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Hydration Changes Accompanying Helix-to-Coil DNA Transitions

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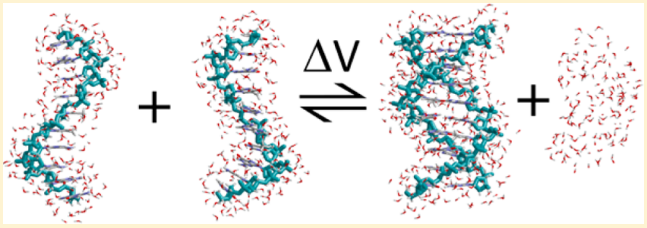
ABSTRACT: We applied ultrasonic velocimetric and high-precision densimetric measurements to characterizing the helix-to-coil transition of the GGCATTACGG/CCGTAATGCC decameric DNA duplex. The transition was induced either by temperature or by mixing the two complementary single strands at isothermal conditions. The duplex dissociation causes increases in volume and expansibility while resulting in a decrease in compressibility. Our volumetric data in conjunction with computer-generated structural information are consistent with the picture in which the duplex dissociation is accompanied by an uptake of ∼180 water molecules from the bulk phase into the hydration shell of the DNA. Analysis of our compressibility and expansibility data reveals that the single-stranded conformation is likely to exist as a heterogeneous mixture of nearly isoenergetic subspecies differing in volume and enthalpy. We use our estimate of the change in hydration to evaluate the hydration and configurational contributions to the helix-to-coil transition entropy. The duplex dissociation is accompanied by an increase in configurational entropy, ΔS_{conf}, of ∼23 cal mol^{-1} K^{-1} per nucleotide, which signifies liberation of manifold frozen degrees of freedom involved in maintaining the conformational stability of the duplex and the related stiffening of the heterocyclic bases and the sugar−phosphate backbone. To the best of our knowledge, this is the first experimental estimate of the change in configurational entropy associated with the helix-to-coil transition of a DNA.



INTRODUCTION

Water, being an integral part of DNA, exerts a strong and ubiquitous influence on its conformational preferences and the thermodynamic and kinetic properties of all nucleic acid recognition events, including ligand and protein binding and helix-to-coil and helix-to-helix transitions.[1−7] Helix-to-coil transitions of nucleic acids, in addition to dramatic changes in structure and dynamics, lead to a ∼25% increase in solvent exposure of functional groups, a decrease in charge density due to strand separation, and the concomitant release of counterions to the bulk. Clearly, these alterations influence the differential distribution of water between the bulk and hydration phases of the helix and coil DNA conformations. The differential hydration, in turn, acts as a contributing factor to modulating the differential thermodynamics of the two conformations.

Despite the importance of hydration as a driving force determining the conformational preferences of nucleic acids, survey of the literature reveals that investigations of hydration changes accompanying duplex-to-single strand transitions of nucleic acids are scarce with no coherent picture emerging.[8−13] The osmotic stress technique has been applied by several researchers to characterization of hydration of DNA in the helix and coil conformations.[10−13] In osmotic stress, the activity of water, a_1, is modulated by adding a preferentially excluded cosolvent, while the apparent equilibrium constant, K, of the reaction under study (in this case, the heat-induced helix-to-coil transition) is measured as a function of a_1.[14] The slope $(\partial \ln K/\partial \ln a_1)$ is related to the reaction-induced changes in the number of water, Δn_1, and cosolvent, Δn_3, molecules preferentially associated with/excluded from the reactants:

$$(\partial \ln K/\partial \ln a_1) = \Delta n_1 - (N_1/N_3)\Delta n_3 \quad (1)$$

where N_1 and N_3 are the numbers of moles of water and cosolvent present in solution, respectively.

Results of osmotic stress measurements have been generally interpreted to suggest more extensive hydration of the double-stranded conformation relative to the single-stranded conformation of polymeric and oligomeric DNA and RNA.[10,11,13] This interpretation is in conflict with the structural picture of DNA dissociation in which a large number of previously buried groups become accessible to water. The main ambiguity of interpretation of osmotic stress results stems from the necessity to separate the first (hydration) and second (cosolvent binding) terms of eq 1. Note that Δn_1 and Δn_3 in eq 1 are not constant but may change with an increase in cosolvent activity, a_3, and the concomitant decrease in water activity, a_1. In addition, Δn_1 and Δn_3 are not independent of each other; an increase in Δn_3 would cause a reduction in Δn_1 in a manner that depends on the steric number of water molecules replaced by a cosolvent molecule upon its association with the DNA. These complexities may lead to misinterpretation of cosolvent-dependent duplex-to-single strand DNA equilibrium data if rationalized solely in terms of Δn_1 ignoring the effect of Δn_3. In a careful and systematic study by Spink et al.,[12] these issues

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have been thoroughly analyzed. The authors have demonstrated that the apparently negative differential hydration of single and double-stranded DNA conformations [$(\partial \ln K/\partial \ln a_1) < 0$] stems from a nonzero value of Δn_3 amplified by the ratio (N_1/N_3), while the actual value of Δn_1 is, probably, positive.[12] In fact, the positive sign of Δn_1 would be consistent with a ~25% increase in solvent-accessible surface area accompanying duplex dissociation.

Low-temperature differential scanning calorimetric (DSC) measurements performed in low-humidity DNA samples have revealed a smaller number of nonfreezing water molecules associated with single-stranded relative to double-stranded DNA.[8,9] While these results have been interpreted as suggesting higher hydration of the duplex DNA, the population of nonfreezing water molecules at the conditions of low humidity represents only a fraction of the full hydration shell of DNA in solution. It is not clear how large this fraction is and what specific water molecules are involved in it. In other words, we have yet to know what specific DNA−water interactions render water molecules nonfreezing and to what extent such waters are representative of DNA hydration as a whole. These ambiguities compromise the quantitative nature of interpretation of low-temperature DSC results in terms of hydration.

