JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

ARTICLE

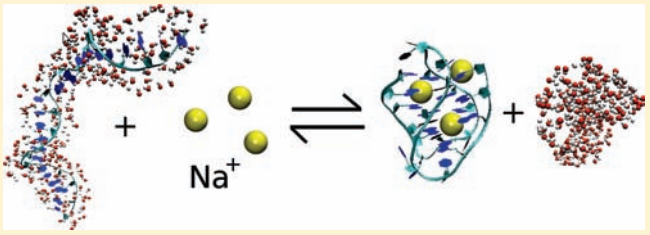
pubs.acs.org/JACS

Volumetric Characterization of Sodium-Induced G-Quadruplex Formation

Helen Y. Fan,[†] Yuen Lai Shek,[†] Amir Amiri, David N. Dubins, Heiko Heerklotz, Robert B. Macgregor, Jr., and Tigran V. Chalikian*

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, Ontario M5S 3M2, Canada

ABSTRACT: Oligodeoxyribonucleotides (ODN) with repeats of the human telomeric sequence can adopt different tetrahelical conformations that exhibit similar energetic parameters. We studied the volumetric properties of the folded and unfolded states of an ODN with four repeats of the human telomeric sequence, d[A(GGGTTA)$_3$GGG], by combining pressure-perturbation calorimetry (PPC), vibrating tube densimetry, ultrasonic velocimetry, and UV melting under high pressure. We carried out our volumetric measurements in aqueous buffers at pH 7 containing 20, 50, and 100 mM NaCl. All of the methods employed yielded volumetric parameters that were in excellent agreement. The molar volume changes, ΔV, of the conformational transition leading to formation of the folded state are large and positive. At 50 mM NaCl, the average transition volume, ΔV_{tr}, obtained from all the methods is 56.4 ± 3.5 cm^3 mol^{-1} at the transition temperature of 47 °C, with ΔV_{tr} decreasing with an increase in temperature. We carried out a molecular dynamics simulation of the change in the intrinsic geometric parameters of the ODN accompanying quadruplex formation. On the basis of the experimental and computational results, the folding transition of the ODN is accompanied by a release of 103 ± 44 water molecules from its hydration shell to the bulk. This number corresponds to ∼18% of the net hydration of the coil conformation.



INTRODUCTION

With the discovery of guanine-rich telomeric sequences of eukaryotic chromosomes, four-stranded nucleic acid structures went from being a biophysical oddity to an important member of the structural family of biologically relevant DNA conformations.[1,2] Earlier studies had shown that poly(G) and poly(dG) adopt four-stranded structures with the guanines on different strands interacting via Hoogsteen base interactions to form a tetrad.[3] There was also evidence, since confirmed by X-ray crystallographic and NMR techniques, that G-tetrads coordinate a cation, usually potassium or sodium, located within the helix.[4]

The telomeric sequence of human chromosomes, dGGGTTA, is similar to that of other species insofar as it has three consecutive guanines separated by a guanine-free sequence. In a human cell, this sequence is repeated many times. Biophysical studies have tended to focus on oligonucleotides containing four repeats of this sequence often with one or two additional bases at one of the ends.[5,6] These oligonucleotides fold into a compact structure in which the guanine residues interact via tetrads and the nonguanine residues form loops between the interacting guanines.[1,2,4,5] The basic structural motif of G-quadruplexes is formed by the planar, cyclic arrangement of hydrogen- bonded guanines with the central cavity incorporating a monovalent cation coordinated to the O6 carbonyls of the guanines forming the tetrad.[4] The structure adopted by these telomeric repeat sequences is dependent on the identity of the cation, the terminal bases, and, perhaps, the concentration of the oligonucleotide.[5] G-quadruplexes are stabilized by interguanine hydrogen bonding, stacking interactions between adjacent tetrads, specific and nonspecific ion−DNA electrostatic interactions, and hydration effects.[5]

Although structural studies suggest that the human telomeric sequence adopts more than one stable conformation, thermodynamic investigations of these molecules have not revealed as much diversity in their conformational energetics.[5] Nor do the reported thermodynamic parameters show any clear trends or correlations with respect to the different structures found.[5] One shortcoming of the existing thermodynamic studies of these structures is the paucity of volumetric data on telomeric sequences in their various conformations. Volumetric observables, such as volume, compressibility, and expansibility, are sensitive to hydration and packing arrangements of nucleic acid structures in their folded and unfolded states and, therefore, may provide important insights into the forces driving the folding of these molecules.[7,8] Hydration is known to provide a major contribution to the stability of G-quadruplexes.[9−12] However, to the best of our knowledge, hydration changes involved in folding/unfolding transitions have not been quantified for any G-quadruplex.

