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Conformational Transitions of Duplex and Triplex Nucleic Acid Helices: Thermodynamic Analysis of Effects of Salt Concentration on Stability Using Preferential Interaction Coefficients

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ABSTRACT For order-disorder transitions of double- and triple-stranded nucleic acid helices, the midpoint temperatures T_m depend strongly on $a_\pm$, the mean ionic activity of uniunivalent salt. Experimental determinations of $\mathrm{d}T_m/\mathrm{d}\ln a_\pm$ and of the enthalpy change (ΔH°) accompanying the transition in excess salt permit evaluation of $\Delta\Gamma$, the stoichiometrically weighted combination of preferential interaction coefficients, each of which reflects thermodynamic effects of interactions of salt ions with a reactant or product of the conformational transition

$$\Delta\Gamma = -\left(\frac{\Delta H^\circ}{2RT_m^2}\right)\frac{\mathrm{d}T_m}{\mathrm{d}\ln a_\pm} = \frac{\Delta H^\circ}{2R}\frac{\mathrm{d}(T_m^{-1})}{\mathrm{d}\ln a_\pm}. \tag{1}$$

Here ΔH° is defined per mole of nucleotide by analogy to $\Delta\Gamma$. Application of Eq. 1 to experimental values of ΔH° and T_m yields values of $\Delta\Gamma$ for the denaturation of B-DNA over the range of NaCl concentrations 0.01–0.20 M (Privalov et al. (1969), Biopolymers 8, 559) and for each of four order-disorder transitions of poly rA·(poly rU)$_n$, $n = 1, 2$ over the range of NaCl concentrations 0.01–1.0 M (Krakauer and Sturtevant (1968), Biopolymers 6, 491). For denaturation of duplexes and triplexes, $\Delta\Gamma$ is negative and not significantly dependent on $a_\pm$, but $\Delta\Gamma$ is positive and dependent on $a_\pm$ for the disproportionation transition of poly rA·poly rU duplexes. Quantitative interpretations of these trends and magnitudes of $\Delta\Gamma$ in terms of coulombic and excluded volume effects are obtained by fitting separately each of the two sets of thermodynamic data using Eq. 1 with $\Delta\Gamma^{PB}$ evaluated from the cylindrically symmetric Poisson-Boltzmann (PB) equation for a standard model of salt-polyelectrolyte solutions. The only structural parameters required by this model are: b, the mean axial distance between the projections of adjacent polyion charges onto the cylindrical axis; and a, the mean distance of closest approach between a salt ion center and the cylindrical axis. Fixing b_{MS} and a_{MS} for the multi-stranded (ordered) conformations, we determined the corresponding best fitted values of b_{SS} and a_{SS} for single-stranded RNA and DNA. The resulting best fitted values of a_{SS} are systematically less than a_{DS} by 2–4 Å. Uncertainty in the best-fitted values of b_{SS} is significantly lower than in the a_{SS}, because b_{MS} is known with relatively high precision and because the larger uncertainty in a_{MS} has a relatively small effect on the best-fitted values of b_{SS}: $b_{SS} = 3.2 \pm 0.6$ Å for single-stranded poly rA and poly rU; and $b_{SS} = 3.4 \pm 0.2$ Å for single-stranded DNA. These values are approximately one-half of those expected for a fully extended single-stranded conformation. With the best fitted values of a_{SS} and b_{SS}, our calculations of $\Delta\Gamma^{PB}$ are in close quantitative agreement with experimental observations on each of five nucleic acid order-disorder transitions.

INTRODUCTION

Conformational and ligand-binding equilibria of nucleic acids are in general strongly dependent on salt activity ($a_\pm$) and concentration (C_3). The effects of $a_\pm$ on these equilibria can be analyzed in terms of various theoretical models that quantify the contribution of coulombic interactions to the nonideality of the reactant(s) and product(s). The prevalent polyelectrolyte theories (reviewed by Anderson and Record, 1982, 1990) predict that the average density of structural charges projected onto the axis of a rod-like (cylindrical) polyion generally is the single most important parameter that determines the dependence on $a_\pm$ of the nonideality resulting from coulombic interactions of salt ions with the polyion. Consequently, any significant alteration in the charge density resulting from a conformational transition or binding of a charged ligand is expected to give rise to an effect of $a_\pm$ on the corresponding equilibrium, which can be quantified by measuring T_m (the midpoint temperature of an order-disorder transition) or K_{obs} (the observed equilibrium constant defined in terms of the concentrations of reactant(s) and product(s)).

Order-disorder transitions of multi-stranded nucleic acids are accompanied by large changes in axial charge density resulting from strand separation, and in addition may involve changes in the average axial charge densities of the individual strands. These transitions have been characterized using calorimetric determinations of the transition enthalpy (ΔH°), and calorimetric and spectroscopic determinations of T_m as a function of $a_\pm$ (cf. Krakauer and Sturtevant, 1968; Privalov et al., 1969; Gruenwedel, 1974; Monaselidze and Mgeladze, 1977; Filimonov and Privalov, 1978; Hopkins et al., 1993a). Representative data sets pertaining to natural polymeric DNA (Privalov et al., 1969) and synthetic polynucleotides (Krakauer and Sturtevant, 1968) are analyzed here. More recent studies of the stabilities of triple helical structures comprising oligoions have been reported by Jin et al. (1993), Hopkins et al. (1993a, b), and

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Singleton and Dervan (1992). These will be the subject of future analysis.

Previously, Manning (1972, 1976) and Record et al. (1975, 1976) analyzed thermodynamic data pertaining to the effects of $a_{\pm}$ on order-disorder transitions of nucleic acid helices in terms of analytic expressions deduced from Manning's original (limiting-law) version of counterion condensation (CC) theory (Manning, 1969). According to this approach, the average axial inter-charge distances (b) of the ordered and disordered states are the *sole structural* determinants of the observed dependence of T_m on $a_{\pm}$ for an order-disorder transition of a cylindrical polyion. The analytic expressions for thermodynamic coefficients that have been deduced from the CC hypothesis were subsequently obtained by Anderson and Record (1983) from the Poisson-Boltzmann (PB) cell model (Marcus, 1955) under conditions of high dilution in all components but without the hypothesis of counterion condensation. More generally, the predictions of the PB cell model do not substantiate the validity of Manning's limiting laws under typical experimental conditions. Specifically, in the experimental range of $a_{\pm}$ where order-disorder transitions of nucleic acids generally are investigated (0.01–1.0 M), the PB cell model predicts that the thermodynamic coefficients pertaining to the individual ordered and disordered states exhibit significant C_3-dependent deviations from the corresponding "limiting law" values. Thus, only if these individual dependences on C_3 effectively cancel could calculations based on the PB equation account for experimental observations that T_m is a linear function of $\ln a_{\pm}$ for various (but not all) nucleic acid order-disorder transitions (Krakauer and Sturtevant, 1968; Privalov et al., 1969).

As an alternative to limiting law expressions, in this paper we use solutions of the cylindrical PB equation to analyze changes in preferential interaction coefficients that are evaluated from experimental measurements of T_m and $\Delta H°$ as functions of $a_{\pm}$. Specifically, we calculate the relevant preferential interaction coefficients for a "standard model" of a polyelectrolyte-electrolyte solution in which the polyion (in the ordered or disordered state) is characterized by only two structural quantities: b, the average spacing between polyion charges projected onto the axis of the cylindrical polyion and a, the distance of closest approach between the center of a small ion and the polyion axis. (Anderson and Record (1990) discuss the standard model assumptions and the conditions where they are valid.)

In the following section, we summarize the experimental results reported by Privalov et al. (1969) and by Krakauer and Sturtevant (1968), and provide background on the use of preferential interaction coefficients to analyze the effect of $a_{\pm}$ on polynucleotide order-disorder transitions. We then describe the model of the polyelectrolyte-salt solution implied by our use of the standard cylindrical PB equation, and describe the numerical methods used to analyze data and fit them using our PB calculations. Next, we present the results of our analyses of experimental data using preferential interaction coefficients calculated by the PB equation. In the concluding section, the physical implications of our results are discussed in the context of previously published analyses of calorimetric and spectroscopic experimental data.

