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Harmonic vibrations and thermodynamic stability of a DNA oligomer in monovalent salt solution

(DNA polymorphism/normal modes)

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ABSTRACT **We compute the full harmonic vibrational spectrum and eigenmodes of a DNA oligomer, d(C-G)$_3$, in optimized B and Z conformations in various ionic environments (0.01–5.0 M NaCl). The statistical interactions of DNA with the diffuse ionic cloud surrounding it in solution are approximately represented within the potential of mean force framework. The lowest eigenfrequency of the B conformation is found to drastically decrease with increased NaCl concentration. This suggests that a *soft mode* mechanism may be a precursor for the B-to-Z conversion. The free energy balance governing the B–Z isomerization of d(C-G)$_3$ is dominated by the solvent-averaged effective phosphate–phosphate interactions due to substantial cancelations between the much larger intramolecular energy contributions.**

Dynamic fluctuations of proteins and nucleic acids around their average conformations are thought to play an important role in many central biological processes, such as enzyme activity, macromolecular recognition, and complex formation (1–3). Furthermore, they contribute to the total free energy balance determining the relative stabilities and structural transitions of the predominant conformations adopted by nucleic acids and proteins.

The two entirely classical methods currently available for studying dynamic fluctuations are normal mode analysis (4) and molecular dynamics (MD) (5, 6). Both methods can, in principle, be used to study biomolecular dynamics (7). Both methods have their strengths, but also both suffer from serious intrinsic limitations, which cannot be overlooked. Harmonic analysis is strictly valid only for small-amplitude motions around an equilibrium structure, but it yields closed-form expressions both for the time evolution of the system and its thermodynamics. The MD method is free from the small-amplitude restriction but is entirely numerical and very inefficient for calculations of crucial thermodynamics quantities.

During the last 5 years the MD technique has been used by several groups (8–12) to study the dynamics of DNA oligomers. A careful study by van Gunsteren *et al.* (12) has simulated the time evolution of a system comprising a d(CGCAACGC) duplex, 1231 simple rigid three-point charge (SPC) water molecules, and 14 counterions (just enough to guarantee overall electroneutrality). Judging from their data, it is clear that the system did not achieve equilibrium. For example, half of the originally intact 22 DNA hydrogen bonds are irreversibly broken at the end of the simulation. If the simulation had run long enough to attain equilibrium, the DNA duplex probably would have completely dissociated into single strands. This speaks for the realistic nature of the simulation because the DNA double helices at room temperature are experimentally known to be unstable in the absence of excess salt (13).

To perform MD simulations of relevance to real DNA/water/salt systems and the conformational properties of DNA one has to (*i*) include large numbers of salt ions, (*ii*) run the simulations for times orders of magnitude longer than 100 ps, and (*iii*) find efficient ways to compute crucial thermodynamic quantities (e.g., free energies) from the data gathered during the stationary part of the run. Because this procedure has to be repeated for many different environments and DNA sequences, we strongly doubt that it will be possible to realize such a program in the near future. It is our opinion that, for the time being, harmonic analysis is the only feasible way to study, under fairly realistic conditions, small-amplitude fluctuations of DNA and their contributions to configurational thermodynamic.

Tidor *et al.* (9) have done a normal mode analysis of d(C-G)$_3$ in B and Z conformations and of d(T-A)$_3$ in B conformation. Subsequently, the same group (14) has used the normal modes data to estimate the vibrational contribution to the B–Z transition entropy of the d(C-G) hexamer, which was found to be 22 cal·K^{-1}·mol^{-1} (or 3.67 cal·K^{-1} per mole of base pairs) at 300 K (1 cal = 4.18 J). The B–Z transition of DNA has been recently reviewed (15), and all available energetic data have been analyzed in detail (16). As discussed in ref. 16, the absolute magnitudes of the B–Z transition entropies fall in the range 3.5–4.5 cal·K^{-1} per mol of base pairs, irrespective of sequence, critical temperature, or solvent conditions; therefore, the value obtained by Irikura *et al.* (14) seems to be very good indeed. However, closer inspection of the computational procedures adopted and the results obtained in refs. 9 and 14 reveals several problems. The authors compute normal modes *in vacuo*, assume a cut-off of 11 Å for all nonbonded interactions, introduce a distance-dependent dielectric constant, and reduce the phosphate charges by 76%. In addition, it is clear that, since the lowest eigenvalue of its vibrational spectrum is negative (9), the Z conformation they obtain is not a minimal energy structure but a saddle point of the DNA energy hypersurface.

