Application of Polyelectrolyte Theories for Analysis of DNA Melting in the Presence of Na$^+$ and Mg^{2+} Ions

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ABSTRACT   Numerical calculations, using Poisson-Boltzmann (PB) and counterion condensation (CC) polyelectrolyte theories, of the electrostatic free energy difference, ΔG^{el}, between single-stranded (coil) and double-helical DNA have been performed for solutions of NaDNA + NaCl with and without added MgCl$_2$. Calculations have been made for conditions relevant to systems where experimental values of helix coil transition temperature (T_m) and other thermodynamic quantities have been measured. Comparison with experimental data has been possible by invoking values of T_m for solutions containing NaCl salt only. Resulting theoretical values of enthalpy, entropy, and heat capacity (for NaCl salt-containing solutions) and of T_m as a function of NaCl concentration in NaCl + MgCl$_2$ solutions have thus been obtained. Qualitative and, to a large extent, quantitative reproduction of the experimental T_m, ΔH_m, ΔS_m, and ΔC_p values have been found from the results of polyelectrolyte theories. However, the quantitative resemblance of experimental data is considerably better for PB theory as compared to the CC model. Furthermore, some rather implausible qualitative conclusions are obtained within the CC results for DNA melting in NaCl + MgCl$_2$ solutions. Our results argue in favor of the Poisson-Boltzmann theory, as compared to the counterion condensation theory.

INTRODUCTION

DNA is a highly negatively charged polyelectrolyte both in double-stranded ("native") and in single-stranded ("denatured") forms and displays considerable sensitivity to ionic surroundings during various structural transitions and in interactions with charged species. Spatially unlocalized electrostatic and specific association of mono- and divalent cations with DNA (in particular, with naturally abundant Na$^+$ and Mg^{2+} ions) is an essential component of all DNA- and RNA-involved biological processes (Anderson and Record, 1982; Eichhorn, 1973; Lohman and Ferrari, 1994; Durlach et al., 1990). Experimental and theoretical studies show that the temperature at the midpoint ("melting temperature," T_m) of the thermally induced order-disorder transition from double helix to the single-strand form of DNA (henceforth called the helix-coil transition) is determined mainly by the charge densities of native and denatured DNA polyions. Furthermore, this transition is highly dependent on the concentrations (C_s) or, more strictly, activities ($a_{\pm}$) and charges of cations in the solution. Despite considerable progress in polyelectrolyte theories, which is mainly related to increased possibilities for detailed computer simulations of complex systems, their applications to the description of the heat-induced polynucleotide helix-coil transitions have previously been limited. Recently, however, this problem has gained new interest (Bond et al., 1994; Diguid and

Bloomfield, 1996; Galindo and Sokoloff, 1996; Esposito et al., 1997).

An obstacle in modeling helix coil equilibria is the absence of reliable data on the structure of single-stranded DNA. Experimental data (Luzatti et al., 1964; Massoulie, 1968; Heinecke et al., 1974; Filimonov and Privalov, 1978; Vesnaver and Breslauer, 1991; Tomac et al., 1996; and references cited in Vesnaver and Breslauer, 1991) give evidence that single-stranded DNA and RNA do not turn into its maximally stretched conformation, even in salt-free solutions, which was earlier believed to be energetically favorable because of the maximum spacing of phosphate group charges. This indicates that hydrophobic ("stacking") interactions between base planes are maintained after separation of the DNA strands, and that base and charge spacings in a single DNA strand are practically the same as in the double helix ($\sim$3.4 Å). Thus denatured DNA should not be considered as a "coil" but rather as a stiff and highly charged "single helix." Additionally, the process of double helix melting is complicated by the fact that this single-helical DNA also melts (i.e., an increase in temperature produces disruption of base stacking). The melting parameters of the single helix-to-coil transition depend strongly on base composition and sequence and on the molecular weight of the polynucleotides (Vesnaver and Breslauer, 1991). Single helix melting usually occurs over a broad temperature range (Luzatti et al., 1964; Massoulie 1968; Heinecke et al., 1974; Filimonov and Privalov, 1978; Vesnaver and Breslauer, 1991), and the enthalpy of transition can reach 12 kJ/mol (Filimonov and Privalov, 1978; Vesnaver and Breslauer, 1991), which means that heat effects are comparable with those for the "double-single helix" transition. However, there is no detailed knowledge about the dependencies of melting parameters on the concentration of electrolytes.

Comparisons of polyelectrolyte theories with experimental data on polynucleotide double helix–single strand thermal transitions have been reported for quantitative theoretical analysis of NaDNA melting in NaCl solutions (Luzatti et al., 1964; Manning, 1972a; Record, 1975; Record et al., 1976b, 1978; Filimonov and Privalov, 1978; Anderson and Record, 1982; Frank-Kamenetskii et al., 1987; Bond et al., 1994). Experimental data for melting of DNA in the presence of NaCl and $MgCl_2$ have also been reported (Dove and Davidson, 1962; Record, 1975; Krakauer, 1974; Manning, 1972b, 1978). Helix-coil transition of DNA in solutions containing univalent salt and in salt-free solutions (Korolev et al., 1994) has been described in a mutually consistent manner by both counterion condensation (CC) theory (Manning, 1972a,b, 1976, 1978; de Marky and Manning, 1975, 1976; Record, 1975; Record et al., 1976b, 1978; Anderson and Record, 1982) and by the Poisson-Boltzmann (PB) theory (Record et al., 1978; Frank-Kamenetskii et al., 1987; Bond et al., 1994; Korolev et al., 1994). Both of these theories describe DNA melting on the basis of an equation obtained from purely thermodynamic (i.e., free of PB or CC model assumptions) considerations (Anderson and Record, 1982):

$$\Delta i = [dT_m/d \ln(a_\pm)] \cdot (\Delta H_m/RT_m^2) \qquad (1)$$

Here ΔH_m is the enthalpy (per mole of nucleotide) of DNA melting and $\Delta i = i_c - i_h$ is the difference between the numbers of thermodynamically dissociated counterions of denatured (i_c) and native (i_h) DNA, quantities that can be calculated from either PB or CC polyelectrolyte theories. By numerical integration and use of experimental values for the relevant parameters of the right-hand side of Eq. 1 that are available from microcalorimetric and spectrophotometric studies of DNA melting, the salt dependence of the transition temperature can be predicted. Analyzing the helix coil transition within the CC model, Manning (1972a) obtained $\Delta i = 1/2 \cdot (1/\xi_c - 1/\xi_h)$. Here c and h are indexes for helix and coil forms of DNA, respectively, and $\xi = q^2/(\epsilon k_B Tb) = b_0/b$ is the well-known charge density parameter. Here q is the proton charge, ϵ the dielectric constant of the solvent, b the average distance between charged groups projected on the axis of the polymer, and $b_0 = q^2/(\epsilon k_B T)$ is called the Bjerrum length; $b_0 = 7.158$ Å at 25°C in water. The CC model gives the best agreement between experimental and theoretical data when b_c is assumed to be equal to 3.8 ± 0.3 Å (Manning, 1976; Record et al., 1976a,b). Recently, numerical calculations of Δi values determined by the PB cylindrical cell model were reported (Bond et al., 1994). Analysis of experimental data is based on calculations of the limiting ($C_P \rightarrow 0$, C_P is DNA concentration) value of the preferential interaction coefficient (Γ^o), which is related to i simply by $i = -2 \cdot \Gamma^o$ in polyelectrolyte solutions containing univalent salt. Data of these (Bond et al., 1994) and other authors (Frank-Kamenetskii et al., 1987; Korolev et al., 1994) based on PB calculations resulted in $b_c \approx 3.4$ Å for denatured DNA from the best fit of

experimental data. However, this difference in estimations of b_c from CC and PB theories cannot be considered as very significant. It should also here be noted that there is a huge spread in reported experimental values for $dT_m/d \ln(a_\pm)$ and ΔH_m (see below).

In this paper we present an approach to the description of helix-coil transitions of polynucleotides that is based on numerical calculations of the difference between the electrostatic free energy (ΔG^{el}) of double-helix and single-strand forms of DNA, using PB and CC polyelectrolyte theories. Our approach is principally related to that proposed earlier (Frank-Kamenetskii et al, 1987; Bond et al., 1994). Therefore it naturally produces the same kind of results, which is a prediction of T_m as a function of salt for DNA systems under different solution conditions. On the other hand, our calculation of ΔG^{el} allowed us to use experimental values of T_m for evaluation of the salt independent nonelectrostatic contribution to the free energy, enthalpy, entropy, and heat capacity changes of the helix-coil transition. Then, by adding together the calculated electrostatic and nonelectrostatic values, the predictions of PB and CC theories can be compared with available microcalorimetric data. Thus we need no calorimetric data in testing polyelectrolyte theories that are necessary in the application of other methods (Manning, 1976; Record et al., 1976a,b, 1978; Bond et al., 1994; Frank-Kamenetskii et al., 1987). It can be noted that our approach of analyzing the salt dependence of DNA helix-coil transition does not make use of Eq. 1 above but is similar to the approach used in studies of polysaccharide conformational transitions, which was also based on calculations of ΔG^{el} (Nilsson et al., 1989; Nilsson and Picullell, 1989).

However, the introduction of the present approach is not the major motivation for this work. Our main concern is to use this method in combination with the PB cell model and apply it to DNA helix-coil transition in solutions containing divalent (Mg^{2+}) as well as monovalent (Na^+) counterions. For this kind of system, experimental data (Dove and Davidson, 1962; Eichhorn and Shin, 1968; Record, 1975) have shown an anomalous destabilization of the double helix upon the addition of NaCl. Our objective is to investigate the capacity of polyelectrolyte theories to analyze and interpret this kind of behavior, and we have therefore also used the CC model and compared it with the PB theory in this respect. In addition, some calculations of ΔG^{el} have also been made using the Monte Carlo self-consistent field simulation method (MCSF) (Vorontsov-Velyaminov and Lyubartsev, 1989, 1992; Lyubartsev et al., 1990).