To better understand the determining role of water in the conformational preferences of nucleic acids, we carry out in this study the volumetric characterization of the helix-to-coil transition of an oligomeric DNA. Compared to other techniques, volumetric measurements offer a complementary and more direct way of sampling DNA hydration and changes in hydration accompanying various processes involving DNA.[15−22] Volumetric observables, including volume, compressibility, and expansibility, are nonselective and "sense" the entire population of water molecules solvating a solute.[17−19,22] In the present work, we employ a combination of volumetric measurements to characterize changes in hydration accompanying the helix-to-coil transition of the decameric DNA duplex GGCATTACGG/CCGTAATGCC. The specific nucleotide sequence of the duplex under study was chosen to meet the following criteria: (i) to be of mixed A/T and G/C composition; (ii) to preclude formation of alternative structures (e. g., hairpins); and (iii) to exhibit an intermediate thermal stability (T_M ~ 50 °C) at moderate salt concentrations (~100 mM NaCl). The latter requirement is important for volumetric melting experiments and is related to the peculiarities of the densimetric and ultrasonic techniques employed in the current study. They provide the optimum precision of measurement at low to moderately high temperatures (up to ~70 °C).

MATERIALS AND METHODS

The non-self-complementary decameric oligonucleotides 5′-GGCATTACGG-3′ and 5′-CCGTAATGCC-3′ were synthesized and cartridge purified by ACGT, Inc. (Toronto, ON, Canada). Sodium chloride and sodium cacodylate were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). EDTA (free acid) was purchased from Fisher Biotech (Fair Lawn, NJ, USA). These reagents were of the highest grade commercially available and used without further purification. All solutions were prepared using doubly distilled water.

Prior to each experiment, the oligonucleotides were dissolved in and exhaustively dialyzed against 10 mM cacodylic acid/sodium cacodylate buffer at pH 6.7, 0.1 mM EDTA, and NaCl adjusted to the desired concentration. Dialysis was carried out in 500 Da molecular weight cutoff Tube-O-Dialyzers from G Biosciences (St. Louis, MO, USA). The final dialysis buffer was retained and used for densimetric and ultrasonic velocimetric experiments.

The concentration of the DNA was determined from the absorbance at 260 nm measured at 25 °C with a Cary 300 Bio spectrophotometer (Varian Canada, Inc., Mississauga, Ontario, Canada) using molar extinction coefficients of 98 200 M^{-1} and 91 400 M^{-1} cm^{-1} for the 5′-GGCATTACGG-3′ and 5′-CCGTAATGCC-3′ sequences, respectively. The extinction coefficient was calculated using an additive nearest-neighbor procedure as described by Dr. Richard Owczarzy (http://www.owczarzy.net/extinctionDNA.htm). To avoid concentration-dependent effects on duplex stability, the DNA concentration was kept similar (on the order 0.06 to 0.08 mM) for all of our temperature-dependent UV, densimetric, and ultrasonic velocimetric experiments.

UV Melting Experiments. UV light absorption at 260 nm was measured as a function of temperature in a DNA sample contained in a 1 mm path-length cuvette. These measurements were performed by a Cary 300 Bio spectrophotometer (Varian Canada, Inc., Mississauga, Ontario, Canada). The temperature was changed at a rate of 1 °C per minute. The helix-to-coil transition temperatures, T_M, along with the van't Hoff enthalpies, ΔH_M, were evaluated from our experimental UV melting profiles using standard procedures for a noncomplementary duplex.[23−25] Specifically, to determine the values of T_M and ΔH_M, the temperature dependence of the fraction unfolded, α, computed from a UV melting profile was approximated by the following analytical function:

$$\alpha = [K(T)/C_t][(1 + 2C_t/K(T))^{0.5} - 1] \quad (2)$$

where $K(T) = (C_t/2)[\alpha^2/(1 - \alpha)] = [(C_t/4)(T/T_M)^{\Delta C_P/R}\exp[(T_M\Delta C_P - \Delta H_M)(T^{-1} - T_M{}^{-1})/R]$ is the dissociation constant for a non-self-complementary duplex; and C_t is the total concentration of DNA strands.

High-Precision Densimetry and Ultrasonic Velocimetry. Solution densities were measured with a precision of $\pm 1.5 \times 10^{-6}$ g cm^{-3} using a vibrating tube densimeter (DMA-5000, Anton Paar, Graz, Austria). The partial molar volume, $V°$, of the DNA was calculated from density values using the relationship $V° = M/\rho_0 - (\rho - \rho_0)/(\rho_0 C)$, where ρ and ρ_0 are the densities of the DNA solution and the neat solvent, respectively; C is the molar concentration of the DNA; and M is the molecular weight of the DNA.

Solution sound velocity measurements were carried out at a frequency of 7.2 MHz using the resonator method and a previously described differential technique.[26−28] The analysis of the frequency characteristics of the ultrasonic resonator cells required for sound velocity measurements was performed by a Hewlett-Packard model E5100A network/spectrum analyzer (Mississauga, ON, Canada). For the type of ultrasonic resonators used in this work, the relative precision of the sound velocity measurements is about $\pm 1 \times 10^{-4}$ %.[26,29] The key characteristic of a solute directly derived from ultrasonic velocimetric measurements is the relative molar sound velocity increment, $[U] = (U - U_0)/(U_0 C)$, where U and U_0 are the sound velocities in the DNA solution and the neat solvent, respectively.

The values of the relative molar sound velocity increment, $[U]$, were used in conjunction with the measured partial molar volume data, $V°$, to calculate the partial molar adiabatic compressibility, $K°_S$, of the DNA from the following relationship:

$$K°_S = \beta_{S0}(2V° - 2[U] - M/\rho_0) \quad (3)$$

where β_{S0} is the coefficient of adiabatic compressibility of the solvent.[16,30,31] The densimetric and ultrasonic velocimetric experiments were performed at least three times. The average values of $[U]$ and $V°$ were used in the analysis.