In previous work (17–20) it has been shown that many ionic effects on DNA structure and structural equilibria can be quantitatively described when one replaces the bare coulomb interactions of DNA phosphates in an aqueous electrolyte environment by approximate potentials of mean force (PMF) (i.e., effective interactions) obtained from statistical treatment of simple models describing aqueous electrolytes. This approach treats the PO_4^- groups in DNA as if they were just salt anions of charge $-e$ located at the center of the group. Among other things, this approach describes the whole experimentally observed salt dependence of both the B–Z

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Abbreviations: PMF, potential of mean force; MD, molecular dynamics; MSA, mean spherical approximation; Exp-MSA, exponential mean spherical approximation; RPM, restricted primitive model.

(17, 18) and B–A (18) transitions induced by increasing NaCl concentrations. This approach also provides a simple explanation for the differential effects of NaCl, KCl, RbCl, and CsCl on the B–Z transition of poly[d(C-G)] (19), and it rationalizes the salt dependence of DNA helix melting temperatures in 1:1 electrolytes from 0.01 M to 7.0 M (20). In a parallel study (21) the PMF framework has been combined with the widely used AMBER (22) force field describing intramolecular interactions. This combination results in an AMBER-PMF effective free energy function for structural computations of DNA and other polyanions in 1:1 aqueous electrolytes. Such free energy function is used below to calculate the normal modes of d(C-G)$_3$ DNA oligomers in optimized B and Z conformations for a wide range of ionic environments.

THEORETICAL BACKGROUND

For the sake of clarity, we now briefly recall the main ideas involved in the PMF approach. Consider one DNA molecule of specified sequence comprising M atoms and immersed in a 1:1 electrolyte of composition $\underline{N} = N_1, N_2, N_w$ (N_1 anions, N_2 cations, and N_w water molecules) at temperature T and pressure P. A given set of all Cartesian atomic positions, r_1, r_2, . . ., defines a DNA conformation denoted $\underline{R}$. Its thermodynamic stability for the state ($\underline{N}$, P, T) is completely determined by Gibbs' free energy:

$$G(\underline{R}; \underline{N}, P, T) = \overline{U}_{in}(\underline{R}) + G_s(\underline{R}; \underline{N}, P, T), \quad [1]$$

where $\overline{U}_{in}$ is the total intramolecular energy and G_s is the free energy of the solvent (water and salt ions) in the external field created by the DNA conformation $\underline{R}$.

The term $\overline{U}_{in}$ in Eq. 1 is approximately described by available semiempirical force fields (22). In view of the fact that it is presently not possible to calculate G_s for a microscopic model of the solvent (e.g., via Monte Carlo or MD), we approximate it by using the PMF framework (17–21), which adopts a simple water-averaged McMillan–Mayer picture of ionic interactions. Thus we set

$$G(\underline{R}; \underline{N}, P, T) \approx F(\underline{R}; c_s, T) \approx \overline{U}_{in}(\underline{R}) + F_1(\underline{R}; c_s, T) \approx \overline{U}_{in}(\underline{R}) + \sum_{i>j}^{n} W_{11}(r_{ij}; c_s, T), \quad [2]$$

where F is now a Helmholtz free energy, c_s is the electrolyte molarity, $\overline{U}_{in}(\underline{R})$ is the molecular mechanics (22) intramolecular energy *minus* the coulomb interactions of the charged phosphate groups, $W_{11}(r_{ij})$ is the PMF of two anions in a homogeneous 1:1 electrolyte characterized by a concentration c_s and temperature T. The total number of phosphates is denoted by n, and r_{ij} is the distance between the centers of the i^{th} and j^{th} phosphate charge distributions. We calculate W_{11} using the exponential mean spherical approximation (Exp-MSA) theory (23, 24) of the restricted primitive model (RPM) for electrolytes whereby:

$$W_{11}(r; c_s, T) = -\kappa_B T[\ln g_o(r, 2c_s) + h_D(r, c_s, T)]. \quad [3]$$

g_o is the exact pair correlation of the uncharged electrolyte (Hard spheres system) (25, 26). The function h_D is obtained from the mean spherical approximation (MSA) (27). There are two parameters entering the RPM Exp-MSA description for any 1:1 electrolyte: the experimentally known bulk dielectric constant of water ε (ε = 78.4 at 298.14 K), and the distance of closest approach of a hydrated anion–cation pair, σ. A consistent set of σ values to be used with DNA alkali halides systems has been found (19) and comprises the following values: 5.20 Å (for LiCl), 4.90 Å (for NaCl), 4.66 Å (for KCl), 4.41 Å (for RbCl), and 3.77 Å (for CsCl).

A distinct advantage of using the Exp-MSA PMF given by Eq. 3, instead of the more accurate PMF values obtained from numerical solution of the hypernetted chain equation (18), is that in the former case using the procedure described above, one has closed-form expressions for W_{11}.

Starting from ideal initial conformations for B and Z DNA and prescribed thermodynamic parameters, c_s and T, we minimize the free energy, F, of Eq. 2 with respect to $\underline{R}$ and obtain the corresponding stable structure in the ionic environment chosen. (We use Greek indices to designate such minimal free energy structures.)

Correct minimization not only requires a vanishing first derivative of the potential but also that the $3M \times 3M$ Hessian matrix (4) has exactly 6 zero eigenvalues (corresponding to overall translation and rotation of the structure), and $3M$-6 *positive* eigenvalues, $\lambda_i(\alpha)$—which, together with the associated eigenvectors, completely characterize the harmonic fluctuations of the molecule around $\underline{R}_\alpha$.

Up to higher order (anharmonic terms), the thermodynamic stability of the minimal energy conformation α is now determined by

$$F(\alpha) = \overline{U}_{in}(\underline{R}_\alpha) + F_1(\underline{R}_\alpha) + F_{vib}(\underline{R}_\alpha), \quad [4]$$

where both F_1 and F_{vib} depend on the thermodynamic parameters, c_s and T. F_{vib} is obtained by using a classical partition function for harmonic oscillators. With certain reservations discussed below, the quadratic form

$$\overline{U}_\alpha(\delta\underline{R}) = \overline{U}_{in}(\underline{R}) + F_1(\underline{R}) + \frac{1}{2}\delta\underline{R}^T \cdot K \cdot \delta\underline{R}, \quad [5]$$

where $\delta\underline{R} = \underline{R} - \underline{R}_\alpha$, and K is the Hessian matrix (4), can be seen as the *potential energy* of a harmonic pseudo-Hamiltonian. In this case the eigenvalues, λ_i, are simply related to the eigenfrequencies, ν_i, of the system, $\lambda_j = \nu_j^2$, which define its experimentally observable vibrational spectrum.

PROCEDURE

In this paper we report the energy minimization and normal modes calculation for 12 NaCl concentrations (0.01–5.0 M) of the molecule [OH-($pCpG$)$_3$]$_2$ in B and Z$_I$ conformation. We represent explicitly the heavy atoms of the molecule and the hydrogen atoms involved in hydrogen bonding.

We use a dielectric constant ε = 78.4 for all electrostatic interactions. This is a first-order approximation that neglects higher order polarization effects due to differences between dielectric properties of the DNA interior and the aqueous solvent. This approximation is consistent with using homogeneous PMF values for the ions and makes all electrostatic terms in $\overline{U}_{in}$ and F_1 to be of the same order of magnitude. In using the Weiner *et al.* (22) force field, we do not rescale one to four interactions, we do not truncate electrostatic interactions or rescale charges, nor do we use a distance-dependent dielectric constant.