METHODS

Thermodynamics

The change in free energy of polynucleotide melting (ΔG_m) is a function of temperature, structural parameters of the denatured and native polynucleotides, and concentrations of ions. The value of ΔG_m is assumed to be a sum of two terms

(Kotin, 1963; Schildkraut and Lifson, 1965; Nagasawa and Muroga, 1972):

$$\Delta G_{m} = \Delta G^{el} + \Delta G^{nel} \qquad (2)$$

The first term (ΔG^{el}) is the difference in electrostatic free energy (calculated per one mole of nucleotide). Assuming $\Delta V = 0$, this term is equal to $\Delta G^{el} = \Delta E^{el} - T \cdot \Delta S^{el}$ and includes the electrostatic energy change ($\Delta E^{el} = E_c^{el} - E_h^{el}$) and the change in electrostatic entropy ($\Delta S^{el} = S_c^{el} - S_h^{el}$). The value of ΔE^{el} accounts for the difference in electrostatic energy of the polyion with all mobile ions between the two states, and ΔS^{el} is a measure of the entropy change due to the difference in entropy of mixing caused by the distribution of all mobile ions in the two states. The numerical procedure for calculation of the terms contributing to ΔG^{el} depends on the theoretical model used for description of the polyelectrolyte system. We have considered three models in the present work:

The counterion condensation (CC) theory (Manning, 1972a, 1978)

The Poisson Boltzmann (PB) cylindrical cell model (Wennerström et al., 1982; Vorontsov-Velyaminov and Lyubartsev, 1989; Lyubartsev et al., 1990)

The Monte Carlo self-consistent field (MCSF) method (Vorontsov-Velyaminov and Lyubartsev, 1989, 1992; Lyubartsev et al., 1990; Lyubartsev, 1991).

The second term in Eq. 2, ΔG^{nel}, contains all nonelectrostatic contributions to the helix-coil transition. This term includes, e.g., changes in free energies of internal rotations, hydrogen bonds, hydrophobic stacking interactions, as well as changes in hydration of the polynucleotides and water structure, and all other contributions that are beyond the consideration of polyelectrolyte theories. The magnitude of ΔG^{nel} is assumed to be a function of temperature only, and is independent of the composition of the solution due to the addition of salt.

Assuming that the change in heat capacity of DNA melting is a constant independent of temperature, the temperature dependence of ΔG^{nel} can be expressed by the following exact thermodynamic relationship:

$$\Delta G^{nel}(T) = \Delta H^{nel}(T_o) - T \cdot \Delta S^{nel}(T_o) + \Delta C_p^{nel}$$
$$\cdot [T - T_o - T \cdot \ln(T/T_o)] \qquad (3)$$

Here ΔH^{nel}, ΔS^{nel}, and ΔC_p^{nel} are changes in nonelectrostatic enthalpy, entropy, and heat capacity, respectively, and T_o is an arbitrary "standard" temperature (we have chosen $T_o = 298.15$). Thus, at standard temperature, $\Delta G_m(T_o) = \Delta G^{el} +$ constant, and the correctness of theoretical calculations can be compared with available calorimetric data on polynucleotide structural transitions. However, experimental values of $\Delta G_m(T_o)$ are not numerous for systems of NaDNA + NaCl and are practically absent for helix-coil transitions of polynucleotides in the presence of divalent cations.

In the present paper, the analysis of DNA helix-coil transitions on the basis of polyelectrolyte theories is performed in the following way:

1. We determine numerically the values of $\Delta G^{el} = f(T, C_{Na}, C_{Mg}, C_P)$ corresponding to different conditions for DNA solutions, using the theoretical models listed above.

2. We determine values of $\Delta G^{nel}(T)$ from calculated values of $\Delta G^{el}(T_m) = f(T_m, C_{Na}, C_P)$ in the absence of Mg^{2+} and from experimental values of T_m at the same concentrations, using the fact that $\Delta G^{nel} = -\Delta G^{el}$ at the melting point. The reason for using the experimental melting temperatures is that these data are more frequently found in literature than calorimetric values of melting enthalpy, entropy, and heat capacity changes. The values of the parameters in Eq. 3 are then determined by a fitting procedure and compared with available calorimetric data.

3. Next we calculate the total free energy change $\Delta G_m(T, C_{Na}, C_{Mg}, C_P) = \Delta G^{el}(T, C_{Na}, C_{Mg}, C_P) + \Delta G^{nel}(T)$ in the presence of Mg^{2+} ions. Setting $\Delta G_m = 0$, we find the concentration dependence of the melting temperature $T_m = T_m(C_{Na}, C_{Mg}, C_P)$. Then we plot theoretical curves of T_m versus log C_{Na} that can be compared with those determined experimentally in the presence of Mg^{2+} ions (Dove and Davidson, 1962; Eichhorn and Shin, 1968; Record, 1975).

Thus any observed differences between experimental and calculated values of T_m can be a measure of the reliability of the theoretical description or may be directly connected with salt effects on the nonelectrostatic free energy in Eq. 2, e.g., due to inner sphere coordination of divalent cations, changes in hydration of DNA and Mg^{2+}, and so on.

It is also assumed in this work that the helix-coil transition is a first-order phase transition between two states, which means that the cooperative unit in the process is equal to the full polynucleotide molecule. If this assumption does not hold and redistribution of ions between melted and unmelted units of a given DNA polyion can proceed during the helix-coil transition, this would influence the calculation of the theoretical T_m curves (Record et al., 1978; McGhee, 1976; Nilsson et al., 1989; Nilsson and Picullel, 1989). This exchange of ions may be of importance for small values of the ratio $r = C_{Mg}/C_P$ (see below).

Numerical calculations of ΔG^{el}

In all of the models used, the solvent is treated as a continuum with a constant dielectric constant that depends only on temperature. The polynucleotides are approximated as infinitely long and uniformly charged cylinders of finite (PB and MCSF models) or infinitely small (CC theory) radius a with unit charge spacing b. The small mobile ions are treated as point charges (CC model) or impenetrable hard spheres (PB and MCSF models) with radii close to those calculated for Na^+ (Fawcett and Tikanen, 1996; Simonin et al., 1996) and Mg^{2+} (MacKerell, 1997) in theoretical models describing thermodynamic properties of simple electrolyte solutions. In the PB approach, the radii of the counterions and coions determine only the distance of closest approach of the small ions to the polyion and do not produce any excluded volume effects near the surface of the cylindrical polyion.

Counterion condensation theory

Manning's polyelectrolyte theory of "condensation" of counterions on a highly charged cylindrical polyion yields "limiting laws" for the properties of polyelectrolyte systems in the case of small concentrations of low-molecular-weight electrolytes and yet much smaller polyelectrolyte concentrations ($C_s \to 0$, $C_P \ll C_s$). Experimental conditions of spectrophotometric studies of DNA thermal denaturation conform ideally to this situation ($C_P \leq 10^{-4}$ M, at any $C_s \geq 0$). The CC theory then predicts qualitatively correct changes of DNA melting temperature in the presence of Mg^{2+} ions in NaCl solutions (Manning, 1972b, 1978; de Marky and Manning, 1975, 1976). The CC theory treatment of DNA counterion binding and structural transitions is here used strictly in accordance with the original formulation of Manning (1972a,b, 1978). The value of $\Delta g^{el} = \Delta G^{el}/RT = \Delta g_{el}^{el} + \Delta g_{mix}^{el}$ can be determined for each b_h and b_c and given values of C_{Na}, C_{Mg}, C_P from the following formulae (Manning, 1978):

$$g_{el}^{el} = E^{el}/RT = -(1 - \theta_1 - Z_2 \cdot \theta_2)^2 \qquad (4)$$
$$\cdot \xi \cdot \ln[1 - \exp(-\kappa \cdot b)]$$

$$g_{mix}^{el} = S^{el}/R = \theta_1 \cdot \ln[(\theta_1 \cdot 10^3)/(V_p \cdot C_1)] \qquad (5)$$
$$+ (C_2/C_P) \cdot \ln(10^{-3} \cdot C_2) + \theta_2 \cdot \ln(e \cdot \theta_2/V_p)$$

Here θ_1 and θ_2 are the degrees of Na^+ and Mg^{2+} ion condensation on the DNA polyion, Z_2 is the Mg^{2+} charge, V_p is the volume (in cm^3/mol) inside which the counterions are treated as "condensed on the polyion," C_1 and C_2 are the concentrations of "free" or "uncondensed" Na^+ and Mg^{2+} ions, respectively, and e is the base of the natural logarithm scale. The value of the Debye screening parameter is determined by the concentrations of free small ions (all coions are regarded as uncondensed): $\kappa^2 = 4 \cdot 10^{-3} \cdot \pi \cdot N_A \cdot b_0 \cdot \Sigma C_i^{free} \cdot Z_i^2$ (N_A is Avogadro's number). The counterion condensation volume, which is equal for both mono- and divalent counterions, is given by $V_p = 8 \cdot \pi \cdot e \cdot N_A b^3 \cdot (\xi - 1)$. Degrees of condensation θ_1 and θ_2, as well as the concentrations of "free" counterions, C_1 and C_2, are determined by an iteration procedure from the equations (Manning, 1978, 1984)

$$1 + \ln[(\theta_1 \cdot 10^3)/(V_p \cdot C_1)] \qquad (6)$$
$$= -2 \cdot (1 - \theta_1 - Z_2 \cdot \theta_2)^2 \cdot \xi \cdot \ln[1 - \exp(-\kappa \cdot b)]$$

$$\ln(\theta_2/C_2) = \ln(V_p \cdot 10^{-3}) + Z_2 \qquad (7)$$
$$\cdot \ln[(10^3 \cdot e \cdot \theta_1)/(V_p \cdot C_1)] + 1$$

In Eqs. 5–7, we assume the value of V_p to be equal for both divalent and monovalent cations, which is valid for the case $C_1 \gg C_2$ (Manning, 1984).

Poisson-Boltzmann theory

The Poisson-Boltzmann theory is probably the most popular approach for studying the ionic environment of charged macromolecules. We consider the "cell model," where the DNA molecule is represented by a hard, uniformly charged cylinder immersed in a coaxial cylindrical cell (Wennerström et al., 1982; Nilsson et al., 1989; Nilsson and Picullel, 1989). The radius of the cell, R, is defined by the polyion concentration:

$$C_P = \xi/(4\pi b_0 N_A R^2) \qquad (8)$$

The charge density of α-species of ions at a distance x from the polyion axis in the PB approximation is

$$\rho_\alpha(x) = C_\alpha^0 \cdot \exp(-Z_\alpha \psi(x)) \qquad (9)$$

Here C_α^0 is the concentration of α ions at the position where the electrostatic potential is defined to be zero, in the present case chosen to be $x = R$, at the outer cell boundary; $\psi(x) = e\phi(x)/kT$ is the reduced electrostatic potential, defined in the case of cylindrical symmetry by

$$\psi(x) = -2\xi \ln(x/R) - 4\pi[\ln(x/R) \cdot \int_a^x x' \sum_\alpha Z_\alpha \rho_\alpha(x')dx'$$
$$+ \int_x^R x' \sum_\alpha Z_\alpha \rho_\alpha(x')\ln(x'/R)dx'] \qquad (10)$$

where a is the polyion radius. Equations 9 and 10 can easily be solved numerically by iteration methods. The resulting data on the electrostatic potential and density distribution of ions can be used to calculate electrostatic contributions to the free energy.

