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Statistical mechanics of the B → Z transition of DNA: Contribution of diffuse ionic interactions

(DNA polymorphism/polyelectrolytes)

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ABSTRACT **A theoretical framework is constructed to treat the effects of salt ions on polyionic structural transitions in the absence of specific ion binding. As an application, the salt concentration-dependent part of the free energy difference governing the B → Z_I transition of [d(C-G)·d(C-G)]$_6$ in 1:1 electrolytes is calculated; quantitative agreement with the experimental findings is obtained. The effects of temperature and multivalent cations are also discussed. Preliminary calculations indicate that the Z_{II} conformation in solution is thermodynamically less favorable than Z_I in the high-salt regime (2.0–5.0 M) but more favorable than Z_I below 2.0 M salt.**

During the 30 years following Watson and Crick's proposition of the classical B-DNA structure, it has been gradually established that DNA exhibits considerable conformational polymorphism (1–3). Depending on the base pair sequence and the environmental conditions present, the molecule may adopt several distinct conformations, undergoing reversible, often very cooperative, structural transitions. Perhaps the most dramatic example of such a structural DNA transition in solution is the highly cooperative salt-induced transition of poly[d(C-G)·d(C-G)] helices observed by Pohl and Jovin in 1972 (4). The CD spectra above a critical salt concentration—2.3 M NaCl or 0.66 M $MgCl_2$ for poly[d(C-G)·d(C-G)]—were found to be inverted with respect to their low-salt forms, indicating the existence of a new, presumably left-handed, high-salt helical structure. Crystallographic proof of a left-handed conformation (named Z) was obtained by Wang *et al.* (5) in 1979, working with [d(C-G)·d(C-G)]$_3$ crystals. Related Z conformations have been found with [d(C-G)·d(C-G)]$_2$ as well (6, 7) and are also consistent with fiber x-ray diffraction patterns of alternating purine-pyrimidine polymers (8). The relationship of the crystal structures to the conformations observed by Pohl and Jovin in solution has been clarified through NMR (9, 10) and Raman spectroscopic studies (11), which provide strong evidence that the low-salt and high-salt conformations in solution are identical (or at least very similar) to the B and Z forms, respectively. As far as we know, an alternating purine-pyrimidine sequence with dinucleotide repeat seems to be a necessary yet not sufficient—a counterexample is the sequence [d(A-T)·d(A-T)]—prerequisite for the occurrence of a left-handed conformation. A given DNA sequence may not undergo a B → Z transition due to an unfavorable free energy balance (e.g., atomic core overlaps in the Z form), too high kinetic barriers, or both. However, when a transition is permitted by sequence it is found to be affected (often very dramatically) by a variety of environmental conditions and chemical DNA modifications such as the type and concentration of ions present (12–15), organic cosolvents (13–16), ligand binding (17, 18), torsional stress in supercoiled DNA (19, 20), methylation (21, 22) and halogenation (23, 24) of the bases, and atomic substitutions in the phosphodiester backbone (25). Compared to this wealth of experimental knowledge, quantitative theoretical understanding of even the simplest B → Z transitions is less than poor.

This is due to the extreme structural complexity of the many-particle systems involved and the lack of working statistical theories for several important interactions (e.g., hydration, specific binding) involved. However, as discussed in this work, one contribution to the total free energy balance controlling the B → Z transition, namely, that due to the interaction between phosphates and the diffuse cloud of ions, can be estimated with fair accuracy thanks to advances in the theory of ionic solutions and the availability of structural DNA data. This contribution is expected to be very important in general, and particularly so in the simplest case of a system exhibiting a B → Z transition—i.e., [d(C-G)·d(C-G)]$_n$–water–alkali halide—since alkali halide ions (with the possible exception of Li^+) do not seem to bind to DNA (26, 27) or perturb DNA hydration. I develop an approximate theoretical framework for treating diffuse ionic effects in structural transitions of polyions and apply it to the case just mentioned, avoiding technical details as much as possible. An extensive technical discussion of the approximations involved as well as additional results will be presented elsewhere.