In titration experiments, aliquots of the 5′-GGCATTACGG-3′ strand (~1 mM) were added to the solution containing the 5′-CCGTAATGCC-3′ strand (~0.2 mM). Densimetric and acoustic titrations were performed at 25 °C and 100 mM NaCl following the previously described experimental protocol.[32] In temperature scanning experiments, high-precision densimetry and ultrasonic velocimetry were employed to measure changes in volume and relative molar sound velocity increment accompanying the coil-to-helix transition of

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the initially unfolded duplex upon a decrease in the temperature of the sample from 60 to 18 °C at 10 mM NaCl.

Computation of Solvent-Accessible Surface Areas and Molecular Volumes. Our analysis is based on the computer-generated canonical B-form DNA structure of the GGCATTACGG/CCGTAATGCC duplex using AmberTools (v1.3) [Nucleic Acid Builder (NAB) T J. Macke, W.A. Svrcek-Seiler, R. A. Brown, I. Kolossváry, Y. J. Bomble, and D. A. Case]. The initial single-stranded conformations of the 5′-GGCATTACGG-3′ and 5′-CCGTAATGCC-3′ oligonucleotides were created by deleting respective complementary strand from the duplex. In order to model a more plausible structures, molecular dynamics was carried out on the single- and double-stranded oligonucleotides using GROMACS (v 4.0.7).[33−36]

In our simulations, the single-stranded DNA molecule was contained in a rectangular box with a 40 Å distance between the solute and box sides; this system contained 37 840 water molecules and 25 sodium ions (volume: 1713.23 nm^3, density 979.409 g/L). Initially, a 100 ps steepest-descent minimization run was conducted to relax each structure [http://www.user.gwdg.de/~ggroenh/SaoCarlos2008/html/build.html#top, accessed July 9, 2013]. A 100 ps solvent equilibration run was performed at 300 K and 1 bar. Subsequently, the conformational space available to the DNA in solution was explored by running a 1 ns MD. The solvent-accessible surface area and molecular volume were calculated following these 1 ns runs. PDB "snapshots" were generated for conformations with the largest and smallest total solvent-accessible surface areas during the 1-ns simulations, which were subsequently employed in subsequent intrinsic volume and surface area calculations.

We calculated the solvent-accessible surface area, S_A, for each double- or single-stranded structure as the sum of the accessible surface areas of all atoms in the structure. The intrinsic volumes, V_M, of the DNA in its duplex and coil conformations were calculated as molecular volumes as described by Richards.[37,38] The program Molecular Surface Package (MSP) version 3.9.3 was obtained from Dr. Michael Connolly at www.biohedron.com and used to calculate the solvent-accessible surface area and molecular volume for each structure, using a 1.4 Å probe radius on a Linux platform.

RESULTS

Figure 1 presents the UV-detected melting profiles of the GGCATTACGG/CCGTAATGCC duplex at NaCl concentrations between 10 and 1000 mM. Inspection of the melting curves shown in Figure 1 reveals that, at and above 50 mM NaCl, the duplex is fully formed and stable at 25 °C. The dependence of the van't Hoff transition enthalpies, ΔH_M (kcal mol^{-1}), on the melting temperature, T_M (°C), is linear and can be approximated by $\Delta H_M(T) = 60.5\ (\pm 0.8) + 0.23\ (\pm 0.02)\ T$. The slope of 0.23 ± 0.02 kcal mol^{-1} K^{-1} is the change in heat capacity, ΔC_P, accompanying duplex dissociation. If normalized per number of base pairs (23 cal mol^{-1} K^{-1}), our measured ΔC_P is in good agreement with calorimetric results obtained on oligomeric and polymeric DNA duplexes.[39−46]

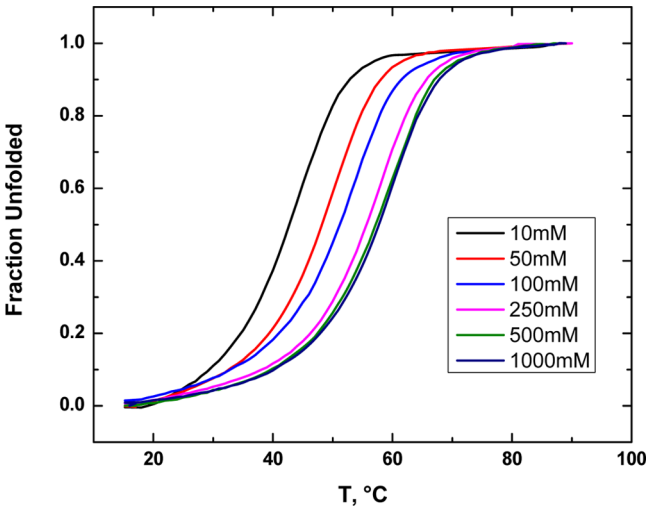

Figure 1. Temperature dependence of the fraction unfolded (single stranded) of the DNA calculated from UV melting profiles at 10, 50, 100, 250, 500, and 1000 mM NaCl. The average DNA concentration was 0.08 mM.

By plotting the transition temperatures, T_M, as a function of $\ln[Na^+]$ (not shown), we calculate the number of sodium ions, Δn_{Na^+}, released to the bulk upon the helix-to-coil transition of the duplex:[47−50]

$$\Delta n_{Na^+} = (\Delta H_M / R T_M^{\,2})(\partial T_M / \partial \ln[Na^+]) \quad (4)$$

The calculated value of Δn_{Na^+} is 1.9 ± 0.2. Thus, duplex dissociation results in a release of 0.095 Na^+ ions per nucleotide, a result typical for oligomeric duplexes.[41,51]

Figures 2 and 3, respectively, plot changes in relative molar sound velocity increment, $\Delta[U]$, and volume, ΔV, accompanying the incremental addition of the 5′-GGCATTACGG-3′ strand into the solution containing the 5′-CCGTAATGCC-3′ strand as a function of the strands' molar ratio, r. The titration experiments were performed at 100 mM NaCl. Inspection of Figures 2 and 3 reveals that decreases in both the relative molar sound velocity increment, $\Delta[U]$, and the partial molar volume, ΔV, accompany the duplex formation at 25 °C. A change in adiabatic compressibility, ΔK_S, accompanying duplex formation was calculated from the modified eq 3: $\Delta K_S = 2\beta_{S0}(\Delta V - \Delta[U])$.

