J. Mol. Biol. (1994) **238**, 245–263

Salt Effects on Ligand–DNA Binding

Minor Groove Binding Antibiotics

Vinod K. Misra[1], Kim A. Sharp[2], Richard A. Friedman[1] and Barry Honig[1]†

[1]*Department of Biochemistry and Molecular Biophysics Columbia University, 630 West 168th Street New York, NY 10032, U.S.A.*

[2]*Department of Biochemistry and Biophysics University of Pennsylvania, Philadelphia, PA 19104, U.S.A.*

Salt dependent electrostatic effects play a central role in intermolecular interactions involving nucleic acids. In this paper, the finite-difference solution to the nonlinear Poisson–Boltzmann (NLPB) equation is used to evaluate the salt dependent contribution to the electrostatic binding free energy of the minor groove binding antibiotics DAPI, Hoechst 33258 and netropsin to DNA using detailed molecular structures of the complexes. For each of these systems, a treatment based on the NLPB equation accurately describes the variation of the experimentally observed binding constant with bulk salt concentration. A solvation formalism is developed in which salt effects are described in terms of three free energy contributions: the electrostatic ion–molecule interaction free energy, $\Delta\Delta G^\circ_{im}$; the electrostatic ion–ion interaction free energy, $\Delta\Delta G^\circ_{ii}$; and the entropic ion organization free energy, $\Delta\Delta G^\circ_{org}$. The electrostatic terms, $\Delta\Delta G^\circ_{im}$ and $\Delta\Delta G^\circ_{ii}$, have both enthalpic and entropic components, while the term $\Delta\Delta G^\circ_{org}$ is purely a cratic entropy. Each of these terms depends significantly on salt dependent changes in the counterion and coion concentrations around the DNA. In each of the systems studied, univalent ions substantially destabilize charged ligand–DNA complexes at physiological salt concentrations. This effect involves a salt dependent redistribution of counterions near the DNA. The free energy associated with the redistribution of counterions upon binding is dominated by the unfavorable change in the electrostatic ion–molecule interactions, $\Delta\Delta G^\circ_{im}$, rather than the change in the cratic entropy of ion organization, $\Delta\Delta G^\circ_{org}$. In addition, the observed slope of the salt dependence of the free energy is determined by electrostatic ion–molecule and ion–ion interactions as well as the cratic entropy of ion release. These findings are in contrast to models in which the cratic entropy of counterion release drives binding.

Keywords: DNA; electrostatics; DNA binding; binding energy; salt

1. Introduction

Understanding the structure and energetics of DNA recognition by proteins and ligands is essential in order to elucidate the molecular basis of gene regulation and expression, and to design DNA binding drugs from structure-based principles. Detailed three-dimensional structures of drug–DNA complexes (Sriram *et al.*, 1992; Quintana *et al.*, 1991; Brown *et al.*, 1990; Frederick *et al.*, 1990; Kennard & Hunter, 1989; Larsen *et al.*, 1989; Teng *et al.*, 1988; Coll *et al.*, 1987; Pjura *et al.*, 1987), DNA-binding proteins and protein–DNA complexes (Brennan, 1991; Harrison, 1991; Phillips, 1991; Rosenberg, 1991; Harrison & Aggarwal, 1990; Steitz, 1990; Pabo & Sauer, 1984) have provided important information about both site-specific and nonspecific nucleic acid recognition. However, a thorough understanding of the interactions of proteins and ligands with DNA requires an evaluation of the thermodynamics of binding as well.

Electrostatic interactions play a crucial role in molecular interactions involving DNA. In particular, the free energy of association of ligand–DNA complexes depends strongly on salt concentration (Record *et al.*, 1978, 1985, 1991). In general, a plot of $-\ln(K_{obs})$ *versus* $\ln[M^+]$ is linear, where K_{obs} is the observed association constant for a DNA–ligand complex and $[M^+]$ is the bulk univalent counterion concentration. The salt dependent properties of binding arise principally in the electrostatic component of the interaction.

† Author to whom all correspondence should be addressed.

0022-2836/94/170245-19 $08.00/0

Salt effects in nucleic acid systems are often described by models based on Manning's counterion condensation (CC†) theory (Manning, 1969*a*, 1978). The CC theory describes the polyion chain as an isolated, infinite linear array of point charges characterized by a dimensionless charge–density parameter $\xi = e^2/\varepsilon kTb$, where ε is the dielectric constant of pure bulk solvent, b is the axial distance between polyion charges, and the other symbols have their standard definitions. The counterion atmosphere around a highly charged polyion ($\xi > 1$) is modeled as two distinct populations: "condensed" counterions which are bound within a well-defined volume around the polyion; and "free" counterions which are treated as a classical Debye–Hückel ion atmosphere. Record *et al.* (1976, 1978) incorporated CC theory into a thermodynamic description of ligand–DNA interactions (deHaseth *et al.*, 1977). They derived a simple expression to describe the linear salt dependence of the observed association constant, K_{obs} (Record *et al.*, 1976):

$$\frac{\partial \ln (K_{obs})}{\partial \ln ([M^+])} = -m'\psi, \qquad (1)$$

where ψ is the fraction of a counterion (M^+) "thermodynamically bound" to each DNA phosphate (for double stranded DNA, $\psi = 0{\cdot}88$) and m' is variously defined as the number of ion pairs formed with DNA (deHaseth *et al.*, 1977; Record *et al.*, 1976) or the net charge on the bound ligand (Braunlin *et al.*, 1982; Record *et al.*, 1981; Lohman *et al.*, 1980). They concluded that the entropic release of counterions from the condensed layer drives binding between cationic ligands and polynucleotides. Manning developed a theory of salt effects on the territorial binding of ligands to polyelectrolytes (Manning, 1978, 1979) in which the slope is explicitly equated with the net charge on the bound ligand. This treatment does not involve a "thermodynamically bound" fraction of counterions, ψ. Manning and co-workers have developed a corresponding theory of site-binding equilibria for polyelectrolytes (Friedman *et al.*, 1990; Friedman & Manning, 1984; Manning, 1981; Manning & Holtzer, 1973). They concluded that a reduction of both the electrostatic and the entropic stress of the polyion helps drive the site-binding reaction and is responsible for the salt effect (Friedman & Manning, 1984).

The Poisson–Boltzmann (PB) equation has been used to model the electrostatic properties of polyelectrolytes for many years. The PB theory models the ionic atmosphere around a polyion as a single population described by a continuous distribution of positive and negative ions. Early analytic and series solutions of the nonlinear PB (NLPB) equation were evaluated for infinitely long cylindrical polyelectrolytes in an electrically neutral volume of solution in the absence of added salt (Alfrey *et al.*, 1951; Fuoss *et al.*, 1951). These early PB results first led to the notion of "counterion condensation" (Oosawa, 1971; Ohnishi, 1963; Ohnishi *et al.*, 1960; Imai & Onishi, 1959). Although the radial distribution of counterions around the polyion differs between CC and PB theory, both the CC model and the PB cell model predict that a relatively fixed local concentration of unbound counterions persistently remains associated with an infinitely long, highly charged, rod-like polyion as the system becomes infinitely dilute (LeBret & Zimm, 1984; Zimm & LeBret, 1983; Anderson & Record, 1982; Gueron & Weisbuch, 1980; MacGillivray, 1972*a*,*b*,*c*,*d*). However, while CC theory predicts that a virtually fixed population of counterions remains within a fixed volume near the polyion surface (Manning, 1979), PB theory predicts that the radius of this ion cloud becomes quite large even at moderate ionic strengths resulting in a dilution of the associated counterions (LeBret & Zimm, 1984; Gueron & Weisbuch, 1980). The present analysis will demonstrate that these salt dependent changes in the concentration of the ion atmosphere can have significant consequences on the energetics of ligand–DNA interactions.

The cylindrical PB equation leads to a self-consistent expression for the total electrostatic contribution to the free energy of a polyelectrolyte in the absence of added salt (the cell model) (Sharp & Honig, 1990*a*; Marcus, 1955). This expression can be used to calculate the polyelectrolyte contribution to the colligative properties of polyelectrolyte solutions (Gueron & Weisbuch, 1980; Gross & Strauss, 1966; Alexandrowicz & Katchalsky, 1963; Marcus, 1955). Exact, closed-form expressions for the osmotic coefficient, mean ion activity coefficient, and Donnan salt exclusion coefficient of rod-like polyions in the presence of excess added uni-univalent salt were subsequently derived using this model (Klein *et al.*, 1981; Anderson & Record, 1980). The Donnan coefficient was subsequently used to evaluate a "preferential interaction parameter", Γ°_{3u} (Anderson & Record, 1982, 1983), which measures the nonuniform distribution of small ions in the domain of the polyion (Timasheff, 1992). It can be shown that Γ°_{3u} is directly related to the salt dependent solvation free energy of a polyion, ΔG°_{s} (Timasheff, 1992). In this paper, we will analyze nonspecific salt effects in terms of ΔG°_{s}.

A general thermodynamic description of salt effects on charged ligand–nucleic acid interactions has been developed in terms of Γ°_{3u} (Anderson & Record, 1993). With some approximations, the salt dependence of binding a Z-valent charged ligand to DNA can be formulated in terms of Γ°_{3u} as (Anderson & Record, 1982, 1983):

$$\frac{\partial \ln (K_{obs})}{\partial \ln ([M^+])} = -Z(1 + 2\Gamma^{\circ}_{3u}). \qquad (2)$$

Since Γ°_{3u} is directly related to the Donnan coefficient, it is clear that models which predict similar

† Abbreviations used: CC, counterion condensation theory; PB, Poisson–Boltzmann; NLPB, non-linear Poisson–Boltzmann; FDPB, finite difference Poisson–Boltzmann; TFD, three-dimensional finite difference; OFD, one-dimensional finite difference; Mes, (2-[*N*-morpholino]ethanesulfonic acid.

colligative properties for polyelectrolyte systems will result in similar salt dependencies. In principle, Γ°_{3u} includes all effects that contribute to the interaction free energy of the mobile ions with the ligand and the DNA (Timasheff, 1992; Anderson & Record, 1982, 1983, 1990; Schellman, 1978). Under limiting law conditions for double stranded DNA, $(1+2\Gamma^{\circ}_{3u})$ equals 0·88 resulting in an expression equivalent to equation (1) (Anderson & Record, 1982, 1983). General theoretical analyses of ligand–DNA binding in terms of preferential interaction coefficients have noted that terms other than the cratic entropy of ion release may contribute to the electrostatic free energy (Anderson & Record, 1993; Record *et al.*, 1978, 1990). However, in the interpretation of equation (2) (which is approximate), it has been concluded, as with equation (1), that the entropic release of bound ions is the dominant force driving the formation of ligand–DNA complexes (Record *et al.*, 1990, 1991; Anderson & Record, 1982, 1983). The analysis presented in this paper will explicitly consider each contribution to the total electrostatic free energy for systems of finite dimensions and salt concentrations without using the approximations of equation (2). We will demonstrate that salt effects on binding can have important electrostatic contributions other than the entropy of ion release.

Both CC and cylindrical PB models, derived under limiting law conditions, have been quite successful in describing the interactions of small counterions with rod-like polyions. Furthermore, each of these theories has provided useful insights into the energetics of the interaction of small cationic ligands with DNA. However, it has been shown that salt effects contribute significantly to the discrimination of DNA binding sites by ligands (Koblan & Ackers, 1991; Senear & Batey, 1991; Terry *et al.*, 1983). Since CC and cylindrical PB models do not explicitly account for detailed molecular structures they cannot account for the salt dependent contribution to the relative stability of ligands bound to different DNA sequences. In addition, since these models do not account for detailed charge distribution, they cannot account for the salt dependent contribution to the relative stability of different ligands with the same charge bound to the same DNA sequence. Such predictions require a theory that specifically relates detailed three-dimensional structure to free energy.

Numerical solutions to the Poisson–Boltzmann (PB) equation for detailed atomic resolution structures of proteins and nucleic acids (Sharp *et al.*, 1990; Jayaram *et al.*, 1989; Klapper *et al.*, 1986; Warwicker & Watson, 1982) have been shown to give accurate descriptions of the electrostatic properties of complex macromolecules. The total electrostatic free energy for any system modeled with the full NLPB equation has been unambiguously defined (Reiner & Radke, 1990; Sharp & Honig, 1990*a*). As such, the electrostatic contribution of polyion–solvent and polyion–counterion interactions on conformation dependent properties of DNA, including binding energies, can be calculated from finite difference solutions to the NLPB equation (Jayaram *et al.*, 1989; Klapper *et al.*, 1986) for detailed molecular geometries. From this information it is possible to derive a detailed physical interpretation of salt dependent electrostatic effects on the energetics of nucleic acid interactions in terms of a complete PB theory.

The PB model provides a rigorous and powerful formalism for describing electrostatic effects in macromolecular systems. Although the PB model has been criticized for neglecting the finite size and the spatial correlations of ions in solution (Soumpasis, 1984; Fixman, 1979), statistical mechanical models have shown that, for small univalent electrolytes, the nonlinear PB (NLPB) equation provides a reasonable approximation to the mean ion distribution around a cylindrical DNA molecule for bulk univalent salt concentrations on the order of at least 0·1 M (Jayaram *et al.*, 1990; Svensson *et al.*, 1990; Bacquet & Rossky, 1988; Murthy *et al.*, 1985; Fixman, 1979). Moreover, errors attributed to PB models for DNA (Soumpasis, 1984) have been shown to be primarily due to overly simplified representations of the nucleic acid rather than to underlying deficiencies in the theory (Gueron & Demaret, 1992).

