$

All of these results lead to the same conclusion. The binding of ions to DNA, including the salt dependence of the association constants, is predicted with fair accuracy by both theories. This also appears to be true for other polycations that possess higher linear charge densities. For example, the fraction of divalent ions associated with polyphosphate and an alternating copolymer of maleic anhydride and methylvinyl ether (trademark Gantrez) (12), both with $\xi \approx 2.8$, has been measured as 0.8–0.9. This can be compared with 0.82 determined using Eq. 1 and ~ 0.8 using the Poisson-Boltzmann method.

As the charge density of the polymer decreases the condensation theory and Poisson-Boltzmann approach become less similar. The condensation theory predicts an abrupt cessation of the binding of monovalent ions at $\xi = 1$. In contrast, as can be seen in Fig. 6, Poisson-Boltzmann theory predicts significant association at high counterion concentrations, with decreasing degrees of association as the counterion concentration goes down. Experiments performed with polymers having ξ near unity should provide a test of the models.

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