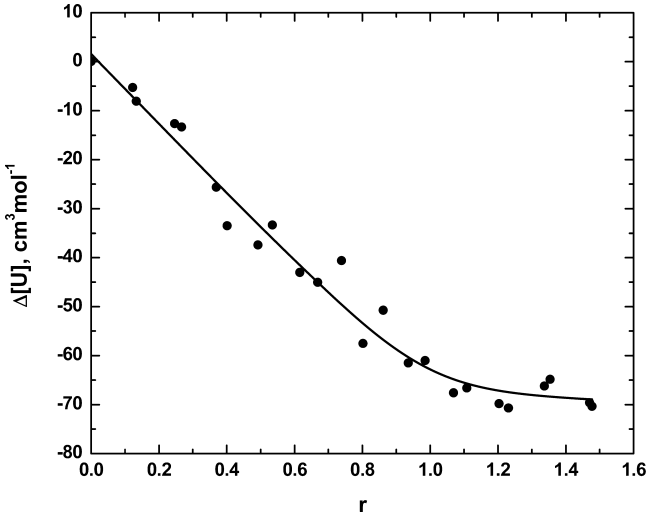

Figure 2. A change in the relative molar sound velocity increment, $\Delta[U]$, of the 5′-CCGTAATGCC-3′ strand (~0.16 mM) accompanying the addition of aliquots of the complementary 5′-GGCATTACGG-3′ strand (~0.93 mM) plotted as a function of the strands' molar ratio, r. The acoustic titration was carried out at 25 °C and 100 mM NaCl. The data were approximated by the analytical function representing a one-to-one stoichiometric binding (solid line).[78]

The values of $\Delta[U]$, ΔV, and ΔK_S determined for the dissociation of the GGCATTACGG/CCGTAATGCC duplex to the complementary 5′-GGCATTACGG-3′ and 5′-CCGTAATGCC-3′ strands are given in the second row of Table 1. At 25 °C, the duplex dissociation results in a decrease in compressibility, while causing an increase in volume.

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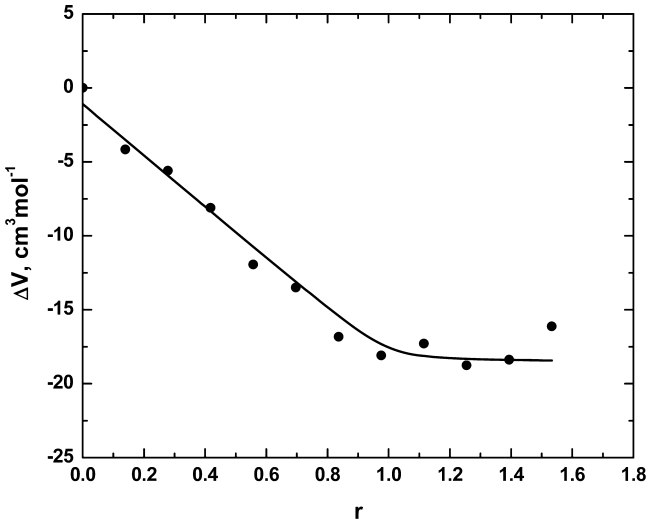

Figure 3. A change in volume, ΔV, accompanying addition of aliquots of the 5′-GGCATTACGG-3′ strand (~0.89 mM) to the solution containing the complementary 5′-CCGTAATGCC-3′ strand (~0.18 mM) plotted as a function of the strands' molar ratio, r. The densimetric titration was carried out at 25 °C and 100 mM NaCl. The data were approximated by the analytical function representing a one-to-one stoichiometric binding (solid line).[78]

Table 1. Volumetric Properties of the Helix-to-Coil Transition

T, °C	$\Delta[U]$, cm^3 mol^{-1}	ΔV, cm^3 mol^{-1}	ΔK_S, 10^{-4} cm^3 mol^{-1} bar^{-1}
25.0	72 ± 5	17 ± 2	−49 ± 6
43.2	57 ± 5	26 ± 3	−27 ± 7

Figures 4 and 5, respectively, present changes in relative molar sound velocity increment, $\Delta[U]$, and volume, ΔV, accompanying duplex formation by the initially dissociated 5′-GGCATTACGG-3′ and 5′-CCGTAATGCC-3′ strands induced the by a decrease in temperature from 60 to 18 °C at 10 mM NaCl. The values of $\Delta[U]$, ΔV, and ΔK_S for the duplex

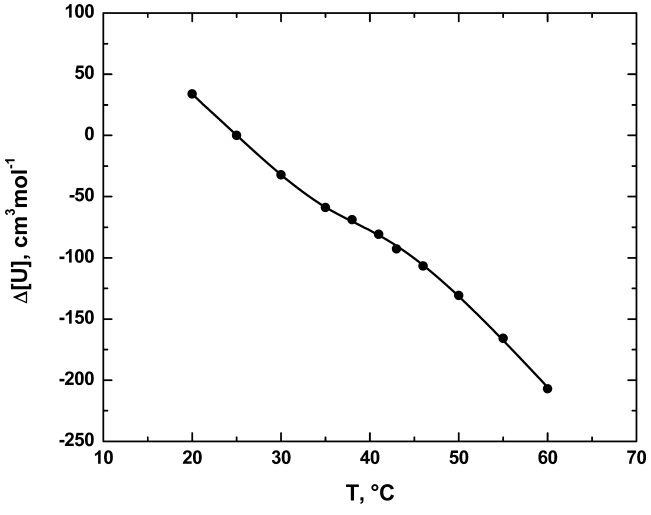

Figure 4. Temperature dependence of change in the relative molar sound velocity increment, $\Delta[U]$, of the duplex DNA at 10 mM NaCl. The DNA concentration was 0.06 mM. The data were approximated by eq 2, an analytical function representing a bimolecular helix-to-coil melting transition of a non-self-complementary duplex (solid line).[23,25]