The electrostatic internal energy per phosphate group, associated with introducing a charged polyion in the system, is calculated by (see, e.g., Stigter, 1995)

$$E^{el}RT = -\frac{1}{2}|\psi(a)| + \frac{1}{2}\int_a^R 2\pi x \sum_\alpha (Z_\alpha \rho_\alpha(x))\psi(x)dx \qquad (11)$$

The electrostatic entropy contribution due to redistribution of ions around the charged cylinder is given by

$$S^{el} = -k_B b_0 \int_a^R 2\pi x \sum_\alpha \left[\rho_\alpha(x)\ln\left(\frac{\rho_\alpha(x)}{C_\alpha^0}\right)\right]dx \qquad (12)$$

Equations 11 and 12 then directly give ΔG^{el}, assuming that ΔV for the helix coil transition is negligible.

Monte Carlo self-consistent field approach

It is well recognized that the PB theory generally satisfactorily describes polyelectrolyte properties if only monovalent ions are present in the solution. In the case of multivalent ions, noticeable differences appear when comparing resulting ion distributions and electrostatic potentials obtained from PB calculations with simulation data (Lyubartsev et al., 1990; Lyubartsev and Nordenskiöld, 1995). To

clarify the role of these discrepancies in the present context, we have calculated the electrostatic potential and ion distribution for relevant polynucleotide systems from computer simulations. Because the considered range of concentrations implies a very large simulation cell, we have applied the combined Monte Carlo self-consistent field (MCSF) method (Vorontsov-Velyaminov and Lyubartsev, 1989, 1992; Lyubartsev, 1991). The essence of this method is a division of the whole cell into two regions, one internal, close to the polyion surface, and one external. In the internal region, which typically has a radius of 20–40 Å, the full Monte Carlo procedure is carried out. In the external zone the PB theory is applied. An exchange of ions between the regions in the manner of the Gibbs ensemble simulation method (Panagiotopoulos, 1987) provides a self-consistent calculation of ion distributions in the whole cell. The details of the Monte Carlo self-consistent field method are given elsewhere (Vorontsov-Velyaminov and Lyubartsev, 1989, 1992). Here we have used this method for calculations of the electrostatic potentials and ion densities, and these data are then used in Eqs. 11 and 12.

It should be noted that Eqs. 11 and 12 are still approximations to the internal energy and entropy, even if exact ion distributions and electrostatic potentials are used. These formulas do not take into account contributions from ion correlations. Correct free energy calculations in computer simulations are computationally much more time demanding. On the other hand, contributions from ion correlations to the free energy may to a large extent be canceled in free energy differences, provided that in each case the ion content is the same. A similar argument should be valid for the solvation contribution to the free energy.

Computational details

PB and MCSF calculations of $\Delta g^{\mathrm{el}} = \Delta G^{\mathrm{el}}/RT$ for each set of parameter values have been calculated by the SFMK program (Lyubartsev, 1991), which is available from the CCP5 program library. Results have been obtained for each type of polyion at fixed C_P and varied values of C_{Na}, ranging from C_P to 200 mM, and variation of $r = C_{\mathrm{Mg}}/C_P$ from 0 to 2.5. Native DNA was modeled as a cylindrical polyion with radius $a_h = 9.5$ Å and charge density $b_h = 1.69$ Å per unit charge. It was previously shown (Bond et al., 1994) that with values of charge spacing $b_c \approx 3.4$ Å and polyion radius $a_c \approx 6$–8 Å, the best agreement between experimental data and PB calculations was obtained. We have used the values $a_c = 7$ Å and $b_c = 3.38$ Å in the majority of our calculations of $\Delta g^{\mathrm{el}} = g_c^{\mathrm{el}} - g_h^{\mathrm{el}}$. We also made some calculations for lower charge density (b_c up to 4.5 Å) and thinner ($a_c = 5$ or 6 Å) single-strand polynucleotide radius. In this way, PB data can be compared with the results of CC theory for which a somewhat lower value for b_c (3.8–4.5 Å) has been predicted (Record et al., 1976a, b; Manning, 1976). The radii of the small ions have been set at $d_{\mathrm{Na}} = d_{\mathrm{Cl}} = 2$ Å. This value of d_{Na} is between the sizes of crystallographic and hydrated Na$^+$ ions and close to the

value estimated in the mean spherical approximation (Fawcett and Tikanen, 1996; Simonin et al., 1996), which produces good theoretical evaluation for osmotic and ionic activity coefficients up to 5 m NaCl (Simonin et al., 1996). The value of the hydrated Mg^{2+} ion radius has been set at $d_{\mathrm{Mg}} = 3$ Å (Corti and Fernandez-Prini, 1986) in the majority of the calculations. We have made some calculations for $d_{\mathrm{Mg}} < 3$ Å and found that the value of d_{Mg} influences Δg^{el} only slightly when $d_{\mathrm{Mg}} \geq d_{\mathrm{Na}}$. At $d_{\mathrm{Mg}} < d_{\mathrm{Na}} = 2$ Å, we have obtained substantially different values of Δg^{el} (these data are not shown). However, it was found in x-ray diffraction (Black et al., 1994) and molecular dynamics simulation (MacKerell, 1997) studies that Mg^{2+} is associated with the phosphate groups of nucleic acids mostly in hydrated form, so $d_{\mathrm{Mg}} < 2$ Å is probably not a realistic value.

It is well known that increasing temperature leads to a decreasing value of the dielectric constant of water and of the product $\epsilon \cdot T$, which determines the values of Bjerrum length, b_o, a key parameter in all polyelectrolyte theories. Although the change in $\epsilon \cdot T$ with temperature is small, the decrease in ϵ nevertheless does not balance exactly the increase in T (for example, $b_o = 7.16$ and 8.05 Å at 25°C and 100°C, respectively, in water). Thus we have made calculations of Δg^{el} for some different temperatures in the range 25–100°C. The dielectric constant of water has been calculated with the following empirical equation (Hasted, 1972):

$$\epsilon(T) = 87.740 - 0.4008 \cdot t + 9.398 \cdot 10^{-4} \tag{13}$$
$$\cdot t^2 - 1.410 \cdot 10^{-6} \cdot t^3$$

where t is the temperature in degrees Celsius.

RESULTS AND DISCUSSION

Dependence of ΔG^{el} on temperature, C_{Na}, C_{Mg}, and C_P

PB results

Dependencies of $\Delta g^{\mathrm{el}} = \Delta G^{\mathrm{el}}/RT$ on different solution conditions, calculated with Eqs. 11 and 12, are presented in Fig. 1 (PB) and Fig. 2 (PB and MCSF). First, it may be noted that the function $\Delta g^{\mathrm{el}} = \Delta G^{\mathrm{el}}/RT$ displays a clear independence on temperature for practically all values of the parameters we have used in our calculations. Only a slight difference in the curves calculated for 25°C (*curves* in Fig. 1) and 100°C (*points* in Fig. 1) can be noticed at small concentrations of NaCl and at $r = 0$ (log $C_{\mathrm{Na}} < -2.5$). However, melting temperatures of DNA under these conditions do not differ significantly from 25°C. On the other hand, the changes with temperature in the different contributions to ΔG^{el} cannot be neglected. For example, ΔG^{el} equals -2.36 and -2.98 kJ/mol at $C_{\mathrm{Na}} = 20$ mM and 1.22 and 1.59 kJ/mol at $C_{\mathrm{Na}} = 200$ mM at 25°C and 100°C, respectively (in the absence of Mg^{2+}). Thus the dependence of the electrostatic free energy on temperature is simply $\Delta G^{\mathrm{el}} = \Delta g^{\mathrm{el}} \cdot RT$, where Δg^{el} is not a function of temperature.
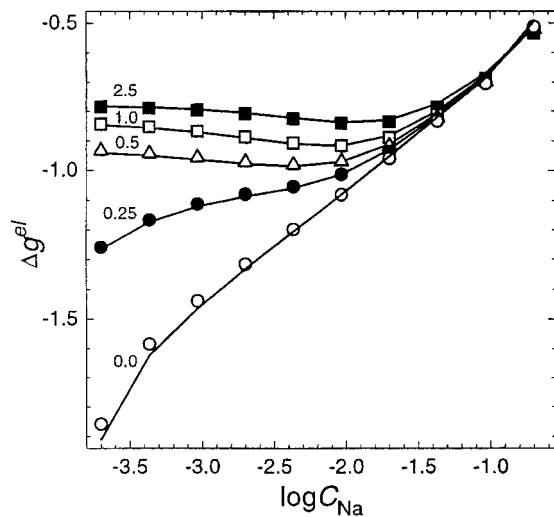
FIGURE 1   Dependencies of $\Delta g^{el} = \Delta G^{el}/RT$ on Na^+ concentration calculated by PB theory for DNA helix coil transition in NaCl + MgCl$_2$ solutions. Values of $r = C_{Mg}/C_P$ are indicated in the figure. Curves and points are for 25°C and 100°C, respectively. The parameters used were $C_P = 0.2$ mM, $b_h = 1.69$ Å, $a_h = 9.5$ Å, $b_c = 3.38$ Å, $a_c = 7.0$ Å, $d_{Na} = d_{Cl} = 2$ Å, $d_{Mg} = 3$ Å.

The most interesting and striking observation regarding the data in Fig. 1 appears when making a qualitative comparison with experimental figures of T_m versus log C_{Na}. Data by Dove and Davidson for calf thymus DNA (figure 2 of Dove and Davidson, 1962) and by Record for T2 and T4 phage DNA (figures 9 and 10 of Record, 1975) contain such curves for solutions containing different contents of MgCl$_2$, and the following observations can be made:

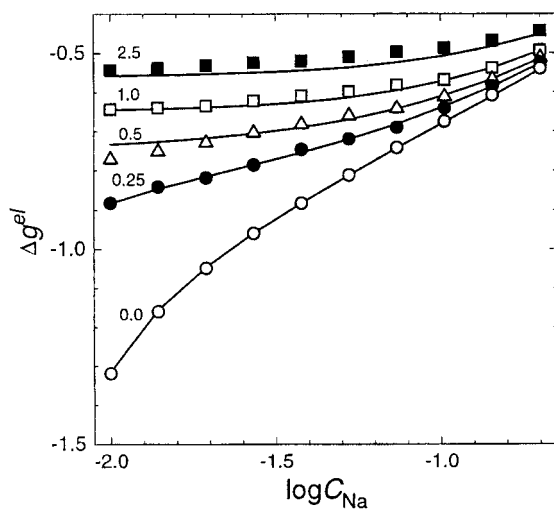FIGURE 2   Dependencies of $\Delta g^{el} = \Delta G^{el}/RT$ on Na^+ concentration calculated by PB theory (*curves*) and Monte Carlo self-consistent field approach (*points*) for DNA helix coil transition in NaCl + MgCl$_2$ solutions. Values of $r = C_{Mg}/C_P$ are indicated in the figure. The parameters used were $C_P = 10$ mM, $b_h = 1.69$ Å, $a_h = 9.5$ Å, $b_c = 3.38$ Å, $a_c = 7.0$ Å, $d_{Na} = d_{Cl} = 2$ Å, $d_{Mg} = 3$ Å.