EXPERIMENTAL AND THEORETICAL BACKGROUND

Determination of $\Delta H°$ and T_m for T2 (B form) DNA denaturation

Privalov et al. (1969) used differential scanning calorimetry to measure $\Delta H°$ and T_m for denaturation of B-DNA at six NaCl concentrations in the range 0.011–0.205 M, all in excess of the variable concentrations (0.6–2 mM) of T2 phage DNA. The mean ionic activities ($a_{\pm}$) of the electrolyte corresponding to the reported concentrations of NaCl and temperatures were interpolated by Privalov et al. (1969) from tabulated activity coefficients. The original data are reproduced as part of Table 1, where the transition enthalpy, $\Delta H°$, is expressed in units of cal (mol nucleotide monomer)$^{-1}$. Over the range investigated, both T_m and T_m^{-1} appear to be linear in $\ln a_{\pm}$ (cf. Fig. 1 below).

Determination of $\Delta H°$ and T_m for order-disorder transitions of complexes of poly rA and poly rU

In solution poly rA and poly rU can form a stable duplex, designated poly rA·poly rU or AU, and/or a stable triplex, designated poly rA·(poly rU)$_2$ or AU$_2$. The stabilities of the duplex and triplex species with respect to various less ordered states are determined by the $a_{\pm}$ and temperature of the solution. Krakauer and Sturtevant (1968) constructed a "phase diagram" (displaying T_m as a function of $\ln a_{\pm}$ for all four transitions) using calorimetric and spectroscopic measurements of T_m. Krakauer and Sturtevant (1968) measured T_m and $\Delta H°$ calorimetrically for the transitions shown in Table 2. Measurements were made at three NaCl concentrations for the denaturation of poly rA·poly rU, and at one

TABLE 1 Determination of $\Delta\Gamma$ for DNA denaturation from the data of Privalov et al. (1969)

C_3 (M)	$\ln a_{\pm}$	$\Delta H°$ (cal/mole base)	$\Delta\Gamma_a$*	$\Delta\Gamma_b$	$\Delta\Gamma_c$	$\Delta\Gamma_d$
0.205	−1.94	4830	−0.0789 (0.0035)	0.0838 (0.0037)	−0.0807 (0.0168)	0.0808 (0.0173)
0.115	−2.44	4710	−0.0786 (0.0034)	0.0818 (0.0036)	−0.0797 (0.0108)	0.0800 (0.0109)
0.057	−3.11	4640	−0.0802 (0.0035)	0.0806 (0.0035)	−0.0804 (0.0047)	0.0804 (0.0047)
0.036	−3.52	4570	−0.0806 (0.0035)	0.0794 (0.0035)	−0.0802 (0.0059)	0.0801 (0.0057)
0.014	−4.21	4580	−0.0834 (0.0037)	0.0795 (0.0035)	−0.0819 (0.0140)	0.0817 (0.0129)
0.011	−4.47	4450	−0.0820 (0.0036)	0.0773 (0.0034)	−0.0802 (0.0169)	0.0800 (0.0154)

* Subscripts on $\Delta\Gamma$ refer to Eq. 8. Numbers in parentheses give 95% confidence intervals.

TABLE 2 Calorimetric data of Krakauer and Sturtevant (1968) used to estimate poly rA and poly rU structural parameters and to construct the phase diagram for solutions of poly rA and poly rU

Reaction	T_m* (K)	$dT_m/d\ln a_\pm$ (K)	$\Delta H°$ (cal(mol base)$^{-1}$)	$\Delta\Gamma$	C_3^{REF} (M)	$RT_m^2/\Delta H°$ (K)
AU → A + U	317.62	8.51	3690	−0.0783		
AU → A + U	324.47	8.51	3975	−0.0809		
AU → A + U	331.40	8.51	4100	−0.0800	0.104	53.4
AU → ½AU$_2$ + ½A	330.62	−6.50	690	+0.0103	0.263	315
AU$_2$ → AU + U	305.23				0.018	159
AU$_2$ → A + 2U	341.02	11.3	4233	−0.1034	0.263	54.6

* T_m^{REF} is the constant of integration necessary to calculate T_m from $dT_m/d\ln a_\pm$ (cf. Eqs. 9 and 10). The integration is from $\ln a_\pm^{REF}$ (which is determined from C_3^{REF}) to $\ln a_\pm$. In the table T_m^{REF} is T_m associated with C_3^{REF}.

NaCl concentration for each of the remaining reactions. With the notable exception of the disproportionation transition of double-helical poly rA·poly rU, T_m increases linearly with $\ln a_\pm$ with a slope that is different for each order-disorder transition investigated. For disproportionation of poly rA·poly rU, T_m decreases in a nonlinear fashion with increasing $\ln a_\pm$ (cf. Fig. 6 below). Krakauer and Sturtevant (1968) also measured T_m and $\Delta H°$ for three of the four transitions in solutions containing KCl rather than NaCl. Although the results are qualitatively similar for the two salts, the curvature of T_m as a function of $\ln a_\pm$ is more pronounced for the disproportionation of poly rA·poly rU in KCl solutions. Because Krakauer and Sturtevant (1968) reported calorimetric measurements for disproportionation of poly rA·poly rU only in the presence of NaCl, we could not evaluate $\Delta\Gamma$ for this transition in the presence of KCl. Krakauer and Sturtevant (1968) raised some doubt about their measurement of $\Delta H°$ for the transition poly rA·(poly rU)$_2$ → poly rA·poly rU + poly rU, so we did not include calorimetric data for this transition in our fitting procedure (described in Numerical Methods).

Preferential interaction coefficients

In a solution consisting of solvent, one polyelectrolyte component, and one electrolyte component, the preferential interaction coefficient, Γ_{3u}, usually is defined as the partial derivative

$$\Gamma_{3u} \equiv (\partial C_3/\partial C_u)_{T,\mu_1,\mu_3}. \tag{1}$$

Here C_3 and C_u are the molar concentrations of the electrolyte and the polyion monomer, respectively, and μ_1 and μ_3 are the chemical potentials of the solvent and of the electrolyte, respectively. The limiting value of this coefficient as $C_u \to 0$ is conventionally designated Γ_{3u}^o. In all cases discussed in this paper, the limit $C_u \to 0$ is implied and the superscript "*o*" is omitted. Similarly, the conventional subscript 3*u*, which designates a salt-polyelectrolyte preferential interaction coefficient expressed per polyion monomer is implied. A superscript *PB* is used to indicate that the PB equation was used to calculate Γ or $\Delta\Gamma$, a subscript lower case letter (*a–d*) is used to designate $\Delta\Gamma$ evaluated from experimental data using Eq. 8a–d below, and upper case subscripts are used to designate the type of polyelectrolyte component to which the preferential interaction coefficient pertains, where necessary to avoid confusion (e.g., Γ_{TS}, Γ_{DS}, Γ_{SS} for triple-, double-, and single-stranded species, respectively).

The Donnan membrane equilibrium provides the most direct experimental measurement of Γ for a three-component system. Measurements of C_3 as a function of C_u are obtained by placing a series of solutions containing salt and various amounts of polyelectrolyte in dialysis equilibrium across a semipermeable membrane with solutions containing only water and salt at a known concentration. Fortunately, Γ ceases to vary detectibly at values of C_u that are experimentally accessible, so that Γ can be evaluated as the slope of a *linear* plot of C_3 vs. C_u.

Extensive discussions of the physical significance of Γ, especially in the context of the Donnan equilibrium, have been given elsewhere (Gross and Strauss, 1966; Anderson and Record, 1980, 1982, 1983). The quantity -2Γ can be considered as the "thermodynamic degree of dissociation" of counterions from (and $1 + 2\Gamma$ the extent of thermodynamic binding to) a polyelectrolyte (Gross and Strauss, 1966; Anderson and Record, 1982, 1983). Therefore, $-2\Delta\Gamma$ can be interpreted as the difference between the thermodynamic degree of counterion dissociation of the product(s) and the reactant(s) involved in a given conformational transition. Where $-2\Delta\Gamma$ is positive for an order-disorder transition, the transition can be interpreted as accompanied by the release of thermodynamically bound counterions (Anderson and Record, 1982, 1983).