Starting from idealized atomic coordinates of Arnott and Hukins (28) for B DNA and Wang *et al.* (29) for Z$_I$ DNA, each structure is first minimized at a salt concentration of 0.01 M NaCl. The second-derivative matrix of the potential energy with respect to Cartesian coordinates (not the mass weighted coordinates) of the minimized structure is diagonalized, and all eigenvalues and eigenvectors are evaluated. An incomplete minimization is identified by the occurrence of negative eigenvalues (implying a saddle point and not a minimum). The corresponding eigenvectors of these negative eigenvalues are the best directions to further minimize the unstable structure. Using a pseudo Newton–Raphson (NR) algorithm for singular matrices, other low-force constant eigenvectors

(usually the lowest 50 to 95 modes) are also used to further minimize the energy. These low-force constant modes involve collective motions of a large number of atoms. Large displacements along the eigenvectors that minimize the total energy of the molecule will increase the first derivative along the *hard* eigenmodes. A first-order minimization (AMBER) that does not involve the projection of the first derivative along the eigenvectors is more efficient in minimizing the energy along these *hard* modes. Thus, after one NR minimization cycle, we resubmitted to AMBER minimization. The NR plus AMBER minimizations were repeated until all eigenvalues of the second-derivative matrix were positive, except for six zeros (to the precision of the calculation, $|\lambda| \approx 10^{-6}$ kcal/Å^2). At this point we evaluated the second derivative of the potential energy with respect to mass-weighted coordinates. The square root of the eigenvalues of this matrix corresponds to the eigenfrequencies of the system. Minimal structures obtained at one salt concentration are used as starting conformation for the next higher salt concentration. When the structure at a different salt concentration is modified, we refine the structure at the lower salt concentration and take the structure that converged to the lowest energy. We calculate all normal modes of oscillation for both structures for each of the 12 salt concentrations considered.

RESULTS AND DISCUSSION

The torsional angles for one strand of optimized B and Z conformations are listed in Tables 1 and 2. The minimized structures preserved the C2 symmetry imposed by the self complementarity of the sequence. All hydrogen-bonded-pair donor-to-acceptor distances and donor-hydrogen-acceptor angles are, for Z-DNA, between 2.94 and 3.05 Å and between 141 and 171°, respectively; and, for B-DNA, between 2.96 and 3.04 Å and between 141 and 163°, respectively.

In Fig. 1 we show the computed density of states $\rho(\nu)$ as a function of frequency ν for the B and Z conformations of the d(C-G)$_3$ hexamer at 0.1 M NaCl. Marked differences in the density of states are found around 1000 cm^{-1}, 500 cm^{-1}, and in the low-frequency regime below 100 cm^{-1}; whereas, above 1500 cm^{-1} the spectra are quite similar. In the high-frequency regime $\rho(\nu)$ is determined by the localized vibrations of molecular substructures (e.g., bases, sugars); whereas, in the very low-frequency regime it is determined by collective delocalized vibrations involving the whole molecule.

At 0.1 M, the lowest eigenfrequency of the B conformation is found to be 2.52 cm^{-1}; whereas, that of the Z conformation is 2.1 cm^{-1}. Tidor *et al.* (9) have obtained a value of 9.66 cm^{-1} for the first mode of the B conformation and an imaginary eigenfrequency of modulus 7.89 cm^{-1} for the Z conformation. The latter result could be a numerical artifact due to incomplete energy minimization of the Z conformation (this also affects the values of all other Z eigenfrequencies reported in ref. 9, especially in the low-frequency regime). In addition, the assumptions of an 11.0 Å cutoff for nonbonded interactions and a distance-dependent dielectric constant (9) affect all low-frequency eigenmodes for both conformations (30). The use of $\varepsilon = 78.5$ will also yield lower eigenfrequencies at the low-frequency end of the spectrum. We find that both the density of states, $\rho(\nu)$, and the eigenmodes (and eigenvectors)