1. The dependence of Δg^{el} versus log C_{Na} is linear and increasing at $C_{Mg} = 0$ for Na^+ ion concentrations from 1 mM to 200 mM. This means an increased double helix stability with added salt. The same qualitative dependence of experimental T_m versus log C_{Na} also holds.

2. The addition of Mg^{2+} leads to an increase in the DNA double-helix stability (increase in Δg^{el}) at small values of $r = C_{Mg}/C_P$. This stabilization effect becomes smaller at $r > 0.5$ when equality of DNA and Mg^{2+} charges is reached. The same behavior of T_m versus log C_{Na} is also seen for this range of MgCl$_2$ content.

3. There are minima in both the curves of Δg^{el} and T_m versus log C_{Na} for $r \geq 0.5$. It means that the addition of NaCl at small concentrations of Na^+ and at $r \geq 0.5$ leads to destabilization of the double helix. The position of the theoretical minimum in thermal stability at constant r is near log $C_{Na} \approx -2$ to -1.5 and corresponds to the minima observed in the experimental curves obtained under similar conditions (see figures 9 and 10 in Record, 1975, and figure 2 in Dove and Davidson, 1962).

Next we consider the dependence of Δg^{el} on Na^+ concentration, for different values of r, but now at different DNA concentrations, C_P. The PB results, displayed in Fig. 2 (*lines*), have been obtained at $C_P = 10$ mM, and the results of Fig. 1 are for $C_P = 0.2$ mM. Data obtained for $C_{Mg} = 0$ indicate that the linearity of Δg^{el} on the logarithm of the Na^+ concentration disappears for $C_{Na} \leq 10 \cdot C_P$. This can be seen in the curves for $r = 0$ in Figs. 1 and 2, where Δg^{el} values are lower than expected from an extrapolation of the linear region at higher Na^+ concentrations. This means that the double helix becomes less stable at a concentration of supporting electrolyte comparable with C_P. This observation coincides well with the data from Record (1975), who found a steeper (than predicted from the linearity of T_m versus log C_{Na}) decrease of T_m for $C_{Na} < 0.5$ mM at $C_P \geq C_{Na}/10$.

The regions of r and C_P where the minimum in the curves Δg^{el} versus log C_{Na} is observed can also be seen in the calculated results. Addition of Na^+ destabilizes the double helix most noticeably at $C_P \leq 1$ mM and for r values from 0.5 to 2.0. The curves of Δg^{el} versus log C_{Na} become "flatter" for $r > 2$ at low polynucleotide concentrations ($C_P \leq 1$ mM). The curves with a minimum change to monotonically increasing ones for $C_P \geq 4$ mM at all values of r (see Fig. 2 obtained for $C_P = 10$ mM). This observation coincides with reported data (Krakauer, 1974), where a reduction of the "depth of minimum" in the curves T_m versus log C_{Na} was noted when r increased from 2 to 10 at $C_P = 0.056$ mM or C_P increased from 0.145 to 0.37 mM at $r = 1.0$, in studies of poly(A)·(poly(U))$_2$ triple helix melting in NaCl + MgCl$_2$ solutions.

MCSF results

Some calculations of Δg^{el} have been done with the MCSF method (Vorontsov-Velyaminov and Lyubartsev, 1989; 1992; Lyubartsev, 1991). Data obtained at $C_P = 10$ mM

from MCSF (*symbols*) and PB (*lines*) are shown in Fig. 2. The results obtained by these two methods are practically equal for all values of C_{Na} and $r \leq 1.0$. There is some deviation between MCSF and PB at $r = 2.5$, with the MCSF results predicting a somewhat higher stability of native DNA than does PB. Monte Carlo calculations can hardly be performed using a reasonable amount of CPU time and still obtain acceptable accuracy at small ($C_P < 10$ mM) DNA concentrations. At the same time, for comparisons of theoretical calculations with experimental data, it is crucial to mimic experimental conditions in the model estimations of Δg^{el} because of the decisive influence of the concentrations of ionic species on the magnitude of the entropy and energy terms (see Eqs. 4, 5 and 11, 12). For this reason we have made only a few calculations with the MCSF method at such small concentrations. These calculations (data not shown) also showed essentially the same values of Δg^{el} as the PB calculations. Previous Monte Carlo simulations on DNA systems (Lyubartsev and Nordenskiöld, 1995; Lyubartsev et al., 1990) have shown considerable deviations for ion distributions of divalent counterions compared to the PB results. The fact that the present results of Δg^{el} are similar for PB and MCSF may to a large extent be due to a cancellation effect when taking the difference of g^{el} between coil and helix. Another reason for this similarity is the large volume of the cylindrical cell (we have made our calculations for small values of C_P to conform with experimental conditions). Under these conditions, contribution from the entropy term to the free energy change becomes decisive and entropy changes are rather similar in the PB and MCSF calculations (data not shown). As noted above, the present way of calculating Δg^{el} from Eqs. 11 and 12 is also an approximation.

CC theory results

Results of Δg^{el} calculations on the basis of Eqs. 4 and 5 are shown in Fig. 3, *A* and *B*, for two values of single-strand DNA charge spacing; $b_c = 3.38$ Å ($\xi_c = 2.1$) and $b_c = 3.9$ Å ($\xi_c = 1.85$), respectively. It can be noted directly that the CC theory also gives a qualitative similarity in the values of Δg^{el} versus $\log C_{Na}$ when compared with T_m versus $\log C_{Na}$ curves from the literature (figure 2 in Dove and Davidson, 1962, and figures 9 and 10 in Record, 1975). Comparing the PB results of Fig. 1 with Fig. 3 *A* (CC theory), it can be noted that the Δg^{el} values obtained for $b_c = 3.38$ Å are similar at low Na$^+$ ion concentrations and at $r \geq 0.5$. However, the following differences in the evaluations of Δg^{el} originating from the CC and PB theories are of importance:

1. In the absence of Mg^{2+}, values of Δg^{el} obtained from the CC theory are much lower than those calculated by PB for both $b_c = 3.38$ Å and $b_c = 3.9$ Å (results obtained with $b_c = 3.9$ Å from the PB approach are not shown).

2. The CC method predicts a steeper decrease in Δg^{el} upon the addition of Na$^+$ at $\log C_{Na} \geq -4$ and $r \geq 0.5$ than does the PB theory (see the left-hand side of the curves in
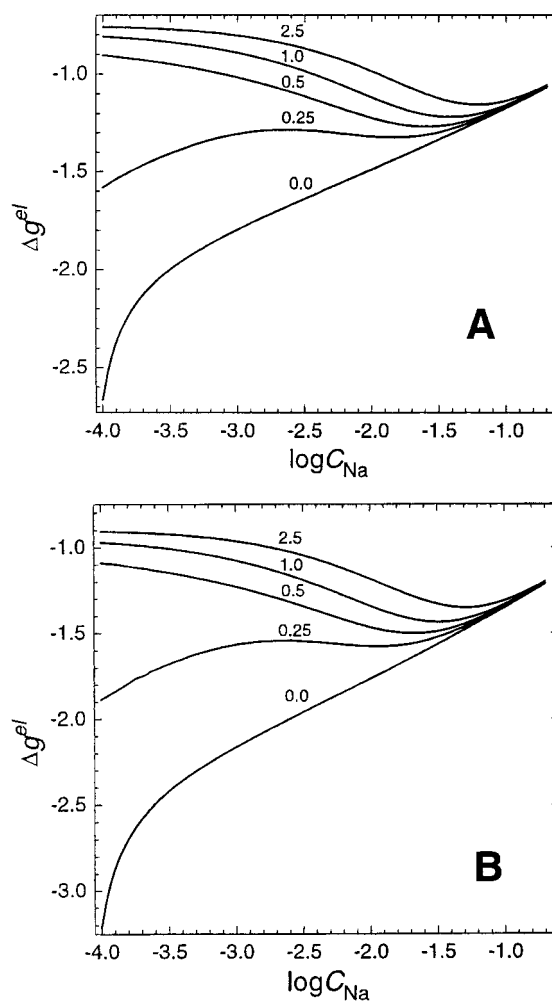

FIGURE 3 Dependencies of $\Delta g^{el} = \Delta g_{el}^{el} + \Delta g_{mix}^{el}$ on Na$^+$ concentration calculated by CC theory for DNA helix coil transition in NaCl + MgCl$_2$ solutions for different values of the denatured polyion charge density, b_c. (*A*) 3.38 Å ($\xi_c = 2.1$), (*B*) 3.9 Å ($\xi_c = 1.85$). Values of $r = C_{Mg}/C_P$ are indicated in the figures. The other parameters were $C_P = 0.1$ mM, $b_h = 1.69$ Å ($\xi_h = 4.2$).

Figs. 1 and 3, *A* and *B*). Manning (1972b) also reported much lower negative values of $dT_m/d \log C_{Na}$ for the left-hand (decreasing) part of T_m versus $\log C_{Na}$ curves than those obtained in the experiments. This means that the PB model can qualitatively reproduce experimental data better than the CC theory.

3. The CC theory calculations show that Δg^{el} for $\log C_{Na} \leq -2.5$ and $r \geq 0.5$ is higher than Δg^{el} at $\log C_{Na} \geq -1.5$ (on the right-hand side of Δg^{el} versus $\log C_{Na}$ curves). At the same time, the PB results show the opposite dependence. Again, PB is in better agreement with experimental data, because at $C_{Na} \approx 100$ mM, the values of T_m are higher than those obtained at $r \leq 1.0$ and $C_{Na} = 0.1-1$ mM.

4. The curves for $r = 0.25$ obtained from CC theory display a maximum that is absent from the corresponding PB curve and is not seen in experimental T_m versus $\log C_{Na}$ curves.