The deviation of Γ from its ideal Donnan value ($\Gamma_{ideal} = -0.5$ for uniunivalent salt) is determined primarily by two classes of interactions: i) long range coulombic attractions and repulsions of mobile salt ions with the fixed structural charges on the polyion; and ii) short range repulsions of salt ions with the polyion caused by their mutual excluded volume. According to the model of the system presupposed by the use of the cylindrically symmetric PB equation, the interaction potential caused by the polyion charge distribution is completely characterized by the distance, *b*, defined as the average axial spacing between structural charges projected onto the cylindrical axis of the (model) polyion. The thermodynamic consequences of coulombic interactions depend not only on *b*, but also on *a*, the ion-polyion distance of closest approach, which is assumed to define a "hard" cylindrical boundary enclosing all the polyion charge.

Preferential interaction coefficients of the type defined in Eq. 1 for a three-component system can be formulated also for *sufficiently dilute* systems containing more than one polyionic species to describe the effects of $a_\pm$ on equilibria involving at least one polyion. The approximations entailed in such applications have been considered in detail by Anderson and Record (1993). Most experimental studies of DNA denaturation (including the ones analyzed here) have been carried out on solutions containing a large enough excess of electrolyte and a low enough monomolar concentration of nucleic acid so that the preferential interaction coefficients of both conformations are well approximated by their corresponding values at low C_{m}.

To an acceptable degree of accuracy for the present analysis, the effect of $a_\pm$ on the order-disorder transition temperature (T_{m}) can be expressed theoretically in terms of $\Delta\Gamma$, the stoichiometrically weighted difference in preferential interaction coefficients (defined per mole of nucleotide monomer) pertaining to the disordered and ordered states

$$\begin{aligned}\Delta\Gamma \equiv \sum_J \frac{\nu_{2J} n_{2J} \Gamma_{2J}}{n_{21}} &= -\left(\frac{\Delta H^\circ}{2\mathrm{R}T_{\mathrm{m}}^2}\right)\frac{\mathrm{d}T_{\mathrm{m}}}{\mathrm{d}\ln a_\pm} \\ &= \frac{\Delta H^\circ}{2\mathrm{R}}\frac{\mathrm{d}(T_{\mathrm{m}}^{-1})}{\mathrm{d}\ln a_\pm},\end{aligned} \tag{2}$$

where R is the gas constant, ν_{2J} is the stoichiometric coefficient of polynucleotide species J in the denaturation reaction, n_{2J} is the number of polynucleotide strands in species J, and $J = 1$ (and thus n_{21}) corresponds to the ordered "reactant" state. In addition to the stoichiometric weight ν_{2J}, each preferential interaction coefficient in the sum in Eq. 2 is weighted by a factor n_{2J}/n_{21} that arises because Γ_{2J} and $\Delta\Gamma$ are expressed per mole of nucleotide monomer. For example, in disproportionation of the poly rA·poly rU double helix, $n_{21} = 2$, $n_{22} = 3$ (triplex), $n_{23} = 1$ (single strand), and $\Delta\Gamma = (0.75)\Gamma_{22} + (0.25)\Gamma_{23} - \Gamma_{21}$. The calorimetric transition enthalpy ΔH° is defined by analogy to $\Delta\Gamma$ as a stoichiometrically weighted sum of terms per mole of nucleotide monomers. The derivation of Eq. (2) is based on the Gibbs-Helmholtz equation (cf. Privalov et al., 1969) and a number of justifiable approximations (Anderson and Record, 1993).

MODEL

Standard model of a nucleic acid polyelectrolyte solution

The single (SS)-, double (DS)-, and triple (TS)-stranded forms of nucleic acids are modeled as impenetrable and inflexible cylinders each of which has a characteristic radius and a uniform continuous axial charge density that are assumed independent of $a_\pm$. The cooperative units of the polynucleotides that undergo a conformational transition are assumed to be sufficiently long so that end effects are negligible. Thus, small ion distributions with respect to the model polyion vary only in the radial direction, perpendicular to the cylindrical axis. The form of the PB equation, therefore, can be simplified, because there is no evidence that the details of angular or axial variation in ion distributions with respect to the polyion have any substantial effect on the thermodynamic coefficients required for the present analysis.

The average charge spacing for double-stranded B form DNA (half of the rise along the helix axis per base pair), b_{DS}, is known from x-ray structures (of both fiber and crystal forms) to be 1.7 Å (cf. Saenger, 1984). Crystallographic information also yields an estimate of the average structural radius of the double helix (~10 Å). However, the appropriate value of a_{DS} (the ion-polyion distance of closest approach) in aqueous solution depends on the extent to which water of solvation excludes ions from the grooves of the helix and from contact with phosphate oxygens. A reasonable upper limit on a_{DS} is 13 Å, deduced by assuming that ions (such as Na^+ and Cl^-) remain hydrated (hard spheres of effective radius 3 Å) and do not penetrate the helix grooves significantly. We use 8.5 Å as a lower limit for a_{DS}, based on the locations of the nonesterified phosphate oxygens (8.9 and 10.3 Å; Arnott, 1976) and on the value of a_{DS} obtained by fitting the membrane dialysis data of Strauss et al. (1967) (see Results). However, use of the "impenetrable cylinder" boundary condition (specified below) in solving the cylindrical PB equation implies that all the structural charge on the polyion must lie within the radius a.

The average axial charge spacing of poly rA·poly rU ($b_{\mathrm{AU}} = 1.4$ Å) and of poly rA·(poly rU)$_2$ ($b_{\mathrm{AU_2}} = 1.0$ Å) are known from x-ray fiber diffraction (Arnott et al., 1973; Arnott and Bond, 1973; Arnott et al., 1976). We investigated values for the distances of closest approach of ions to poly rA·poly rU in the range 9–13 Å and for poly rA·(poly rU)$_2$ in the range 10–14 Å based, as for the case of B-DNA, on the locations of the nonesterified phosphate oxygens and the possibility that water of solvation results in a larger distance of closest approach of the ions to the RNA. For denatured B-DNA and for single-stranded poly rA and poly rU, experimental values of the average axial charge spacing in solution have not been determined with precision comparable with those available for the ordered nucleic acid species. Estimation of this parameter by fitting experimental data to the predictions of the PB cell model for the preferential interaction coefficients is one principal goal of this paper.

The entire solution is modeled as a dielectric continuum, with dielectric constant ϵ given as a function of temperature for pure water by (Hasted, 1972)

$$\begin{aligned}\epsilon = 87.740 &- 0.4008T \\ &+ 9.398 \times 10^{-4}T^2 - 1.410 \times 10^{-6}T^3.\end{aligned} \tag{3}$$

Inclusion in Eq. 3 of a salt concentration-dependent correction to the dielectric constant (Hasted et al., 1948) did not have a significant effect on results obtained.

PB cell model

For the "primitive" model of a salt-polyelectrolyte solution, Monte Carlo (MC) simulations of the thermodynamic properties of polyelectrolyte solutions indicate that the (relatively computationally efficient) PB equation is of acceptable accuracy for the purpose of calculating preferential interaction coefficients (Mills et al., 1986; Paulsen et al., 1987; Vlachy and Haymet, 1986). Preliminary results (J. P. Bond, unpublished data) obtained at salt concentrations greater than 1 M indicate that the relatively small differences between PB and MC predictions of coion distributions cancel significantly when these distributions are integrated to evaluate Γ. In the present study, we have utilized exclusively PB calculations of $\Delta\Gamma$.

At sufficiently high dilutions of both salt and cylindrical polyelectrolyte, the PB cell model (*without* the condensation hypothesis) ratifies the prediction of CC theory (Manning, 1969) that Γ has the following "limiting law" form:

$$\text{If} \quad \xi > 1: \qquad \lim_{C_3 \to 0} \Gamma^{\mathrm{PB}} = 1/4\xi. \tag{4}$$

In Eq. 4 $\xi \equiv e^2/\epsilon kTb$, where e is the protonic charge (conventionally expressed in e.s.u.), ϵ is the dimensionless dielectric constant of the solvent, and kT has its usual significance. Therefore, the limiting value of Γ^{PB} is directly proportional to b and is independent of a or any other structural parameters.

