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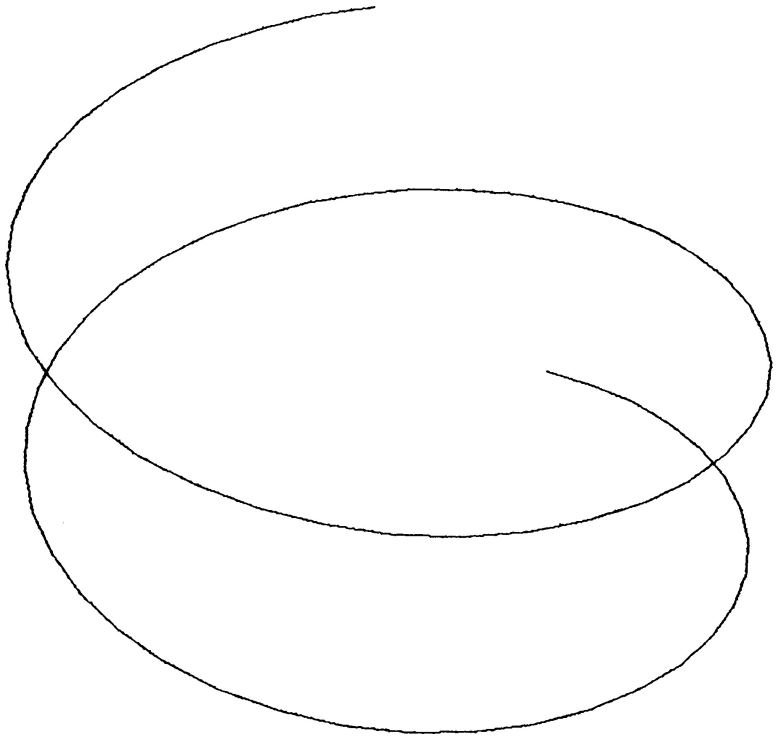
J. Am. Chem. Soc., **2003**, 125 (49), 15087-15092• DOI: 10.1021/ja030320t • Publication Date (Web): 14 November 2003



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Published on Web 11/14/2003

Is a Small Number of Charge Neutralizations Sufficient to Bend Nucleosome Core DNA onto Its Superhelical Ramp?

Gerald S. Manning

Contribution from the Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, 610 Taylor Road, Piscataway, New Jersey 08854-8087

Received May 27, 2003; E-mail: gmanning@rutchem.rutgers.edu

Abstract: X-ray diffraction structures of the nucleosome core particle along with a variety of experiments are consistent with the idea that an important source of the free energy holding DNA to the superhelical ramp on the histone octamer surface is obtained from a relatively small amount of electrostatic neutralization of the DNA phosphate charge by positively charged histone groups, especially arginine residues. Here we present a theoretical analysis of a simple model that emphasizes the competition between the high degree of bending of the stiff DNA molecule required for its tight curvature on the histone octamer and the neutralization of the DNA phosphate charge by basic histone residues. Our calculation accounts for the strong influence of condensed counterions on the electrostatic interactions. We find that the minimum amount of free energy required to bend DNA into axial conformity with the superhelical ramp at physiological salt concentration can be provided by a scant 6% neutralization of the phosphate charge, in close correspondence to the stoichiometric neutralization of phosphate charge by the arginine side chain that intrudes into the inward-facing minor groove of each DNA double helical turn.

1. Introduction

The nucleosome core particle consists of 145−147 base pairs of DNA organized into 1.65−1.75 turns of a flat left-handed superhelix around an octamer of histone proteins.[1−3] The DNA superhelix is irregular, but the best fit of an ideal superhelix to the DNA double helical axis yields a radius of ∼4.2 nm.[1] The root-mean-square radius of curvature of free DNA, determined from its persistence length, is ∼35 nm. The much smaller superhelical radius of nucleosomal DNA must therefore be stabilized against a significant amount of stored bending energy.