We report here on the application of the finite difference PB (FDPB) method to evaluate the salt dependent contribution to the electrostatic free energy of binding of ligands to DNA. Specifically, we consider the interaction of the minor groove binding antibiotics 4′,6-diamidino-2-phenylindole (DAPI), 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1H-benzimidazole (Hoechst 33258), and netropsin to their respective DNA fragments (Figure 1). We find that the NLPB model provides an accurate description of nonspecific salt effects in each of these systems. However, the interpretation of these effects is quite different from descriptions based on standard CC theories. A detailed physical interpretation of salt dependent electrostatic effects on ligand interactions with DNA will be presented. Salt effects on the minor groove binding antibiotics and simple cylindrical models calculated with the NLPB equation will be related to general solutions of CC theory. The accompanying paper will analyze salt effects in protein–DNA interactions (Misra *et al.*, 1994).

2. Methods

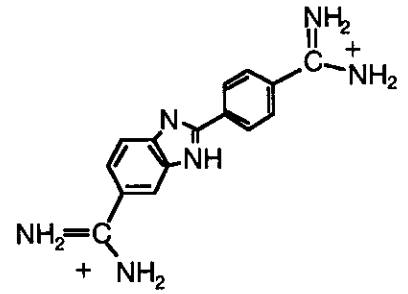
(a) *Theory*

(i) *The NLPB theory*

The electrostatic behavior of a macromolecular system in a 1:1 salt solution is given by the NLPB equation:

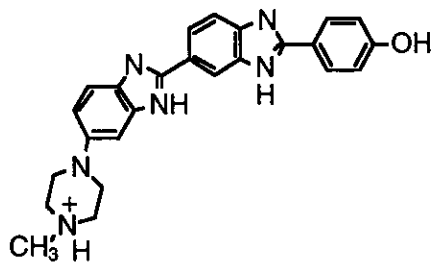
$$\nabla\cdot[\varepsilon(\mathbf{r})\nabla\cdot\phi(\mathbf{r})]-\varepsilon\kappa^2\sinh[\phi(\mathbf{r})]+4\pi e\rho^{f}(\mathbf{r})/kT=0, \quad (3)$$

where ϕ is the dimensionless electrostatic potential in units of kT/e in which k is Boltzmann's constant, T is the absolute temperature, and e is the proton charge. In addition, ε is the dielectric constant and ρ^{f} is the fixed charge density. The term $\kappa^2=1/\lambda^2=8\pi e^2I/\varepsilon kT$, where λ is the Debye length and I is the ionic strength of the bulk



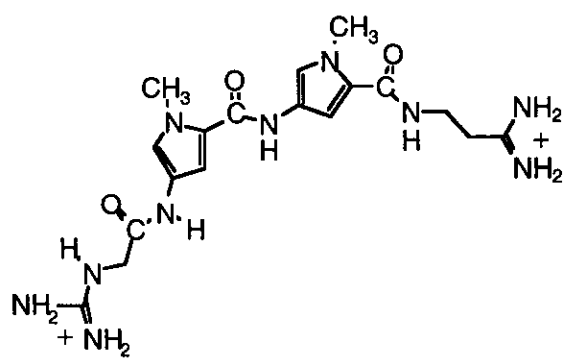
DAPI

(a)



Hoechst 33258

(b)



Netropsin

(c)

Figure 1. Chemical structures of the minor groove binding antibiotics used in this study. a, DAPI; b, Hoechst 33258; c, netropsin.

solution. The quantities ϕ, ε, κ and ρ are all functions of the position vector **r** in the reference frame centered on a fixed macromolecule.

The total electrostatic free energy of a system, ΔG^{el}, described by the NLPB equation may be derived from the calculus of variations (Reiner & Radke, 1990; Sharp & Honig, 1990*a*). The variational approach involves identifying a nonlinear scalar function $F = F(\phi, \nabla\phi)$ which satisfies a local extremum condition (Reiner & Radke, 1990). ΔG^{el} is given by the volume integral of F (Sharp & Honig, 1990*a*):

$$\Delta G^{el} = \int \{\rho^f\phi^f/2 + \rho^f\phi^m + \rho^m\phi^m/2 - (\rho^m\phi + \Delta\Pi)\}\, dv, \quad (4)$$

where the potential, ϕ, and charge density, ρ, have been split up into contributions from the fixed, f, and mobile, m, charges. The term $\Delta\Pi$ is the excess concentration of ions at any point in solution relative to the bulk concentration of ions, c^b, such that:

$$\Delta\Pi = kTc^b[2\cosh(\phi) - 2]. \quad (5)$$

The physical interpretation of evaluating the total electrostatic free energy of a macromolecule as described above is given by following the thermodynamic pathway in Figure 2 (Gilson & Honig, 1988). An initial state is defined where the macromolecule and its associated mobile ions and solvent are infinitely separated (Figure 2a). In this initial state, the mobile ions are uncharged and uniformly distributed in solvent, while the macromolecule is uncharged in a medium corresponding to the dielectric constant of the molecule, ε_m, containing no mobile ion atmosphere. In the first step of the pathway, the macromolecule is charged in a medium of dielectric constant ε_m to its final state q^f (the first step in Figure 2a). The change in free energy associated with this step is the coulombic interaction among the fixed macromolecular charges, ΔG°_c. Next, pure solvent lacking any mobile ions is assembled from infinity to a position defined by the macromolecular surface (the second step in Figure 2a). This charge–solvent interaction energy of the system in the absence of mobile ions is given by ΔG°_p. The sum of the first 2 terms is the salt independent contribution to the total electrostatic free energy of the macromolecule, ΔG°_{ns}, given by (Gilson & Honig, 1988; Gilson *et al.*, 1985):

$$\Delta G^\circ_{ns} = \Delta G^\circ_c + \Delta G^\circ_p = \int (\rho^f\phi^f/2)\, dv. \quad (6)$$

The macromolecule is subsequently solvated by its mobile ion atmosphere (Figure 2b). The salt dependent contribution to the electrostatic solvation free energy of the fixed macromolecule, ΔG°_s, is given by the remaining terms in equation (4):

$$\Delta G^\circ_s = \int \{\rho^f\phi^m + \rho^m\phi^m/2 - (\rho^m\phi + \Delta\Pi)\}\, dv. \quad (7)$$

The electrostatic free energy of solvating the fixed charges with the mobile ion atmosphere, ΔG°_s, can be subdivided into 3 terms described by the thermodynamic pathway in Figure 2b. The uncharged mobile ions, independent of the macromolecule, are first redistributed to their final average positions corresponding to the fully charged state. The free energy associated with this process is the purely entropic (cratic) work of organizing the ion atmosphere, ΔG°_{org}; this has been confirmed by a van't Hoff analysis of the total electrostatic free energy (K.A.S. *et al.*, unpublished results). The ions, fixed in their final average positions, are then charged against a potential ϕ^m to their final state, q^m. This free energy is given by the electrostatic self energy of charging the ion atmosphere, ΔG°_{ii}. Finally, the fully charged macromolecule is transferred from pure solvent to its final position within the fully charged and assembled ion atmosphere. This electrostatic free energy is ΔG°_{im}. We emphasize that the electrostatic free energy terms ΔG°_{ii} and ΔG°_{im} can have significant enthalpic and entropic components; the latter arising from the temperature dependence of the dielectric constant of water (Bockris & Reddy, 1970). The relative contributions of the electrostatic enthalpy, cratic entropy, and dielectric entropy will be discussed in a subsequent paper. From equation (7), the 3 terms contributing to ΔG°_s are identified as:

$$\Delta G^\circ_{im} = \int (\rho^f\phi^m)\, dv, \quad (8)$$

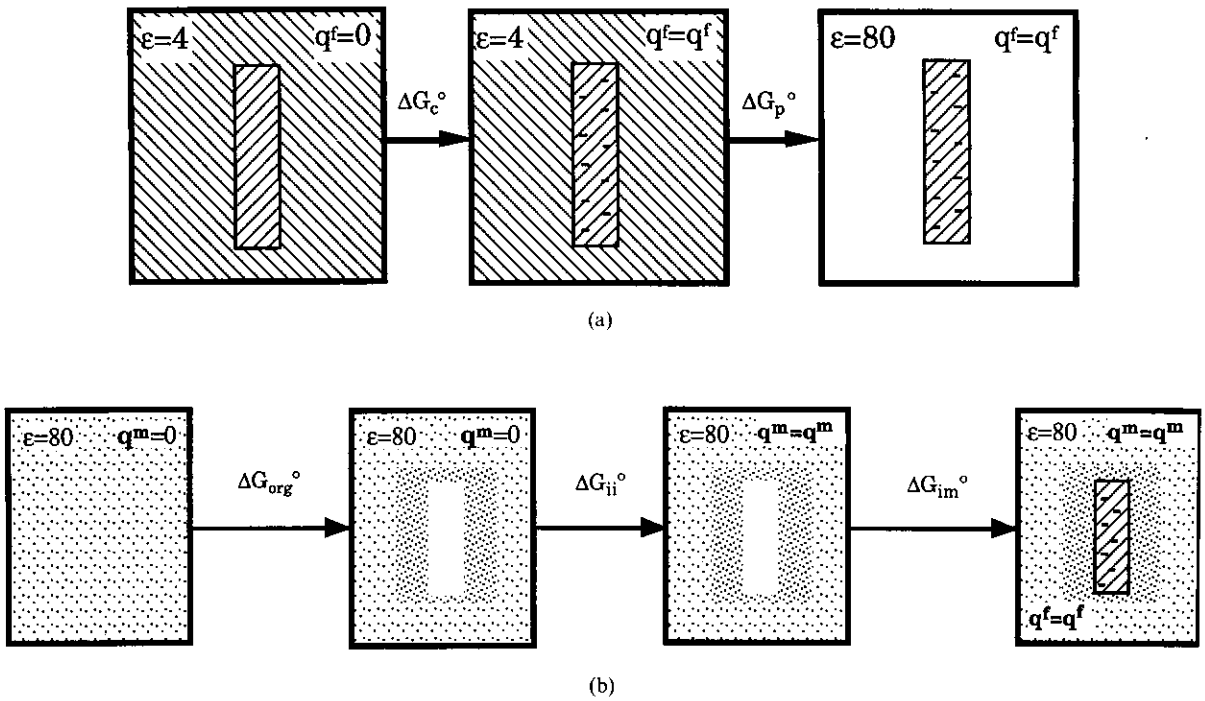


Figure 2. Thermodynamic pathway for calculating the total electrostatic free energy of a macromolecule. (a) Calculation of the total electrostatic free energy at zero ionic strength, ΔG_{ns}°, involving the charging against intramolecular coulombic interactions, ΔG_c°, and the transfer into pure nonionic solvent, ΔG_p°. (b) Calculation of the solvation free energy of the macromolecule upon transferring it from pure water to a salt solution, ΔG_s°. This process consists of ion assembly, ΔG_{org}°; ion charging, ΔG_{ii}°; and ion-molecule interaction, ΔG_{im}°. The crosshatched areas represent a dielectric constant of 4, while the stippling represents mobile ions in $\varepsilon = 80$.

$$\Delta G_{ii}^\circ = \int (\rho^m \phi^m/2)\, dv, \tag{9}$$

$$\Delta G_{org}^\circ = \int \{-(\rho^m \phi + \Delta\Pi)\}\, dv. \tag{10}$$

(ii) *The salt dependence of binding*

The binding reaction:

$$A + B \overset{K_{obs}}{\longleftrightarrow} A \cdot B \tag{11}$$

is described by the equilibrium condition:

$$\mu_{AB} - \mu_A - \mu_B = 0, \tag{12}$$

where μ_i, the chemical potential of the ith component, can be evaluated and interpreted in terms of the interaction free energy of the charged molecules with the solvent (i.e. the solvation free energy). In this formalism, the chemical potential of each species in the reaction (11), μ_i, is expressed as (Sharp & Honig, 1990*b*):

$$\mu_i = \mu_i^g + \Delta\mu_i^{g\to p} + \Delta\mu_i^{p\to s} + kT \ln (c_i), \tag{13}$$

where μ_i^g is the standard state chemical potential defined in the gas phase (i.e. no solute–solvent interaction energies are present); c_i is the concentration; $\Delta\mu_i^{g\to p}$ is the free energy of transfer from the gas phase into pure (salt free) solvent; and $\Delta\mu_i^{p\to s}$ is the transfer free energy from pure solvent into a salt solution. The latter 2 terms describe the total solvation free energy of the macromolecule.

Each of the terms, μ_i^g, $\Delta\mu_i^{g\to p}$ and $\Delta\mu_i^{p\to s}$, contains both electrostatic and nonelectrostatic components. In the NLPB model, the electrostatic contribution to μ_i^g is simply equal to ΔG_c°; while $\Delta\mu_i^{g\to p}$ equals ΔG_p° and $\Delta\mu_i^{p\to s}$ equals ΔG_s°. The free energy of binding can be expressed in terms of the solvation free energies as:

$$\begin{aligned}\Delta\Delta G_{bind}^\circ &= \Delta\Delta G_{bind}^g + \Delta\Delta G_{bind}^{g\to p} + \Delta\Delta G_{bind}^{p\to s} \\ &= -kT \ln (K_{obs}),\end{aligned} \tag{14}$$

where

$$\Delta\Delta G_{bind}^g = \mu_{AB}^g - \mu_A^g - \mu_B^g \tag{15}$$

is the gas phase binding free energy; and:

$$\Delta\Delta G_{bind}^{g\to p} = \Delta\mu_{AB}^{g\to p} - \Delta\mu_A^{g\to p} - \Delta\mu_B^{g\to p} \tag{16}$$

is the change in the solvation free energy in pure solvent upon binding; and:

$$\Delta\Delta G_{bind}^{p\to s} = \Delta\mu_{AB}^{p\to s} - \Delta\mu_A^{p\to s} - \Delta\mu_B^{p\to s} \tag{17}$$

is the change in the solvation by salt upon binding. If the salt dependence of the reaction is assumed to be entirely in the electrostatic part of the binding free energy, the variation of $\ln K_{obs}$ with $\ln [M^+]$ is given by:

$$-\frac{\partial \ln (K_{obs})}{\partial \ln ([M^+])} = \frac{\partial(\Delta\Delta G_s^\circ)}{kT\, \partial \ln ([M^+])}. \tag{18}$$

The term $\Delta\Delta G_s^\circ$ is the change in the salt dependent contribution to the total electrostatic free energy upon binding:

$$\Delta\Delta G_s^\circ = \Delta G_s^\circ(AB) - \Delta G_s^\circ(A) - \Delta G_s^\circ(B). \tag{19}$$

The quantity $\Delta\Delta G_s^\circ$ can be further partitioned into differences in ion–molecule, $\Delta\Delta G_{im}^\circ$; ion–ion, $\Delta\Delta G_{ii}^\circ$; and organizational, $\Delta\Delta G_{org}^\circ$, free energies as described by equations (8) and (10). This formulation emphasizes how the change in counterion distribution around each molecule, governed by the NLPB equation, affects the solvation free energy upon binding.