More generally, Anderson and Record (1983) used the PB cylindrical cell model to show that at sufficient dilution of polyion and in a sufficient excess of salt ($C_3 \gtrsim 5C_u$):

$$\Gamma^{\mathrm{PB}} = -[2\xi - \xi^2 + \mathrm{S_a}]/4\xi. \tag{5}$$

In Eq. 5, S_a is proportional to the sum of the local concentrations of electrolyte ions at the polyion surface: $S_a \equiv 2\xi\bar{V}_u[C_+(a) + C_-(a)]$, where $\bar{V}_a \equiv N_A\pi a^2b/10^3$ (*not* a partial molar volume) is the volume per polyion monomer excluded at contact between a small ion (modeled as a hard sphere) and the polyion (modeled as a hard cylinder); N_A is Avogadro's number, and $C_+(a)$ and $C_-(a)$ denote the local "contact" concentrations (at a) of univalent cations and anions, respectively. As $C_3 \rightarrow 0$, there are two limiting values of S_a, depending on the magnitude of ξ. For $\xi > 1$, $S_a \rightarrow (\xi - 1)^2$, so that Eq. 5 acquires the simple form of Eq. 4, wherein Γ is directly proportional to b and independent of a (Anderson and Record, 1980). At very high salt concentrations, the contact concentrations both approach C_3, so that $S_a \rightarrow 4\xi C_3$ and $\Gamma^{\mathrm{PB}} \rightarrow -C_3\bar{V}_a$. Thus, in this case also Γ^{PB} is directly proportional to b, but now has a quadratic dependence on a as well. In the experimental range of C_3, $C_3\bar{V}_a$ contributes significantly to Γ, but generally is not the dominant contribution, and the quantity $\Gamma - C_3\bar{V}_a$ also depends significantly on C_3.

At salt concentrations in the typical experimental range, $C_+(a)$ and $C_-(a)$ can be evaluated by solving numerically the PB equation in the form appropriate for a uniformly and continuously charged cylindrical polyanion:

$$\frac{1}{r}\frac{\mathrm{d}}{\mathrm{d}r}\left(r\frac{\mathrm{d}y(r)}{\mathrm{d}r}\right) = \frac{4\pi N_A e^2}{10^3\epsilon kT}[C_+(R)e^{y(r)} - C_-(R)e^{-y(r)}], \tag{6}$$

where $y = -e\Psi/kT$, and Ψ is the mean electrostatic potential minus its value at the reference surface of radius R. At $r = a$, $C_+(a) = C_+(R)e^{y(a)}$ and $C_-(a) = C_-(R)e^{-y(a)}$. According to the cell model, the magnitude of R is determined by the concentration of polyion monomers: $C_u = 10^3/\pi bR^2N_A$. (A more complete description of the mathematical form of the cylindrical PB equation appropriate for the cell model, and its relationship to the conventional hyperbolic sine form, appropriate for the isolated polyion, has been given elsewhere (Anderson and Record, 1983).)

For the standard cell model, numerical solution of Eq. 6 is based on the following boundary conditions. To ensure the electroneutrality of the cell, the outer boundary condition stipulates that the field vanishes at R:

$$\left.\frac{\mathrm{d}y}{\mathrm{d}r}\right|_R = 0. \tag{7a}$$

At the distance of closest approach of an ion center to the axis of a uniformly charged impenetrable polyion, the field is related to the polyion structural charge density (entirely contained within a) by Gauss' Law:

$$\left.\frac{\mathrm{d}y}{\mathrm{d}r}\right|_a = \frac{-2\xi}{a}. \tag{7b}$$

This boundary condition is the ultimate origin of the appearance of ξ in Eq. 5, which implies, therefore, that the PB potential and its gradient, both evaluated at the ion-polyion distance of closest approach, suffice to determine Γ^{PB}.

NUMERICAL METHODS

Calculation of Γ^{PB}

Multiple iterative computations of Γ^{PB} are required to determine the values of the structural parameters for the different conformational states that yield optimal fittings of the calorimetric data. To expedite the fitting procedure, we have used the isolated polyion model to calculate Γ^{PB} in a single step instead of employing the conventional PB cell model evaluation as the slope of a plot of C_3 vs. C_u (see, for example, Mills et al., 1986). Specifically, we truncate the PB equation at a suitably chosen "boundary point," at and beyond which the Debye-Hückel linearization of the PB equation is taken to be valid. The approach is similar to that of Stigter (1975). Details are available upon request from J. P. Bond. Determinations of Γ^{PB} reported here agree to 8 decimal places with Γ^{PB} obtained using substantially (10-fold) larger R and with Γ^{PB} obtained by use of the conventional PB cell model (from the slope of a plot of C_3 vs. C_u with sufficiently large R). The current approach is more efficient for the present application, because iterative evaluations of Γ^{PB} as a function of a, b, and C_3 are required. Using a VAXstation 3100, each calculation of Γ by the "truncation method" required 1 s.

Calculation of dT_m/d ln $a_\pm$

The extent (if any) of linearity of T_m as a function of ln $a_\pm$ is not known a priori. We investigated four functional forms for T_m (ln $a_\pm$), given by Eq. 8a–d, which are indicated as plausible by the extant experimental data.

$$T_m = A_a + B_a \ln a_\pm \tag{8a}$$

$$1/T_m = A_b + B_b \ln a_\pm \tag{8b}$$

$$T_m = A_c + B_c \ln a_\pm + C_c(\ln a_\pm)^2 \tag{8c}$$

$$1/T_m = A_d + B_d \ln a_\pm + C_d(\ln a_\pm)^2 \tag{8d}$$

where the coefficients A_i, B_i, and C_i are fitting parameters. In general, both Eq. 8a and Eq. 8b are capable of describing the dependence of T_m on $\ln a_\pm$ for DNA denaturation data (Cantor and Schimmel, 1980), but yield significantly different estimates of the derivatives in Eq. 2 at the extremes of $\ln a_\pm$. Therefore, we also investigated the use of Eq. 8c and Eq. 8d, which include the next terms in Taylor expansions of T_m and T_m^{-1} as functions of $\ln a_\pm$, and which necessarily yield broader (more conservative) confidence intervals than Eq. 8a and Eq. 8b, respectively (Seber, 1977). To estimate $\Delta\Gamma$, we performed unweighted linear regression on data fitted to each of Eq. 8a–d, differentiated with respect to $\ln a_\pm$ to obtain $dT_m/d \ln a_\pm$ or $d(T_m^{-1})/d \ln a_\pm$ and used the resulting derivative with $\Delta H°$ in the appropriate form of Eq. 2 to obtain "experimental" values of $\Delta\Gamma$.

Nonlinear least-squares analysis

Unknown parameters in Eq. 7b and 8a–d were estimated by minimizing the sum of weighted squared differences between the experimental measurement of Γ, T_m, and $\Delta H°$ and the value predicted by the fitting equations (Draper and Smith, 1966; Press et al., 1986; Bates and Watts, 1988). In fitting the membrane dialysis data of Strauss et al. (1967), weights were obtained from the SE (reported by Strauss et al., 1967) of each estimate of Γ. Calorimetric measurements of T_m and $\Delta H°$ by Privalov et al. (1969) were fitted simultaneously. For this weighted least-squares analysis, weights were obtained from the SEs (assumed independent of $\ln a_\pm$) of the measurements of T_m and $\Delta H°$, which were estimated from unweighted linear regression of $\Delta H°$ and T_m on $\ln a_\pm$. Marginal confidence intervals were obtained using the "linear approximation" (Bates and Watts, 1988), which was explicitly tested for several cases. Calorimetric measurements of Krakauer and Sturtevant (1968) were analyzed by fitting $\Delta\Gamma$ directly using Eq. 2 with $\Delta H°$ and $dT_m/d \ln a_\pm$ as shown in Table 2. Details are available from J. P. Bond.