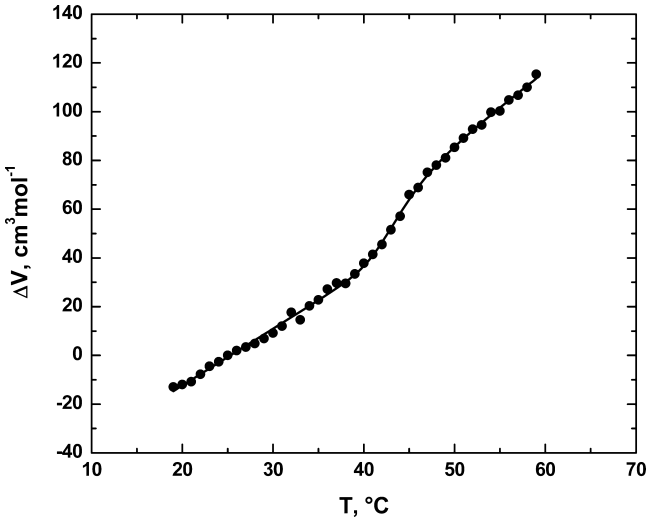

Figure 5. Temperature dependence of the change in volume, ΔV, of the duplex DNA at 10 mM NaCl. The DNA concentration was 0.06 mM. The data were approximated by eq 2, an analytical function representing a bimolecular helix-to-coil melting transition of a non-self-complementary duplex (solid line).[23,25]

dissociation at the transition temperature, T_M, of 43.2 ± 0.1 °C are presented in the third row of Table 1. The positive sign and the order of ΔV (26 ± 3 cm^3 mol^{-1}) are consistent with the pressure-perturbation calorimetric (PPC) results from the Privalov lab obtained on three dodecameric duplexes (two with mixed AT/GC composition and one with an all-GC composition).[52] Our measured ΔV is also in good agreement with the high-pressure data from the Macgregor lab.[53] In fact, for our oligomeric sequence, we calculate a transition volume of 18.4 cm^3 mol^{-1} using the additive approach and the nearest-neighbor parameters presented by Dubins and Macgregor.[53] This value is in excellent agreement with our results presented in Table 1.

Inspection of Figure 5 reveals that the duplex melting is accompanied by an increase in the slope of the baseline (the post-denaturational baseline is steeper than the pre-denaturationall baseline). A change in expansibility, $\Delta E = (\partial\Delta V/\partial T)_P$, associated with the melting transition can be determined as the differential baseline slope which equals 0.6 ± 0.2 cm^3 mol^{-1} K^{-1}. This value is in excellent agreement with 0.5 ± 0.2 cm^3 mol^{-1} K^{-1}, the value of $\Delta E = \Delta\Delta V/\Delta T_M$ determined from the data presented in Table 1 [(26 − 17)/(43.2 − 25)]. Thus, at the experimental conditions employed in this study, duplex dissociation brings about increases in volume and expansibility, while causing a decrease in compressibility. It should be noted that the sign of our determined ΔE for the decamer dissociation is in agreement with positive changes in expansibility reported for the melting transitions of polymeric duplexes.[39,54] Thus, an increase in expansibility may be a universal volumetric signature of duplex melting.

Table 2 shows our computed solvent-accessible surface areas, S_A, and intrinsic volumes, V_M, of the DNA in the coil and helix conformations. The uncertainties of the data in Table 2 correspond to the standard deviations of S_A and V_M computed for the MD-simulated conformational substates of the single- and double-stranded DNA. Inspection of Table 2 reveals that the helix-to-coil transition of the DNA is accompanied by a significant increase in solvent-accessible surface area, ΔS_A, of 1270 ± 53 Å^2 (~25%) and an insignificant decrease in intrinsic

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Table 2. Molecular, V_M, Volumes and Solvent-Accessible Surface Areas, S_A, of the DNA in the Coil and Helix Conformations

conformation	S_A, Å^2	V_M, Å^3
coil	5031 ± 40	5083 ± 6
helix	3759 ± 35	5148 ± 6

volume, V_M, of 65 Å^3 or 39 ± 7 cm^3 mol^{-1} (~1%). The increase in solvent-accessible surface area, S_A, accompanying duplex-to-single strand transition of 127 Å^2 per base pair is in qualitative, although not quantitative, agreement with an estimate of 200 Å^2 per base pair made for a polymeric duplex.[55]

DISCUSSION

Change in Hydration Accompanying Duplex Dissociation. We use our measured change in volume, ΔV, accompanying the duplex dissociation to evaluate the number of water molecules exchanged between the bulk and hydration phases. The partial molar volume, V°, of a solute is the sum of the following contributions:[56−58]

$$V^\circ = V_M + V_T + V_I + \beta_{T0}RT \quad (5)$$

where V_M is the intrinsic volume of a solute; V_T is the thermal volume; V_I is the interaction volume; β_{T0} is the coefficient of isothermal expansibility of the solvent; R is the universal gas constant; and T is the temperature.