Table 1. Backbone dihedral angles for B-DNA

	α	β	γ	δ	ε	ζ	χ
C	290.8	174.2	56.2	146.3	189.2	274.4	241.2
G	279.3	179.9	51.8	126.5	186.3	277.3	235.1
C	287.6	170.6	55.8	99.2	179.2	274.5	231.2
G	292.9	174.0	56.6	123.1	178.7	267.3	231.6
C	293.3	178.8	57.9	128.9	181.5	267.3	237.0
G	288.8	179.2	54.4	130.7	—	—	243.4

Table 2. Backbone dihedral angles for Z_I-DNA

*	α	β	γ	δ	ε	ζ	χ
C	180.5	177.5	59.1	133.5	282.0	67.6	218.4
G	66.6	182.9	181.9	94.6	284.1	302.0	48.4
C	211.2	177.6	66.6	148.5	290.7	68.1	212.5
G	68.5	189.0	185.0	92.3	285.2	303.5	48.6
C	204.5	179.0	67.3	149.8	284.8	61.7	214.1
G	63.5	187.2	176.9	85.6	—	—	64.6

*Sequences in the 5′ to 3′ direction.

above 20 cm^{-1} are independent of the salt concentration. However, several of the low-frequency eigenmodes and eigenfrequencies (below 20 cm^{-1}) exhibit rather strong dependence on salt concentration as can be seen in Figs. 2 and 3.

A particularly interesting finding is that the lowest eigenfrequency of the B conformation drastically *softens* as the salt molarity increases, particularly so in the region around 2.0 M NaCl where the B–Z transition occurs—in contrast to the corresponding mode of the Z form, which is essentially salt independent.

The kinetic pathway through which the B conformation of a d(C-G) sequence converts into the left-handed Z conformation when the former becomes thermodynamically unstable is unknown. All pathways proposed to date (for review, see ref. 15) are simple schemes based on intuition and stereochemical modeling. The decrease found here of the lowest B eigenfrequency with increasing salt concentration suggests that the transition may be initiated via a mechanism well known in solid-state physics [e.g., in displacive transitions of ferroelectrics (31)]—namely, via a *soft mode*. The distribution of vibrational energy (amplitudes) over the atoms in the structure for this lowest frequency B mode involves primarily large motions of the end phosphates and smaller amplitude motions of the internal phosphates. This supports the idea of a soft mode mechanism primarily involving the sugar phosphate backbone.

Some years ago, Prohofsky and coworkers (32) proposed, on the basis of lattice dynamic calculations, that the A-to-B transition of long DNA in hydrated fibers takes place by way of mode softening. Such mode softening has been experi-

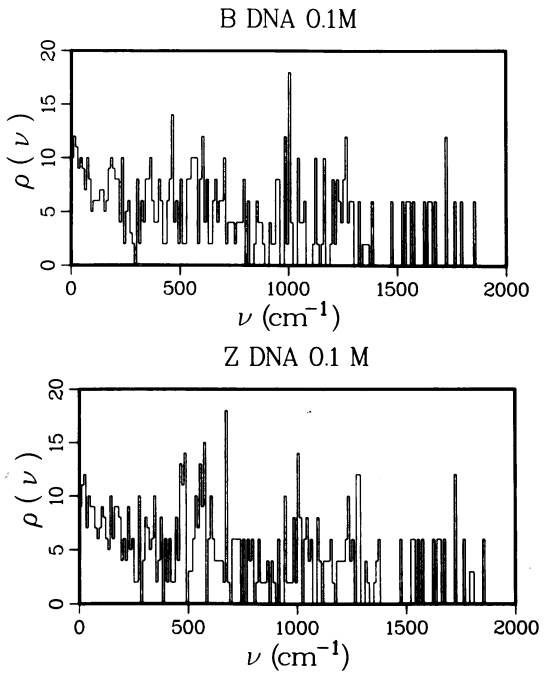

FIG. 1. The unnormalized density of vibrational states of d(C-G)$_3$ in B and Z conformation at 298.14 K and 0.1 M NaCl.