Construction of phase diagrams of stability of native DNA and complexes of poly rA and poly rU as a function of ln $a_\pm$

We took somewhat different approaches to the experimental measurements of T_m and $\Delta H°$ for DNA denaturation by Privalov et al. (1969) (Table 1) as compared with those reported for transitions of complexes of poly rA and poly rU by Krakauer and Sturtevant (1968) (Table 2). Privalov et al. (1969) measured $\Delta H°$ at six values of $a_\pm$ for a single transition, whereas Krakauer and Sturtevant (1968) measured $\Delta H°$ at one $a_\pm$ for three of the four transitions of poly rA·(poly rU)$_n$, $n = 1,2$, and measured $\Delta H°$ at three $a_\pm$ for the melting of poly rA·poly rU. Krakauer and Sturtevant (1968) obtained additional spectroscopic measurements of T_m for each of the four transitions. We analyzed the data of Privalov et al. (1969) by simultaneously fitting the entire set of $\Delta H°$ and T_m measurements, where T_m was described as a function of $\ln a_\pm$ using each of Eq. 8a–d. We chose to analyze the data of Krakauer and Sturtevant (1968) by first fitting to $\Delta\Gamma$ *only* at T_m associated with the calorimetric measurements of $\Delta H°$ and then comparing the resulting predictions of $dT_m/d \ln a_\pm$ with the spectroscopic data. Given structural parameters *a* and *b* for reactant and product species, $dT_m/d \ln a_\pm$ for transitions of complexes of poly rA and poly rU was evaluated using Eq. 2:

$$\frac{dT_m}{d \ln a_\pm} = -2\Delta\Gamma^{\mathrm{PB}} \frac{RT_m^2}{\Delta H°}, \tag{9}$$

where $T_m^2/\Delta H°$ (Table 2) was assumed constant for each reaction on the basis of calorimetric data for melting of poly rA·poly rU and other experimental data (Krakauer and Sturtevant, 1968; cf. Record et al., 1978). The mean ionic activity ($a_\pm$) of NaCl corresponding to a specified choice of C_3 and T_m was obtained by two-dimensional interpolation of the activity coefficients of Robinson and Stokes (1965) as a function of concentration and of temperature. At the experimental values of ln $a_\pm$, T_m was obtained by numerical integration:

$$T_m(\ln a_\pm) = T_m^{\mathrm{REF}} + \int_{\ln a_\pm^{\mathrm{REF}}}^{\ln a_\pm} \frac{dT_m}{d \ln a_\pm} d \ln a_\pm, \tag{10}$$

where $T_m^{\mathrm{REF}}$and $\ln a_\pm^{\mathrm{REF}}$ (which was calculated from C_3^{REF}) were taken from the (exclusively calorimetric) data reported by Krakauer and Sturtevant (1969), and appear here in Table 2. Because T_m appears in Eq. 9 (implicitly through Eqs. 6 and 7b) as ϵT_m, calculation of $dT_m/d \ln a_\pm$ requires knowledge of T_m and, therefore, prediction of T_m should be done self-consistently. However, the dependence of ϵ on T given by Eq. 3 indicates that the product ϵT is quite insensitive to T over the range of differences between the value of T_m that was used as input and that which was obtained from Eq. 10. Thus, it is assumed that the predictions of T_m are self-consistent

RESULTS

Calculation of ΔΓ for denaturation of B-DNA from the data of Privalov et al. (1969)

On the basis of Eq. 2, the experimental measurements of $\Delta H°$ and T_m reported by Privalov et al. (1969) (Table 1) permit calculation of $\Delta\Gamma$ at each $\ln a_\pm$, provided that $dT_m/d \ln a_\pm$ or $d(T_m^{-1})/d \ln a_\pm$ can be estimated. We assume four different functional forms for the dependence of T_m on salt activity, Eq. 8a–d, and find that all four are capable of fitting the experimental data well (Fig. 1). When the linear or nonlinear fitting to each of the four functional forms is differentiated to obtain $dT_m/d \ln a_\pm$ or $d(T_m^{-1})/d \ln a_\pm$, the values of $\Delta\Gamma$ obtained using Eqs. 2 and 8a–d are all approximately constant (Table 1), but $\Delta\Gamma_a$ (from Eq. 8a) and $\Delta\Gamma_b$ (from Eq. 8b) are significantly different at the extreme (high and low) salt activities. The values of $\Delta\Gamma$ estimated by using either of the quadratic functional forms, Eq. 8c or 8d, are the same: $\Delta\Gamma_c \approx \Delta\Gamma_d \approx -0.0803$ (with errors shown in Table 1), independent of $a_\pm$. Eq. 8c and 8d provide fittings of essentially equivalent quality.

Estimation of *a* for B-DNA from membrane dialysis equilibrium data using the PB equation

Donnan equilibrium studies provide a direct measurement of Γ_{DS} for B-DNA. The best data appear to be those reported by Strauss et al. (1967). By equilibrium dialysis at 25°C, Strauss et al. (1967) determined values of the Donnan coefficient (equivalent to the preferential interaction coefficient defined in Eq. 1) for helical B-DNA and NaBr at four salt concentrations (0.0095, 0.0889, 0.2316, and 0.982 M) using ratios of DNA to added salt of 0.4, 0.6, and 0.8. Using PB calculations to fit these data by nonlinear least-squares analysis, we fixed $b_{DS} = 1.7$ Å and found that $a_{DS} = 9.4 \pm 0.7$ Å (95% confidence interval). To illustrate the quality of this fitting, we calculated Γ^{PB} as a function of C_3 with $a_{DS} = 9.4$ Å (Fig. 2). The residual errors are all within one SE (as reported by Strauss et al., 1967), and no systematic error is apparent. Thus, the PB equation is capable of describing accurately the experimental measurement of Γ within the experimental error reported by Strauss et al. (1967)

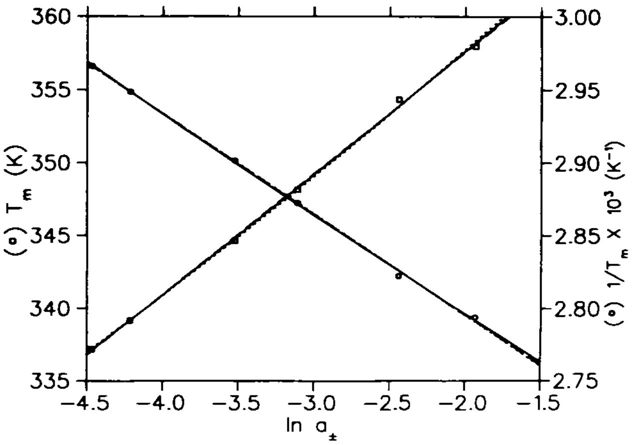

FIGURE 1 Dependences of mid-point transition temperature T_m (□) and of T_m^{-1} (○) for phage T2 (B form) DNA (Privalov et al., 1969) as a function of the logarithm of the NaCl mean ionic activity ($\ln a_\pm$) at pH 7.0. Fits to a linear (——) and a quadratic (- - - -) function of $\ln a_\pm$ are shown. The calorimetric data (T_m and $\Delta H°$) of Privalov et al. (1969) were fit simultaneously using Eq. 2 (with $\Delta\Gamma^{PB}$) and Eq. 8c (– · – ·).

Estimation of structural parameters for denatured DNA using the PB equation and experimental data for $\Delta H°$ and T_m

Eqs. 2 (with $\Delta\Gamma^{PB}$) and 8c were used to obtain the unknown parameters a_{DS}, a_{SS}, b_{SS}, A_c, B_c, and C_c by fitting the experimental measurements of $\Delta H°$ and T_m (Table 1). We fixed a_{DS} at various values in the range 8.5–13 Å, and in each case determined the remaining parameters (a_{SS}, b_{SS}, A_c, B_c, C_c) by minimizing the sum of the squared deviations of the predictions from the experimental data. Estimates of Γ and $\Delta\Gamma$ are shown in Fig. 3. The fittings of the $\Delta H°$ (Fig. 4) and the T_m (Fig. 1) data indicate that there are no obvious systematic deviations and, thus, that the standard model is capable of describing the data.

The quality of the fitting of the PB standard model to the calorimetric data (as indicated by the magnitude of the sum of squared errors) does not depend significantly on the value assigned to a_{DS}; the sum of squared errors changes by less than 5% over the range 8.5 Å $\leq a_{DS} \leq$ 13 Å. The estimate of a_{SS} depends significantly on a_{DS} (a_{SS} is always approximately 2–4 Å less than a_{DS} over the interval 8.5 Å $\leq a_{DS} \leq$ 13 Å), but the estimate of b_{SS} does not depend on a_{DS} (<1.2% variation), as shown in Fig. 5.

The experimental data of Privalov et al. (1969) were analyzed alternatively using three versions of the counterion condensation (CC) theory and Eqs. 2, 3, and 8c. The original (limiting-law) form of the CC theory (Eq. 4) was found to predict $b_{SS} = 4.2 \pm 0.1$ Å, consistent with the result of Record et al. (1976). The quality of the fitting (assessed using s, the SD of the fitting) was comparable with that obtained using the PB equation. In addition, the preferential interaction coefficient was obtained using the molecular CC theory (Manning, 1978b). Differentiating the expression for the free energy given by Manning (i.e., the sum of contributions defined by his Eqs. 8 and 9), we found $b_{SS} = 3.9 \pm 0.2$ Å, but the quality of the fitting was poorer (s was larger by a factor of 2.7). Finally, a modified version of the molecular CC theory (Manning, 1978a; in which Γ_{LL} is supplemented by a term arising from exclusion of coions from both the polyion structural volume and the volume containing condensed counterions) was not capable of describing the denaturation data (s was larger than that obtained using the original form of the CC theory by a factor of 540).