THEORETICAL BACKGROUND

Consider the system consisting of (*i*) a single DNA polyanion of specified sequence—e.g., [d(C-G)·d(C-G)]$_n$—bearing its full stoichiometric charge, $-eM$, in which M is the number of phosphates present, (*ii*) N_1 anions and $M + N_2$ cations stemming from complete dissociation of a simple salt (e.g., NaCl) as well as the DNA phosphates, and (*iii*) N_w water molecules, at temperature T and pressure p. Assume that the DNA may exist in conformations X, Y, ... defined as specified sets of the positions of all DNA atoms denoted $\{\mathbf{R}(X)\}$, $\{\mathbf{R}(Y)\}$, ... with probabilities $P(X)$, $P(Y)$, ... Introducing the corresponding Gibbs free energies of the entire system, $G(X)$, $G(Y)$, ..., the relative probability for occurrence of any two conformations (say X and Y) is given by

$$\frac{P(Y)}{P(X)} = \exp -\beta\Delta G(X, Y), \qquad [1]$$

in which $\beta = (k_B T)^{-1}$ (k_B being the Boltzmann constant) and $\Delta G(X, Y) = G(Y) - G(X)$. In the case of dilute DNA solutions and whenever the $X \rightarrow Y$ conformational transition is of the all-or-none type (which is usually the case for segments shorter than about 100 base pairs due to the cooperativity of the transitions), either side of Eq. 1 is equal to the apparent equilibrium constant of the $X \rightarrow Y$ isomerization, which may

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Abbreviations: pmf, potential of mean force; RPM, restricted primitive model; HNC, hypernetted chain; EXP-MSA, exponential mean spherical approximation; CC, counter-ion condensation; PB, Poisson–Boltzmann; KSA, Kirkwood superposition approximation.

be measured spectroscopically or calorimetrically (for the case of the B → Z transition see refs. 4 and 28). The condition

$$\Delta G(X, Y; \mathring{\mathbf{N}}, \mathring{T}, \mathring{p}) = 0, \quad [2]$$

in which the dependence of the Gibbs functions on the thermodynamic state parameters ($\mathring{\mathbf{N}} = (\mathring{N}_w, \mathring{N}_1, \mathring{N}_2 + M), \mathring{T}, \mathring{p}$) has been explicitly introduced, defines the set $\mathring{\mathbf{N}}, \mathring{T}, \mathring{p}$ for which X and Y are equiprobable—i.e., the so-called midpoint of the $X \rightarrow Y$ transition. If no such set can be found when the state parameters are varied in a given range of values, there is no transition in that range, one of the conformations being always more favorable there. To proceed further we now split $\Delta G(X, Y)$ into two parts:

$$\Delta G(X, Y) = \Delta G_o(X, Y) + \Delta G_1(X, Y). \quad [3]$$

$\Delta G_1(X, Y)$ is the contribution due to the interactions of DNA phosphates and the ions only. $\Delta G_o(X, Y)$ is the contribution due to all other interactions in the system (e.g., DNA chemical bonds, base stacking, steric repulsions of DNA groups other than the phosphates, DNA hydrophobic and hydrophilic interactions, dispersion forces, etc.). This term is obviously hopelessly complex and cannot be dealt with at present. Even the "simpler" many-body problem involved in the evaluation of the term $\Delta G_1(X, Y)$ is intractable without introducing a series of approximations both at the model and the statistical averaging level. In this first attack of the problem we proceed as follows:

(*i*) Using the classical McMillan–Mayer (MM) strategy (29) for treating multicomponent systems, one can show that when total volume changes accompanying the $X \rightarrow Y$ transition are negligible

$$\Delta G_1(X, Y) = \Delta F_1(X, Y) = F_1(Y) - F_1(X). \quad [4]$$

$F_1(X)$ and $F_1(Y)$ are the Helmholtz-like free energies obtained by statistical averaging over ion configurations with the phosphates fixed at conformations X and Y, respectively. The effective pair potentials $\psi^{\alpha\beta}_{MM}(r)$ thereby used as an input are also obtained from statistical averaging albeit over water configurations with both the phosphates and the ions fixed. They depend on T and the water chemical potential μ_w as well as the distance r between two particles of species α and β.