The strong ionic-strength dependence of nucleosome conformation[4] suggests that a large part of the energy stabilizing the DNA superhelix comes from interaction of the DNA phosphate groups with basic histone residues on the histone octamer surface. On the basis of an analysis of the salt-dependence of nucleosomal DNA melting, McGhee and Felsenfeld estimated that only ∼15% of the DNA phosphates may be involved in intimate charge−charge interactions with histones.[5] Such a small number is consistent with the positioning of the DNA on the outside of the histone octamer; as the double helical trajectory of the sugar−phosphate backbone is traced, only a small number of phosphate groups on each double helical turn can come close to the histone surface.

A lysine and arginine accessibility experiment with striking results was performed by Ichimura et al.[6] The interpretation quantitatively consistent with the data was that removal or loosening of the DNA freed up exactly 14 arginines (and no lysines) on the histone octamer surface, suggesting one close arginine−DNA contact for each DNA double helical turn, or 5% electrostatic neutralization of the DNA phosphate charge.

Recent years have seen reports of high-resolution diffraction structures of the core particle[1−3] that are consistent with the hypothesis that much of the free energy that stabilizes the DNA superhelix on the histone octamer is conferred by a relatively small number of salt-dependent electrostatic interactions. According to Harp et al.,[2] binding of DNA to the octamer surface is primarily mediated by the insertion of a cationic guanidino side chain of an arginine residue into the DNA minor groove, where salt bridges are formed to two phosphates on either side of the groove. There are 14 such binding sites, distributed more or less uniformly along a superhelical ramp on the surface of the histone octamer. Luger et al.[1] observe of their structure that binding is primarily to the two DNA phosphodiester chains as they face the protein on the outside of the octamer. On each helical turn of each chain, only two consecutive phosphate groups interact closely with the protein. An arginine residue is inserted into the DNA minor groove between phosphate chains in each of the 14 times the minor groove faces the histone octamer. Protein side chains facing the major groove "generally hydrogen-bond to phosphate groups or create a complementary, positive electrostatic charge density".

The methods of structural biology can lead to strongly suggestive but inherently inconclusive assessments of sources of stabilizing free energy. In the crystal structures there are in fact

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many close approaches between DNA atoms and both main chain and side chain protein atoms. The histone tails pass either between or over DNA gyres in their outward trajectories, and in doing so may interact energetically with the DNA. The positively charged arginine side chains are a central feature, however, and "it would appear that the function of the remainder of the histone octamer is to place the arginines at appropriate positions".[2] We therefore ask of ionic free energy theory whether only a few charge-neutralizing contacts with DNA phosphates can in principle be sufficient to stabilize the strong bending required to wind a stiff DNA molecule onto the superhelical ramp provided by the histone octamer.

Among the results of a Debye−Hückel electrostatic calculation by Kunze and Netz[7,8] is the pertinent conclusion that a uniformly charged sphere modeling the histone octamer can wrap a bendable line model of DNA at physiological ionic strength by opposing a charge of only +10 against the 300 negative charges on the DNA, corresponding to a mere 3% neutralization. In this paper we go beyond Debye−Hückel, with a full treatment of the effect of counterion condensation. By reaching a conclusion not very different from Kunze and Netz, we provide further support to the idea that the dominating global structural feature of nucleosomal DNA, namely, its tightly wound superhelical trajectory on the histone octamer, can be stabilized by a small number of charge neutralizations.

2. Description of the Model

2.1. Preliminary Remarks. In previous theoretical modeling of the wrapping of DNA around an inner core of histone proteins, we allowed the possibility of partially wound states but found that nucleosome length DNA was either fully wrapped or fully unwrapped,[9,10] an all-or-none transition-like behavior in agreement with existing experiment.[11,12] Our results subsequently proved useful to others in providing an interpretive framework for the all-or-none transitions indicated by their measurements as well.[13−15] The more detailed but still idealized theoretical model of Netz and Joanny[16] and Kunze and Netz[7] generates transitions between partially wrapped states caused by electrostatic repulsion of different parts of the DNA molecule at low salt. However, a strongly discontinuous wrapping−unwrapping transition does appear at high salt in their modeling also. If the goal, then, is to formulate a simple theory with focus on physiological salt concentration, a reasonable beginning is the assumption of two states, bound and free, the former corresponding to the native nucleosome core particle and the latter defined as the intact protein octamer with DNA attached to it at only one site (the strong binding site at the dyad axis)[1−3] but otherwise completely unwound from the superhelical ramp on the octamer surface.