A change in volume, ΔV, accompanying duplex dissociation is given by changes in the intrinsic, thermal, and interaction terms:

$$\Delta V = \Delta V_M + \Delta V_T + \Delta V_I \quad (6)$$

A change in intrinsic volume, ΔV_M, accompanying duplex dissociation is −39 ± 7 cm^3 mol^{-1} as determined from our computational data presented in Table 2. A change in thermal volume, ΔV_T, can be estimated by multiplying a change in solvent-accessible surface area, ΔS_A, by δ, the thickness of the thermal volume around DNA.[58,59] For DNA, the value of δ is ~0.5 Å.[22,59] With ΔS_A of 1270 ± 53 Å^2, we estimate ΔV_T = 635 ± 27 Å^3 (0.5 × 1270) or 382 ± 16 cm^3 mol^{-1}. Hence, the interaction contribution, $\Delta V_I = \Delta V - \Delta V_M - \Delta V_T$, is negative and equal to −326 ± 18 cm^3 mol^{-1} (17 + 39 − 382).

The interaction term, ΔV_I, reflects exchange of water between the bulk and hydration phases. The link between ΔV_I and the number of water molecules, Δn_h, released or taken up upon duplex dissociation is given by $\Delta V_I = \Delta n_h(V_h - V_0)$, where V_h and V_0 are the partial molar volumes of water of DNA hydration and bulk water, respectively. For a DNA, the partial molar volume of water of hydration is ~10% smaller than that of bulk water; hence, the differential partial molar volume, $(V_h - V_0)$, is ~-1.8 cm^3 mol^{-1}.[60] With this value, we determine that duplex dissociation causes an uptake of 180 ± 10 (−326/1.8) water molecules from the DNA hydration shell to the bulk. This value is ~25% larger than 140, the number of first coordination-sphere water molecules taken up upon duplex formation. The latter can be computed by dividing the change in solvent-accessible surface area, ΔS_A, by 9 Å^2, the effective cross-section of a water molecule (1270/9 ≈ 140). The observed disparity suggests that DNA dehydration involves water molecules not only from the first but, partly, also the second hydration layers. This finding is consistent with the picture in which some DNA domains are solvated by more than one layer of water molecules, a notion suggested by a number of researchers.[20,55,61−63]

The uptake of ~180 water molecules (or 9 water molecules per nucleotide) represents ~30% of 560 (5031/9) or 28 waters molecules per nucleotide involved within the first hydration layer of the single-stranded conformation. The overall increase in hydration associated with duplex DNA dissociation is intuitively appealing and consistent with an increase by ~25% of solvent-accessible surface area. This result is in qualitative disagreement with the conclusions reached in several works in which osmotic stress results have been rationalized in terms of water release/uptake ignoring the possible effect of cosolvent binding.[10,11,13,64] Cosolvent-dependent data on thermal stability of polymeric and oligomeric DNA and RNA duplexes have been interpreted in these works as suggesting higher hydration of the duplex compared to the single-stranded conformation. The difference between the volumetric and osmotic stress-based estimates may be related to the difference in criteria of detection and, hence, the sampled pools of hydration water. For example, some of the waters of preferential hydration which are detected by the osmotic stress technique may be volumetrically "silent" or *vice versa*. As mentioned above, another possibility may be related to the complexities of molecular interpretations of osmotic stress results. In the osmotic stress analysis, only completely excluded cosolvents provide the correct estimate of hydration/dehydration accompanying the molecular event under investigation.[65] However, many conventionally employed cosolvents are not completely excluded from the nucleic acid surface but interact with it.[12,55,66,67] In fact, nonzero cosolvent−DNA interactions may alter the magnitude and even the sign of the measured slope $(\partial \ln K/\partial \ln a_1)$ which, understandably, would compromise the validity of the proposed interpretation (an increase in hydration may be confused with a decrease).[12]

Relaxation Contribution to Compressibility. The uptake of ~180 water molecules from the bulk should be reflected in the measured changes in adiabatic compressibility and expansibility accompanying the helix-to-coil transition of the decamer. Compressibility and expansibility are volumetric observables known to be strongly sensitive to the hydration properties of a solute and/or changes in these properties upon binding reactions and conformational transitions.[15,16,19−22,52,54,68]

A change in compressibility associated with a conformational transition of DNA can be presented as the sum:

$$\Delta K_s = \Delta K_M + \Delta K_h + \Delta K_{rel} \quad (7)$$

where ΔK_M, ΔK_h, and ΔK_{rel} are, respectively, the changes in the intrinsic, hydration, and relaxation contributions to the partial molar adiabatic compressibility of a solute.

A double-stranded DNA is a rigid molecule with a negligible intrinsic compressibility, K_M.[18,19,22,69] The same should be true for a single-stranded DNA which lacks compressible interior voids. Therefore, the helix-to-coil transition-induced change in the intrinsic compressibility of a DNA, ΔK_M, in eq 7 can be set to zero. The hydration change in compressibility, ΔK_h, is proportional to the number of water molecules, Δn_h, taken up from the bulk upon the transition; $\Delta K_h = \Delta n_h(K_{Sh} - K_{S0})$, where K_{Sh} and K_{S0} are the partial molar adiabatic compressibilities of water of DNA hydration and bulk water, respectively. For charged solutes, such as DNA, the partial molar adiabatic compressibility of water of hydration, K_h, is roughly ~25% smaller than that of bulk water, K_0.[60] Thus, we use the value of $(K_h - K_0)$ of approximately −2 × 10^{-4} cm^3

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mol^{-1} bar^{-1}. However, as will be discussed below, the real value of $(K_h - K_0)$ may be higher (less negative). With $(K_h - K_0)$ of -2×10^{-4} cm^3 mol^{-1} bar^{-1}, we calculate a value of ΔK_h of $-(360 \pm 20) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} $(-180 \times 2 \times 10^{-4})$.