Depending on the identity of the central cation (Na^+ or K^+), the human telomeric oligonucleotide, d[A(GGGTTA)$_3$GGG], may exist in one of its two polymorphic forms. In the presence of Na^+ ions, d[A(GGGTTA)$_3$GGG] forms a basket structure

Received: November 22, 2010
Published: March 03, 2011

dx.doi.org/10.1021/ja110495c | J. Am. Chem. Soc. 2011, 133, 4518–4526

featuring antiparallel strands with one diagonal and two lateral loops.[13] When crystallized, the K^+ form is a parallel-stranded propeller with three double-chain reversal loops.[14] However, in solution, the K^+ form is probably an interconverting mixture of parallel and antiparallel structures.[15,16] The Na^+ form of d[A(GGGTTA)$_3$GGG] exhibits lower thermal and thermodynamic stabilities relative to the K^+ form.[17] The binding of Na^+ to the oligonucleotide is cooperative with the Hill coefficient 2.38 ± 0.05 and a half-saturation concentration of 4.40 ± 0.04 mM.[18]

In this work, we employ a range of volumetric techniques including pressure-perturbation calorimetry (PPC), high-precision densimetry, ultrasonic velocimetry, and UV melting under high pressure to determine changes in volume, expansibility, and compressibility accompanying the coil-to-G-quadruplex transition of the human telomeric oligonucleotide, d[A(GGGTTA)$_3$GGG]. Changes in the volumetric properties accompanying nucleic acid transitions are notoriously small in magnitude and, therefore, difficult to measure with high precision. Approaching this task in a multitechnique way allows us to compare directly the results of each of the techniques we use without introducing ambiguity arising from experiments being performed in different laboratories on different sample preparations. This approach will increase the credence of the parameters we measure and their subsequent interpretations. To the best of our knowledge, this is the first time the results of each of the techniques we employ in this study are compared directly on the same system under identical environmental conditions. Because of our focus on volumetric methods, we have used our results to probe the role of water in the stability of the structures.

MATERIALS AND METHODS

Materials. The oligodeoxyribonucleotide (ODN), d[A(GGGTTA)$_3$GGG], containing four repeats of the human telomeric DNA sequence was synthesized and cartridge purified by ACGT, Inc. (Toronto, ON, Canada). Sodium chloride, phosphoric acid, sodium azide, and tetrabutylammonium hydroxide 30 hydrate were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). EDTA (free acid) was purchased from FisherBiotech (Fair Lawn, NJ). These reagents were of the highest grade commercially available and used without further purification. All solutions were prepared using doubly distilled water.

For all temperature scanning experiments, the oligonucleotide was dissolved in and exhaustively dialyzed against a buffer consisting of 10 mM sodium phosphate titrated to pH 7.0, 0.1 mM EDTA, and 0.1 mM NaN_3. The ionic strength of the buffer was adjusted to the desired level by adding known amounts of NaCl. To form the equilibrium structure, the DNA-containing solution was placed in a boiling water bath and allowed to cool to room temperature. Dialysis was carried out in 1000 Da molecular weight cutoff Tube-O-Dialyzers from G Biosciences (St. Louis, MO). The final dialysis buffer was retained for PPC and high-precision densimetric temperature scanning experiments.

For circular dichroism (CD), high-precision densimetric, and ultrasonic velocimetric titration experiments, the DNA was dissolved in and dialyzed against a buffer in which the ODN does not fold and which consists of 10 mM tetrabutylammonium phosphate, 0.1 mM EDTA, and 0.1 mM NaN_3 titrated to pH 7.0. The buffer was chosen in order to ensure that the initial conformation of the oligonucleotide was unfolded. Due to its large size, a tetrabutylammonium cation cannot stabilize quadruplex structures. The titrations were carried out with the same buffer into which sodium chloride was added at concentrations ranging between 0.2 and 1 M.