DNA is a highly ionized polymer. The charge density of its phosphate groups is over four times greater than the threshold for counterion condensation,[17] and about 76% of the phosphate charge is compensated by condensed counterions, visualized in computer modeling as a dense layer with double helical structure adhering to the phosphate-sugar backbones.[18] The sharp boundary seen in the computer graphics between the population of condensed counterions and the more distant and diffuse layer of uncondensed counterions[18] has validated the assumption of counterion condensation theory that the condensed counterion phase is a distinct population of counterions physically separate from uncondensed counterions. Another central assumption, that the electrostatic charge of the polyion holding the condensed counterions is "renormalized", or reduced, by an amount exactly equal to the charge of the counterion condensate, has been supported by recent scattering measurements.[19] The classical experimental literature indicating the sharp onset of counterion condensation at a critical polyelectrolyte charge density has been reviewed.[20] There must then be thermodynamic consequences of the release of some of the condensed counterions when a fraction of the DNA phosphates are neutralized, including free energy changes in the layer of condensed counterions as well as the more obvious entropy increase due to the released counterions, and a quantitative theory should account for them.

Since inclusion of the statistical thermodynamics of condensed counterions is an important feature of our free energy calculations, it bears emphasis that the presence of condensed counterions has an influence well beyond simple reduction of the polyelectrolyte charge.[17] The condensed layer is characterized by a partition function Q (in an earlier version of the theory,[21] Q was described as the volume of the condensed phase), and a self-consistency condition determines an expression for Q in terms of known parameters of the system, such as the Debye screening length, the linear charge density of the polyion, and other structural parameters of the polyion. Equations 7, 11, and 18 of ref 17 give formulas for the condensed layer partition function for, respectively, polyelectrolyte charge assemblies corresponding to a line, a single helix, and a double helix. If some of the charges on the polyelectrolyte are neutralized, these expressions for Q will change accordingly and provide free energy contributions separate from the entropy of counterion release.

2.2. Detailed Description. To allow some scope for consideration of various (not all) possible cases, we consider the wrapping of "polyelectrolyte A" onto "macroion B". In our primary application, polyelectrolyte A is DNA and macroion B is the histone octamer.

We model polyelectrolyte A and macroion B in idealized fashion, retaining only the features necessary to capture the energetics of smooth axial bending and long-range electrostatics. Briefly, polyelectrolyte A is a bendable charged rod, while macroion B is a rigid oppositely charged circle. A complex is formed when the rod is forced to bend onto the circle by the attraction of opposite charges. The simplified geometry precludes consideration of histone tails. We proceed to a detailed description.

Polyelectrolyte A in its free state is a straight linear lattice of length L, bearing N_A charge sites with charge spacing $b_A =$

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L/N_A and dimensionless charge density $\xi_A = l/b_A$, where Bjerrum's length $l = q^2/\epsilon kT$, q is the unit charge, ϵ is the dielectric constant of the solvent, and kT is Boltzmann's constant times temperature. Macroion B is a rigid curvilinear lattice of N_B charge sites with arc length L (same length as the polyelectrolyte lattice), charge spacing along the arc $b_B = L/N_B$, and corresponding dimensionless charge density $\xi_B = l/b_B$. The curvilinear shape of macroion B is the simplest possible, the arc of a circle, with radius of curvature R. Note that we do not model the histone octamer as a spherical or cylindrical surface. We model it even more abstractly as a one-dimensional circular arc, which is enough to generate bending free energy when the axially stiff DNA lattice is forced to bend around it. The charge sites on polyelectrolyte A and macroion B are univalent and of opposite sign. We assume that polyelectrolyte A has a higher charge density than macroion B, that is, $b_A < b_B$ and $\xi_A > \xi_B$.