This estimate of ΔK_h is much more negative than $-(49 \pm 6) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, the net change in compressibility accompanying the duplex dissociation at 25 °C. The observed discrepancy is suggestive of the presence of a large positive relaxation contribution, ΔK_{rel}. Based on eq 7, the value of ΔK_{rel} is $(310 \pm 20) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} $(360 \times 10^{-4} - 49 \times 10^{-4})$. It originates from the pressure-induced shift in the populational distribution of solute species from conformations with a large volume to those characterized by a smaller volume.[70] Clearly, conformational flexibility and, hence, relaxation compressibility, K_{rel}, is larger for the single-stranded relative to the duplex conformation of DNA. Importantly, to be detected in ultrasonic velocity measurements conducted at a frequency ~7 MHz, the relaxation times of the pressure-induced high-to-low volume structural transitions of the DNA should be on the order of μs or faster. Assuming conformational rigidness of the duplex conformation, the relaxation term, ΔK_{rel}, in eq 7 originates predominantly from the conformational diversity of the single-stranded conformation.

The relaxation contribution to compressibility is given by $K_{rel} = (\langle \Delta V^2 \rangle - \langle \Delta V \rangle^2)/RT$, where $\langle \Delta V \rangle$ is the ensemble average change in volume relative to the "ground state" conformation.[71] The single-stranded conformation is, obviously, extremely heterogeneous with respect to its structural and volumetric properties. Therefore, determination of the ensemble average value of ΔV is extremely difficult if not impossible without simplifications. As an extreme case of simplification, the populational distribution of the single strands can be operationally presented as consisting of two states separated by the differential volume, ΔV. Given the two-state assumption, the relaxation contribution to compressibility, ΔK_{rel}, can be presented as follows:[60]

$$\Delta K_{rel} = (\Delta V^2/RT)\alpha(1 - \alpha) \quad (8)$$

where α is the fractional composition of the low-volume conformational states.

To estimate the order of ΔV, we calculate its minimum value by setting the fractional population, α, equal to 0.5. With α of 0.5, the value of ΔV is ~55 cm^3 mol^{-1}. Thus, the differential volume of the two effective single-stranded populations is at least ~55 cm^3 mol^{-1}. This number is larger than the differential volume of the double- and single-stranded DNA conformations being on the order of ~2% of the partial molar volume of the duplex decamer. While this is not unreasonable, an alternative view may originate from the possibility that the value of $(K_h - K_0)$ used in our analysis was too large (too negative). The value we used $(-2 \times 10^{-4}$ cm^3 mol^{-1} $bar^{-1})$ is characteristic of highly charged solutes of which DNA is, undoubtedly, a representative. However, the new surface that becomes exposed to solvent upon duplex dissociation is uncharged. It contains polar (uncharged) and nonpolar atomic groups which are characterized by much less negative contributions to hydration.[17] While the hydration contribution to compressibility of nonpolar groups is slightly negative at room temperature,[17] the contributions of polar groups in the uncharged nucleic acid analogs nucleic acid bases, ribonucleosides, and deoxyribonucleosides may be positive or negative depending on the individual group.[72] Using a less negative value of $(K_h - K_0)$ would decrease the absolute magnitudes of ΔK_h and, hence, ΔK_{rel}. In fact, if $(K_h - K_0)$ is chosen to be -0.25×10^{-4} cm^3 $mol^{-1}bar^{-1}$ (a value not unreasonable for a mixture of polar and nonpolar groups), ΔK_h would become equal to the net change in compressibility, ΔK, thereby resulting in a near-zero relaxation term, ΔK_{rel} [see eq 7]. Clearly, further studies are required to better understand the relative magnitudes of the hydration and relaxation contributions to a change in compressibility accompanying DNA association/dissociation transitions.

Relaxation Contribution to Expansibility. A change in expansibility associated with DNA dissociation is given by the sum:

$$\Delta E = \Delta E_M + \Delta E_h + \Delta E_{rel} \quad (9)$$

where ΔE_M, ΔE_h, and ΔE_{rel} are, respectively, the changes in the intrinsic, hydration, and relaxation contributions to expansibility.

Analogous to compressibility, the change in intrinsic expansibility, ΔE_M, can be assumed to be zero. DNA, in its duplex or single-stranded conformations, lacks expandable voids. The hydration change in expansibility, ΔE_h, is proportional to the number of water molecules, Δn_h, taken up from the bulk upon the transition; $\Delta E_h = \Delta n_h(E_h - E_0)$, where E_h and E_0 are the partial molar expansibilities of water of DNA hydration and bulk water, respectively. Charged, polar, and nonpolar functional groups all contribute positively to the partial molar expansibility of small solutes.[73,74] Thus, the differential partial molar expansibility, $(E_h - E_0)$, of water of hydration and bulk water is positive for charged, polar, and nonpolar groups. The lesser heterogeneity of the differential partial molar expansibility, $(E_h - E_0)$, enhances the veracity of the analysis of our expansibility results compared to the analysis of compressibility data presented above.

For a charged solute, such as the zwitterionic amino acid glycine, the differential partial molar expansibility, $(E_h - E_0)$, of water of hydration and bulk water is ~0.0045 cm^3 mol^{-1} K^{-1}.[59] Assuming $(E_h - E_0)$ to be equal to 0.0045 cm^3 mol^{-1} K^{-1}, we calculate a ΔE_h of 0.81 ± 0.05 cm^3 mol^{-1} K^{-1} (180×0.0045). This value is somewhat larger than 0.6 ± 0.2 cm^3 mol^{-1} K^{-1}, the net change in expansibility accompanying the duplex dissociation. The disparity is suggestive of the presence of a negative relaxation contribution, ΔE_{rel}, of −0.2 ± 0.2 cm^3 $mol^{-1}K^{-1}$. This number may be statistically significant, or it may reflect an overestimate of the value of $(E_h - E_0)$ used in the analysis. If a 30% lower value of $(E_h - E_0)$ (~0.003 cm^3 $mol^{-1}K^{-1}$) is used, the relaxation contribution, ΔE_{rel}, would reduce to zero. As in the case of compressibility, we emphasize the necessity of further studies to evaluate the relative magnitudes of the hydration and relaxation expansibility contributions in DNA transitions.