(b) *Molecular model*

The details of the model have been described (Jayaram *et al.*, 1989; Gilson *et al.*, 1988). The bound and free molecules were described by the 3-dimensional structure of the ligand–DNA complexes listed below. The locations of all charges were defined by the coordinates of the appropriate atoms. Charges were assigned to the center of each atom and were treated as being embedded in a low dielectric medium (ε_m) consisting of the volume enclosed by the solvent-accessible surface of the macromolecule (probe radius = 1·4 Å). For the cases studied here, results were calculated for $\varepsilon_m = 2$, 3 and 4 (Sharp & Honig, 1990*b*; Harvey, 1988). The molecular charges for each complex were derived from the CVFF (Hagler *et al.*, 1979), OPLS (Pranata *et al.*, 1991; Jorgensen & Tirado-Rives, 1988) and AMBER (Weiner *et al.*, 1986) forcefield parameters. The surrounding solvent was treated as a continuum of dielectric constant 80 with a 1:1 electrolyte behaving according to the NLPB equation. A 2·0 Å ion exclusion radius (corresponding roughly to the radius of a hydrated sodium ion) was included (Klapper *et al.*, 1986). The calculations presented here neglect differential protonation effects upon binding, although they can be included in more detailed calculations of binding free energies (V. K. Misra & B. Honig, unpublished results).

The atomic coordinates of the minor groove binding drugs were obtained as follows: the coordinates of the DAPI–d[(CGCGAATTCGCG)$_2$] complex were provided by Dr Richard E. Dickerson (Larsen *et al.*, 1989); the coordinates of 3 forms of the Hoechst 33258–d[(CGCGAA TTCGCG)$_2$] complex were obtained from the Protein Data Bank (PDB) at Brookhaven National Laboratory (Abola *et al.*, 1987; Bernstein *et al.*, 1977) as deposited by Teng *et al.* (1988) and Pjura *et al.* (1987); the coordinates of the netropsin–d[(CGCGAATTCGCG)$_2$] complex were provided by Dr Andrew H.-J. Wang (Sriram *et al.*, 1992); the coordinates of 2 forms of the netropsin–d[(CGCGAT ATCGCG)$_2$] complex were obtained from the PDB as deposited by Coll *et al.* (1987). Before assigning partial charges to each atom, protons were added to each molecule and the conformations were minimized using the molecular simulation program DISCOVER (Biosym Technologies, Inc.) with all heavy atoms fixed according to the X-ray structures.

For each system studied, the salt dependence of the DNA transition from the *B* conformation to the bound conformation was calculated. The conformation of *B*-form d[(CGCGAATTCGCG)$_2$] was defined by the single-crystal X-ray diffraction structure (Drew *et al.*, 1981). The coordinates of the other *B*-DNA double helices were generated from the idealized local coordinates of Arnott & Hukins (1972) using the Insight II/DISCOVER software package (Biosym Technologies, Inc.). Beyond this, no attempt was made to explicitly account for structural changes upon binding. Since the structures of the free and bound antibiotics are expected to be quite similar, the salt dependence of these transitions should be very small due to their relatively low charge densities. These effects may later be included in more sensitive analyses of binding energies.

The NLPB equation was also solved for cylindrical models of the polyelectrolyte chain. In this case, the DNA was modeled as a 1000 Å long cylinder characterized by a linear charge density of 1 charge every 1·7 Å and a radius of 10 Å with $\varepsilon_m = 2$ or 4. The binding of a divalent ligand to the polyelectrolyte was modeled as the neutralization of 2 discrete charges at the center of the linear array.

(c) *Numerical methods*

Details of the finite difference procedure to calculate electrostatic potentials with the NLPB equation have been reported (Nicholls & Honig, 1991; Jayaram *et al.*, 1989; Gilson *et al.*, 1988). All finite difference calculations were done with the DelPhi software package (Nicholls *et al.*, 1990) using both a 65^3 and a 129^3 lattice. The potentials were calculated using 3-step focussing (Gilson *et al.*, 1988). In the initial calculation, the largest dimension of the macromolecule fills 16 to 23% of the grid and the potentials at the lattice points on the boundary of the grid are approximated analytically using the Debye–Hückel equation such that $\phi(\infty) = 0$ (Klapper *et al.*, 1986). This condition ensures that the system is electroneutral and is confirmed numerically to within 1%. The final potentials on the lattice are calculated in 2 steps in which the grid is made 4 times finer, such that the largest dimension of the macromolecule fills 64 to 92% of the grid with the boundary conditions interpolated from the previous step. The maximum final resolution for the ligand–DNA complexes were 1·4 grids/Å for the 65^3 lattice and 2·8 grids/Å for the 129^3 lattice. Salt dependent electrostatic effects do not depend on the lattice resolution above about 0·9 grid/Å. Potentials around the cylindrical DNA models were calculated after 5 focussing runs, such that the final resolution of the grid was 1·04 grids/Å.

Each of the integrals of salt dependent free energy terms (eqns (8) to (10)) were evaluated as summations over discrete lattice points with specified charges, q. The salt dependent free energy terms were calculated from the difference of 2 nonlinear finite-difference calculations of the electrostatic potentials. In the first calculation, the charged macromolecule with an internal dielectric constant ε_m is embedded in a solvent of dielectric constant ε_s with no salt ($\kappa = 0$ everywhere). The second calculation includes added salt of ionic strength κ. The difference in the electrostatic potentials with and without salt at each point in space results from the change in the mobile ion distribution. The electrostatic ion–molecule interaction free energy (eqn (7)) is calculated as a sum over all macromolecular charges:

$$\Delta G^{\circ}_{im} = \sum_f q_f\{\phi(\kappa) - \phi(0)\}, \tag{20}$$

where $\phi(\kappa)$ and $\phi(0)$ are the calculated potentials at the macromolecular charges, q_f, with and without added salt.

The mobile ion charge density, q_m, excess cation concentration, q_m^+, and excess anion concentration, q_m^-, were calculated directly from the Boltzmann distribution of the electrostatic potentials with added salt, $\phi(\kappa)$, at each grid point in solution (Jayaram *et al.*, 1989):

$$q_m = 2c_b \sinh(\phi(\kappa)), \tag{21}$$

$$q_m^+ = c_b \exp(-\phi(\kappa)), \tag{22}$$

$$q_m^- = c_b \exp(+\phi(\kappa)). \tag{23}$$

The electrostatic ion–ion interaction free energy was calculated as the sum over all lattice points in solution:

$$\Delta G^{\circ}_{ii} = \sum_m q_m\{\phi(\kappa) - \phi(0)\}, \tag{24}$$

where $\phi(\kappa)$ and $\phi(0)$ are the calculated potentials at each lattice point in solution with and without added salt. The free energy of organizing the ion atmosphere was also calculated as the sum over all lattice points in solution:

$$\Delta G^{\circ}_{org} = -\sum_m \{q_m\phi(\kappa) + q_m^+ + q_m^- - 2\}. \tag{25}$$

(d) *Numerical accuracy*

The numerical accuracy of the 3-dimensional finite-difference (TFD) method for calculating the salt dependent charge–solvent interaction energies for an infinite cylinder was estimated as a function of the salt concentration of the solution and the linear charge density of a cylinder. The results of our TFD solution to the NLPB equation for a charged cylinder were compared to the exact 1-dimensional finite-difference (OFD) solution for the cylinder (Sharp & Honig, 1990*a*). For 1:1 salt concentrations from 0·001 M to 1·00 M, the TFD solution is numerically accurate to within 5% of the exact OFD solution. The error increases substantially below about 10^{-3} M 1:1 salt due to the finite lattice size. All calculations reported here use at least 10^{-3} M 1:1 salt. The error in the TFD solution of the linearized PB equation (LPB) approaches 50% for highly charged cylinders (1 charge/1·7 Å). The LPB equation is clearly not applicable to the systems studied here.

3. Results

(a) *Minor groove binding antibiotics*

Our initial objective was to test the accuracy of the finite difference NLPB model for calculating the salt dependence of binding small ligands to DNA. The minor groove binding antibiotics DAPI, Hoechst 33258 and netropsin were chosen because they induce very little structural distortion in the DNA upon binding. Because these structural changes were small, the calculated salt dependent free energy of the DNA transition from the *B*-form to the bound form was found to be negligible. This greatly simplifies our theoretical analysis. The interaction of the minor groove binding antibiotics with DNA is often heterogeneous. Therefore, we have also analyzed the effects of different conformations of the drugs bound to the DNA when structural data were available.

(i) *The DAPI–DNA complex*

The salt dependent electrostatic free energy, $\Delta\Delta G_s^\circ$ (in units of kT), for the binding of DAPI to d[(CGCGAATTCGCG)$_2$] calculated with the finite-difference NLPB equation increases linearly with ln [M$^+$] (Figure 3(a)) with a slope of 2·1 over the experimental salt range (Table 1). Wilson *et al.* (1990) evaluated $-\partial(\ln K_{obs})/\partial(\ln [M^+])$ for DAPI binding to both poly[d(A-T)]$_2$ and poly[d(G-C)]$_2$ in Mg^{2+}-free buffer containing 0·01 M Mes, 0·001 M EDTA and NaCl (pH 6·2). Our results agree with the linear experimental plot of $-\ln K_{obs}$ *versus* ln [Na$^+$] with a slope of 2·3 for both poly[d(A-T)]$_2$ and poly[d(G-C)]$_2$ (Wilson *et al.*, 1990). Our calculations vary by less than 5% with internal dielectric constant and charge set. Results are given for the CVFF charge set with $\varepsilon_m = 4$. The salt dependent components of the electrostatic free energy have been individually evaluated and analyzed in both the free and bound state.

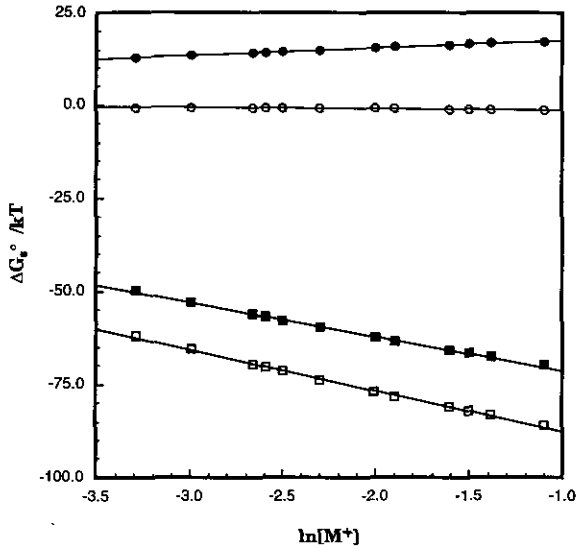
The ion–molecule interaction free energy, ΔG_{im}°, of the isolated oligonucleotide is favorable and decreases linearly with ln [M$^+$] (Figure 3(b)). The large negative electrostatic potential around DNA drives the formation of a cationic counterion atmosphere. As the bulk salt concentration increases, more and more counterions accumulate in the vicinity of the DNA (Figure 4) making ΔG_{im}° progressively more favorable (Figure 3(b)). A similar phenomenon is observed with the isolated DAPI molecule. The positive potential surrounding the antibiotic drives the accumulation of anions resulting in a favorable ΔG_{im}°. However, due to the low charge density of DAPI, the effects are much smaller (Figure 3(b)).