In the bound state the rigid macroion B retains its shape as a circular arc of length L and radius of curvature R, but polyelectrolyte A, also of length L, is fully wrapped along the arc of macroion B and therefore has been bent from its initially straight shape to radius of curvature R. There are fewer charges on macroion B than on polyelectrolyte A, so all of the positive charges on macroion B are neutralized by negative charges on polyelectrolyte A. Conversely, the charges of macroion B neutralize the fraction α of the charges on polyelectrolyte A, $0 < \alpha < 1$. We also have the stoichiometric relations,

$$N_B/N_A = \xi_B/\xi_A = b_A/b_B = \alpha \tag{1}$$

Finally, our solvent is aqueous and contains uni:univalent salt of molarity c. At room temperature a numerical formula for κ, the Debye screening parameter (inverse Debye length) is,[21]

$$\kappa = 3.29c^{1/2}\,\mathrm{nm}^{-1} \tag{2}$$

Our model omits the interaction between adjacent gyres of the DNA superhelix, which has a pitch of 23.9 Å.[1] In our line model for DNA, the charge is on the axis of the double helix, and adjacent gyres are then largely screened at physiological salt (0.1 M), with corresponding Debye length 9.6 Å.

3. Free Energy Calculations

In this section, G_A^{free} and G_A^{bound} are the free energies of polyelectrolyte A, for example, DNA, in its free and bound states, respectively. Correspondingly, G_B^{free} and G_B^{bound} are the free energies of macroion B (histone octamer) in its free and bound states. We calculate the free energy difference Δg between the overall bound and free states, per charge on the free polyelectrolyte and in units of kT, that is,

$$\Delta g = \frac{\Delta G}{N_A kT} \tag{3}$$

where,

$$\Delta G = [G_A^{bound} + G_B^{bound}] - [G_A^{free} + G_B^{free}] \tag{4}$$

Of course, when two particles are bound to each other, they are energetically coupled, and the free energy of the complex should not be written as a sum of free energies for each particle. In the context of our model, however, we can determine separate free energies for bound polyelectrolyte A and bound macroion B in a consistent way.

There are several cases, but all of them have in common the vanishing of G_B^{bound},

$$G_B^{bound} = 0 \tag{5}$$

since there is no electrostatic charge on bound macroion B—all of it has been used to neutralize charge on polyelectrolyte A in the binding reaction. Furthermore, macroion B is rigid, and there is no deformation energy associated with its passage from free to bound state.

Another common thread through all cases is an assumption about the free state of macroion B. Since we have in mind the idea that macroion B represents a protein in our primary application, and since ionized amino acids in most proteins are of relatively low density (compared to the phosphate charge density of DNA, for example), it is reasonable to assume that there are no counterions condensed on macroion B. Therefore, we take $\xi_B < 1$ in all cases. This subcritical value of the charge density on the macroion allows us to use linear Debye−Hückel theory in writing the free energy of macroion B,

$$G_B^{free} = -N_B kT\xi_B \ln(1 - e^{-\kappa b_B}) + \frac{N_B kT\xi_B}{8\kappa^2 R^2} \tag{6}$$

Notice the signature of electrostatic linearity in this expression; the coefficients of both terms are linear in the charge density ξ_B. The formula is obtained as the first two terms of an expansion in square curvature R^{-2} of the sum of screened Coulomb potentials among all pairs of charges on macroion B.[22] The second term is identical to the Odijk−Skolnick−Fixman formula for the work against electrostatic repulsion required to bend a charged rod.[23,24] It should be kept in mind, however, that macroion B has a rigidly curved shape, and the physical meaning of eq 6 is that it gives the work required to assemble electrostatic charge from infinity onto this preexisting shape. The range of validity of the two-term expansion is restricted to radii of curvature greater than the Debye screening length.[8,25] In our application to the nucleosome system, we will not work below salt concentration 0.1 M, and then the Debye length is less than a fourth of the 4.2 nm radius of the nucleosome DNA superhelix.