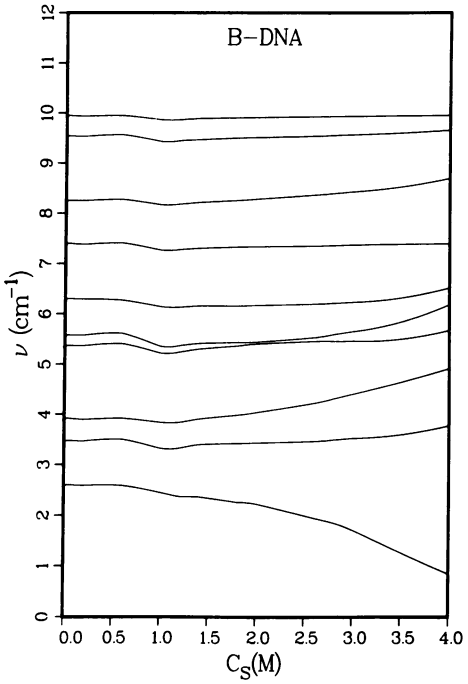

FIG. 2. Dependence of the 10 lowest eigenfrequencies of d(C-G)$_3$ in B conformation on NaCl concentration at 298.14 K.

mentally observed to occur in low-frequency Raman studies of hydrated DNA fibers (33, 34). However, it has been currently shown (35) that the Raman mode softening is strongly affected by interhelical interactions present in ordered samples. Nevertheless, considering the fact that certain sequences undergo salt-induced B–A transitions in solution (36) [as predicted when using the PMF approach (18)], it seems possible that the mechanism could be a general one for solvent-induced order–order transitions of DNA.

Within our treatment the relative stability of the B and Z conformations of a DNA oligomer at any salt molarity, c_s, and temperature, T, is determined by the free energy difference, $\Delta F(B, Z) = F(Z) - F(B)$, computed using Eq. 4 for each conformation. The contribution to ΔF_{vib} from the classical formula in Eq. 4 is negligible. The quantum mechanical harmonic oscillator partition function gives ΔF = 0.53 kcal/mol, with an entropy of 3.16 cal·K^{-1} per mol (or 0.53 cal·K^{-1} per mol of base pairs).

In Fig. 4 we have plotted both the total free energy difference [$\Delta F(B, Z)$] and the PMF contribution [$\Delta F_1(B, Z)$] versus salt molarity for the hexamer we study here. In the case at hand, one sees that the whole effect of including the intramolecular interactions and vibrational contributions in the free energy balance is a curve shifted relative to the PMF curve by a small salt-independent constant. This result completely corroborates the earlier assertion (16–19) that in the canonical case of salt-induced B–Z transition [i.e., that of a d(C-G) helix in alkali halide solutions] the total free energy balance is dominated by DNA phosphate-ionic cloud statistical interactions—due to cancellations between the other, individually much larger, contributions that occur when one forms free energy differences. Although the PMF contributions only account for ≈10% of the total energy in both the B and Z conformations, it is essentially all that matters in the free energy difference that governs the relative stability of the two conformations.

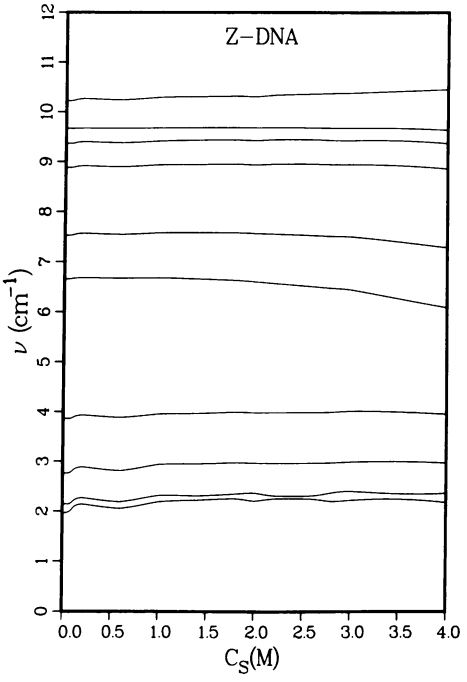

FIG. 3. Dependence of the 10 lowest eigenfrequencies of d(C-G)$_3$ in Z conformation on NaCl concentration at 298.14 K.