(*ii*) The ith DNA phosphate in conformation X is treated as if it were just another salt anion of charge $-e$, located at the charge center of gravity $\mathbf{r}_i(X)$ of the group, as determined from the atomic coordinates and the partial charges. Within the approximate framework just constructed, $\Delta F_1(X, Y)$ is nothing else but the amount of reversible work spent in bringing M of the anions from positions $\{\mathbf{r}(X)\}$ to positions $\{\mathbf{r}(Y)\}$ in a simple salt solution. It can be shown that

$$\beta\Delta F_1(X, Y) = \beta[W^{(M)}_{1\ldots1}(\mathbf{r}_1(Y), \ldots, \mathbf{r}_M(Y)) - W^{(M)}_{1\ldots1}(\mathbf{r}_1(X), \ldots, \mathbf{r}_M(X))], \quad [5]$$

in which $\beta W^{(M)}_{1\ldots1} = -\ln g^{(M)}_{1\ldots1}$ is the M-anion potential of mean force and $g^{(M)}_{1\ldots1}$ is the M-anion correlation function (30, 31).

(*iii*) Unfortunately, almost nothing is known about many-particle correlations in fluids, but very much has been learned in the past 20 years or so concerning two-particle correlations (30, 31). To derive a working expression for ΔF_1 I introduce the well-known Kirkwood superposition approximation (KSA) (30), replacing the M-particle potential of mean force (pmf) by the sum over two-particle pmfs. Eq. **5** then becomes

$$\beta\Delta F_1(X, Y) = \beta\sum_{\substack{ij \\ i>j}}^{M} [W^{(2)}_{11}(r_{ij}(Y)) - W^{(2)}_{11}(r_{ij}(X))], \quad [6]$$

$r_{ij}(X) = |\mathbf{r}_i(X) - \mathbf{r}_j(X)|$ being the distance between phosphates i and j in conformation X.

(*iv*) The solvent-averaged potential $\psi^{\alpha\beta}_{MM}(r)$ can in principle be accurately determined by using computer-based techniques (Monte Carlo, molecular dynamics) in conjunction with nowadays available potentials of the "vacuum" interactions involved (water–water, ion–ion, ion–water). However, in view of the other approximations introduced above, such an accuracy is not required here. The dominant ion–ion interactions (after solvent averaging) will be (*a*) dielectrically screened coulomb and (*b*) core repulsions of the hydrated ions. They are approximately described by the well-known potential defining the most studied theoretical model of ionic solutions—namely, the so-called restricted primitive model (RPM) (32, 33).

$$\psi^{\alpha\beta}_{MM}(r) = \psi^{0}_{\alpha\beta}(r) + \frac{Z_\alpha Z_\beta e^2}{\varepsilon r}$$

$$\psi^{0}_{\alpha\beta}(r) = \begin{cases} \infty & r < \sigma \\ 0 & r > \sigma. \end{cases} \quad [7]$$

Here $\alpha = 1$ ($\alpha = 2$) for anions (cations), Z_α is valency, ε is dielectric constant of water, and σ is average effective distance of closest approach of the ions (ionic diameter). Thus the ions are treated as equal-size hard spheres embedded in a dielectric continuum characterized by ε. As mentioned above, the parameters ε and σ depend both on T and on salt concentration (via μ_w). For ε one uses available experimental data for pure water, the salt dependence being a second-order effect. An unambiguous *a priori* choice for σ is not possible. However, it is clear that it must depend on the type of ions present and has to be larger than the sum of the ionic crystal radii (2.5–4.0 Å) and smaller than the sum of the hydrated ionic radii (6.0–8.0 Å) of an anion–cation pair. It is used as an adjustable parameter in the range 3.0–7.0 Å. Items *i–iv* define the theoretical framework used here to determine the free energy contribution $\Delta F_1(X, Y)$. The RPM potential of mean force W_{11} is calculated as a function of distance, salt concentration, and temperature. This quantity is subsequently used in conjunction with DNA structural data to determine ΔF_1 for the B → Z transition, by means of Eq. **6**.

RESULTS

Anion–anion pmfs were calculated by means of the following three statistical mechanical approximations: (*i*) the so-called hypernetted chain (HNC) integral equation, known to yield excellent results for simple RPM electrolytes (32, 33); (*ii*) the so-called exponential mean spherical approximation (EXP-MSA) of Andersen and Chandler (34, 35); and (*iii*) a simple semi-analytic expression suggested by Olivares and McQuarrie (36, 37), namely,

$$\beta W_{11}(\tilde{r}) = -\ln g_o(\tilde{r}; \tilde{\rho}) + \frac{\lambda}{1 + \chi} \frac{\exp -\chi(\tilde{r} - 1)}{\tilde{r}}, \quad [8]$$

in which $\tilde{r} = r/\sigma$, $\tilde{\rho} = \rho\sigma^3$, $\rho = (N_1 + N_2 + 2M)/V$, $\chi = \kappa\sigma$, ρ is the ionic number density,

$$\kappa = \left(\frac{4\pi\beta e^2}{\varepsilon} \sum_{\alpha=1}^{2} \rho_\alpha Z_\alpha^2\right)^{1/2}$$

is the Debye–Hückel screening parameter, $\lambda = \beta e^2/\varepsilon\sigma$ is a dimensionless coupling constant, V is the volume, and g_o is the exact pair correlation function of the hard sphere fluid obtained when all ions are uncharged. In this work we use g_o values calculated by Barker and Henderson (38) using the