3.1. Polyelectrolyte with Subcritical Charge Density. For the sake of completeness and also to set the pattern for the calculation, we begin with a treatment of the Debye−Hückel case for polyelectrolyte A, $\xi_A < 1$, that is, a sparsely charged polyelectrolyte with no condensed counterions. For free polyelectrolyte A (a straight one-dimensional assembly of charge sites with spacing b_A),

$$G_A^{free} = -N_A kT\xi_A \ln(1 - e^{-\kappa b_A}) \tag{7}$$

Equation 7 is obtained by summing screened electrostatic potentials from linear Debye−Hückel theory over all pairs of charge sites on polyelectrolyte A. The right-hand side is, of course, strictly analogous to the first term of eq 6. Note in

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particular the linear dependence of the coefficient on the free polyelectrolyte charge density ξ_A.

In the bound state of polyelectrolyte A, a fraction $\alpha < 1$ of its charges are neutralized. It therefore carries $(1 - \alpha)N_A$ charges, its charge density is $(1 - \alpha)\xi_A$, and its average charge spacing has increased to $b_A/(1 - \alpha)$. Moreover, the polyelectrolyte has been bent to radius of curvature R in order to conform to the shape of the macroion. For the free energy G_A^{bound} of polyelectrolyte A in its bound state, then, we have,

$$G_A^{bound} = -(1-\alpha)^2 N_A kT\xi_A \ln(1 - e^{-\kappa b_A/1-\alpha}) + \frac{(1-\alpha)^2 N_A kT\xi_A}{8\kappa^2 R^2} + \frac{N_A kT b_A \lambda}{2R^2} \quad (8)$$

The first two terms on the right-hand side of eq 8 account for electrostatic repulsions among unneutralized charge sites on the bound polyelectrolyte, taking into account its bent shape. The third term represents the nonelectrostatic work required to bend the polyelectrolyte onto the macroion. Thus, it equals the bending free energy of a rod of length $L = N_A b_A$ and elastic bending rigidity $kT\lambda$. In this expression for the bending rigidity, λ is the bare polymer persistence length, that is, it does not include the effect of electrostatic repulsions among the polymer charge sites, represented by the second term in eq 8.

Equations 1−8 combine to give the desired result for the reduced free energy difference between bound and free states,

$$\Delta g = \frac{b\lambda}{2R^2} + \frac{(1-2\alpha)\xi}{8\kappa^2 R^2} - (1-\alpha)^2\xi \ln(1 - e^{-\kappa b/1-\alpha}) + \xi \ln(1 - e^{-\kappa b}) + \alpha^2\xi \ln(1 - e^{-\kappa b/\alpha}) \quad (9)$$

In this formula we have made use of the stoichiometric relations in eq 1 to eliminate explicit reference to the electrostatic parameters of macroion B. We are therefore able to streamline the notation by defining the unsubscripted quantities $b = b_A$ and $\xi = \xi_A$. The quantities b, ξ, and λ are then the charge spacing, reduced charge density, and bare persistence length of the free polyelectrolyte.

3.2. Polyelectrolyte with Condensed Counterions. For postcritical polyelectrolyte charge densities $\xi_A > 1$, the fraction $1 - (1/\xi_A)$ of the polyelectrolyte charge sites is neutralized by a closely held, but largely mobile, layer of condensed counterions.[17] Some of these counterions are released when neutralization is instead accomplished by the charges on the binding macroion, and the free energy associated with this process is not captured by linear Debye−Hückel theory.