In the salt range studied, $\Delta\Delta G_{im}^\circ$ opposes binding (Figure 3(b); Table 2). Like the isolated oligonucleotide, the complex has a large, favorable ΔG_{im}°, although it is reduced in magnitude (Figure 3(b)). The smaller ion–molecule interaction seen in the complex has two causes. First, the binding of DAPI to the DNA decreases the large negative electrostatic potentials around the nucleic acid. Second, the presence of DAPI in the minor groove excludes cations from a high potential region near the DNA. As a result, the concentration of ions vicinal to the DNA is reduced. This is shown by the decrease in the radially averaged charge density of ions near the DNA surface (at about 8 to 15 Å from the helix axis) in the complex (Figure 5). This redistribution of counterions makes ΔG_{im}° less favorable upon binding (Figure 3(b); Table 2). This effect becomes more pronounced as the salt concentration increases and proportionally more ions around the DNA are

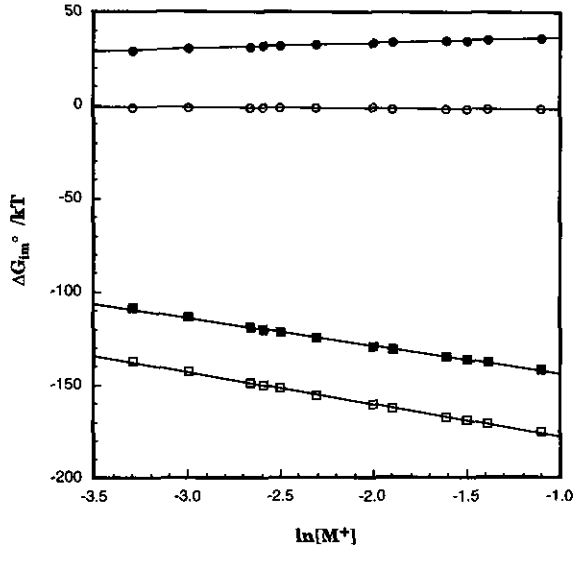
Table 1
Salt dependence of drug–DNA interactions

	DAPI	Hoechst 33258	Netropsin	Cylinder[a]
Experimental				
$-\partial \ln K_{obs}/\partial \ln [M^+]$	2·3[b]	0·7 to 0·9[c]	1·5 to 1·6[d]	—
Calculated[e]				
$\partial\Delta\Delta G_s^\circ/\partial \ln [M^+]$	2·1	0·6 to 1·1[f]	2·1 to 2·2[f]	2·1

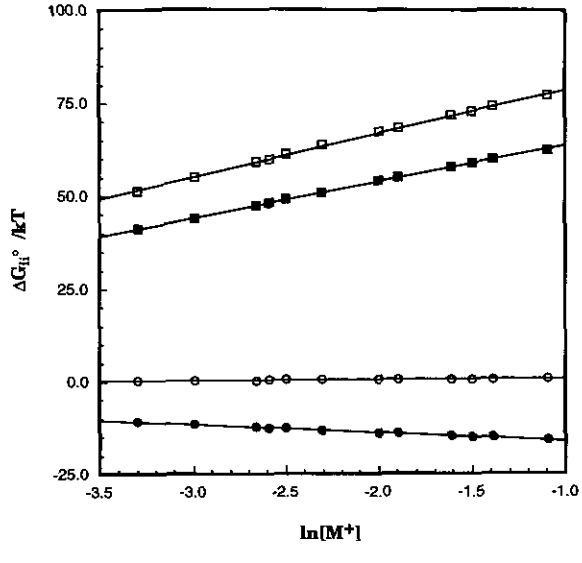
[a] Cylindrical model of divalent ligand binding (see the text for details).
[b] From Wilson *et al.* (1990).
[c] From Loontiens *et al.* (1989, 1990).
[d] From Breslauer *et al.* (1987) and Marky & Breslauer (1987).
[e] Calculated with the TFD solution to the NLPB equation with the CVFF charge set and $\varepsilon_m = 4$ (see the text for details). Units of free energy are kT.
[f] Calculated for several different forms of the drug–DNA complex (see the text for details).



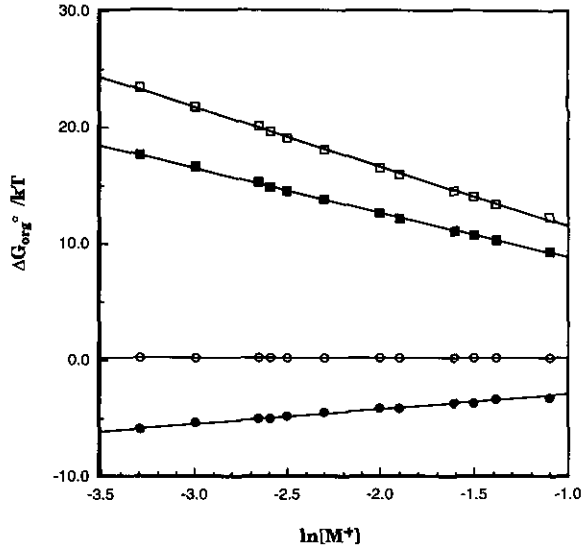
(a)



(b)



(c)



(d)

Figure 3. The salt dependence of the free energy terms calculated with the NLPB equation for the DAPI–d[(CGC GAATTCGCG)$_2$] complex for: (■), the DAPI–DNA complex; (□), the isolated oligonucleotide; (○), the isolated DAPI molecule; and (●), the change in the free energy calculated for the binding of DAPI to the oligonucleotide. Each line represents a linear least-squares regression analysis of the data points. (a) ΔG_s°; (b) ΔG_{im}°; (c) ΔG_{ii}°; (d) ΔG_{org}°.

perturbed by the ligand (Figure 3(b); Table 3). Thus, relatively small salt dependent changes in the counterion concentration near the DNA significantly affect the salt dependence of binding through electrostatic ion–molecule interactions. Because of the low charge density of DAPI, the energetic consequences of anion redistribution around the drug are small, although they are formally included in our calculation (Figure 3(b)).

The ion–ion interaction free energy, ΔG_{ii}°, for the solitary deoxyoligonucleotide is unfavorable and increases linearly with $\ln[M^+]$ (Figure 3(c)). This results from electrostatic ion–ion repulsions as counterions build up around the DNA with increasing bulk salt concentration. Since complex formation diffuses the ion atmosphere, binding of DAPI to DNA decreases ΔG_{ii}° of the system (Figure 3(c)), so that $\Delta\Delta G_{ii}^\circ$ favors binding (Table 2). As before, this effect grows as bulk salt concentration increases and proportionally more ions are affected upon complexation (Figure 3(c); Table 3).

Finally, the free energy associated with assembling the ion atmosphere around DNA, ΔG_{org}°, is unfavorable and decreases linearly with $\ln[M^+]$ (Figure 3(d)). This term is understood in terms of a phenomenon analogous to counterion condensation. A large concentration of counterions accumulates around DNA to minimize ΔG_{im}°. Large changes in bulk salt concentration are accompanied by relatively small changes in the local counterion concen-

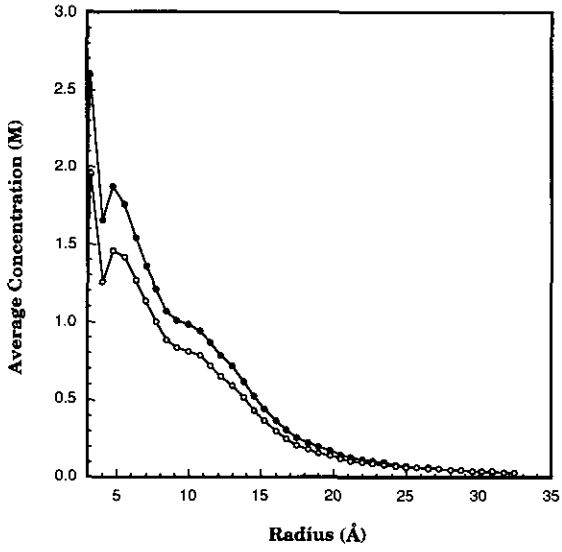


Figure 4. Radially averaged excess salt concentration around d[(CGCGAATTCGCG)$_2$] as a function of distance from the helix axis. (●), Calculated at 0·100 M bulk salt concentration; (○), calculated at 0·037 M bulk salt concentration.

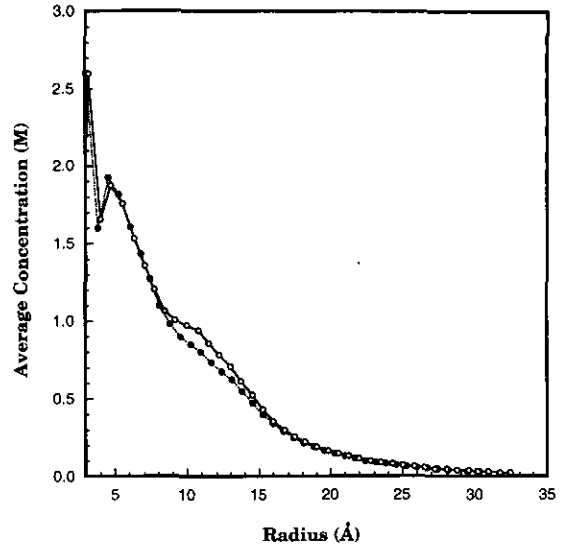


Figure 5. Radially averaged excess salt concentration around d[(CGCGAATTCGCG)$_2$] in the free and bound states at 0·100 M bulk salt concentration. (○), Calculated for the isolated oligonucleotide; (●), calculated for the DAPI–d[(CGCGAATTCGCG)$_2$] complex.

Table 2

Values of the free energy terms for minor groove binding drugs at 0·1 M M^+

	DAPI[a]		Hoechst 33258[b]			Netropsin[c]			
Free energy (kcal/mol)	12 bp	57 bp	Complex I	Complex II	Complex III	Complex I	Complex II	Complex III	Cylinder[d]
$\Delta\Delta G^\circ_s$	8·9	17·2	4·4	3·2	4·9	9·4	9·5	9·2	28·2
$\Delta\Delta G^\circ_{im}$	19·2	36·3	9·5	6·8	11·0	20·8	21·0	20·2	58·1
$\Delta\Delta G^\circ_{ii}$	−7·8	−16·5	−3·9	−2·7	−4·4	−8·3	−8·5	−8·2	−27·3
$\Delta\Delta G^\circ_{org}$	−2·6	−2·6	−1·3	−0·9	−1·7	−3·1	−3·0	−2·8	−2·6

[a] 12 bp: DAPI–d[(CGCGAATTCGCG)$_2$] complex (Larsen *et al.*, 1989).
57 bp: DAPI–57 bp DNA complex (see the text for details).
[b] Complex I and II: Hoechst 33258–d[(CGCGAATTCGCG)$_2$] complex (Pjura *et al.*, 1987).
Complex III: Hoechst 33258–d[(CGCGAATTCGCG)$_2$] complex (Teng *et al.*, 1987).
[c] Complex I and II: netropsin–d[(CGCGATATCGCG)$_2$] complex (Coll *et al.*, 1989).
Complex III: netropsin–d[(CGCGAATTCGCG)$_2$] complex (Sriram *et al.*, 1992).
[d] Cylindrical model for divalent ligand binding (see the text for details).

Table 3

Salt dependence of the free energy terms for minor groove binding drugs

	DAPI[a]		Hoechst 33258[b]			Netropsin[c]			
Free energy (kcal/mol)	12 bp	57 bp	Complex I	Complex II	Complex III	Complex I	Complex II	Complex III	Cylinder[d]
$\partial(\Delta\Delta G^\circ_s/\partial(\ln[M^+])$	2·1	2·0	1·0	0·6	1·1	2·2	2·2	2·1	2·1
$\partial(\Delta\Delta G^\circ_{im}/\partial(\ln[M^+])$	3·0	3·0	1·5	0·9	1·6	3·3	3·3	3·2	2·6
$\partial(\Delta\Delta G^\circ_{ii}/\partial(\ln[M^+])$	−2·2	−2·0	−1·0	−0·7	−1·1	−2·2	−2·2	−2·1	−2·1
$\partial(\Delta\Delta G^\circ_{org}/\partial(\ln[M^+])$	1·3	1·0	0·5	0·4	0·6	1·1	1·1	1·1	1·6

[a] 12 bp: DAPI–d[(CGCGAATTCGCG)$_2$] complex (Larsen *et al.*, 1989).
57 bp: DAPI–57 bp DNA complex (see the text for details).
[b] Complex I and II: Hoechst 33258–d[(CGCGAATTCGCG)$_2$] complex (Pjura *et al.*, 1987).
Complex III: Hoechst 33258–d[(CGCGAATTCGCG)$_2$] complex (Teng *et al.*, 1987).
[c] Complex I and II: netropsin–d[(CGCGATATCGCG)$_2$] complex (Coll *et al.*, 1989).
Complex III: netropsin–d[(CGCGAATTCGCG)$_2$] complex (Sriram *et al.*, 1992).
[d] Cylindrical model for divalent ligand binding (see the text for details).

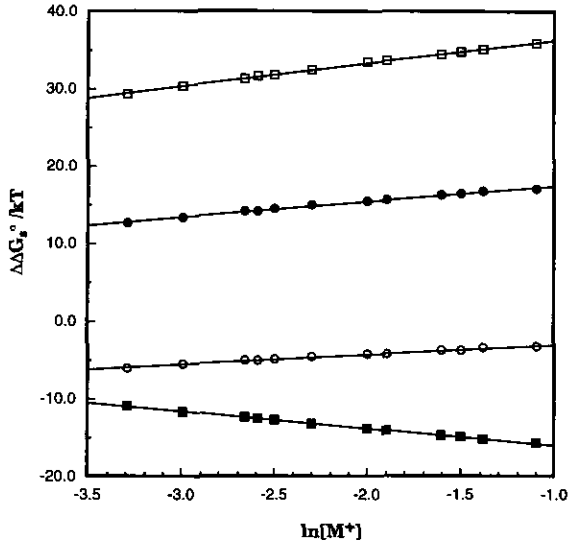


Figure 6. The salt dependent contributions to the total electrostatic free energy of binding for the DAPI–d[(CGCGAATTCGCG)$_2$] complex calculated with the NLPB equation. (●), $\Delta\Delta G^\circ_s$; (□), $\Delta\Delta G^\circ_{im}$; (■), $\Delta\Delta G^\circ_{ii}$; (○), $\Delta\Delta G^\circ_{org}$.

tration around the DNA. Organization of this ion atmosphere around the nucleic acid is entropically unfavorable. As bulk salt concentration increases, the concentration difference between the ions around DNA and in bulk solution decreases, thus reducing ΔG°_{org}. For DAPI alone, ΔG°_{org} is negligible due to its small net charge (Figure 3(d)). Since binding of DAPI to DNA decreases the net concentration of counterions around the DNA, $\Delta\Delta G^\circ_{org}$ favors binding (Figure 3(d); Table 2). This effect is smaller at higher salt concentrations when the difference between local counterion concentrations and bulk salt concentration becomes small (Figure 3(d); Table 3).

The salt dependent contributions to the binding free energy are compared in Figure 6 and Table 2. At physiological ionic strength (0·10 to 0·20 M), the largest salt dependent contribution to $\Delta\Delta G^\circ_s$ is the increase of $\Delta\Delta G^\circ_{im}$. The sum of $\Delta\Delta G^\circ_{ii}$ and $\Delta\Delta G^\circ_{org}$, the favorable salt dependent contributions to $\Delta\Delta G^\circ_s$, is smaller than $\Delta\Delta G^\circ_{im}$. Consequently, $\Delta\Delta G^\circ_s$ is unfavorable. Salt destabilizes the DAPI–d[(CGCGAATTCGCG)$_2$] complex by almost 9 kcal/mol at 0·1 M bulk salt concentration.