Monte Carlo method supplemented by data obtained from numerical solution of the Percus–Yevick integral equation (39). Representative pmfs are shown in Fig. 1 for a low (0.39 M) and a high (2.31 M) salt concentration of 1:1 electrolyte (σ = 6.0 Å) at room temperature. At the low salt concentration all three approximations yield essentially identical results (only the HNC pmf is depicted). The effective anion–anion interaction is repulsive everywhere and monotonically decreasing with distance due to the predominance of screened Coulomb interactions. At the high salt concentration, the situation is dramatically different. The many-body hard core contribution to the effective interaction W_{11} dominates over the now heavily screened Coulomb repulsions and gives rise to the highly nonmonotonic behavior shown. All three approximations yield qualitatively similar results, the largest quantitative differences occurring in the region of the peak ($1.3 \leq \tilde{r} \leq 2.0$). The quality of the approximations improves in the order *iii* < *ii* < *i* due to a progressively better description of Coulomb screening effects, particularly in the region of the peak. However, it turns out that in the case of the B and Z structures most distances involved lie predominantly outside the region of quantitative discrepancy and therefore even the simplest approximation—i.e., *iii*—suffices to obtain reasonably accurate results. With this in mind, we now turn to the calculation of ΔF_1 for the B → Z transition. Fig. 2 schematically depicts the phosphate backbone charge configurations of DNA in the right-handed classical B form (low salt) and the most commonly found left-handed conformation, Z_I (high salt). The drawing is based on computer-generated graphs of the structures kindly provided by T. Jovin and F. Eckstein (Göttingen, F.R.G.). All calculations reported here are for the DNA dodecamer [d(pCpG)·d(pCpG)]$_6$ (i.e., a full helical turn in the Z_I and slightly more than one helical turn in the B conformation). We use the atomic coordinates published by Arnott and Hukins (40) for B and those of Wang *et al.* (41) for Z_I DNA, to obtain all positions of the 24 univalent charges representing the phosphates and the 276 distances involved. Due to the symmetry of the structures not all distances are distinct. When the distinct distances are numbered in increasing order, the kth distance r_k, (k = 1, ..., S) appears ν_k times. The set $\{r_k, \nu_k, S; k = 1, ..., S\}$ depends on the conformation and contains all structural information entering our calculation. Using approximation *iii*, Eq. 7 becomes

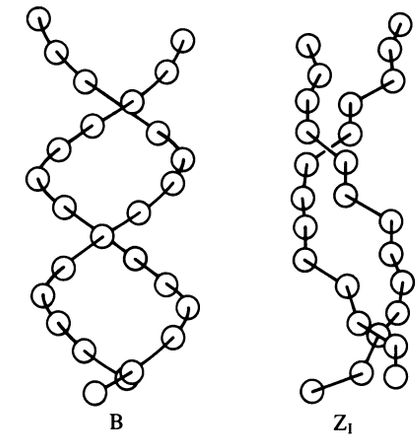

FIG. 2. Schematic phosphate configurations in the B and Z_I conformations. Each phosphate is modeled as a negatively charged hard sphere.

$$\beta\Delta F_1(\mathrm{B}, \mathrm{Z_I}) = \sum_{t=1}^{2} (-1)^t \left[\sum_{k=1}^{S(X_t)} \nu_k \left\{ -\ln g_o(\tilde{r}_k(X_t)) + \left(\frac{\lambda \exp \chi}{1+\chi} \right) \frac{\exp -\chi \tilde{r}_k(X_t)}{\tilde{r}_k(X_t)} \right\} \right] \quad [9]$$