Phase diagram for solutions containing poly rA and poly rU

We analyzed the experimental estimates of $\Delta\Gamma$ obtained from the calorimetric data of Krakauer and Sturtevant (1968) (Table 2) using the PB equation for the standard model, as described in Numerical Methods. We found that assigning single values to a_{SS} and to b_{SS} for both poly rA and poly rU permits fitting of all five $\Delta\Gamma$ (Table 2) within 0.0025. The resulting best fittings were obtained when distances of closest approach for the three RNA species were (without con-

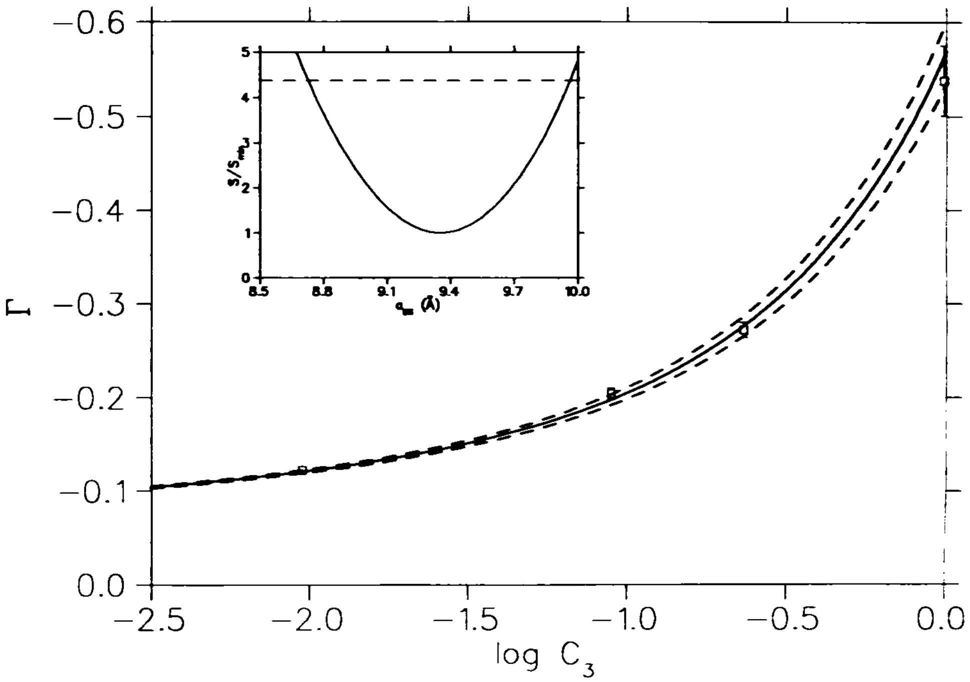

FIGURE 2 DNA-NaBr preferential interaction coefficient Γ as a function of the common logarithm of the NaBr concentration, log C_3. (□) Experimental determination of Γ_{DS} from membrane dialysis data (with SEs) by Strauss et al. (1967). (——) Γ^{PB} obtained using the standard model and adjusting the structural parameter a_{DS} to fit the data (a_{DS} = 9.36 Å). (– – –) Γ^{PB} obtained with a_{DS} at the 95% confidence boundaries (a_{DS} = 8.8 Å, a_{DS} = 9.78 Å). (*inset*) The approximate 95% confidence interval is defined by the range of a_{DS} where S/S_{min} (——) < 1 + $F(1, 3, 0.05)/3$ (– – –), where S and F have their conventional meanings (Bates and Watts, 1988) and S_{min} is the minimum value of S.

fidence intervals) a_{DS} = 11.8 Å, a_{TS} = 13 Å, a_{SS} = 7.5 Å, and when b_{SS} (95% confidence) was 3.2 ± 0.6 Å. (The confidence intervals for a_{DS}, a_{TS}, and a_{SS} are large because of correlation, as in Fig. 5 for B-DNA. For example, for a range of a_{SS} it is possible to find a_{DS} and a_{TS} that result in good fits.) Using this set of structural parameters and the assumption that $RT_m^2/\Delta H^\circ$ is constant (justified on the basis of the calorimetric data for dissociation of poly rA·poly rU (Table 2) and other experimental data (Krakauer and Sturtevant, 1968; cf. Record et al., 1978)), we calculated melting temperatures, T_m, as a function of ln $a_\pm$ for the four reactions of poly rA and poly rU (Fig. 6). The resulting phase diagram quantitatively reproduces two salient features of the experimental phase diagram that were not included in the fitting procedure, specifically: the increase in T_m as a linear function of ln $a_\pm$ (at high salt concentration) for the transition poly rA·(poly rU)$_2$ → poly rA + 2poly rU; and the nonlinear decrease in T_m with ln $a_\pm$ (also at high salt concentration) for the disproportionation reaction, poly rA·poly rU → ½poly rA·(poly rU)$_2$ + ½poly rA.

DISCUSSION

At least below ~0.2 M, effects of $a_\pm$ on helix stability are primarily coulombic in origin, and represent an entropic contribution to helix stability in water (Filimonov and Privalov, 1978; cf. review of Record et al., 1981). Manning (1972) first incorporated modern polyelectrolyte thermodynamic results into a rigorous, albeit limiting-law (low C_3), analysis of the experimentally observed linear dependence of T_m on $a_\pm$ for the denaturation of various nucleic acids. Record and collaborators (Record, 1975; Record et al., 1976) generalized

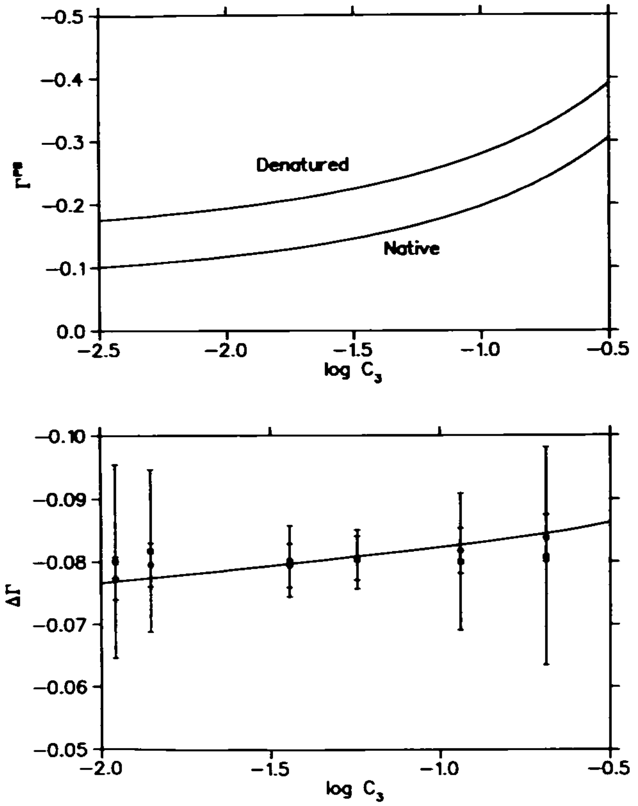

FIGURE 3 (*A*) Preferential interaction coefficient Γ^{PB} for single- or double-stranded phage T2 DNA as a function of log C_3. The structural parameters of double-stranded DNA were taken to be a_{DS} = 9.4 Å and b_{DS} = 1.7 Å and those of the denatured DNA were determined by nonlinear least-squares analysis to be a_{SS} = 6.9 Å and b_{SS} = 3.4 Å. (*B*) The difference of preferential interaction coefficients, ΔΓ, of single and double-stranded phage T2 DNA (at the melting temperature) as a function of log C_3. ΔΓ was determined from the data of Privalov et al. (1969) ($\Delta\Gamma_b$, ○; $\Delta\Gamma_c$, □) or from the PB equation ($\Delta\Gamma^{PB}$, ——).