CONCLUSIONS

Within the approximations introduced to cope with the otherwise intractable many-body problems involved, both the low-frequency modes and the thermodynamic stability of the DNA oligomer studied have been found to depend on solvent-averaged coulomb interactions in a conformation-specific manner. We believe that this behavior is typical not only for DNA but also for other heavily charged biomolecules (e.g., the RNA and some proteins) in aqueous electrolytes. It is likely that simple environmental changes (e.g, variation of salt concentration) do not only render structural transitions thermodynamically possible (via energy changes) but may

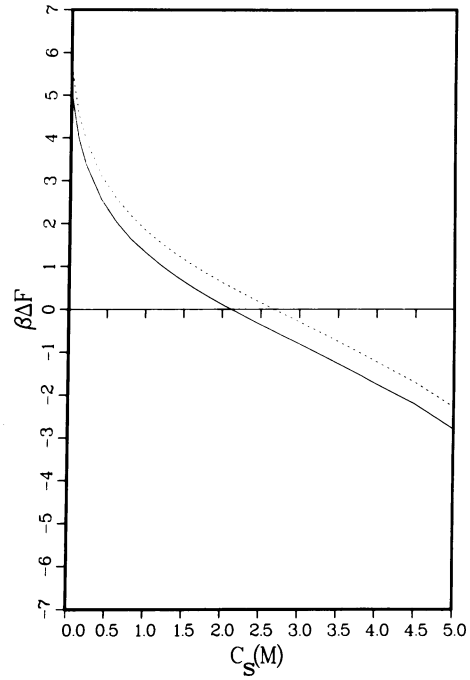

FIG. 4. Dependence of the total B/Z free energy difference ΔF(B, Z) (——) and PMF contribution ΔF_1(B, Z) (– – –) of d(C-G)$_3$ on NaCl concentration.

also provide a kinetic pathway (via mode softening) for salt-induced transitions of charged biomolecules. However, nonlinearities that cannot be treated within the harmonic approximation may also be important (37).

Cancellations of the individually big intramolecular energies, such as those obtained here for the B and Z_I DNA conformations, must probably take place whenever structural transitions can be induced by solvent changes. Such changes generally give rise to rather small free energy changes that cannot compensate large intramolecular energy differences. An obvious limitation of our theory at its present state of development is that water is treated only as a dielectric continuum. As far as the relative thermodynamic stabilities of DNA conformations are concerned, we expect this to be a serious problem only in the case of sequences (e.g., dA·dT stretches) where the geometry of DNA hydrogen donors and acceptors in some of the conformations envisaged (e.g., the B form) permits formation of stable structural water networks (38). In the absence of such sequence-specific hydration patterns, the contribution of the short-range DNA–water energies should be similar and therefore does not influence structural transitions due to cancellations.

However, due to both mass loading of the structure and changes in the effective force constants (stiffening), a microscopic treatment of water (and DNA–water interactions) would most likely yield somewhat different eigenfrequencies of the system. We do not expect such a treatment to yield qualitatively different results concerning the salt dependence (e.g., mode softening) of the vibrational spectrum. On physical grounds it is expected that coupling some of the low-frequency DNA modes to the collective water degrees of freedom will lead to additional frequency shifts and damping. These dynamic effects cannot be treated within the PMF framework, which, strictly speaking, is valid only in quasi-static equilibrium (i.e., when the DNA eigenfrequencies are either much smaller or larger but not comparable to the solvent characteristic frequencies). In the latter case, the quadratic form of Eq. 5 cannot be interpreted as an effective harmonic potential energy.

In principle, molecular dynamics could be the method of choice for studying the coupled DNA–solvent dynamics at the microscopic level, but, as we have already discussed in the introduction, the MD apparatus in its present state of development is simply not powerful enough to handle complex multicomponent charged systems such as DNA in an aqueous electrolyte. We feel that approximate, physically oriented theories such as the one presented above will help us treat many problems in the field of biomolecular structure and dynamics, at least semiquantitatively.

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