For $\xi_A > 1$ the free energy of free polyelectrolyte A is

$$G_A^{free} = -N_A kT\left(2 - \frac{1}{\xi_A}\right)\ln(1 - e^{-\kappa b_A}) - N_A kT\left(1 - \frac{1}{\xi_A}\right) \quad (10)$$

The derivation of this equation may be found in ref 17. Notice its nonlinear character; the coefficient of the first term on the right-hand side is not linear in polyelectrolyte charge density ξ_A, and neither is the second term. But a deeper observation is that the equation is also not a simple "renormalized" version of its linear counterpart eq 7, which would be obtained by the replacements $\xi_A \to 1$, $N_A \to (1/\xi_A)N_A$, and $b_A \to l$ (the constant value of Bjerrum's length) in eq 7. As discussed in the Introduction, full consideration of condensed counterions in the polyelectrolyte free energy requires proper handling of the internal partition function of the condensed layer.[17]

When the charge density ξ_A of free polyelectrolyte A exceeds unity, and counterions are consequently condensed on free A, there are two possibilities for the state of bound A. Either the extent of neutralization α is large enough to lower the net charge density $(1 - \alpha)\xi_A$ of bound A to values smaller than unity or the net charge density remains in excess of unity. In the first case, all condensed counterions have been released, and in the second case, some counterions remain condensed on the bound polyelectrolyte. We derive the corresponding free energies for the wrapping transition and their ranges of validity.

3.2.1. Complete Release of Condensed Counterions. For the case of complete release of condensed counterions, $(1 - \alpha)\xi_A < 1$, or $\alpha > 1 - (1/\xi_A)$. But also $\xi_B = \alpha\xi_A$, and we have assumed $\xi_B < 1$, so $\alpha < 1/\xi_A$. Thus, for complete release of condensed counterions, the extent of neutralization of the polyelectrolyte on binding is in the range $1 - (1/\xi_A) < \alpha < 1/\xi_A$. The range exists if ξ_A is itself restricted to values greater than 1 but less than 2 ($\xi_A \approx 4$ for DNA, so this case is excluded from our primary application).

To find the free energy in the case of complete release, we observe that the charge density on bound polyelectrolyte A, $(1 - \alpha)\xi_A$, is less than unity, so Debye−Hückel theory is applicable. In this case, G_A^{bound} is given by eq 8. The four free energy components of eq 4 are then given by eqs 5, 6, 8, and 10, and for the reduced free energy difference between bound and free states, defined by eq 3, we get,

$$\Delta g = \frac{b\lambda}{2R^2} + \frac{(1-2\alpha)\xi}{8\kappa^2 R^2} - (1-\alpha)^2\xi \ln(1 - e^{-\kappa b/1-\alpha}) + \alpha^2\xi \ln(1 - e^{-\kappa b/\alpha}) + \left(2 - \frac{1}{\xi}\right)\ln(1 - e^{-\kappa b}) + 1 - \frac{1}{\xi} \quad (11)$$

where the unsubscripted b, ξ, and λ refer, as before, to the free polyelectrolyte. Note that eqs 9 and 11 are the same at $\xi = 1$. Since at $\xi = 1^+$, any amount of neutralization $\alpha > 0$, no matter how small, is enough to carry the polyelectrolyte below the condensation threshold, it is indeed the present case of complete release that interfaces the Debye−Hückel case.

3.2.2. Partial Release of Condensed Counterions. For partial release of condensed counterions, $(1 - \alpha)\xi_A > 1$, or $\alpha < 1 - (1/\xi_A)$. But for the same reason as in the case of complete release, $\alpha < 1/\xi_A$. The restriction on α is therefore $\alpha < \min[1/\xi_A, 1 - (1/\xi_A)]$, and the only restriction on ξ_A is $\xi_A > 1$.