$$\tilde{r}_k = r_k/\sigma, \; X_1 = \mathrm{B}, \; X_2 = \mathrm{Z_I}.$$

This expression has been used to calculate the salt dependence of the free energy contribution $\beta\Delta F_1$. Representative results for three values of the hard core diameter σ (4.25 Å, 5.0 Å, 6.0 Å) and T = 298.15 K and ε = 78.4 are depicted in Fig. 3. It is seen that above a critical salt concentration (3.36 M for σ = 4.25 Å, 2.05 M for σ = 5.0 Å, 1.32 M for σ = 6.0 Å) the B form becomes thermodynamically unfavorable [$\beta\Delta F_1(\mathrm{B}, \mathrm{Z_I}) < 0$] as far as phosphate-ion interactions are concerned. The critical salt concentration (at which $\beta\Delta F_1$ = 0) depends very strongly on σ (not shown). Since we do not calculate ΔG_o of Eq. 3 here, the predictions of our theory cannot be directly compared with the experimentally determined total free energy difference for the B → Z transition

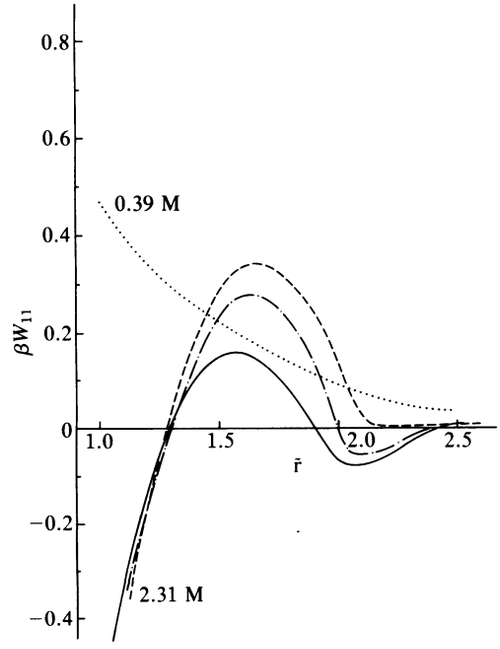

FIG. 1. Anion–anion potentials of mean force of a 1:1 RPM electrolyte at 25°C. Salt concentrations are indicated; reduced distance $\tilde{r} = r/\sigma$, σ = 6.0 Å; ···· and —, HNC; –·–·, EXP-MSA; ----, Eq. 8 (see text).

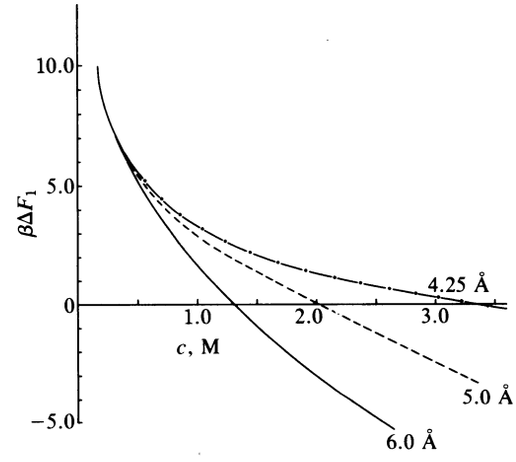

FIG. 3. Salt concentration dependence of the free energy difference $\beta\Delta F_1(\mathrm{B}, \mathrm{Z_I})$ due to diffuse ionic interactions. Calculations are for 1:1 electrolyte at 25°C for three effective ionic diameters (σ). c, Salt concentration.

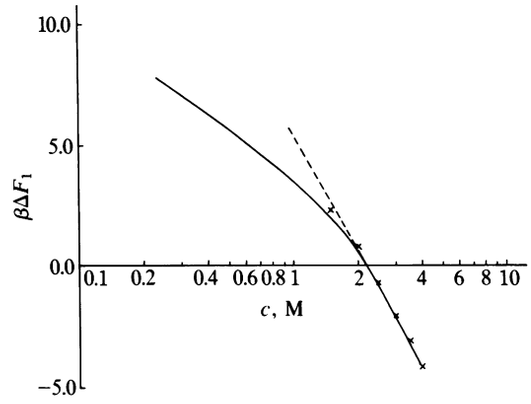

FIG. 4. Comparison of theory with experiment. DNA in NaCl solution at 25°C. —, Eq. **9** with σ = 4.90 Å; ×, experimental data; $12\Delta G^\circ/RT$ of ref. 28.

but rather its salt-dependent part [i.e., $N\Delta G^\circ/RT$ with N = 12 and R being the gas constant, in Pohl's notation (28)]. As shown in Fig. 4, we obtain excellent agreement with experiment if we set the value of the only adjustable theoretical parameter, namely, σ, equal to 4.90 Å. For concentrations (c) above 2 M the same linear dependence on ln c found experimentally (slope: $-7.20 = -12 \times 0.6$) is also predicted theoretically (slope: -6.9 for σ = 4.90 Å, strongly dependent on σ in general). Furthermore, deviations from this linear behavior occur in the same direction and range in both the experimental and theoretical cases. (Compare figure 1b of ref. 28.)