The relaxation contribution to expansibility is given by $E_{rel} = (\langle \Delta V \Delta H \rangle - \langle \Delta V \rangle \langle \Delta H \rangle)/RT^2$, where $\langle \Delta H \rangle$ is the ensemble average change in enthalpy relative to the "ground-state" conformation.[71] Provided that the relaxation contribution, ΔE_{rel}, of −0.2 ± 0.2 cm^3 mol^{-1} K^{-1} is statistically significant, it can be analyzed within the framework of the two-state assumption of the population of the single-stranded conformation. For a system that may exist in two states, the relaxation contribution to expansibility is given by:

$$\Delta E_{rel} = (\Delta V \Delta H/RT^2)\alpha(1 - \alpha) \quad (10)$$

With α = 0.5 and ΔV = 55 cm^3 mol^{-1}, the differential partial molar enthalpy, ΔH, of the single-stranded populations is ~2.6

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± 2.3 kcal mol^{-1}. Given the negative sign of ΔE_{rel}, the state with a lower volume is characterized by a higher enthalpy.

Entropic Considerations. At 100 mM NaCl (the ionic strength of our densimetric and ultrasonic velocimetric titration experiments), the transition temperatures, T_M, and van't Hoff enthalpies, ΔH_M, are 53.9 ± 0.1 °C and 72.4 ± 1.4 kcal mol^{-1}, respectively. Extrapolated to 25 °C, changes in entropy, $\Delta S^\circ = \Delta H^\circ/T_M + \Delta C_P \ln(T/T_M) + R \ln(C_T/4)$, and free energy, $\Delta G^\circ = \Delta H^\circ(1 - T/T_M) + \Delta C_P[T - T_M - T \ln(T/T_M)] - RT \ln(C_T/4)$, accompanying the helix-to-coil transition are 181 ± 5 cal mol^{-1} K^{-1} and 11.7 ± 1.5 kcal mol^{-1}, respectively.

A change in entropy, ΔS_b, caused by an association/dissociation event is the sum of the intrinsic (configurational), hydration, and rotational and translational terms:[75]

$$\Delta S^\circ = \Delta S_{conf} + \Delta S_{hyd} + \Delta S_{rt} \quad (11)$$

where ΔS_{conf} is the change in the configurational entropy of the two interacting species (in this, case the two DNA single strands); ΔS_{hyd} is the contribution due to a change in DNA hydration; and ΔS_{rt} is the change in entropy due to the rotational and translational degrees of freedom. For a 1:1 stoichiometric dissociation event, ΔS_{rt} equals 8 cal mol^{-1} K^{-1}.[75]

The duplex dissociation-induced uptake of ∼180 water molecules should have a profound impact on the energetics of the conformational stability of the duplex state. With the average differential partial molar entropy of water of DNA hydration and bulk water of −1.6 cal mol^{-1} K^{-1},[76] the uptake of 180 ± 10 water molecules is accompanied by a negative change in the hydration contribution, ΔS_{hyd}, of −288 ± 16 cal mol^{-1} K^{-1} (−1.6 × 180).

To be rigorous, eq 9 should include a polyelectrolyte term, ΔS_{pe}, signifying the release to the bulk of 1.9 ± 0.2 Na^+ ions. However, given the 100-fold excess of the number of absorbed water molecules over the number of released Na^+ ions (180 versus 1.9), ΔS_{pe} should be much smaller than ΔS_{hyd} in absolute magnitude and, therefore, can be ignored. A more rigorous estimate of the effect of ΔS_{pe} is based on the following consideration. As a first approximation, ΔS_{pe} is given by the mixing entropy $\Delta S_{mix} = \Delta n_{Na^+} R \ln([Na^+]_l/[Na^+]_b)$, where $[Na^+]_l$ are $[Na^+]_b$ are the local (in the vicinity of the duplex) and bulk sodium concentrations, respectively. With the local sodium concentration of ∼1 M,[77] one estimates the polyelectrolyte term, ΔS_{pe}, to be on the order of ∼9 cal mol^{-1} K^{-1} at 100 mM NaCl. This value is much smaller that our estimate of the hydration entropy, ΔS_{hyd}, of −288 ± 16 cal mol^{-1} K^{-1} and, therefore, can be ignored.

With ΔS_{hyd} of −288 ± 16 cal mol^{-1} K^{-1}, we compute a change in configurational entropy, ΔS_{conf}, accompanying the helix-to-coil transition of the DNA of 461 ± 17 cal mol^{-1} K^{-1} (181 + 288 − 8). This large increase in entropy (∼23 cal mol^{-1} K^{-1} per nucleotide) signifies liberation of manifold frozen degrees of freedom involved in duplex formation and the related stiffening of heterocyclic bases and the sugar−phosphate backbone. To the best of our knowledge, this is the first estimate of the change in configurational entropy associated with DNA dissociation.

CONCLUSION

The helix-to-coil transition of the decameric GGCATTACGG/CCGTAATGCC DNA duplex is accompanied by increases in volume and expansibility and a decrease in compressibility. Molecular interpretation of our volume data in conjunction with computer-generated structural information reveals that duplex dissociation is accompanied by an uptake of ∼180 water molecules from the bulk phase into the hydration shell of the DNA. Analysis of our compressibility and expansibility data suggests that the single-stranded conformation is likely to exist as a heterogeneous mixture of structural subspecies differing in volume and enthalpy which may give rise to the relaxation contributions to the partial molar compressibility and expansibility. We use our determined change in hydration to evaluate the hydration and configurational contributions to the change in entropy accompanying the helix-to-coil transition of the decamer.

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Notes

The authors declare no competing financial interest.

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