Concentration Determination. The concentration of the ODN was determined from the absorbance at 260 nm measured at 25 °C with a Cary 300 Bio spectrophotometer (Varian Canada, Inc., Mississauga, Ontario, Canda) using a molar extinction coefficient of 228 500 M^{-1} cm^{-1} for the unfolded conformation.[18] For the PPC, densimetric, and ultrasonic velocimetric experiments, DNA concentration was on the order of ~0.3 mM. For CD measurements and pressure-dependent UV light absorption measurements, the DNA concentrations were ~0.05 and ~0.01 mM, respectively.

Circular Dichroism Spectroscopy. CD spectra were recorded at 25 °C using an Aviv model 62 DS spectropolarimeter (Aviv Associates, Lakewood, NJ). In titration experiments, CD spectra were measured after each addition of an aliquot of sodium chloride solution to a cuvette containing a known amount of DNA (initially, in the coil form in the absence of Na^+ ions). To ensure a matching background chemical environment of the DNA sample, the NaCl solution was prepared using the buffer solution from DNA dialysis. In addition to titration, CD spectroscopy is used prior to each ultrasonic and densimetric titration to confirm that the DNA is in a coil conformation.

Pressure-Perturbation Calorimetry. PPC measurements were conducted on a MicroCal VP-DSC instrument with a PPC accessory (MicroCal, LLC, Northampton, MA). We analyzed our measured PPC profiles to determine transition temperatures, T_M, and volumes, ΔV_{tr}.[19−21] The coefficient of thermal expansibility of the DNA [$\alpha(T) = (1/V^\circ)(\partial V^\circ/\partial T)_P$, where V° is the partial molar volume of the DNA] is determined from the changes in the heat of the calorimetric cell during the water-versus-buffer scan, $\Delta Q_{w/b}$, and the DNA sample-versus-buffer scan, $\Delta Q_{s/b}$, following small pressure jumps, ΔP, of ~5 bar

$$\alpha = \alpha_w - \frac{\Delta Q_{w/b}}{T\Delta P V_c} - \frac{\Delta Q_{s/b}}{T\Delta P V_c C V^\circ} \quad (1)$$

where α_w is the coefficient of thermal expansibility of water, $V_c \approx 0.5$ mL is the volume of the calorimetric cell, and C is the DNA concentration.

Before each experiment, control runs of water-versus-water, buffer-versus-water, and buffer-versus-buffer were carried out. The PPC experiments were set up in such a way that the samples were analyzed from 3 to about 95 °C with the first scan followed by the second. Before the DNA sample was loaded into the instrument, water−water (1 °C intervals), buffer−water (1 °C intervals), and buffer−buffer (2 °C intervals) runs had been conducted in succession. The sample against the buffer was subsequently run with 0.5 °C intervals for the temperatures ±5 °C from the T_M, 1 °C intervals for the temperatures ranging from ±5 to ±10 °C from T_M, and 2 °C intervals for the rest of the scanned temperature range. Two up and down pressure jumps were performed at each experimental temperature. The PPC experiments were run at 20, 50, and 100 mM NaCl concentration.

Data were analyzed with Origin software's PPC routine, which calculates the coefficient of expansibility of the DNA by taking into account water and buffer effects. Points obtained for the same sample and temperature from different scans were averaged with the mean values subsequently used in the volumetric analysis. The coefficient of thermal expansibility of DNA [$\alpha = (1/V^\circ)(\partial V^\circ/\partial T)_P$] was multiplied by its partial specific volume, V°, to obtain the partial molar expansibility, $E^\circ = (\partial V^\circ/\partial T)_P$. Integration of the expansibility-versus-temperature profile yields the volume change accompanying the temperature-induced quadruplex-to-coil transition of the oligonucleotide.