Comparisons of experimental and calculated values of thermodynamic functions

Dependence of ΔG_m on NaCl concentration

The similarity of the Δg^{el} and T_m versus $\log C_{Na}$ curves obtained within the frames of both PB and CC theories clearly indicates that the salt dependence of the helix-coil transition of DNA solutions containing both NaCl and MgCl$_2$ can be explained mainly by the change in the electrostatic component of the free energy of double helix and single strand, respectively. At the same time, the values of Δg^{el} are *negative* at all concentrations of NaCl and MgCl$_2$. Clearly, this means that to conform to experimental data, the nonelectrostatic term in Eq. 2 must be positive and exceed the absolute values of ΔG^{el} for all temperatures that are lower than the experimentally observed values of T_m.

First, let us compare the results of our calculations with available experimental data on the free energy difference ΔG_m at a fixed temperature, as a function of the amount of added univalent salt, NaCl. We are not aware of such data for DNA containing MgCl$_2$, but some results in the presence of only monovalent counterions do exist in the literature. Experimental values of $\Delta G_{m,0}(C_{Na})$ at the standard temperature T_o can be determined from the dependence of melting enthalpy (ΔH_m) on melting temperature from the following relation (Shiao and Sturtevant, 1973):

$$\Delta G_{m,0}(C_{Na}) = \Delta H_{m,0}(C_{Na}) - \Delta S_{m,0}(C_{Na}) \cdot T_o - \Delta C_p \qquad (14)$$
$$\cdot [T_m - T_o + T_o \cdot \ln(T_m/T_o)]$$

Here $\Delta S_{m,0}(C_{Na}) = \Delta H_{m,0}(C_{Na})/T_m$ is the entropy of melting, and ΔC_p is the change in heat capacity. The subscript 0 indicates constant temperature T_0, which we take to be 298 K. We neglect here the dependence on DNA concentration, C_P, which is correct if $C_{Na} \gg C_P$.

Some literature data on experimental melting enthalpy, entropy, and heat capacity changes are collected in Table 1. The value of ΔC_p can be determined either from the slope

$\Delta C_p = d[\Delta H_{m,0}(C_{Na})]/dT_m$ or directly from the difference in heat capacities of native and denatured DNA obtained from experimental melting curves during scanning microcalorimetry experiments (Filimonov and Privalov, 1978; Privalov and Potekhim, 1986). Such direct determination of ΔC_p demands application of very sensitive calorimetric equipment, and experiments must be performed with high precision (Filimonov and Privalov, 1978). Experimental values of $\Delta G_{m,0}$ at 298 K, calculated with Eq. 14 from literature data, are presented in Fig. 4, *A* and *B*.

To obtain theoretical values of $\Delta G_{m,0}$ for comparison with those experimentally measured, the value of $\Delta G^{nel}(298)$ in Eq. 2 must be evaluated. This was done in the following manner:

1. We used the experimental $\Delta G_{m,0}$ data obtained by Privalov et al. for T2 phage DNA in NaCl solutions (Privalov et al., 1969) (*solid squares* in Fig. 4 *A*) and the results reported for salt-free LiDNA from chicken blood (Korolev et al., 1994) (*solid circles* in Fig. 4 *B*).

2. The linear dependencies of $\Delta G_{m,0}$ versus $\log C_{Na}$ (Privalov et al., 1969) and $\Delta G_{m,0}$ versus $\log C_P$ (Korolev et al., 1994) were then extrapolated to the values $\Delta G_{m,0} = 0$ to find C_{Na} or C_P for which DNA melts at 25°C. It was determined that $\Delta G_{m,0} = 0$ at $\log C_{Na} = -4.17$ and $\log C_P = -3.77$.

3. Then the values of $\Delta G^{nel}(298) = -\Delta G^{el}(298) = \Delta g^{el} \cdot R \cdot 298.15$ were determined from the theoretically calculated values of Δg^{el} at the concentrations indicated above for each set of modeled structural parameters.

4. Finally, the theoretically calculated values of ΔG^{el} as a function of salt or DNA concentration, together with the obtained values of $\Delta G^{nel}(298)$ for the two cases, were used to construct the theoretical curves of $\Delta G_{m,0}$ in Fig. 4, *A* and *B*.

It can be seen that the PB model, together with the assumption of independence of ΔG^{nel} on salt content, provides a self-consistent description of available calorimetric data (Fig. 4, *A* and *B*). Some deviation between experimen-

TABLE 1 Scanning microcalorimetry data on the helix-coil transition of double-stranded, highly polymerized polynucleotides at neutral pH (6.5–8)

Polynucleotide	$\Delta H°(298)$ (kJ/mol)	$\Delta S°(298)$* (J/(mol $\cdot$ K))	ΔC_p (J/(mol $\cdot$ K))	Range of T_m (°C)	Range of C_s (mM)	Reference
T2 phage NaDNA (35% GC)	16.0 ± 0.3	47.4; 43.5	72 ± 6	64 ÷ 85	11–204	Privalov et al. (1969)[‖]
Calf thymus NaDNA	10.7 ± 0.2	31.2; 30.1	86 ± 11	58 ÷ 77	1–52	Shiao and Sturtevant (1973)
Calf thymus NaDNA	10.7 ± 0.3	31.7; 26.3	137 ± 12	63 ÷ 100	3–285	Gruenwedel (1974)**
Poly(A) $\cdot$ poly(U)	17.0	55; 51	67 ± 5[#]	37 ÷ 58	10–100	Filimonov and Privalov (1978)
			63 ± 8[§]			
Chicken blood NaDNA	14.0	46.8	73 ± 17	50 ÷ 85	1–2000	Chipev and Angelova (1983)
Calf thymus NaDNA	15.6 ± 0.3	46.7; 43.2	74 ± 7	57 ÷ 87	4–205	Vlasov (1993)
			83 ± 4[#]			
Chicken blood LiDNA, salt free	12.3 ± 0.6	39.3; 33.5	160 ± 35	41 ÷ 68	1.2–32[¶]	Korolev et al. (1994)

*The highest value at low C_s and the lowest one at high C_s were taken from references when available.

[#]Direct determination.

[§]After correction for single-stranded poly(A) melting.

[¶]Concentration of DNA.

[‖]One experimental point was omitted in the determination of ΔC_p.

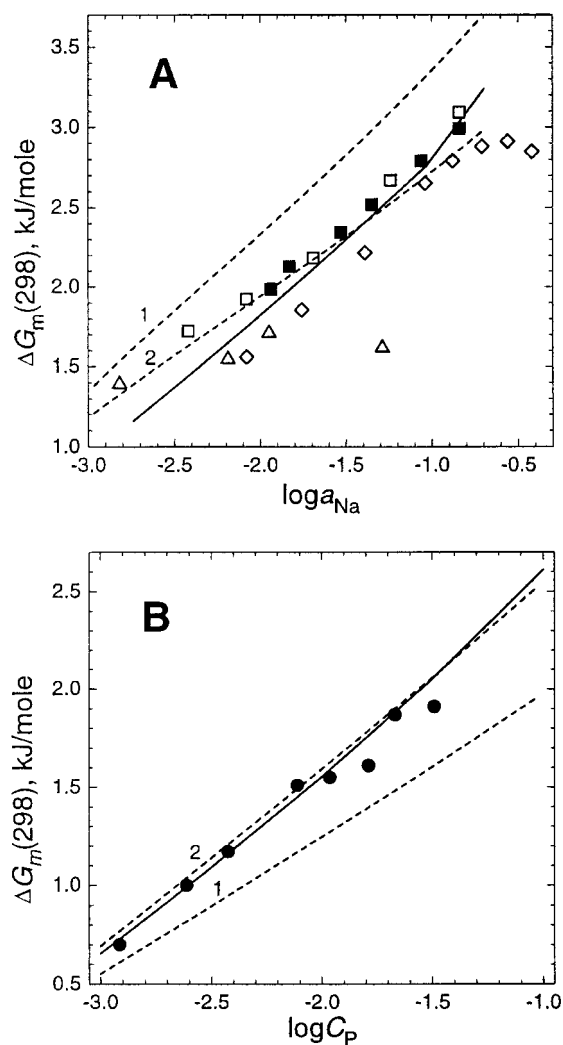**Points at $C_s > 160$ mM were omitted in the determination of ΔC_p.

FIGURE 4 Comparisons of experimental (*points*) and theoretical (*curves*) dependencies of $\Delta G_{m,0}$ (*A*) on Na^+ activity (a_{Na}) in NaDNA + NaCl solutions and (*B*) on DNA concentration (C_P) in salt-free solutions of LiDNA. Solid curves are for PB theory (a_h = 9.5 Å, b_c = 3.38 Å, a_c = 7.0 Å); dotted curves are for CC theory. Curve 1: b_c = 3.38 Å (ξ_c = 2.1); curve 2: b_c = 3.9 Å (ξ_c = 1.85). The charge spacing for native DNA, b_h, is 1.69 Å (ξ_h = 4.2) in all calculations. Experimental data were taken from (*A*) Privalov et al. (1969) (■), Gruenwedel (19740 (◇), Vlasov (1993) (□), Shiao and Sturtevant (1973) (△). (*B*) Korolev et al. (1994) (●). Details are given in the text.

tal data and the solid curve can be noted at low NaCl (C_{Na} ≤ 10 mM) concentrations. This may be related to the larger uncertainty of both the microcalorimetry (Vlasov, 1993) and the other type of measurements (Shiao and Sturtevant, 1973; Privalov and Potekhim, 1986; Gruenwedel, 1974) at low concentrations of DNA and salt. It should also be noted that our method for the evaluation of $\Delta G^{nel}(298)$ is expected to be quite accurate, because it is obtained from extrapolation of the total dependencies of $\Delta G_m(298)$ versus log $a_\pm$ or log C_P. This is in contrast to the procedure in, e.g., Frank-Kamenetskii et al. (1987), where only one experimental point is used. We are of the opinion that the present treatment (by applying extrapolated and highly distant values of

the total linear dependencies of $\Delta G_m(298)$ versus log $a_\pm$ or log C_P) gives a somewhat stronger credit to the correctness of our theoretical predictions than the usually applied procedure. The reason is that if any of the two variables (i.e., the absolute values $\Delta G_m(298)$ or slope $\Delta G_m(298)$ versus logarithm of concentration) were incorrect, the divergence between experimental data and the theoretical curves should become noticeable in Fig. 4, *A* and *B*, which is not the case.