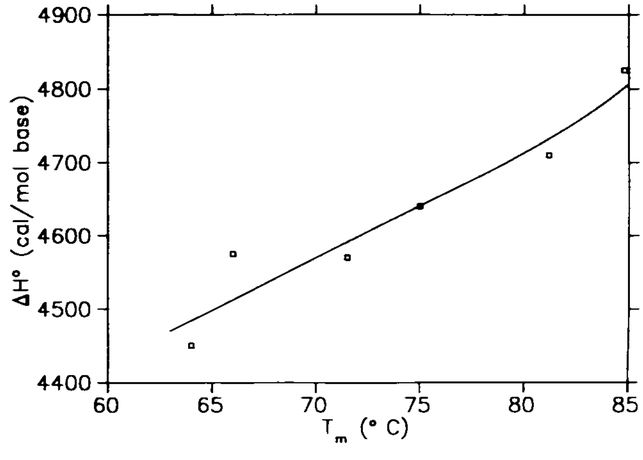

FIGURE 4 Transition enthalpy for phage T2 DNA denaturation $\Delta H°$ as a function of the melting temperature T_m. (□) Calorimetric data of Privalov et al. (1969). (——) Estimate of $\Delta H°$ obtained using nonlinear least-squares analysis.

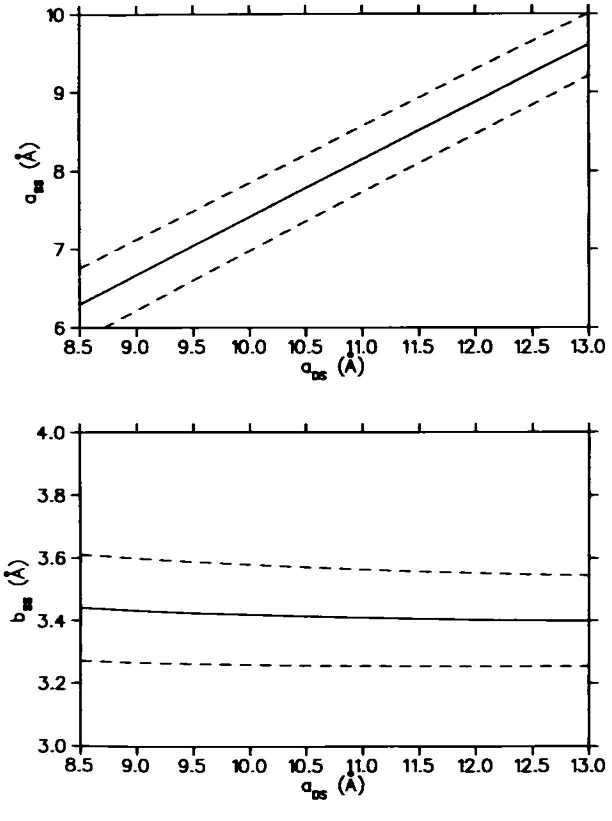

FIGURE 5 Estimated structural parameters (a_{SS} and b_{SS}) for denatured phage T2 DNA as a function of the assumed distance of closest approach of ions to native DNA, a_{DS}: (*A*) a_{SS} (——) with 95% confidence interval (– – –); (*B*) b_{SS} (——) with 95% confidence interval (– – –).

and provided a stoichiometric interpretation of this result, and analyzed experimental values of $dT_m/d \ln a_\pm$ for a variety of nucleic acid order/disorder transitions. Calculations based on the CC limiting-law theory (Eq. 4), in which the axial intercharge distance b is the only polyion structural quantity, have been shown to be capable of explaining the following qualitative observations: a) for transitions of DNA or a polynucleotide from a helix to a completely denatured single-stranded state, $dT_m/d \ln a_\pm$ increases as the number of strands (1, 2, 3) in the helix increases; b) for DNA, $dT_m/d \ln a_\pm$ decreases monotonically with increasing pH, becoming negative above pH 11; and c) $dT_m/d \ln a_\pm$ is negative for disproportionation of the polyrA·polyrU duplex into a triplex poly rA·(poly rU)$_2$ and a single strand (poly rA).

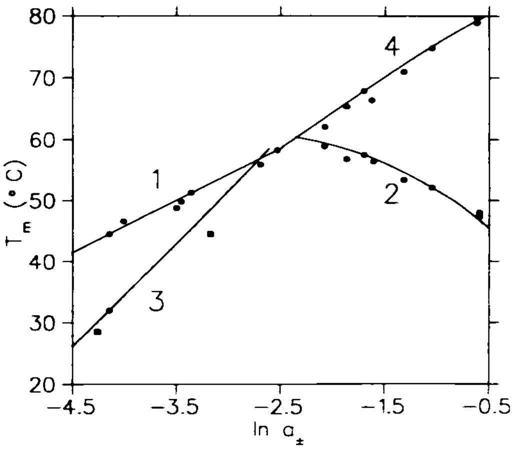

FIGURE 6 Phase diagram of stability of ordered states of poly rA and poly rU in aqueous NaCl solutions. The dependences of melting temperatures T_m on the logarithm of NaCl activity ($\ln a_\pm$) for the transitions: (1) $AU \rightarrow A + U$; (2) $AU \rightarrow \frac{1}{2}AU_2 + \frac{1}{2}A$; (3) $AU_2 \rightarrow AU + U$; and (4)-$AU_2 \rightarrow A + 2U$ were determined calorimetrically (○) and spectrophotometrically (●, transitions 1, 2, 4; ■, transition 3) by Krakauer and Sturtevant (1968). The solid lines are predictions of the PB thermodynamic analysis, as described in Numerical Methods.

The applicability of CCLL theory at salt concentrations in the range 0.01–1.0 M has no clear theoretical foundation, as indicated by PB calculations (Fixman, 1979; Gueron and Weisbuch, 1980; Stigter, 1978) and GCMC simulations (Mills et al., 1985, 1986) for essentially the same model. Hence, it is important to apply more rigorous theoretical approaches to analyze values of $\Delta\Gamma$ derived from thermodynamic data ($dT_m/d \ln a_\pm$ and $\Delta H°$) for the order-disorder transitions of nucleic acids. Frank-Kamenetskii et al. (1987) reported that the cylindrical PB equation for the standard model suffices to predict the dependence on $a_\pm$ of the coulombic component of the free energy difference between native and denatured states of polymeric DNA. Recently, we (Olmsted et al., 1991) used grand canonical Monte Carlo simulations at low $a_\pm$ for a standard model of oligoelectrolyte solutions to predict $\Delta\Gamma$ for comparisons with values of $\Delta\Gamma$ obtained from experimental measurements of the dependence on $a_\pm$ of T_m for denaturation of oligomer dimer and hairpin helices. On the basis of these calculations, Olmsted et al. (1991) proposed a resolution of the apparently conflicting conclusions about the importance of oligoelectrolyte end effects for oligonucleotide helix denaturation. In the present work, we have demonstrated the quantitative applicability of PB calculations of $\Delta\Gamma$ to analyze coulombic and excluded volume effects of $a_\pm$ on all of the above-mentioned order-disorder transitions of multistranded nucleic acid helices at neutral pH.

Dependence of $\Delta\Gamma$ on $a_{\pm}$

From a thermodynamic analysis of their calorimetric measurements for DNA denaturation, Privalov et al. (1969) concluded that the empirical quantity Δn (according to Eq. 2 equal to $4\Delta\Gamma$) is insensitive to the salt concentration. We have applied statistical methods to fit the dependence of T_m on $a_{\pm}$ to several different functional forms (cf. Eq. 8), and demonstrate that $\Delta\Gamma = -0.0803$ over the entire range (0.01–0.2 M NaCl) investigated (errors indicated in Table 1).

The experimental results reported by Krakauer and Sturtevant (1968) permit conclusions about the constancy of $\Delta\Gamma$ for the transitions of poly rA and poly rU. Their calorimetric measurements of ΔH° (Table 2) for denaturation of poly rA·polyrU indicate that $\Delta H^{\circ}/RT_m^2$ is approximately constant. Their calorimetric and spectroscopic measurements of T_m for this transition (Fig. 6) demonstrate that T_m is approximately a linear function of ln $a_{\pm}$ (i.e., dT_m/d ln $a_{\pm}$ is approximately constant). Thus, from Eq. 2, $\Delta\Gamma$ for denaturation of poly rA·poly rU is approximately constant. For denaturation of poly rA·(poly rU)$_2$, the melting temperature T_m is a linear function of the NaCl activity. Assuming that $\Delta H^{\circ}/RT_m^2$ is constant, $\Delta\Gamma$ for the melting of the triplex is also independent of $a_{\pm}$. In contrast, for the disproportionation reaction poly rA·poly rU → ½poly rA·(poly rU)$_2$ + ½poly rA, T_m is not a linear function of the salt activity, so (if $\Delta H^{\circ}/T_m^2$ is constant) $\Delta\Gamma$ varies significantly with salt concentration over the range of salt activities (0.1–0.8 M).