(ii) *The Hoechst 33258–DNA complex*

The salt dependence of Hoechst 33258 binding to d[(CGCGAATTCGCG)$_2$] was calculated for three different structures of the complex (Teng *et al.*, 1988; Pjura *et al.*, 1987). In the crystal structure of Pjura *et al.* (1987), the drug molecule lies in the minor groove such that the piperazine ring protrudes into the groove of the adjacent GC region. In this structure, the piperazine ring adopts two different conformations in roughly equal populations: buried in the minor groove (complex I) and extended out of the groove into solution (complex II). In the independent crystal structure analysis of Teng *et al.* (1988), the same Hoechst–DNA complex shows a different binding mode in which the drug completely covers the AATT base pairs in the minor groove (complex III). In this structure, there is no structural disorder associated with the piperazine ring. The salt dependence of $\Delta\Delta G^\circ_s$ calculated for each of these structures with the finite-difference NLPB equation is linear with a slope between 0·6 and 1·1 in the experimental salt range (Tables 1 and 3). Loontiens *et al.* (1989, 1990) have determined the salt dependence of binding Hoechst 33258 to calf thymus and chicken erythrocyte DNA, and poly[d(A-T)]$_2$ in Mg^{2+}-free buffer containing 20 mM bis-Tris and NaCl (pH 7·0). Our calculations are in excellent agreement with the experimental value of $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ which is between 0·7 and 0·9 (Loontiens *et al.*, 1989, 1990).

The salt dependent electrostatic effects calculated for the Hoechst 33258–DNA complexes are qualitatively similar to the effects observed in the DAPI–DNA complex (Tables 2 and 3). At physiological salt concentrations, the largest salt dependent contribution to the binding free energy is ΔG°_{im} (Table 2). The terms ΔG°_{ii} and ΔG°_{org} are relatively small. Salt destabilizes the Hoechst 33258–d[(CGCGAATTCGCG)$_2$] complex by 3·2 to 4·9 kcal/mol at 0·1 M M^+ (Table 2).

(iii) *The netropsin–DNA complex*

The salt dependence of netropsin binding to DNA was also calculated for three different structures of the complex. Two forms of the netropsin–d[(CGCGATATCGCG)$_2$] complex have been reported by Coll *et al.* (1989). These two forms differ in the orientation of the drug bound in the minor groove (complexes I and II). A single conformation of the netropsin–d[(CGCGAATTCGCG)$_2$] complex has been described by Sriram *et al.* (1992: complex III). The calculated salt dependence of $\Delta\Delta G^\circ_s$ calculated for each of these structures is linear with a slope between 2·1 and 2·2 in the experimental salt range (Tables 1 and 3). Breslauer *et al.* (1987) have reported $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ for netropsin binding to both poly[d(A-T)]$_2$ and poly[d(G-C)]$_2$ in Mg^{2+}-free buffer containing 0·01 M sodium phosphate, 1 mM EDTA, and NaCl (pH 7·0) (Breslauer *et al.*, 1987; Marky & Breslauer, 1987). Our calculated values agree reasonably well with the experimental value of 1·5 to 1·6 (Breslauer *et al.*, 1987; Marky & Breslauer, 1987).

The salt dependent electrostatic effects calculated for the netropsin–DNA complexes are qualitatively similar to the effects observed in both the DAPI and the Hoechst–DNA complexes (Tables 2 and 3). Once again, at moderate salt concentrations, $\Delta\Delta G^\circ_s$ is dominated by ΔG°_{im} (Table 2), while ΔG°_{ii} and ΔG°_{org} are small. Salt destabilizes the netropsin–DNA complex by almost 9·5 kcal/mol at 0·1 M M^+ (Table 2).

(iv) *The role of oligoelectrolyte end effects in NLPB calculations*

The NLPB calculations on the minor groove binding antibiotics were performed on 12 bp oligo-

nucleotides, whereas the corresponding experimental studies used polynucleotides. Since end effects can play an important role in determining the electrostatic properties of oligonucleotides (Olmsted *et al.*, 1991; Record & Lohman, 1978), we have tried to evaluate their influence on our calculation of the salt dependent free energy of binding. The salt dependence of the DAPI–DNA interaction was calculated for drug binding to a 57 bp DNA fragment built around a central d[(CGCGAATTCGCG)$_2$] binding site. The role of end effects in this construct are expected to be minimal (Olmsted *et al.*, 1989).

For the DAPI–57 bp DNA complex, the plot of $\Delta\Delta G_s^\circ$ *versus* ln[M$^+$] is linear with a slope of 2·1 (Table 1). The salt dependence of the individual components of $\Delta\Delta G_s^\circ$ are identical to those in the DAPI–d[(CGCGAATTCGCG)$_2$] complex (Table 3). However, the magnitudes of the electrostatic components of $\Delta\Delta G_s^\circ$, $\Delta\Delta G_{im}^\circ$ and $\Delta\Delta G_{ii}^\circ$ are substantially increased (Table 2). These changes reflect the larger interaction, ΔG_s°, of the ion atmosphere with the longer DNA chain. The largest salt dependent contribution to $\Delta\Delta G_s^\circ$ is still the increase of $\Delta\Delta G_{im}^\circ$ (Table 2). Thus, accounting for the contribution of end effects does not substantially alter the conclusions reached by our calculations.

A full NLPB evaluation of the axial dependence of the electrostatic potential for cylindrical oligomers found that the length of the electrostatic end effect was about half the Debye screening length, $0{\cdot}5\,\kappa^{-1}$ (Katoh & Ohtsuki, 1982). This finding was supported by a subsequent grand canonical Monte Carlo analysis (Olmsted *et al.*, 1989). This length corresponds to a maximum of about 11 Å from each end of an oligomer at 0·02 M salt. The minor groove binding antibiotics studied here bind at least 13 Å from each end of d[(CGCGAATTCGCG)$_2$]. Therefore, it is expected that the electrostatic interaction of the antibiotics is effectively screened from the end effects. The insensitivity of the calculated salt dependence to oligonucleotide lengths above 12 bp reflects this expectation.

(b) *The cylindrical PB model*

Since most theoretical descriptions of polyelectrolytes to date have modeled DNA as a uniformly and continuously charged cylinder of infinite length, we have calculated the salt dependent electrostatic free energy terms for a cylindrical DNA model. In this model, DNA is described by a cylinder with a linear charge density of one charge every 1·7 Å and a radius of 10 Å. Binding of a divalent cation is described by the neutralization of two charges at a specific binding site. This model corresponds to earlier simple descriptions of ligand–DNA binding (Olmsted, 1991; Wilson *et al.*, 1980). The dependence of $\Delta\Delta G_s^\circ$ on ln[M$^+$], calculated with the TFD solution to the finite difference NLPB equation, is linear with a slope of 2·1 in the salt range 0·01 M to 0·7 M (Tables 1 and 3). This value is consistent with experimentally observed values for the binding of small divalent ligands to DNA (Manning, 1978; Record *et al.*, 1976, 1978).

The salt dependent electrostatic effects observed in this simple model are qualitatively similar to those observed in the specific ligand–DNA complexes (Tables 2 and 3). At physiological ionic strengths, binding is accompanied by a dispersion of ions which significantly increases ΔG_{im}° (Table 2). The resulting changes in ΔG_{ii}° and ΔG_{org}° are smaller than $\Delta\Delta G_{im}^\circ$ but contribute significantly to $\Delta\Delta G_s^\circ$ (Table 2). For the cylindrical model, salt opposes the formation of the divalent ligand–DNA complex by 28·2 kcal/mol at 0·1 M M$^+$ (Table 2).

4. Discussion

We have shown that the NLPB equation describes the nonspecific salt dependent effects in a variety of ligand–DNA systems with considerable accuracy (Table 1). The finite difference NLPB/solvation model for nucleic acid systems represents an advance over previous treatments for several reasons. First, the finite-difference NLPB model incorporates detailed molecular models of both DNA and its ligands in the calculations so that three-dimensional structural effects on electrostatic interactions are explicitly taken into account. The NLPB analysis confirms the usefulness of simple cylindrical models in evaluating the overall salt dependence of binding reactions for molecules with approximately cylindrical symmetry (e.g. drug–DNA complexes). However, cylindrical models cannot be used to properly describe the interaction of complex ligands, such as proteins, with DNA (see accompanying paper). A second advantage of the NLPB/solvation model is that it explicitly considers each term in the total electrostatic free energy without assuming that limiting law conditions must be satisfied or that binding is a simple cation exchange process. Finally, the NLPB equation can be used to calculate the magnitude of a well-defined electrostatic binding free energy. This provides a degree of generality not offered by theories that only treat the slope of the free energy as a function of salt concentration. These points are discussed in detail in the following sections.

(a) *The origin of salt effects on ligand–DNA interactions in the NLPB model*

In the NLPB/solvation model, nonspecific salt effects can be interpreted in terms of the difference in the salt dependent solvation free energy between the bound and free molecules. The total solvation free energy of a macromolecule is directly related to the distribution of small ions around the molecule (Timasheff, 1992). Poisson–Boltzmann theory describes the interaction of a continuously distributed ion atmosphere with a macromolecule in terms of three salt dependent free energy terms: the electrostatic ion–molecule interactions, ΔG_{im}°; the electrostatic ion–ion repulsions, ΔG_{ii}°; and the

entropic free energy of organizing the ion atmosphere, ΔG°_{org}. These three terms constitute the salt dependent solvation free energy of the macromolecule, ΔG°_s. The large, favorable ΔG°_{im} drives the accumulation of counterions around the polyion. The terms ΔG°_{ii} and ΔG°_{org} favor the complete dissipation of the ion atmosphere. At equilibrium, the distribution of ions around the polyion reflects the balance between ΔG°_{im}, ΔG°_{ii} and ΔG°_{org}. The spontaneous formation of an ion atmosphere around the DNA reflects the dominant role of ΔG°_{im} (Reiner & Radke, 1990; Verwey & Overbeek, 1948) on the solvation of the individual macromolecules. The salt dependent binding free energy reflects changes in each of these salt dependent terms upon binding.

The change in each salt dependent contribution to the binding free energy results from a dispersion of the ion atmosphere surrounding the DNA. In addition, each salt dependent contribution to the binding free energy is affected by salt dependent changes in the ionic distribution around the DNA (Figures 3 and 4). In the physiological salt range, the dominant salt dependent contribution to binding for the ligand–DNA complexes is the unfavorable change in ΔG°_{im} (Table 2). At these salt concentrations, ligand binding to DNA disrupts the large favorable electrostatic interactions of the highly organized ion atmosphere with the free polyion. Conversely, the dispersion of the ion atmosphere reduces ion–ion repulsions within the ion atmosphere, so $\Delta\Delta G^\circ_{ii}$ provides a small favorable driving force for binding. The magnitude of each of the purely electrostatic effects, $\Delta\Delta G^\circ_{im}$ and $\Delta\Delta G^\circ_{ii}$, increases with bulk salt concentration as proportionally more ions are displaced by the ligand. A relatively small favorable increase in the cratic entropy of ion organization, $\Delta\Delta G^\circ_{org}$ (Figure 3(d)), accompanies the redistribution of ions into bulk solution upon binding. This term becomes progressively smaller at high salt concentrations when the difference between local counterion concentrations around the DNA and bulk salt concentration decreases.

Changes in the electrostatic ion–molecule, $\Delta\Delta G^\circ_{im}$, and ion–ion, $\Delta\Delta G^\circ_{ii}$, as well as ion organization, $\Delta\Delta G^\circ_{org}$, terms are all found to be important in describing salt dependent effects in charged ligand–nucleic acid interactions. Although the term $\Delta\Delta G^\circ_{org}$ is a purely entropic free energy, the terms $\Delta\Delta G^\circ_{im}$ and $\Delta\Delta G^\circ_{ii}$ can have significant enthalpic and entropic components. Therefore, according to the NLPB model, both the enthalpy and the entropy of binding can be salt dependent. The relative contributions of each of these terms to the free energy of binding will be treated in a subsequent paper.

The total salt dependent contribution to the electrostatic free energy of binding, $\Delta\Delta G^\circ_s$, is unfavorable at physiological salt concentrations. The unfavorable change in ΔG°_s upon binding reflects the dominant role of the change in the electrostatic ion–molecule interaction, $\Delta\Delta G^\circ_{im}$, with the DNA. This finding indicates that the molecules are better solvated by salt in the separated state than in the bound state. From this standpoint, $\Delta\Delta G^\circ_s$ is a salt dependent "desolvation" penalty for binding in this salt range.

(b) *The interpretation of salt effects on ligand–DNA binding in CC theory*

The CC description of salt effects has been widely used and is quite successful in describing the salt dependence of small ligand–DNA interactions. Indeed, the experimental salt-linked effects on the minor groove binding antibiotics studied here are consistent with the predictions of CC theory. Record and co-workers' extension of CC theory predicts that $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ for the interaction of a charged ligand with DNA is equal to $m'\psi$ (eqn (1)) where m' is equal to the number of phosphate charges neutralized by a charged ligand (ion-pairs) (deHaseth *et al.*, 1977; Record *et al.*, 1976). For simple charged ligands, the value of m' is assumed to be equal to the net charge on the ligand (Braunlin *et al.*, 1982; Record *et al.*, 1981; Lohman *et al.*, 1980), although formally no ion-pairs are found in the structures of the minor groove binding antibiotics studied here. As such, the calculated value of the slope for the divalent antibiotics DAPI and netropsin is 1·76, while the univalent antibiotic Hoechst 33258 is 0·88. The territorial binding model of polyion associated counterions predicts that the binding of a Z-valent cationic ligand within the condensation layer will replace Z condensed univalent counterions (Friedman & Manning, 1984; Manning, 1978). In this model, $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ is given by the net charge on the ligand. Therefore, this model also makes reasonable predictions of $-\partial(\ln K_{obs})/\partial(\ln[M^+])$ for each of the systems studied here. No distinction in salt effects is made among similarly charged ligands and different DNA sequences in these models.