The same procedure was repeated for the CC theory. Comparisons of the CC theory with experimental data in NaDNA + NaCl solutions lead to the conclusion that Manning's theory produces a good estimation of the absolute values of $\Delta G_{m,0}$ for b_c = 3.9 Å (*curve 2* in Fig. 4 *A*) and a reasonable value for the slope $\Delta G_{m,0}$ versus log a_{Na} for b_c = 3.38 Å (*curve 1* in Fig. 4 *A*). It should be noted that experimental values of $\Delta G_{m,0}$ determined at log a_{Na} < -2 may be unreliable because of the instability of the DNA secondary structure at these salt concentrations (Record, 1975; Record et al., 1976b; Korolev et al., 1994). Furthermore, there is an uncertainty in the Na^+ ion activity calculations at comparable concentrations of NaDNA and NaCl (Gruenwedel, 1974). On the other hand, agreement between theoretical and experimental values of $\Delta G_{m,0}$ is observed in salt-free solutions of LiDNA only for the value b_c = 3.9 Å (*curve 2* in Fig. 4 *B*), and not for the value b_c = 3.38 Å (*curve 1* in Fig. 4 *B*).

Our results produce estimates for values of the charge spacing of the denatured DNA polyion that are in agreement with literature data: b_c = 3.9 and 3.4 Å, respectively, for the CC theory (Manning, 1976; Record et al., 1976a,b) and the PB calculations (Bond et al., 1994; Frank-Kamenetskii et al., 1987; Korolev et al., 1994).

Estimation of ΔG^{nel} temperature dependence: analysis of microcalorimetric data

In this section we will focus on using the calculated values of Δg^{el} to evaluate the temperature dependence of the non-electrostatic component of ΔG_m in Eq. 2 (i.e., ΔG^{nel}). We start with a set of experimental data on the dependence of T_m on supporting NaCl electrolyte concentration, in the absence of divalent ions. We calculate values of Δg^{el} for these electrolyte concentrations at corresponding melting temperatures, T_m. Then the values $\Delta G^{nel} = -\Delta g^{el} \cdot RT_m$ are determined from these data, using the fact that at the melting point $\Delta G_m = 0$.

Numerous experimental data on the dependence of T_m on the concentration of the supporting electrolyte can be used for this determination. We have used values of T_m obtained by Record for T4 phage DNA in the range $C_{Na} \geq 1$ mM (Record, 1975). We have also applied our analysis to the empirical formula proposed for dependence of T_m versus log C_s at different GC contents and based on numerous experiments with DNA from different sources (Schildkraut and Lifson, 1965; Wada et al., 1980):

$$T_m = k \cdot \log C_s + 0.41 \cdot X_{GC} + 81.5 \qquad (15)$$

In the present case the value of the GC-pair content $X_{GC} = 0.41$, and $k = 17.5°C$ (this value of k is believed to be most reliable; Wada et al., 1980).

In the next step, the obtained set of data ΔG^{nel} versus temperature is fitted by least-squares analysis to Eq. 3, with $\Delta H^{nel}(298)$, $\Delta S^{nel}(298)$, and ΔC_p^{nel} as fitting parameters. After evaluation of these parameters, we can sum up the electrostatic and nonelectrostatic components of the changes in enthalpy, entropy, and heat capacity, and compare them with the experimental calorimetric values presented in Table 1. Below we will discuss the results of this comparison.

Experimental literature data on thermodynamic parameters of melting of highly polymerized double-helical polynucleotides are collected in Table 1. We have chosen the data that report the values of ΔC_p and were obtained at neutral pH. One can see from Table 1 that despite the high accuracy achieved in modern scanning microcalorimetry (Privalov and Potekhim, 1986), there is a considerable discrepancy in reported thermodynamic parameters, even in determination of $\Delta H°(298)$. The main reason for this is the ambiguity in evaluations of ΔC_p. Extremes of reported data on ΔC_p (not given in Table 1) oscillate from zero (Breslauer, 1995) to values higher than 200 J/(mol·K) (Filimonov and Privalov, 1978; Mrevlishvili et al., 1992). Such large discrepancies in values of ΔC_p are probably due to a combination of limited accuracy in the experiments and problems caused by effects of single-strand melting (Filimonov and Privalov, 1978), varying pH (Mrevlishvili et al., 1992), and DNA molecular weight (Chipev and Angelova, 1983). In summary, however, the majority of the experimental determinations of ΔC_p obtained with highly sensitive microcalorimetric equipment report ΔC_p values between 60 and 90 J/(mol·K) for highly polymerized double-stranded polynucleotides in neutral pH solutions of univalent counterion salt.

An example of the least-squares fitting ΔG^{nel} versus temperature curve obtained on the basis of PB calculations and using T_m values determined from Table 1 of the reference (Record, 1975) is shown in Fig. 5. Resulting thermodynamic quantities obtained from several fitting procedures under different conditions, and performed on the basis of both PB and CC models, are collected in Table 2. It can be seen from Table 2 that PB and CC theories produce generally similar evaluations of the nonelectrostatic components of the melting free energy.

Calculated dependencies of changes of electrostatic energy and entropy on log C_{Na} at 25°C and 100°C are shown in Fig. 6, A and B, respectively. Usually, microcalorimetric data are available in the range $C_{Na} = 10–200$ mM (see Table 1). It can be seen from Fig. 6, A and B, that ΔE^{el} and ΔS^{el} decrease from ~0 to -400 J/mol and from 9 to 4 J/(mol·K), respectively, in this range of salt concentration. Because ΔE^{el} is small and its change with Na^+ concentration is in the range of experimental error and within the significance of fitting of ΔG^{nel} versus T_m, the experimental and theoretical values of melting enthalpies in Tables 1 and
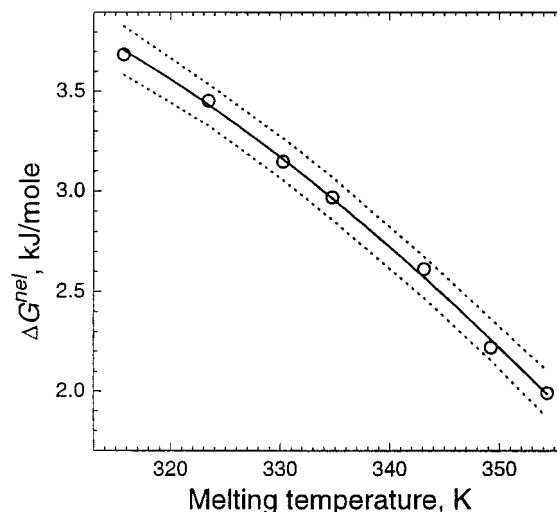

FIGURE 5 Determination of temperature dependence of ΔG^{nel} from calculated (PB theory) values of $\Delta g^{nel} = \Delta G^{nel}/RT$ and experimental values of T_m (Record, 1975). Structural parameters for double helix (h) and coil (c) DNA are $b_h = 1.69$ Å, $a_h = 9.5$ Å, $b_c = 3.38$ Å, $a_c = 7.0$ Å. The solid line is the least-squares fitted curve obtained for Eq. 3. Dotted curves are limits of predicted intervals for a confidence value of 0.95. Calculated parameters of Eq. 3 are listed in Table 2 (the first line of Table 2 is for data presented in this figure). See text for more details.

2 can be compared directly without correction from the electrostatic term. One can see that the theoretically estimated values of ΔH^{nel} are within the range of reported experimental determinations. The electrostatic component of the change in heat capacity (ΔC_p^{el}) can be evaluated from Fig. 6 A from the relation

$$\Delta C_p^{el} = d\Delta E^{el}/dT_m = (d\Delta E^{el}/d \log C_{Na}) \cdot (dT_m/d \log C_{Na})^{-1}$$

$$\approx -30 \text{ to } -20 \text{ J/(mol·K)}$$

for

$$dT_m/d \log C_{Na} \approx 15 - 20°C.$$

Some experimental melting entropy data in the second column of Table 1 contain two values of $\Delta S°(298)$, obtained at the lowest and at the highest salt concentrations. One can see a tendency of the standard melting entropy to decrease with salt concentration. The same behavior is also predicted by the PB model (see Fig. 6 B). It is interesting to note that this relatively small change in melting entropy (3–5 J/(mol·K) per decimal logarithm unit) is responsible for the noticeable linear dependence of $\Delta G_m(298)$ on the logarithm of C_s or C_P (see Fig. 4, A and B). Values of ΔS_m calculated with PB theory are lower (when ΔG^{nel} fits the UV spectrophotometry data; Record, 1975) than those determined in calorimetric experiments.

To improve the error limits of the fitted parameters in the expression for ΔG^{nel}, we also made some fittings of the data using values of ΔS^{nel} fixed at 30, 35, and 40 J/(mol·K) and fitting only two parameters, $\Delta H^{nel}(298)$ and ΔC_p^{nel}. Results are given in rows 2–4 in Table 2. In that way, a good

TABLE 2 Evaluation of nonelectrostatic parameters of helix-coil transition from experimental values T_m and theoretical calculations of Δg^{el} with Eq. 3

Model	Source of T_m values	Structural parameters of denatured polyion	$\Delta H^{nel}(298)$ (kJ/mol)	$\Delta S^{nel}(298)$ (J/(mol · K))	ΔC_p^{nel} (J/(mol · K))
PB theory	Record (1975)	$b_c = 3.38$ Å, $a_c = 7$ Å	10.9 ± 2.2	22.5 ± 6.9	191 ± 58
			13.3 ± 0.1	30^*	128 ± 9
			14.8 ± 0.1	35^*	86 ± 11
			16.4 ± 0.1	40^*	44.1 ± 13.7
	Wada et al. (1980)	$b_c = 3.38$ Å, $a_c = 7$ Å	13.9 ± 0.2	31.3 ± 0.6	170 ± 5
CC theory	Record (1975)	$b_c = 3.38$ Å ($\xi_c = 2.1$)	8.0 ± 1.6	10.3 ± 5.0	174 ± 42
		$b_c = 3.9$ Å ($\xi_c = 1.85$)	10.3 ± 2.0	14.5 ± 6.4	232 ± 54
		$b_c = 3.9$ Å ($\xi_c = 1.85$)	15.1 ± 0.1	30^*	102 ± 11
		$b_c = 4.5$ Å ($\xi_c = 1.6$)	12.7 ± 2.7	18.7 ± 8.7	315 ± 73

*Adjustment was made of values $\Delta H^{nel}(298)$ and ΔC_p^{nel} at fixed value $\Delta S^{nel}(298)$.

agreement between the results of the PB-based treatment and experimental data in Table 1 can be seen when the value of $\Delta S^{nel}(298)$ is fixed between 30 and 35 J/(mol·K).

In row 5 of Table 2, we display the results of fitting ΔG^{nel}, obtained using the empirical relation (Wada et al., 1980) for the salt dependence of T_m according to Eq. 15. The most noticeable feature of this result is the improved error limits obtained with this treatment. The fitted parameter values for ΔS^{nel}, ΔH^{nel}, and ΔC_p^{nel} are good, although the value for ΔC_p^{nel} is somewhat high.