Having shown that the salt-dependent part of $\beta\Delta G$ is correctly described by the theory proposed here, I identify the remaining term $\beta\Delta G_o$ for the system at hand to be

$$\beta\Delta G_o = -\ln \beta_Z/\beta_B = 0.799 \qquad [10]$$

by comparison to experiment (28) and the thermodynamic model for the transition, proposed by Pohl and Jovin (4, 28). In this model β_Z and β_B are the nucleation parameters for nucleation of a Z conformation at the end of a B form DNA and B conformation at the end of a Z form DNA, respectively. The experimental critical concentration $\overset{*}{c}$ at the midpoint of the transition of the [d(C-G)·d(C-G)]$_6$ dodecamer in NaCl solution is 2.51 M. Using Eqs. **10**, **9** with σ = 4.90 Å, **3**, and **2**, we obtain $\overset{*}{c}$ = 2.48 M. This is not too far from the value 2.20 M one obtains ignoring $\beta\Delta G_o$ altogether (i.e., using the condition $\beta\Delta F_1 = 0$ instead of Eq. **2**). In other words, whenever diffuse ionic interactions are prevalent, useful first-order estimates of the critical concentrations $\overset{*}{c}$ may be obtained by considering the term $\beta\Delta F_1$ alone. An example for the usefulness of this conjecture is provided by calculations concerning the effects of multivalent salt cations on the transition, always assuming that no specific site binding occurs.

Typical results are shown in Fig. 5 for 1:1, 1:2, and 1:3 electrolytes and the same σ = 5.0 Å in all three cases. The critical concentrations are 1.0 M for the 1:2 electrolytes and 0.7 M for the 1:3. The value 1.0 M is not too far from the experimental value 0.66 M for poly[d(C-G)·d(C-G)] (4) in $MgCl_2$ solution. Better agreement can be obtained if one takes into account the binding of one Mg^{2+} to the terminal phosphate of the Z_I form, seen in the x-ray work (41). The accompanying charge neutralization leads to a lower estimate for $\overset{*}{c}$. If σ is assumed to be temperature independent, the dependence of $\beta\Delta F_1$ on temperature is found to be entirely negligible. This is due to the fact that the product εT entering Eq. **9** is essentially constant, its variation in the range 20–70°C being of the order of only 10% (42). Precisely this kind of behavior is also observed experimentally. For

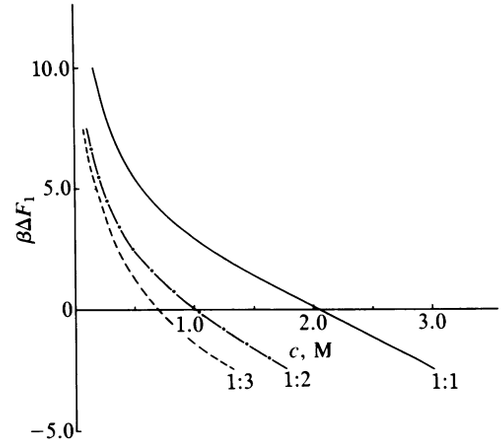

FIG. 5. Salt concentration dependence of $\beta\Delta F_1(B, Z_I)$ in 1:1, 1:2, and 1:3 electrolytes at 25°C; σ = 5.0 Å.

the system at hand, the transition characteristics do not depend on temperature, at least in the range 25–50°C (4).

Finally, we have also calculated the relative stability of the other Z conformation described by Wang *et al.* (41)—namely, Z_{II}—using the same approach and parameters as above. The B → Z_{II} transition in 1:1 electrolyte is found to occur at $\overset{*}{c}$ = 3.50 M. In the range 2.0–5.0 M the Z_{II} is thermodynamically less favorable than Z_I, but below 2.0 M the situation is reversed, the Z_{II} being only slightly less favorable than B [e.g., for 0.4–1.8 M, $0 < \beta\Delta F_1(B, Z_{II}) < 1.0$ while $1.0 < \beta\Delta F_1(B, Z_I) < 7.0$]. These preliminary results indicate that the Z_{II} free energy lies between the B and Z_I free energies and therefore Z_I is likely to be found at B/Z interfaces, as suggested by Wang *et al.* (41).