The total electrostatic free energy of the polyion–counterion system, ΔG^{el}, modeled with CC theory can be expressed as the sum of two contributions, g_{el} and g_{mix} (Manning, 1978). The term g_{el} is the free energy of charging the condensed polyion system in a Debye–Hückel ion atmosphere fixed in its final average position. Thus, g_{el} represents the Debye–Hückel screened coulombic potential between the effective charges on the polyion, q_{net}, where q_{net} represents the total charge at each polyion site, q, reduced by a factor $(1-N\theta_N)$ related to the extent of counterion condensation, θ_N (for univalent ions $N=1$). The term g_{mix} is the free energy of assembling the uncharged condensed ions from bulk solution to their final average positions around the polyion. The term g_{mix} is the cratic free energy of mixing of the free and condensed counterions and solvent. The CC formalism does not explicitly separate the salt independent interpolyion coulombic interactions from the salt dependent electrostatic ion–polyion interactions in g_{el}.

Minimizing the expression for ΔG^{el} with respect to θ_N results in a salt invariant condensed layer of counterions within a fixed volume around the DNA

(Manning, 1977, 1978). At equilibrium, the condensed state represents a balance between g_{el} and g_{mix}. By itself, the term g_{el} would lead to the complete neutralization of polyion charges by condensed counterions ($N\theta_N = 1$) to minimize the free energy of charging the polyion. The term g_{mix} favors the complete dissociation of condensed counterions ($\theta_N = 0$) to maximize the entropy of the counterion atmosphere. The formation of the condensed state reflects the dominant role of the electrostatic ion–polyion interactions in stabilizing the polyelectrolyte system (Manning, 1969*b*; Manning & Zimm, 1965). However, the magnitude of these interactions cannot be theoretically determined for infinite rod-like systems.

A fundamental conclusion of the CC analyses is that charged ligand–DNA reactions are driven by the entropic release of univalent counterions associated with the charged phosphates for processes that maintain g_{el} (Record *et al.*, 1976, 1978, 1991; Anderson & Record, 1982; Manning, 1978). The formation of a condensed layer leads to a polyion–counterion system "potentiated" by a large entropy increase that accompanies the transfer of counterions from the concentrated condensation layer to the more dilute bulk solution (Manning, 1978). This phenomenon has been called the "polyelectrolyte effect" (Record *et al.*, 1991). Manning and Friedman have defined the polyelectrolyte effect to include the electrostatic effect of the Debye–Hückel layer on the screening of phosphate charges as a function of salt (Friedman *et al.*, 1990; Friedman & Manning, 1984).

The energetic consequences of ligand binding to DNA as described by CC theory depend on two basic features of the model. First, the change in g_{el} upon ligand binding to the DNA is assumed to be very small (Manning, 1978; Record *et al.*, 1976). Record and co-workers have assumed that the change in g_{el} upon ligand binding to the DNA is small since changes in the interaction of counterions with the nucleic acid phosphates upon binding are exactly compensated by coulombic interactions between the ligand and the DNA independent of bulk salt concentration (Record *et al.*, 1976). In other words, the electrostatic ion–polyion interactions are not explicitly included in the overall description of salt effects. Second, the counterion concentration within the condensed or "bound" layer is invariant to changes in the bulk salt concentration (Manning, 1969*a*, 1978). As a result, the electrostatic ion–polyion interaction free energy is essentially salt independent. Since the change in electrostatic ion–polyion interactions upon binding are treated as a small, salt-invariant term, the dominant contribution to the electrostatic binding free energy and its salt dependence must necessarily be g_{mix} (Record *et al.*, 1976, 1978, 1991; Anderson & Record, 1982; Manning, 1978). In other words, according to these interpretations of the CC model, the salt dependent variation of the free energy of ligand–DNA binding is a consequence of changes in the cratic entropy of ion release from the bound layer. In contrast, if the salt dependence of g_{el} is recognized, then the salt dependence of the binding free energy can have a significant electrostatic component even within the context of CC theory (Friedman *et al.*, 1990; Friedman & Manning, 1984).

(c) *Thermodynamic binding models of salt effects on ligand–DNA binding*

The salt dependence of ligand–DNA interactions is sometimes expressed in terms of a chemical equilibrium (Record *et al.*, 1976, 1978):

$$L + D \overset{K_T}{\longleftrightarrow} L \cdot D + \Delta q M^+, \qquad (26)$$

where K_T is the thermodynamic equilibrium constant for the binding of a ligand, L, to DNA, D, resulting in the release of Δq univalent salt ions, M^+. In these models, the slope of the plot of $-\ln K_{obs}$ *versus* $\ln [M^+]$ gives information about the stoichiometry of the release of thermodynamically bound counterions upon ligand–DNA complexation (Record *et al.*, 1978). It must be emphasized that these competitive binding models provide only a phenomenological description of salt dependent effects on ligand binding equilibria. They are thermodynamically accurate only in describing the stoichiometric site binding of each species in the reaction. However, it is well known that simple univalent salts interact with DNA in a delocalized mode (Anderson & Record, 1990; Leyte, 1990; Bleam *et al.*, 1983; Anderson *et al.*, 1978; Manning, 1978). The long-range salt dependent effects on macromolecular equilibria arising from the delocalized interaction of simple univalent ions with DNA cannot be properly described by a mass action relationship in either CC theory (Manning, 1978) or NLPB theory (Wyman & Gill, 1990). In this context, it has been shown using the hypernetted chain integral equation that the interaction of Mg^{2+} with DNA results in the stoichiometric release of only about one Na^+, although $-\partial (\ln K_{obs}^{Mg^{2+}})/\partial (\ln [Na^+])$ is equal to about 2, where $K_{obs}^{Mg^{2+}}$ is the observed equilibrium binding constant for Mg^{2+} (Bacquet & Rossky, 1988).

A general description of the thermodynamic binding model has been developed in terms of the preferential interaction parameter, Γ_{3u}° (Anderson & Record, 1993). In this context, the term "thermodynamic ion release" has been used to represent any redistribution of ions around DNA that accompanies binding (Record *et al.*, 1990, 1991). The formulation in terms of preferential interaction parameters makes no *a priori* statement about the breakdown of free energy into entropic and enthalpic contributions for a particular system. Indeed, this formulation can be shown to be thermodynamically equivalent to the one developed in this work (K. Sharp, R. Friedman, V. Misra, J. Hecht & B. Honig, unpublished results). However, in keeping with earlier conclusions based on counterion condensation theory, the process of ion release has been identified explicitly as the entropic force driving cationic ligand–DNA interactions as

well as DNA denaturation (Record *et al.*, 1990, 1991; Mascoti & Lohman, 1990; Lohman, 1985). It is this interpretation rather than the general formulation which is inconsistent with the conclusions of the present study.

In thermodynamic binding models, the free energy of binding is often written (Record *et al.*, 1976, 1991):

$$\Delta G^\circ_{obs} = \Delta G^\circ_0 + NRT \ln [M^+], \qquad (27)$$

where the standard free energy change, ΔG°_0, contains both electrostatic ion-pairing and nonelectrostatic binding contributions; and the salt dependent electrostatic terms are included entirely in the entropic $NRT \ln [M^+]$ term, where N is the number of "thermodynamically released counterions" (Record *et al.*, 1976, 1991). The notion that binding is driven by the entropic release of counterions rests on two underlying assumptions. First, it is assumed that the electrostatic free energy change of ion-pair formation with the ligand is small since similar interactions with thermodynamically released univalent ions are lost. That is, binding is viewed as a cation exchange process. Thus, the change in the electrostatic interaction of the released univalent ions with DNA is not included in the overall description of salt effects. Second, the change in the electrostatic interaction of the thermodynamically bound ions with the DNA is bulk salt independent. As a result, the variation of free energy with salt concentration, "the polyelectrolyte effect", is entirely given by a purely entropic term which goes as $NRT \ln [M^+]$ (Record *et al.*, 1991). These assumptions are essentially equivalent to those made in CC models (see above) which attribute salt effects to the entropic release of counterions from the condensed layer (Manning, 1978; Record *et al.*, 1976). Thus, although the release of "thermodynamically bound ions" is a more general concept than the release of condensed ions, in practice the same conclusions have been reached regarding an entropic driving force.

The reference state used in treatments that emphasize counterion release is very different from the one used in this work. The reference state for the electrostatic free energy in the NLPB treatment, like that in standard Debye–Hückel theory (McQuarrie, 1976), is the well-defined hypothetical state in which the solutes are completely discharged so that the electrostatic free energy is zero (Figure 2(a)). The electrostatic contribution to binding in this reference state is, of course, also zero. When binding takes place at zero salt, there will generally be a favorable coulombic interaction between DNA and an oppositely charged ligand; and an unfavorable interaction resulting from the desolvation of charges and dipoles that occurs upon binding. Depending on the relative magnitudes of these two effects, the total electrostatic free energy at zero salt, ΔG°_{ns}, may be either positive or negative. As salt is added to the system, ΔG°_{ns}, of course, remains unchanged, but there are additional salt dependent electrostatic forces given by ΔG°_s. Thus, the overall electrostatic free energy of binding represents the effect of both $\Delta\Delta G^\circ_{ns}$ and $\Delta\Delta G^\circ_s$. The solvation formalism developed here says nothing *a priori* as to whether electrostatics drive or oppose binding. However, $\Delta\Delta G^\circ_s$ will always oppose the coulombic term, so, for all the systems examined here, the net effect of salt is to oppose binding.

The reference state generally used in treatments that emphasize ion release is one where the concentration of univalent ions is 1 M (Record *et al.*, 1976, 1978, 1991). In this state, the entropic driving force for binding, $NRT \ln [M^+]$, is zero by convention (Record *et al.*, 1976, 1978, 1991). Since the electrostatic interactions included in the standard free energy change of binding, ΔG°_0 (including coulombic attractions between the DNA and its ligands as well as its counterions), are assumed to cancel, whatever binding is observed at 1 M is usually attributed to nonelectrostatic forces. The observation that ligand–DNA interactions are often weak at high salt ($\Delta G^\circ_{obs} \approx 0$ in eqn (27)) only implies that the combination of forces (both electrostatic and nonelectrostatic) driving binding are approximately cancelled by salt dependent terms that favor dissociation.

5. Conclusions

The NLPB and ion release models provide very different descriptions of the physical origins of salt effects on ligand–DNA interactions. The slope of the salt dependence of $-\ln K_{obs}$ represents changes in the free energy of interaction of small ions with the polyion (Timasheff, 1992; Anderson & Record, 1990; Wyman & Gill, 1990; Schellman, 1978). However, the breakdown of the free energy into entropic and enthalpic contributions depends on the model used to describe the electrostatic and nonelectrostatic free energies of the polyelectrolyte system.

The essential difference in the description of salt effects between the CC and NLPB models arises from their different descriptions of the radial distribution of small ions around the polyion. CC theory assumes that ions are distributed in two distinct populations around the DNA: a salt invariant condensed layer vicinal to the nucleic acid and a more diffuse, salt dependent ion atmosphere; in contrast, NLPB theory assumes that the ions are continuously distributed in a single population defined by a Boltzmann factor governed by the electrostatic potentials. As such, in NLPB theory, the concentration of ions at any point in space is fundamentally salt dependent. Consequently, in the NLPB model, salt dependent effects in nucleic acid equilibria include not only changes in the cratic entropy of ion organization, but also changes in the electrostatic ion–molecule and ion–ion interactions. These latter two terms can have significant enthalpic and entropic components. Thermodynamic binding models entail the same generality as the NLPB solvation model. However, these have usually been applied so as to emphasize the same type of entropic driving forces that arise in CC

theory (Mascotti & Lohman, 1992; Record *et al.*, 1991; Anderson & Record, 1982).

The NLPB model rigorously describes systems of finite dimensions without limiting law assumptions. This makes it possible to explicitly define and quantify each and every contribution to the electrostatic binding free energy. As a result, univalent ions are found to substantially destabilize charged ligand–DNA complexes at physiological salt concentrations due to a large unfavorable change in electrostatic ion–molecule interactions. That salt weakens binding at all concentrations is inconsistent with the concept of a dominant salt dependent force favoring binding.

The variation of the free energy of ligand–DNA binding is often thought to be a consequence of the changes in cratic entropy arising from the release of counterions from a bound layer (Mascotti & Lohman, 1992; Anderson & Record, 1982; Manning, 1978; Record *et al.*, 1976, 1978). The attribution of the salt dependence of ligand–DNA binding primarily to the entropy of ion release arises from a mechanistic interpretation of the thermodynamic contributions to ion–nucleic acid interactions based on the CC model. Experimental data on the binding of oligolysines to single-stranded RNA systems show that the salt dependence of the binding free energy can sometimes arise exclusively from the binding entropy (Mascotti & Lohman, 1992). However, the relevance of these studies, on highly flexible polyelectrolytes, to more rigid DNA systems has been questioned (Ray & Manning, 1992). For many DNA intercalating ligands, both the enthalpy and entropy of binding are observed to be strongly dependent on salt concentration (Chakraborty *et al.*, 1990; Barcelo *et al.*, 1988; Chaires, 1985; LePecq & Paoletti, 1967). Although the CC formalisms accurately describe the salt dependence of the binding free energy of these ligands to DNA, they cannot account for the observed salt dependence of both the enthalpy and the entropy of binding (Chaires, 1985). In contrast, since the NLPB model makes no *a priori* assumptions about the magnitude and salt dependence of the components of the free energy, the salt dependence of both the enthalpy and the entropy of binding observed for some drugs is not inconsistent with the NLPB model. An experimental evaluation of these theories will require a careful evaluation of the salt dependence of both the enthalpy and entropy of binding for minor groove binding ligands and proteins to double stranded DNA.