Similar fittings have been made for the CC theory. The results are shown in Table 2. Generally, the results from the CC theory produce reasonable correspondence with experimental data. However, the agreement of the CC results with experimental values is not as good as that observed for the PB model, despite the fact that only one set of values, $b_c = 3.38$ Å and $a_c = 7$ Å, was used in our comparisons of PB and experimental results, whereas in the CC calculations the b_c value was varied. The best result is observed for $b_c = 3.9$ Å ($\xi_d = 1.85$).

Thus theoretical values of thermodynamic functions are in reasonable agreement with available calorimetric data. This means that the basic assumption of our considerations, that ΔG^{nel} does not depend on concentration of salt, is basically correct for solutions of NaDNA + NaCl (or similar systems). The term ΔG^{nel} mainly represents the change in internal molecular properties of DNA during melting (i.e., ΔG^{nel} accounts for changes in hydrogen bonding, stacking interactions, internal motions, changes in hydration of bases, sugar, and phosphate residues). It can be concluded that the influence of counterions with charge +1 on the thermodynamic parameters of DNA melting can be effectively calculated by polyelectrolyte theory, as in the rather simple PB model with all approximations that are inherent in this treatment. More subtle features of the ionic interactions do not seem to influence the helix-coil transition characteristics noticeably (otherwise we would obtain dependence of ΔG^{nel} on C_{Na}). These contributions are either small enough or, most likely, they balance each other in solutions of native and denatured DNA.

Comparison of experimental and theoretical values of T_m in the presence of Na⁺ and Mg²⁺ ions

PB results

The DNA melting temperatures in solutions of NaCl + MgCl$_2$ can now be predicted from calculated values of Δg^{el} (determined in the presence of Mg²⁺) and by using the dependence of ΔG^{nel} on temperature obtained by the fitting procedure described in the previous section. In practice, values of T_m were determined from the plot of $\Delta g^{nel} = \Delta G^{nel}/RT$ versus temperature for every calculated $\Delta g^{el} = f(C_{Na}, C_{Mg}, C_P)$, using $\Delta g^{nel} = -\Delta g^{el}$ at $T = T_m$. Values of Δg^{el} were calculated for varying values of C_{Na} and for fixed values of C_P and $r = C_{Mg}/C_P$, the latter (C_P and r) being taken equal to the concentrations used in the experimental studies ($C_P = 0.042$ mM, $r = 0.0, 0.15, 0.3, 0.5,$ and 1.0) (Record, 1975). Results of our PB calculations are compared with Record's experimental data in Fig. 7. The PB results have been obtained using the dependence of ΔG^{nel} on temperature defined by the fitted thermodynamic parameters of the first row in Table 2.

It is clear that the predictions based on the PB calculations of Δg^{el} reproduce all of the qualitative features of the experimental data. Specifically, the anomalous NaCl salt dependence of T_m at high values of $r = C_{Mg}/C_P$, where T_m decreases with increasing salt, is borne out by the calculations. Using the fitted thermodynamic parameters of the fifth row in Table 2 (based on Eq. 15) gives only slightly better agreement between the theoretical curves and experimental points (data not shown). Even though the general correspondence between experimental and calculated T_m values is rather good, the discrepancy is still not negligible, and reasons for this should be discussed. There are two main approximations within the model for calculating T_m that may be expected to cause deviations between theory and experiment.

Limitations of the "two-state model". The free energy difference, Δg^{el}, has been calculated on the basis of assuming that the helix-coil transition is a first-order phase transition between two solution states, containing only pure
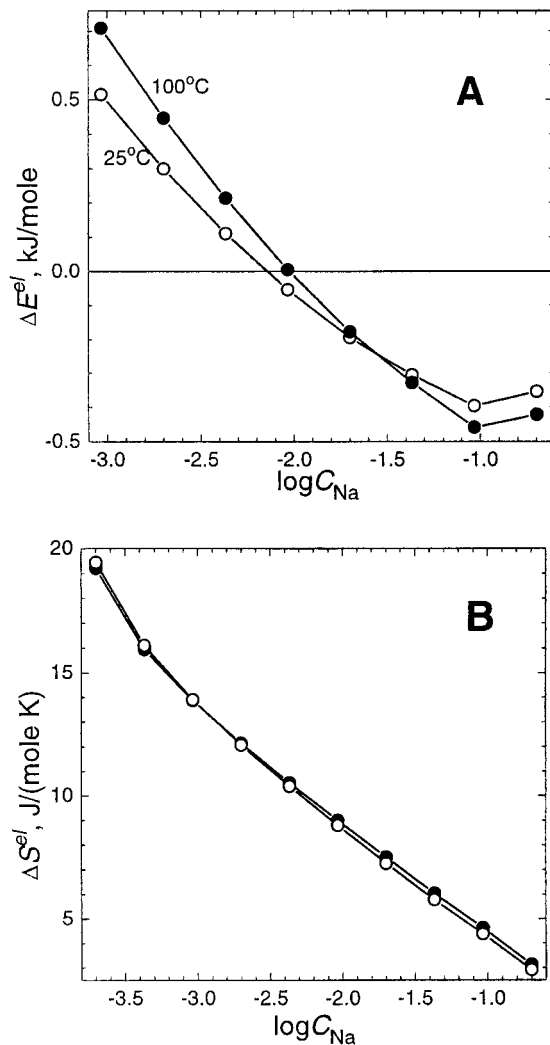
FIGURE 7 Comparison of experimental (*points*) (Record, 1975) and calculated PB theory (*curves*) values of T_m dependence on Na^+ concentration for different values of $r = C_{Mg}/C_P$ (indicated near the corresponding theoretical curves). Experimental points are marked for different r as follows: 0.0 (●, T_m values were used in calculation of ΔG^{nel}), 0.15 (■), 0.3 (○), 0.5 (△), 1.0 (□). The parameters used in the calculations are $C_P = 0.042$ mM, $b_h = 1.69$ Å, $a_h = 9.5$ Å, $b_c = 3.38$ Å, $a_c = 7.0$ Å, $d_{Na} = d_{Cl} = 2$ Å, $d_{Mg} = 3$ Å.

FIGURE 6 Changes in (*A*) electrostatic energy, ΔE^{el}, and (*B*) entropy, ΔS^{el}, calculated at 25°C (○) and 100°C (●) in the frame of PB theory for helix coil transition of DNA as a function of Na^+ concentration. The values used in the calculations are $C_P = 0.2$ mM, $b_h = 1.69$ Å, $a_h = 9.5$ Å, $b_c = 3.38$ Å, $a_c = 7.0$ Å, $d_{Na} = d_{Cl} = 2$ Å.

helix or pure coil, and with no mixing of coil and helix units. If this assumption does not hold (i.e., within the interval of helix-coil transition), redistribution of ions between melted and unmelted units of a given DNA polyion can proceed during the transition. This redistribution would influence the calculation of the theoretical T_m curves (McGhee, 1976; Record et al., 1978; Nilsson et al., 1989; Nilsson and Picullel, 1989; Korolev et al., 1994). It still should be noted that this effect cannot shift the melting temperature more that the width of the melting transition.

A modified treatment of calculating Δg^{el} that takes these effects into account and allows determination of thermodynamic quantities in solutions representing the mixture of polyions of different charge densities has been proposed by Nilsson et al. (1989) and Nilsson and Picullel (1989). This treatment introduces a quite complicated iteration procedure in the calculations and, given all other approximations
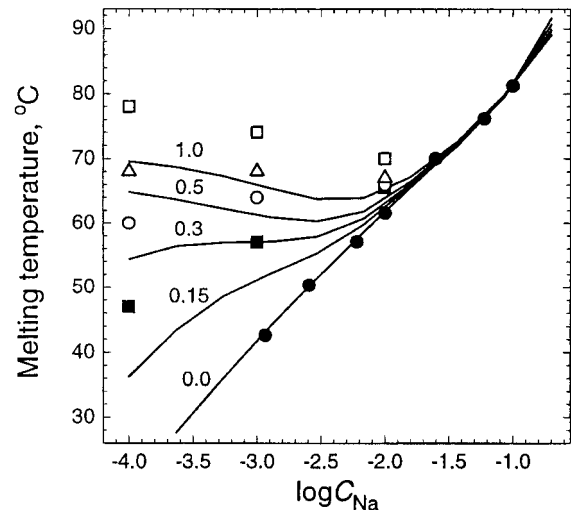
within the model, we have refrained from such a treatment. Instead we have concentrated on the behavior of Mg^{2+} concentration at the cell boundary and at the polyion surface.

Calculated values of Mg^{2+} concentration in the vicinity of polyion ($C_{Mg}(a_h)$) as well as the degree of dissociation ($i_{Mg} = C_{Mg}(R_h)/C_{Mg}^{tot}$) are listed in Table 3 for solutions of native DNA at three values of C_{Na}. Concentrations of Mg^{2+} ions at the cell boundaries of native and denatured DNA are also compared in Table 3 (ratio $C_{Mg}(R_c)/C_{Mg}(R_h)$). It can be seen that values of $C_{Mg}(a_h)$ rise noticeably when r changes from 0.15 to 0.5 and do not change significantly when r is between 0.5 and 1.0. At $r \geq 0.5$ saturation of the polyion vicinity with Mg^{2+} ions takes place. Simultaneously, the differences in Mg^{2+} concentrations at the cell boundaries of native and denatured DNA (ratio $C_{Mg}(R_c)/C_{Mg}(R_h)$) are large for low values of r and C_{Na} (7.73 and 5.85 at $r = 0.15$ and 0.3, respectively) but decrease rapidly with increase in both r and C_{Na}. It is clear that both the difference in Mg^{2+} ion concentration at the cell boundaries and the absence of "saturation" with Mg^{2+} in the vicinity of native DNA should lead to a transfer of Mg^{2+} from the cell occupied by coiled DNA to that containing native DNA. It is also possible that this redistribution of Mg^{2+} during the DNA melting transition in solutions of $MgCl_2 + NaCl$ at low values of C_{Na} and r is the main reason for the observed increase in the width of the melting transition (see figure 8 of Record, 1975).