Bound polyelectrolyte A in this case has net charge density above the condensation threshold, so its free energy must account both for the presence of condensed counterions and for its bending stress,

$$G_A^{bound} = -(1-\alpha)N_A kT\left[2 - \frac{1}{(1-\alpha)\xi_A}\right]\left[\ln(1 - e^{-\kappa b_A/1-\alpha}) - \frac{1}{8\kappa^2 R^2}\right] - \left(1 - \alpha - \frac{1}{\xi_A}\right)N_A kT + \frac{N_A kT b_A \lambda}{2R^2} \quad (12)$$

The first two terms give the electrostatic contribution, including the electrostatic free energy of curvature, and the last term is the nonelectrostatic work of bending. Section 2 of ref 22 contains the derivation of eq 12 in the context of zero α, as well as a discussion of how the electrostatic curvature term differs from a charge-renormalized Odijk−Skolnick−Fixman persistence

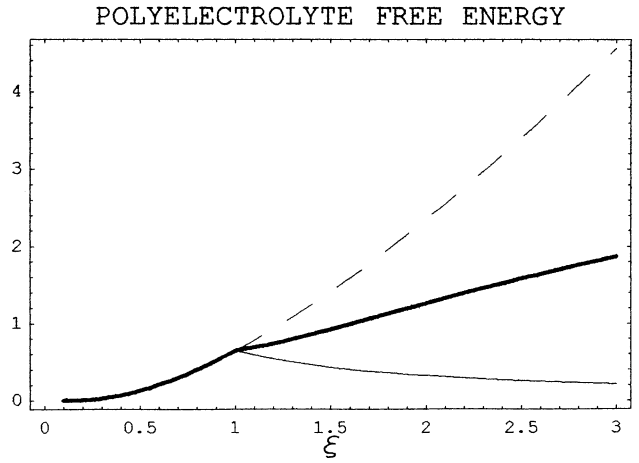

Figure 1. Polyelectrolyte free energy (per charged group on the polymer, in units of kT) as a function of dimensionless charge density ξ. For $\xi < 1$, the thick curve is eq 7, and for $\xi > 1$, it is eq 10 (in both cases, divided by $N_A kT$). The dashed curve is the extension of the Debye−Hückel eq 7 into the range $\xi > 1$, where it is not valid. The thin curve results from charge-renormalization of eq 7, which is also an invalid way of computing the free energy. In all plots the salt concentration is physiological, $c = 0.1$ M.

length. In particular, although there is no uptake of condensed counterions when the polyelectrolyte is bent subject to the condition $\kappa R > 1$, the internal partition function of the condensed layer does change.[22]

The four components of eq 4 are now taken from eqs 5, 6, 10, and 12, and our result for the reduced free energy difference Δg of eq 3 is,

$$\Delta g = \frac{\lambda b}{2R^2} + \alpha + \left(2 - \frac{1}{\xi}\right)\ln(1 - e^{-\kappa b}) - (1-\alpha)\left[2 - \frac{1}{(1-\alpha)\xi}\right]\left[\ln(1 - e^{-\kappa b/1-\alpha}) - \frac{1}{8\kappa^2 R^2}\right] + \alpha^2\xi\left[\ln(1 - e^{-\kappa b/\alpha}) - \frac{1}{8\kappa^2 R^2}\right]$$

where once more the unsubscripted parameters λ, b, and ξ refer to the free polyelectrolyte.

4. Numerical Results and Discussion

We have placed some emphasis on the need in a calculation of this type to go beyond linear Debye−Hückel electrostatic theory, even as corrected by charge renormalization. When the reduced charge density of the binding polyelectrolyte exceeds unity (the value for DNA is four times greater than that), counterions are condensed on the polyelectrolyte, and some of them are released when the polyelectrolyte wraps onto the macroion. Both the entropy of release and the perturbation of the free energy of the condensed layer must be considered.

To illustrate these statements, we show in Figure 1 a plot of the polyelectrolyte free energy as used in our calculations, that is, eqs 7 and 10, along with two invalid variants, which we did not use. The free energy is plotted as a function of polyelectrolyte charge density ξ. The thick curve follows the Debye−Hückel eq 7 for $\xi < 1$ and the counterion condensation eq 10 for $\xi > 1$. The break in slope is commonly reflected in the experimental and computational polyelectrolyte literature.[20] The dashed curve extends Debye−Hückel eq 7 into the high charge density range $\xi > 1$, where it is not valid. The correct free energy values (thick curve) are much lower. The thin curve is