Although the total entropy of ligand–DNA binding is often positive, this does not imply that the salt dependent free energy of binding is determined by the cratic entropy. The positive entropy of binding in aqueous solution reflects the cumulative contributions of many effects, including: changes in water structure, changes in ion distribution, entropic contributions to electrostatic interactions, hydrogen bonding, van der Waals interactions, and changes in conformational and vibrational entropy. The large variation observed in the binding entropy for systems with similar salt dependencies (Breslauer *et al.*, 1987) suggests that the salt dependent free energy is not the only factor that contributes to the observed positive binding entropy. Indeed, the hydrophobic interaction has been shown to be quite important for complexation of several minor groove binding ligands (Ding & Ellestad, 1991; Boger *et al.*, 1990) and proteins (Lundback *et al.*, 1993; Ha *et al.*, 1989) to DNA. The relative contribution of each of these effects to the entropy of binding remains to be determined.

We thank Mr Jonathan Hecht and Drs An-Suei Yang and Gerald Manning for many stimulating and insightful discussions. In addition, we thank Drs Timothy Lohman and Martin Zacharias for helpful discussions; Dr Richard E. Dickerson for providing the coordinates of the DAPI–DNA complex; and the referees for their comments on an earlier version of this manuscript. This work was supported by the NIH (GM-41371) and the NSF (MCB-92-20477).

References

Abola, E. E., Bernstein, F. C., Bryant, S. H., Koetzle, T. F. & Weng, J. (1987). Protein data bank. In *Crystallographic Databases - Information Content, Software Systems, Scientific Applications* (Allen, F. H., Bergerhoff, G. & Sievers, R., eds), pp. 107–132, Data Commission of the International Union of Crystallography, Bonn/Cambridge/Chester.

Alexandrowicz, Z. & Katchalsky, A. (1963). Colligative properties of polyelectrolyte solutions in excess of salt. *J. Polymer. Sci. (A)*, **1**, 3231–3260.

Alfrey, T. J., Berg, P. W. & Morawetz, H. (1951). The counterion distribution in solutions of rod-shaped electrolytes. *J. Polymer Sci.* **7**, 543–547.

Anderson, C. F. & Record, M. T. (1980). The relationship between the Poisson–Boltzmann model and the condensation hypothesis: an analysis based on the low salt form of the Donnan coefficient. *Biophys. Chem.* **11**, 353–360.

Anderson, C. F. & Record, M. T. (1982). Polyelectrolyte theories and their applications to DNA. *Annu. Rev. Phys. Chem.* **33**, 191–222.

Anderson, C. F. & Record, M. T. (1983). The thermodynamic effects of polyelectrolyte–electrolyte interactions. In *Structure and Dynamics: Nucleic Acids and Proteins* (Clementi, E. & Ramaswamy, H. S., eds), pp. 301–318, Adenine Press, New York.

Anderson, C. F. & Record, M. T. (1990). Ion distributions around DNA and other cylindrical polyions: theoretical descriptions and physical implications. *Annu. Rev. Biophys. Biophys. Chem.* **19**, 423–465.

Anderson, C. F. & Record, M. T. (1993). Salt dependence of oligoion–polyion binding: a thermodynamic description based on preferential interaction coefficients. *J. Phys. Chem.* **97**, 7116–7126.

Anderson, C. F., Record, M. T. & Hart, P. A. (1978). Sodium-23 NMR studies of cation–DNA interactions. *Biophys. Chem.* **7**, 301–316.

Arnott, C. F. & Hukins, D. W. (1972). Optimised parameters for *A*-DNA and *B*-DNA. *Biochem. Biophys. Res. Commun.* **47**, 1504–1509.

Bacquet, R. J. & Rossky, P. J. (1988). Ionic distributions and competitive association in DNA/mixed salt solutions. *J. Phys. Chem.* **92**, 3604–3612.

Barcelo, F., Martorell, J., Gavilanes, F. & Gonazlez-Ros, J. M. (1988). Equilibrium binding of daunomycin and adriamycin to calf thymus DNA: temperature and ionic strength dependence of thermodynamic parameters. *Biochem. Pharmacol.* **37**, 2133–2138.

Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. F., Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). The protein data bank: a computer-based archival file for macromolecular structures. *J. Mol. Biol.* **112**, 535–542.

Bleam, M. L., Anderson, C. F. & Record, M. T. (1983). Sodium-23 nuclear magnetic resonance studies of cation-deoxyribonucleic acid interactions. *Biochemistry*, **22**, 5418–5425.

Bockris, J. O. & Reddy, A. K. N. (1970). *Modern Electrochemistry. I.* Plenum Press, New York.

Boger, D. L., Invergo, B. J., Coleman, R. S., Zarrinmayer, H., Kitos, P. A., Thompson, S. A., Leong, T. & McLaughlin, L. W. (1990). A demonstration of the intrinsic importance of stabilizing hydrophobic binding and non-covalent van der Waals contacts dominant in the non-covalent CC-1065/B–DNA binding. *Chem.-Biol. Interact.* **73**, 29–52.

Braunlin, W. H., Strick, T. J. & Record, M. T. (1982). Equilibrium dialysis studies of polyamine binding to DNA. *Biopolymers*, **21**, 1301–1314.

Brennan, R. G. (1991). Interactions of the helix-turn-helix binding domain. *Curr. Opin. Struct. Biol.*. **1**, 80–88.

Breslauer, K. J., Remeta, D. P., Chou, W.-Y., Ferrante, R., Curry, J., Zaunczowski, D., Snyder, J. G. & Marky, L. A. (1987). Enthalpy–entropy compensations in drug–DNA binding studies. *Proc. Nat. Acad. Sci., U.S.A.* **84**, 8922–8926.

Brown, D. G., Sanderson, M. R., Skelly, J. V., Jenkins, T. C., Brown, T., Garman, E., Stuart, D. I. & Neidle, S. (1990). Crystal structure of a berenil–dodecanucleotide complex: the role of water in sequence-specific ligand binding. *EMBO J.* **9**, 1329–1334.

Chaires, J. B. (1985). Thermodynamics of the daunomycin–DNA interaction: ionic strength dependence of the enthalpy and entropy. *Biopolymers*, **24**, 403–419.

Chakraborty, S., Nandi, R. & Maiti, M. (1990). Thermodynamics of the interaction of aristololactam-β-D-glucoside with DNA: ionic strength dependence of enthalpy and entropy. *Biochem. Pharmacol.* **39**, 1181–1186.

Coll, M., Frederick, C. A., Wang, A. H.-J. & Rich, A. (1987). A bifurcated hydrogen-bonded conformation in the d(A·T) base pairs of the DNA dodecamer d(CGCAAATTTGCG) and its complex with distamycin. *Proc. Nat. Acad. Sci. U.S.A.* **84**, 8385–8389.

Coll, M., Ayomi, J., van der Marel, G. A., Van Boom, J. H., Rich, A. & Wang, A. H. J. (1989). Molecular structure of the netropsin-d(CGCGATATCGCG) complex: DNA conformation in an alternating AT segment. *Biochemistry*, **28**, 310–320.

deHaseth, P. L., Lohman, T. M. & Record, M. T. (1977). Nonspecific interaction of *lac* repressor with DNA: an association reaction driven by counterion release. *Biochemistry*, **16**, 4783–4790.

Ding, W.-D. & Ellestad, G. A. (1991). Evidence for hydrophobic interaction between calichaemacin and DNA. *J. Amer. Chem. Soc.* **113**, 6617–6620.

Drew, H. R., Wing, R. M., Takano, T., Broka, C., Tanaka, S., Itakura, K. & Dickerson, R. E. (1981). Structure of a *B*-DNA dodecamer: conformation and dynamics. *Proc. Nat. Acad. Sci., U.S.A.* **78**, 2179–2183.

Fixman, M. (1979). The Poisson–Boltzmann equation and its application to polyelectrolytes. *J. Chem. Phys.* **70**, 4995–5005.

Frederick, C. A., Williams, L. D., Ughetto, B., van der Marel, G. A., van Boom, J. H., Rich, A. & Wang, A. H.-J. (1990). Structural comparison of anticancer drug–DNA complexes. Adriamycin and daunomycin. *Biochemistry*, **29**, 2538–2549.

Friedman, R. A. & Manning, G. S. (1984). Polyelectrolyte effects on site-binding equilibria with application to the intercalation of drugs into DNA. *Biopolymers*, **23**, 2671–2714.

Friedman, R. A., Manning, G. S. & Shahin, M. A. (1990). The polyelectrolyte correction to site-exclusion numbers in drug–DNA binding. In *Chemistry and Physics of DNA–Ligand Interactions* (Kallenbach, N. R., ed.), pp. 37–64, Adenine Press, New York.

Fuoss, R. M., Katchalsky, A. & Lifson, S. (1951). The potential of an infinite rod-like molecule and the distribution of counter ions. *Proc. Nat. Acad. Sci., U.S.A.* **37**, 579–589.

Gilson, M. K. & Honig, B. (1988). Calculation of the total electrostatic energy of a macromolecular system: solvation energies, binding energies, and conformational analysis. *Proteins: Struct. Funct. Genet.* **4**, 7–18.

Gilson, M. K., Rashin, A., Fine, R. & Honig, B. (1985). On the calculation of electrostatic interactions in proteins. *J. Mol. Biol.* **183**, 503–516.

Gilson, M. K., Sharp, K. A. & Honig, B. H. (1988). Calculating the electrostatic potential of molecules in solution: method and error assessment. *J. Comput. Chem.* **9**, 327–335.

Gross, L. M. & Strauss, U. (1966). In *Chemical Physics of Ionic Solutions* (Conway, B. E. & Barradas, R. G., eds), pp. 361–389, John Wiley and Sons, Inc., New York.

Gueron, M. & Demaret, J.-P. (1992). A simple explanation of the electrostatics of the *B*-to-*Z* transition of DNA. *Proc. Nat. Acad. Sci., U.S.A.* **89**, 5740–5743.

Gueron, M. & Weisbuch, G. (1980). Polyelectrolyte theory. I. Counterion accumulation, site-binding, and their insensitivity to polyelectrolyte shape in solutions containing finite salt concentrations. *Biopolymers*, **19**, 353–382.

Ha, J.-H., Spolar, R. S. & Record, M. T. (1989). Role of the hydrophobic effect instability of site-specific protein–DNA complexes. *J. Mol. Biol.* **209**, 801–816.

Hagler, A. T., Stern, P. S., Sharon, R., Becker, J. M. & Naider, F. (1979). Computer simulation of the conformational properties of oligopeptides. Comparison of theoretical methods and analysis of experimental results. *J. Amer. Chem. Soc.* **101**, 6842–6852.

Harrison, S. C. (1991). A structural taxonomy of DNA-binding domains. *Nature (London)*, **353**, 715–719.

Harrison, S. C. & Aggarwal, A. K. (1990). DNA recognition by proteins with the helix-turn-helix motif. *Annu. Rev. Biochem.* **59**, 933–969.

Harvey, S. (1988). Treatment of electrostatic effects in macromolecular modeling. *Proteins: Struct. Funct. Genet.* **5**, 78–92.

Imai, N. & Onishi, T. (1959). Analytical solution of Poisson–Boltzmann equation for two-dimensional many-center problem. *J. Chem. Phys.* **30**, 1115–1116.

Jayaram, B., Sharp, K. A. & Honig, B. (1989). The electrostatic potential of DNA. *Biopolymers*, **28**, 975–993.

Jayaram, B., Swaminathan, S., Beveridge, D. L., Sharp, K. A. & Honig, B. (1990). Monte Carlo simulation studies on the structure of the counterion atmosphere

of *B*-DNA. Variations on the primitive dielectric model. *Macromolecules*, **23**, 3156–3165.

Jorgensen, W. L. & Tirado-Rives, J. (1988). The OPLS potential functions for proteins. Energy minimizations for crystals of cyclic peptides and crambin. *J. Amer. Chem. Soc.* **110**, 1657–1666.

Katoh, T. & Ohtsuki, T. (1982). End effects for a rodlike polyelectrolyte molecule in salt solution. *J. Polymer. Sci. Polymer Phys. Ed.* **20**, 2167–2175.

Kennard, O. & Hunter, W. N. (1989). Oligonucleotide structure: a decade of results from single crystal X-ray diffraction studies. *Quart. Rev. Biophys.* **22**, 327–379.

Klapper, I., Hagstrom, R., Fine, R., Sharp, K. & Honig, B. (1986). Focusing of electric fields in the active site of CuZn superoxide dismutase: effects of ionic strength and amino acid modification. *Proteins: Struct. Funct. Genet.* **1**, 47–59.

Klein, B. K., Anderson, C. F. & Record, M. T. (1981). Comparison of Poisson–Boltzmann and condensation model expressions for the colligative properties of cylindrical polyions. *Biopolymers*, **20**, 2263–2280.

Koblan, K. S. & Ackers, G. K. (1991). Cooperative protein–DNA interactions: effects of KCl on lambda cI binding to OR. *Biochemistry*, **30**, 7822–7827.

Larsen, T. A., Goodsel, D. S., Cascio, D., Grzeskowiak, K. & Dickerson, R. E. (1989). The structure of DAPI bound to DNA. *J. Biomol. Struct. Dynam.* **7**, 477–491.

LeBret, M. & Zimm, B. H. (1984). Distributions of counterions around a cylindrical polyelectrolyte and Manning's condensation theory. *Biopolymers*, **23**, 287–312.

LePecq, J. B. & Paoletti, C. (1967). A fluorescent complex between ethidium bromide and nucleic acids. *J. Mol. Biol.* **27**, 87–106.