It is clear, however, that values of Δg^{el} that would be calculated in a modified procedure should be somewhat higher than those obtained in the two-state model. Transfer of Mg^{2+} ions from denatured to native DNA cells should

TABLE 3　PBCM results on the counterion distribution in solutions of DNA in the presence of Na^+ and Mg^{2+} ions

C_{Na} (mM)	r = 0.15			r = 0.3			r = 0.5			r = 1.0		
	$C_{Mg}(a_h)$ (M)	i_{Mg}	$\frac{C_{Mg}(R_c)}{C_{Mg}(R_h)}$	$C_{Mg}(a_h)$ (M)	i_{Mg}	$\frac{C_{Mg}(R_c)}{C_{Mg}(R_h)}$	$C_{Mg}(a_h)$ (M)	i_{Mg}	$\frac{C_{Mg}(R_c)}{C_{Mg}(R_h)}$	$C_{Mg}(a_h)$ (M)	i_{Mg}	$\frac{C_{Mg}(R_c)}{C_{Mg}(R_h)}$
0.1	0.87	0.0017	7.73	1.57	0.017	5.85	1.87	0.21	1.51	1.93	0.56	1.07
1.26	0.67	0.23	2.12	1.04	0.37	1.59	1.29	0.52	1.31	1.53	0.70	1.12
15.9	0.04	0.95	1.03	0.08	0.95	1.03	0.13	0.95	1.03	0.24	0.96	1.03

Parameters used in calculations: $C_P = 0.042$ mM, $a_h = 9.5$ Å, $b_h = 1.69$ Å, $a_c = 7$ Å, $b_c = 3.38$ Å.
Abbreviations: $C_{Mg}(a_h)$ is Mg^{2+} ion concentration near the polyion surface (mean value of three points of PB equation solution at $x > (a_h + d_{Mg})$, x is radial coordinate); $i_{Mg} = C_{Mg}(R_h)/C_{Mg}^{tot}$ (C_{Mg}^{tot} and $C_{Mg}(R_h)$ are values of total and cell boundary concentration of Mg^{2+} ions determined for native polyion); $C_{Mg}(R_c)$ is Mg^{2+} ion concentration on the denatured polyion's cell boundary.

lower g_h^{el} and increase g_c^{el}. As a result, the theoretical curves should come closer to the experimental values of T_m. On the other hand, the small effects for $r \geq 0.5$ show that, in this interesting region where an anomalous dependence of the melting temperature on the NaCl concentration is observed, the present approach should be reliable.

Influence of Mg^{2+} ions on ΔG^{nel}

The values of T_m^{PB} are lower than T_m^{exp} data obtained at all concentrations of NaCl and MgCl₂. These values of T_m^{PB} have been obtained from PB calculations and from the temperature dependence of ΔG^{nel} evaluated from data measured in Mg^{2+}-free solutions. Thus it is assumed that there is no effect of the presence of Mg^{2+} on ΔG^{nel}. The observed deviations (especially at $r = 1.0$ and 0.5) cannot be explained by the inapplicability of the "two-state model" alone. Therefore the possibility that ΔG^{nel} is dependent on the presence of Mg^{2+} is likely to contribute to the disagreement between experimental and calculated data. The positive sign of the quantity $\delta T_m = T_m^{exp} - T_m^{PB}$ indicates that Mg^{2+} ions produce some additional nonelectrostatic stabilization of the DNA double helix in comparison with single-stranded DNA. The difference between observed and calculated values of melting temperatures (δT_m) can now, within our treatment, be considered as a measure of that part of ΔG^{nel} that arises from the difference in specific (nonelectrostatic) interactions of Mg^{2+} with the helix and coil forms.

Experimental observations report that cations of all alkali earth metals (and especially Mg^{2+}) stabilize the double-helix conformation of DNA (Eichhorn and Shin, 1968; Record, 1975; Eichhorn, 1973; Vlasov, 1993). However, no literature data are available in which electrostatic (polyelectrolyte) and specific modes of this stabilization effect have been separated. Thus there are no direct measurements of this specific association of Mg^{2+} to double helix in comparison with single-stranded DNA. It was shown (Anderson and Record, 1990; Rouzina and Bloomfield, 1996) that previously observed (Braunlin et al., 1989, 1991, 1992) Mg^{2+} and Ca^{2+} binding to native DNA exceed values calculated with the PB cell model. Raman spectroscopy studies carried out in concentrated ($C_P \approx 165$ mM) solutions of short (150 bp) DNA fragments demonstrated that Mg^{2+} and other earth alkali metal cations have only a weak influence on the spectra of denatured DNA (Diguid et al., 1995). Therefore, it is reasonable to assume that the specific mode of Mg^{2+}-DNA interaction is stronger with the double helix than with the single-strand form.

CC theory results

We have carried out calculations analogous to those above, to obtain the dependence of T_m versus $\log C_{Na}$ from CC theory. Results are shown in Fig. 8, *A* and *B*, for two values of the charge spacing parameter of the coil form, $b_c = 3.38$ and 4.5 Å, respectively. Comparison of PB and CC data (Figs. 7 and 8, *A* and *B*) leads to the observation that these models give the opposite (i.e., opposite signs of δT_m) conclusion regarding the specific interactions of Mg^{2+} with native and denatured DNA polyions. Values of the quantity δT_m in the CC theory are about $-20°$ at low C_{Na}, and the difference of the T_m evaluations between the CC and PB approaches reaches almost 30°C at low NaCl concentrations (compare Figs. 7 and 8 *A*). Moreover, CC theory leads to the conclusion that Mg^{2+} has a more specific nonelectrostatic affinity for single-stranded than for double-stranded DNA. Evidently, in the case of transition metal ions (but not alkali earth metal cations), numerous studies have reported significant a decrease in T_m values (see Eichhorn, 1973, and references cited therein; Eichhorn and Shin, 1968; Diguid et al., 1995). These studies indicate clearly that this decrease in thermal stability is usually accompanied by coordination of the metal cations with DNA bases, which become more accessible after disintegration of the double helix. On the other hand, the conclusion of a stronger nonelectrostatic interaction of Mg^{2+} with denatured DNA than with the native form does not seem plausible, however, in light of the differential Raman spectra obtained for MgDNA mentioned above (Diguid et al., 1995). Attempts to improve the comparison between CC theory and experiment by increasing the b_c value reduce T_m slightly (see Fig. 8 *B*) but do not alter the principal contradiction between CC and PB theory concerning the sign of δT_m.

CONCLUSIONS

Numerical calculations, using polyelectrolyte theories (PB and CC), of the electrostatic free energy difference, ΔG^{el},
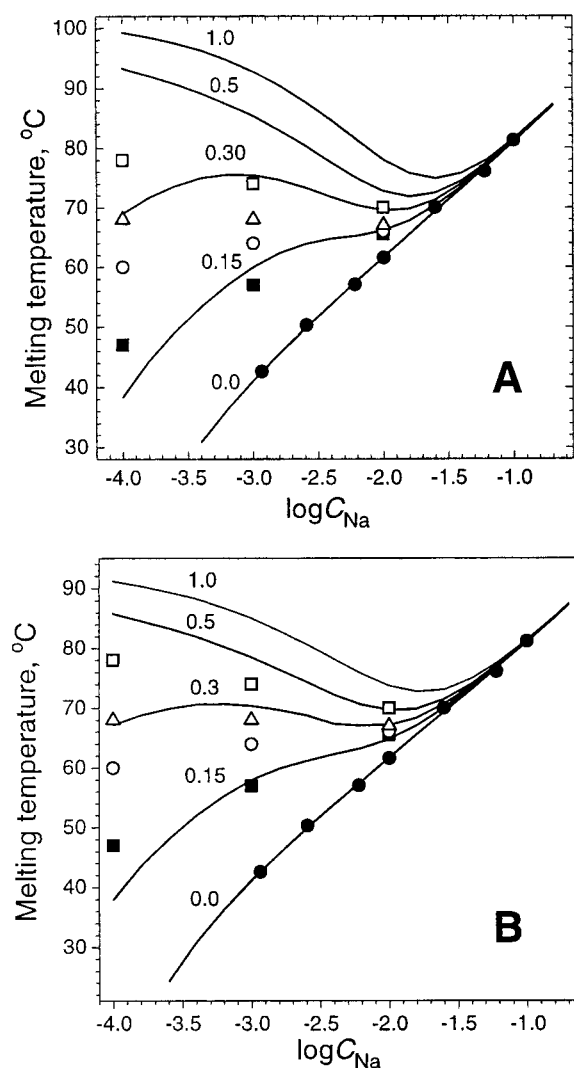
FIGURE 8 Comparison of experimental (*points*) (Record, 1975) and calculated CC theory (*curves*) values of T_m dependence on Na^+ concentration for different values of $r = C_{Mg}/C_P$ (indicated near the corresponding theoretical curves). A and B are for the following values of the denatured polyion charge density, b_c: (A) 3.38 Å ($\xi_c = 2.1$); (B) 4.5 Å ($\xi_c = 1.6$). The other values are $C_P = 0.042$ mM, $b_h = 1.69$ Å ($\xi_h = 4.2$). Experimental points are marked as in Fig. 7.

between single-strand (coil) and double-helix forms of DNA have been performed for solutions of NaDNA + NaCl with and without added $MgCl_2$. Calculations have been made for conditions relevant to systems where experimental values of T_m as well as thermodynamic quantities for this structural transition have been measured. Comparison with experimental data has been possible by invoking values of DNA melting temperatures for solutions containing NaCl salt only. Resulting theoretical values of thermodynamic functions (ΔH_m, ΔS_m, ΔC_p) for NaCl salt-containing solutions and of melting temperatures T_m as a function of NaCl concentration in solutions containing varying amounts of added $MgCl_2$ have thus been obtained. Qualitative and, to a large extent, quantitative reproduction of the experimental

results has been found from the results of polyelectrolyte theories. It is clear, however, that the quantitative resemblance with experiments is in general considerably better with the PB theory, as compared to the CC model. Furthermore, some rather implausible qualitative conclusions are obtained within the CC results: namely, comparison of the CC theory predictions of DNA melting temperature in the presence of Mg^{2+} ions with experimental data leads to the doubtful conclusion that electrostatic stabilization of the DNA double helix in the presence of divalent cations is reduced because of the effect of Mg^{2+} on ΔG^{nel}. We consider our results to be a strong argument in favor of the Poisson-Boltzmann theory, as compared to the counterion condensation theory. It is interesting to note that our results were obtained for conditions where the applicability of the CC theory is believed to be most reliable and correct ($C_s \gg C_P$, and C_P is small). It is also interesting to note that δT_m is small when comparing experimental and Poisson-Boltzmann theory results. Finally, it should be emphasized that there are several simplifications and approximations within the PB cylindrical cell model that we have used. The molecular nature of the water is ignored by treating the solvent as a dielectric continuum. The atomic structure of DNA, particularly the grooved nature of its surface and the point-like nature of the phosphate charge distribution, is not taken into account. The flexibility of the single- and double-stranded DNA and any salt effects on this has not been taken into account. Although these factors may affect the electrostatic free energy difference between helix and coil, we do not believe these effects would qualitatively change the conclusions of this work.

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