DISCUSSION

In contrast to other currently used approximate theories of chain polyelectrolytes such as the counter-ion condensation (CC) theory (reviewed in ref. 26) and the so-called Poisson–Boltzmann (BP) equation approach (reviewed in ref. 43), which, as noticed by Behe and Felsenfeld (21), do not seem to describe the electrostatics of the B → Z transition well, the approximate approach outlined above seems to explain the experimentally observed salt dependence of the simplest B → Z transition with a perhaps surprising accuracy. Since this may be the case with other salt-induced polyionic structural transitions as well, it is appropriate, as suggested by one referee, to briefly discuss the main approximations involved as well as the possible reasons for the inadequacy of previous theories and apparent success of the present one. Both the CC and PB theories are simple versions of what may be called the inhomogeneous fluid approach, viewing the polyion structure (in our case the DNA phosphates in conformation X) as the source of an external field giving rise to space-dependent ionic distributions $\{\rho_i(\mathbf{r}, X)\}$. The total free energy determining the probability $P(X)$ is then the sum of the energy to create X *in vacuo* and the free energy of the salt solution in the external DNA field, which is a functional of the $\{\rho_i(\mathbf{r}, X)\}$. This general picture is qualitatively exact but does not lead to a realistic quantitative description at present, since neither the integral equations determining the $\rho_i(\mathbf{r}, X)$ nor the form of the free energy functional are known well enough to deal with the DNA–salt solution system, which in addition is characterized by considerable structural complexity. To obtain results within the inhomogeneous fluid framework, one has to: (*i*) drastically simplify the geometry of the phosphate charge configuration [e.g., model it as an infinitely long uniformly charged cylinder (PB) or line (CC)], (*ii*) neglect ionic core repulsions (which are as impor-

tant as Coulomb interactions at high salt concentrations), and (*iii*) use linearized (CC) or nonlinearized (PB) mean field equations to determine the ionic distributions. These approximations still lead to reasonable estimates for colligative properties of the DNA–salt system (26, 43), but they are simply too drastic for treating problems such as the B → Z transition. Although the CC equation may be cast in a form capable of describing complex discrete charge configurations (44) and the PB equation may be modified to include hard core repulsions, I could not find a practicable way to overcome limitations *i*, *ii*, and *iii* simultaneously, adopting the usual inhomogeneous fluid point of view. In the approach used here, the problem is first conceptually "homogenized" by treating the phosphates as if they were simply salt anions. After this model approximation has been introduced, a rigorous result by Percus (45) leads us directly to the exact statistical relationship (5), which expresses the free energy difference of interest to us in terms of many-particle pmfs of the homogeneous salt solution, quantities which *a priori* depend only on bulk ionic concentrations and not on the high (unknown) local ionic densities near the phosphates. Introduction of the KSA in conjunction with fairly accurate approximate two-particle pmfs for the RPM model finally led to expressions suitable for obtaining quantitative results. *A priori*, one simply doesn't know how good an approximation the KSA is. On the basis of experience with triplet correlations in hard core and Lennard–Jones fluids (30, 31) I expect it to be reasonable when the fixed particles are not too closely bunched, which is roughly the case with extended "linear" polyions such as DNA. In addition, one must keep in mind that we fortunately calculate only free energy differences and not absolute free energies and therefore profit from extensive cancellation of errors and terms neglected in the expansions. Several refinements of the theory can be introduced at the price of more computing—e.g., the charged particles can be modeled as hard spheres of different diameters and HNC pmfs can be used throughout. Such work will be reported elsewhere. Going beyond the KSA and RPM levels is not as straightforward and must await further advances in the theory of electrolytes. It is clear that a complete theory should also determine the complex term ΔG_o in Eq. **3** (which in fact contains all the sequence dependence). I hope that the present approach could in principle be combined with so-called "force-field" methods used in large-scale computer simulations of DNA structures in the absence of solvent, to yield the complete ΔG of Eq. **3** for the B → Z and other DNA structural transitions in the course of the next few years.

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