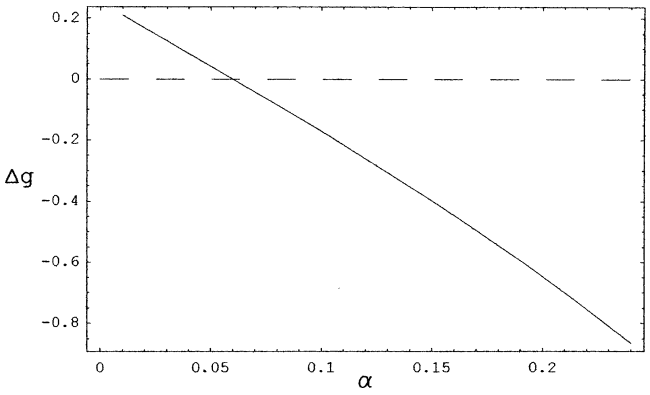

Figure 2. A plot of the binding free energy (per DNA phosphate, in units of kT) as a function of the fractional extent of neutralization α of the DNA phosphate charge by positive charges on the histone octamer. The negative values for $\alpha > 0.06$ indicate the range of stability of the bound state (native nucleosome core particle) for the following parameter values: DNA average axial charge spacing $b = 0.1688$ nm ($\xi = 4.227$); bare persistence length of DNA $\lambda = 50$ nm; radius of curvature of bound DNA superhelix $R = 4.18$ nm; salt concentration $c = 0.1$ M.

the result of an attempt to correct Debye−Hückel eq 7 in the high charge density range by renormalizing the polyelectrolyte charge, as indicated in the remarks immediately following eq 10. The qualitative trend is wrong. After all, charge renormalization at very high charge density states that the polyelectrolyte approaches a state of zero charge (no dissociation of counterions) and therefore zero electrostatic free energy. The correct eq 10 (thick curve) does not behave like that.

We now pursue the central result of our paper. For free B-form DNA, the average phosphate axial spacing b equals 0.1688 nm, and the corresponding value of the reduced axial charge density ξ is 4.227 at room temperature in aqueous solvent. Since ξ is greater than unity, there are condensed counterions on DNA, and either eq 11 or eq 13 may be applicable. But since ξ is also greater than 2, consideration is limited to eq 13, the case of incomplete release of condensed counterions. The value of $1/\xi$ is 0.24, so the range of neutralization fraction α to which eq 13 is limited is from zero to min-(0.24, 0.76), or $0 < \alpha < 0.24$. Figure 2 presents a plot of eq 13 in this range at physiological salt, $c = 0.1$ M. The bare persistence length λ is taken as the high-salt value 50 nm.[26]

We see from Figure 2 that the onset of stability of the fully wrapped bound state (transition from positive to negative values of Δg) requires only 6% neutralization of the DNA charge at physiological salt, or about one neutralized phosphate for every double helical turn. The monovalent cationic arginine side chain that inserts between phosphates across the minor groove of each double helical turn of DNA in the native nucleosome core particle[1,2] would then provide just sufficient electrostatic free energy to wrap the DNA along the superhelical ramp on the histone octamer surface, at least as concerns the required amount of axial bending.

The importance of the 14 groove-inserting arginines is thus underlined by our energy calculation as well as suggested by their prominence in the core particle structure. However, there are many other apparently net stabilizing protein−DNA interactions in the native structure, including other electrostatic interactions.[1−3] About 60 cationic side chains have been counted

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as within 6 Å of DNA phosphate oxygen atoms in a 146 bp nucleosome, although the negative charges on nearby carboxylates could energetically mask up to a third of them.[27] Moreover, the distortion of DNA structure on the nucleosome involves more than simple axial bending. Accurate assessment of the complete energetics of the core particle awaits analysis on the atomistic level.

Acknowledgment. The author extends his thanks to Gerard Bunick, Jeffrey Hayes, and Roland Netz for valuable correspondence.

JA030320T

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