On the basis of our PB calculations for the standard model, the experimentally based inference that $\Delta\Gamma$ is independent of $a_{\pm}$ for DNA denaturation can be accounted for via compensation of the $a_{\pm}$ dependences of Γ_{DS} and Γ_{SS}, with structural parameters for denatured DNA that can be specified within a narrow range (especially in the case of b_{SS}) when the corresponding parameters for the ordered state are fixed at known (or most probable) values. However, in contrast to any expectation based on the apparent extended validity of Manning's limiting-law expressions, the individual preferential interaction coefficients are predicted to vary substantially with $a_{\pm}$ in the experimental range. The PB cell model and the CC theory agree at (limiting) low salt concentrations (Anderson and Record, 1983). The extent to which compensation of Γ_{DS} and Γ_{SS} can yield a relatively constant value of $\Delta\Gamma$ is sensitive to the values of a_{DS} (fixed) and a_{SS} (fitted). That is, the PB equation would predict values of $\Delta\Gamma$ that were highly dependent on $a_{\pm}$ for other values of a_{DS} and a_{SS}.

In our PB analysis, we did not specify a fixed value for a_{DS}, but investigated a range of values, and the resulting estimate of a_{SS} depends on a_{DS}. Thus, although a_{SS} is not predicted uniquely by our analysis, all values of a_{SS} as a function of a_{DS} were found to correspond to $\bar{V}_{SS} \approx 1.1\ \bar{V}_{DS}$ (where $\bar{V}_{SS}$ and $\bar{V}_{DS}$ are defined as in Eq. 5). Although the notation $\bar{V}$ is not intended to imply that this "excluded volume" contribution to Γ^{PB} is equivalent to a partial molar volume, it is of interest that Chapman and Sturtevant (1969) found that the partial molar volumes of native and denatured DNA are the same within experimental error. From our analysis of the data of Krakauer and Sturtevant (1968) we found that for the RNA species the estimates of the structural parameters yield $\bar{V}_{SS} \approx 0.95\bar{V}_{TS} \approx 0.9\bar{V}_{DS}$.

We found that PB calculations based on the standard model are capable of fitting the calorimetric data reported by Krakauer and Sturtevant (1968) for all four order-disorder transitions of poly rA and poly rU; and, assuming $\Delta H^{\circ}/RT_m^2$ is constant, the constant value of $\Delta\Gamma$ for the dissociation of poly rA·poly rU can be fitted with reasonable structural parameters for the ordered and disordered states. Our PB calculations successfully predict the linearity of T_m as a function of ln $a_{\pm}$ for the dissociation of poly rA·(poly rU)$_2$, and the curvature of T_m as a function of ln $a_{\pm}$ for the disproportionation of poly rA·poly rU → ½poly rA·(poly rU)$_2$ + ½poly rA. For the disproportionation transition, both $\Delta\Gamma$ and $\Delta H^{\circ}/RT_m^2$ are smaller in magnitude than for the other reactions, and from Eq. 9, dT_m/d ln $a_{\pm}$ is proportional to their ratio. Thus, variations in the magnitude of $\Delta\Gamma$ caused by changes in $a_{\pm}$ correspond to larger variations in dT_m/d ln $a_{\pm}$ relative to the other transitions (a twofold variation of dT_m/d ln $a_{\pm}$ is observed). Even though $\Delta\bar{V}_m$ for this reaction is small, C_3 is large enough so that the contribution to $\Delta\Gamma$ arising from the term $-C_3\Delta\bar{V}_m$ accounts for a significant fraction (55–75%) of the change in the preferential interaction coefficients, and this contribution is directly proportional to the salt concentration. For the disproportionation reaction, the variation in $\Delta\Gamma$ is caused in large part by the excluded volume contribution expressed by $C_3\Delta\bar{V}_m$.

Estimation of nucleic acid structural parameters from thermodynamic data

Several studies have presented analyses of the effect of $a_{\pm}$ on T_m of order-disorder transitions of various nucleic acid helices as a basis for estimating the mean axial distance between phosphate charges in the denatured form (cf. reviews of Record et al., 1978; Manning, 1978). From CC-based limiting-law (low-salt) analyses of DNA denaturation data, Record et al. (1976) and Manning (1976) obtained values of b_{SS} (4.1 and 3–4 Å, respectively) that are similar to those obtained here from the more rigorous PB analysis (3.2 and 3.4 Å) and significantly less than b_{SS} expected for a fully extended strand (~7 Å). Frank-Kamenetskii et al. (1987) used coulombic free energies obtained from the cylindrical PB equation to deduce that $b_{SS} = 3.4$ Å provided a better fit to DNA denaturation data than $b_{SS} = 6.5$ Å. The approximate agreement between the limiting-law and the PB predictions for b_{SS} must result from the compensation in $\Delta\Gamma^{PB}$ between $a_{\pm}$-dependent corrections to Γ^{LL} for both reactant and product species. (Recall that Γ^{LL} (eq. 4) is specified entirely by the structural quantity b.)

Record et al. (1976) analyzed the data of Krakauer and Sturtevant (1968) with analytic expressions deduced from Manning's limiting laws (1969) and obtained axial intercharge distances of the individual poly rA and poly rU strands that are substantially less than those of the extended single strands ($b_U = 4.5$ Å, $b_A = 3.1$ Å). We find that use of a single value of the axial charge distance, $b_{SS} = 3.2$ Å, results in values of Γ^{PB} that are dependent on C_3 for ordered and disordered forms and accurately describes the thermodynamic data.

Experimental information about the axial charge spacing of single-stranded nucleic acids

By fitting the (effectively constant) experimental value of $\Delta\Gamma$ with the preferential interaction coefficients predicted by the (standard) cylindrical PB equation, we find that the average axial inter-phosphate distance of denatured DNA is 3.4 ± 0.2 Å and that of single-stranded RNA (poly rA, poly rU) is 3.2 ± 0.6 Å. Manning (1976, 1978) has argued that a value of b_{SS} in the range 3–4 Å is consistent with experimental information obtained from x-ray scattering (Gulik et al., 1970). (A review of experimental studies of the structure of single-stranded nucleic acids can be found in Saenger (1984) and Cantor and Schimmel, 1980.) Information about the axial charge spacing of single-stranded polynucleotides is available from spectroscopic studies of homopolymers, which do not form double-stranded helices at neutral pH. The fact that both poly rA and poly rC are hypochromic relative to their respective nucleotides at low temperatures (as is denatured DNA, to a lesser extent) suggests that adjacent bases stack (argued in greater detail in Cantor and Schimmel, 1980) and, therefore, that the average axial charge spacing must be close to 3.4 Å, the optimal stacking distance. As temperature is increased, the extinction coefficient approaches that of the monomeric units, indicating an unstacking transition. Spectroscopic (Barszcz and Shugar, 1964; Riley et al., 1966; see analysis by Record, 1967; Record et al., 1976) and calorimetric (Filimonov and Privalov, 1978) studies have shown that the melting temperature for single-stranded RNA unstacking is insensitive to $a_\pm$. ΔH° (reported for the unstacking of poly rA by Filimonov and Privalov, 1978) and $dT_m/d \ln a_\pm$ can be used to obtain an estimate of $\Delta\Gamma$ for the unstacking transition of poly rA. Because $dT_m/d \ln a_\pm$ is small in magnitude and ΔH° is comparable with those of the other transitions analyzed here, $\Delta\Gamma$ for single strand unstacking is small in magnitude compared with other transitions. Therefore, we conclude that the change in the axial charge spacing upon unstacking is small, and that both stacked and unstacked single-stranded nucleic acids have axial charge spacings that are much smaller than the extended (all-*trans*) single strand. Indeed the all-*trans* conformation has been considered an improbable conformation because of the unfavorable consequences for solvation of charged phosphates (Alden and Kim, 1979) and unfavorable intramolecular interactions (Kim et al., 1973).

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