Leyte, J. C. (1990). Nuclear magnetic relaxation in polyelectrolyte solutions. *Makromol. Chem. Macromol. Symp.* **34**, 81–111.

Lohman, T. M. (1985). Kinetics of protein–nucleic acid interactions: use of salt effects to probe mechanisms of interaction. *CRC Crit. Rev. Biochem.* **19**, 191–245.

Lohman, T. M., deHaseth, P. L. & Record, M. T. (1980). Pentalysine–deoxyribonucleic acid interactions: a model for the general effects of ion concentrations on the interactions of proteins with nucleic acids. *Biochemistry*, **19**, 3522–3530.

Loontiens, F. G., Regenfuss, P., Zechel, A. & Clegg, R. M. (1989). Binding properties in solution of Hoechst 33258 and Dapi with CCGGAATTCCGG, poly[d(A−T)] and other DNAs. *Arch. Int. Physiol. Biochem.* **97**, B105.

Loontiens, F. G., Regenfuss, P., Zechel, A., Dumortier, L. & Clegg, R. M. (1990). Binding characteristics of Hoechst 33258 with calf thymus DNA, poly[d(A−T)], and d(CCGGAATTCCGG): multiple stoichiometries and determination of tight binding with a wide spectrum of site affinities. *Biochemistry*, **29**, 9029–9039.

Lundback, T., Cairns, C., Gustafsson, J.-A., Carlstedt-Duke, J. & Hard, T. (1993). Thermodynamics of the glucocorticoid receptor–DNA interaction: binding of wild-type GR DBD to different response elements. *Biochemistry*, **32**, 5074–5082.

MacGillivray, A. D. (1972*a*). Analytic description of the condensation phenomenon near the limit of infinite dilution based on the Poisson–Boltzmann equation. *J. Chem. Phys.* **56**, 83–85.

MacGillivray, A. D. (1972*b*). Bounds on solutions of the Poisson–Boltzmann equation near infinite dilution – the moderately charged case. *J. Chem. Phys.* **57**, 4075–4078.

MacGillivray, A. D. (1972*c*). Lower bounds on solutions of the Poisson–Boltzmann equation near the limit of infinite dilution. *J. Chem. Phys.* **57**, 4071–4075.

MacGillivray, A. D. (1972*d*). Upper bounds on solutions of the Poisson–Boltzmann equation near the limit of infinite dilution. *J. Chem. Phys.* **56**, 80–83.

Manning, G. S. (1969*a*). Limiting laws and counterion condensation in polyelectrolyte solution. I. Colligative properties. *J. Chem. Phys.* **51**, 924–933.

Manning, G. S. (1969*b*). Limiting laws and counterion condensation in polyelectrolyte solutions. III. An analysis based on the Mayer ionic solution theory. *J. Chem. Phys.* **51**, 3249–3252.

Manning, G. S. (1977). Limiting laws and counterion condensation in polyelectrolyte solutions. IV. The approach to the limit and the extraordinary stability of the charge fraction. *Biophys. Chem.* **7**, 95–102.

Manning, G. S. (1978). The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Quart. Rev. Biophys.* **11**, 179–246.

Manning, G. S. (1979). Counterion binding in polyelectrolyte theory. *Acc. Chem. Res.* **12**, 443–449.

Manning, G. S. (1981). Limiting laws and counterion condensation in polyelectrolyte solutions. 6. Theory of the titration curve. *J. Phys. Chem.* **85**, 870–877.

Manning, G. S. & Holtzer, A. (1973). Application of polyelectrolyte limiting laws to potentiometric titration. *J. Phys. Chem.* **77**, 2206–2211.

Manning, G. S. & Zimm, B. H. (1965). Cluster theory of polyelectrolyte solutions. I. Activity coefficients of the mobile ions. *J. Chem. Phys.* **43**, 4250–4259.

Marcus, R. A. (1955). Calculation of thermodynamic properties of polyelectrolytes. *J. Chem. Phys.* **23**, 1057–1068.

Marky, L. A. & Breslauer, K. J. (1987). Origins of netropsin binding affinity and specificity: correlations of thermodynamic and structural data. *Proc. Nat. Acad. Sci., U.S.A.* **84**, 4359–4363.

Mascotti, D. P. & Lohman, T. M. (1990). Thermodynamic extent of counterion release upon binding oligolysines to single-stranded nucleic acids. *Proc. Nat. Acad. Sci., U.S.A.* **87**, 3142–3146.

Mascotti, D. P. & Lohman, T. M. (1992). Thermodynamics of single-stranded RNA binding to oligolysines containing tryptophan. *Biochemistry*, **31**, 8932–8946.

McQuarrie, D. A. (1976). *Statistical Mechanics*. Harper & Row, Publishers, Inc., New York.

Misra, V. K., Hecht, J. L., Sharp, K. A., Friedman, R. A. & Honig, B. (1994). Salt effects on protein–DNA interactions: the λcI repressor and *Eco*RI endonuclease. *J. Mol. Biol.* **238**, 264–280.

Murthy, C. S., Bacquet, R. J. & Rossky, P. J. (1985). Ionic distributions near polyelectrolytes. A comparison of theoretical approaches. *J. Phys. Chem.* **89**, 701–710.

Nicholls, A. & Honig, B. (1991). A rapid finite difference algorithm, utilizing successive over-relaxation to solve the Poisson–Boltzmann equation. *J. Comp. Chem.* **12**, 435–445.

Nicholls, A., Sharp, K. A. & Honig, B. (1990). Delphi 3.0. Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York.

Ohnishi, T. (1963). Properties of double-stranded DNA as a polyelectrolyte. *Biophys. J.* **3**, 459–468.

Ohnishi, T., Imai, N. & Oosawa, F. (1960). Interaction

between rod-like polyelectrolytes. *J. Phys. Soc. Japan*, **15**, 896–905.

Olmsted, M. (1991). Grand canonical Monte Carlo analysis of the thermodynamics of processes involving oligomeric and polymeric DNA. Ph.D. thesis, University of Wisconsin, Madison.

Olmsted, M. C., Anderson, C. F. & Record, M. T. (1989). Monte Carlo description of oligoelectrolyte properties of DNA oligomers: range of the end effect and the approach of molecular and thermodynamic properties to the polyelectrolyte limits. *Proc. Nat. Acad. Sci., U.S.A.* **86**, 7766–7770.

Olmsted, M. C., Anderson, C. F. & Record, M. T. (1991). Importance of oligoelectrolyte end effects for the thermodynamics of conformational transitions of nucleic acid oligomers: a grand canonical Monte Carlo analysis. *Biopolymers*, **31**, 1593–1604.

Oosawa, F. (1971). *Polyelectrolytes*. Marcel Dekker, New York.

Pabo, C. O. & Sauer, R. T. (1984). Protein–DNA recognition. *Annu. Rev. Biochem.* **53**, 293–321.

Phillips, S. E. V. (1991). Specific β-sheet interactions. *Curr. Opin. Struct. Biol.* **1**, 89–98.

Pjura, P. E., Grzeskowiak, K. & Dickerson, R. E. (1987). Binding of Hoechst 33258 to the minor groove of *B*-DNA. *J. Mol. Biol.* **197**, 257–271.

Pranata, J., Wierschke, S. G. & Jorgensen, W. L. (1991). OPLS potential functions for nucleotide bases. Relative association constants of hydrogen-bonded base pairs in chloroform. *J. Amer. Chem. Soc.* **113**, 2810–2819.

Quintana, J. R., Lipanov, A. A. & Dickerson, R. E. (1991). Low-temperature crystallographic analyses of the binding of Hoechst 33258 to the double-helical DNA dodecamer C-G-C-G-A-A-T-T-C-G-C-G. *Biochemistry*, **30**, 10294–10306.

Ray, J. & Manning, G. S. (1992). Theory of delocalized ionic binding to polynucleotides: structural and excluded-volume effects. *Biopolymers*, **32**, 541–549.

Record, M. T. & Lohman, T. M. (1978). A semiempirical extension of polyelectrolyte theory to the treatment of oligoelectrolytes: application to oligonucleotide helix–coil transitions. *Biopolymers*, **17**, 159–166.

Record, M. T., Lohman, T. M. & deHaseth, P. (1976). Ion effects on ligand–nucleic acid interactions. *J. Mol. Biol.* **107**, 145–158.

Record, M. T., Anderson, C. F. & Lohman, T. M. (1978). Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. *Quart. Rev. Biophys.* **11**, 103–178.

Record, M. T., Mazur, S. J., Melancon, P., Roe, J.-H., Shaner, S. L. & Unger, L. (1981). Double helical DNA: conformations, physical properties, and interactions with ligands. *Annu. Rev. Biochem.* **50**, 997–1024.

Record, M. T., Anderson, C. F., Mills, P., Mossing, M. & Roe, J.-H. (1985). Ions as regulators of protein–nucleic acid interactions *in vitro* and *in vivo*. *Advan. Biophys.* **20**, 109–135.

Record, M. T., Olmsted, M. & Anderson, C. F. (1990). Theoretical studies of the thermodynamic consequences of interaction of ions with polymeric and oligomeric DNA: the preferential interaction coefficient and its application to the thermodynamic analysis of electrolyte effects and ligand binding. In *Theoretical Chemistry and Molecular Biophysics* (Beveridge, D. L. & Lavery, R., eds), pp. 285–308, Adenine, Schenectady.

Record, M. T., Ha, J.-H. & Fisher, M. A. (1991). Analysis of equilibrium and kinetic measurements to determine thermodynamic origins of stability and specificity and mechanism of formation of site specific complexes between proteins and helical DNA. *Methods Enzymol.* **208**, 291–343.

Reiner, E. S. & Radke, C. J. (1990). Variational approach to the electrostatic free energy in charged colloidal suspensions: general theory for open systems. *J. Chem. Soc. Faraday Trans.* **86**, 3901–3912.

Rosenberg, J. M. (1991). Structure and function of restriction endonucleases. *Curr. Opin. Struct. Biol.* **1**, 104–113.

Schellman, J. A. (1978). Solvent denaturation. *Biopolymers*, **17**, 1305–1322.

Senear, D. F. & Batey, R. (1991). Comparison of operator-specific and nonspecific DNA binding of the lambda cI repressor: [KCl] and pH effects. *Biochemistry*, **30**, 6677–6688.

Sharp, K. A. & Honig, B. (1990*a*). Calculating total electrostatic energies with the nonlinear Poisson–Boltzmann equation. *J. Phys. Chem.* **94**, 7684–7692.

Sharp, K. A. & Honig, B. (1990*b*). Electrostatic interactions in macromolecules: theory and applications. *Annu. Rev. Biophys. Biophys. Chem.* **19**, 301–332.

Sharp, K. A., Honig, B. & Harvey, S. C. (1990). Electrical potential of transfer RNAs: codon-anticodon recognition. *Biochemistry*, **29**, 340–346.

Soumpasis, D.-M. (1984). Statistical mechanics of the $B \rightarrow Z$ transition of DNA: contributions of diffuse ionic interactions. *Proc. Nat. Acad. Sci., U.S.A.* **81**, 5116–5120.

Sriram, M., van der Marel, G. A., Roelen, H. L. P. F., van Boom, J. & Wang, A. H.-J. (1992). Structural consequences of a carcinogenic alkylation lesion on DNA: effect of O^6-ethylguanine on the molecular structure of the d(CGC[e^6G]AATTCGCG)-netropsin complex. *Biochemistry*, **31**, 11823–11834.

Steitz, T. A. (1990). Structural studies of protein–nucleic acid interaction: the sources of sequence specific binding. *Quart. Rev. Biophys.* **23**, 205–280.

Svensson, B., Jonsson, B. & Woodward, C. E. (1990). Monte Carlo simulations of an electric double layer. *J. Phys. Chem.* **94**, 2105–2113.

Teng, M.-K., Usman, N., Frederick, C. A. & Wang, A. H.-J. (1988). The molecular structure of the complex of Hoechst 33258 and the DNA dodecamer d(CGCGAATTCGCG). *Nucl. Acids Res.* **16**, 2671–2690.

Terry, B. J., Jack, W. E., Rubin, R. A. & Modrich, P. (1983). Thermodynamic parameters governing interaction of *Eco*RI endonuclease with specific and nonspecific DNA sequences. *J. Biol. Chem.* **258**, 9820–9825.

Timasheff, S. N. (1992). Water as ligand: preferential binding and exclusion of denaturants in protein unfolding. *Biochemistry*, **31**, 9857–9864.

Verwey, E. J. W. & Overbeek, J. G. (1948). *Theory of the Stability of Lyophopic Colloids*. Elsevier, Amsterdam.

Warwicker, J. & Watson, H. C. (1982). Calculation of the electric potential in the active site cleft due to alpha-helix dipoles. *J. Mol. Biol.* **157**, 671–679.

Weiner, S. J., Kollman, P. A., Nguyen, D. T. & Case, D. A. (1986). An all atom force field for simulations of proteins and nucleic acids. *J. Comput. Chem.* **7**, 230–252.

Wilson, R. W., Rau, D. C. & Bloomfield, V. A. (1980). Comparison of polyelectrolyte theories of the binding of cations to DNA. *Biophys. J.* **30**, 317–326.

Wilson, W. D., Tanious, F. A., Barton, H. J., Jones,

R. L., Fox, K., Wydra, R. L. & Strekowski, L. (1990). DNA sequence dependent binding modes of 4′,6-diamidino-2-phenylindole (DAPI). *Biochemistry*, **29**, 8452–8461.

Wyman, J. & Gill, S. J. (1990). *Binding and Linkage. Functional Chemistry of Biological Macromolecules*. University Science Books, Mill Valley.

Zimm, B. H. & LeBret, M. (1983). Counter-ion condensation and system dimensionality. *J. Biomol. Struct. Dynam.* **1**, 461–471.

Edited by P. von Hippel

(Received 6 July 1993; accepted 20 January 1994)