High-Pressure Experiments. We measured the effect of hydrostatic pressure on the quadruplex-to-coil transition temperature using the equipment that has been previously described.[22] The instrument allows us to monitor and regulate the temperature and pressure inside the high-pressure vessel containing the DNA sample while measuring the optical absorption of the sample. In these experiments, we monitor the temperature-induced change in the populations of the coil and quadruplex conformations by measuring UV absorption at 295 nm in a DNA sample contained in a 0.5-cm cylindrical cuvette. To obtain the helix−coil transition temperature, T_M, we subtract the low- and

high-temperature baselines from the raw data (absorption versus temperature) to obtain $\theta(T)$, the extent of the transition as a function of temperature

$$\theta(T) = \frac{A(T) - A_L(T)}{A_H(T) - A_L(T)} \quad (2)$$

where $A(T)$, $A_L(T)$, and $A_H(T)$ are the optical density of the sample at the experimental temperature, T, and the low-temperature and high-temperature base lines linearly extrapolated to T, respectively. The G-quadruplex-to-coil transition temperature, T_M, is the temperature corresponding to $\theta(T) = 0.5$. The temperature was changed at a rate of 0.9 °C/min.

The volume change of the reaction was calculated from the pressure dependence of T_M using the Clapyeron equation $\Delta V_{tr} = (\Delta T_M/\Delta P)$-$(T_M/\Delta H_M)$, where the transition enthalpy, ΔH_M, and temperature, T_M, are those determined at atmospheric pressure. The transition enthalpies were calculated from the van't Hoff analysis of the UV melting profiles.[23]

Vibrating Tube Densimetry and Ultrasonic Velocimetry. All 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

$$V^\circ = \frac{M}{\rho_0} - \frac{(\rho - \rho_0)}{\rho_0 C} \quad (3)$$

where ρ and ρ_0 are the densities of the nucleic acid solution and the neat solvent, respectively, C is the molar concentration of the nucleic acid, and M is the molecular weight of the nucleic acid.

Solution sound velocity measurements were carried out at a frequency of 7.2 MHz using the resonator method and a previously described differential technique.[24,25] 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 $\pm 1 \times 10^{-4}$ %.[24,26] A 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

$$K_S^\circ = \beta_{S\circ}\left(2V^\circ - 2[U] - \frac{M}{\rho_0}\right) \quad (4)$$

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

Densimetric and acoustic titrations were performed at 25 °C following the previously described experimental protocol.[27] The titration experiments were performed by adding aliquots of a 0.2−1 M sodium chloride solution to the initially sodium-free DNA solution containing tetrabutylammonium phosphate. High-precision densimetry was additionally used to measure directly changes in volume accompanying the coil-to-quadruplex transition upon a decrease in temperature of the sample. The oligonucleotide was dialyzed in a buffer containing either 20 or 50 mM NaCl to form the folded quadruplex, as described above. The densities of the dialysate and the nucleic acid sample were measured separately as the temperature was lowered from 70 to 15 °C.

Computation of Solvent-Accessible Surface Areas and Molecular Volumes. Our analysis is based on the NMR-determined sodium-induced quadruplex structure of the ODN (PDB entry 143D) obtained from the RCSB Protein Databank. As 143D contained six models, each was treated as a distinct structure. We generated an unfolded single-stranded structure for the ODN by first creating B-form DNA with AmberTools (v1.3) [NAB (Nucleic Acid Builder) Macke, T J.; Svrcek-Seiler, W.A.; Brown, R. A.; Kolossváry, I.; Bomble, Y. J.; Case, D. A.] and then deleting the complementary strand. In order to model a more plausible single-stranded structure, molecular dynamics was carried out on the single-stranded structure using GROMACS (v 4.0.7).

In our simulations the DNA molecule was contained in a rectangular box with a 40-Å distance between the solute and the box sides; this system contained 50 953 water molecules and 38 sodium ions (volume, 1575 nm^3; density, 975.494 g/L). Initially, a 100-ps steepest-descent minimization run was conducted to relax each structure [http://wwwuser.gwdg.de/~ggroenh/SaoCarlos2008/html/build.html#top, accessed Aug 2010]. A 100-ps solvent equilibration run was performed at 300 K and 1 atm. Subsequently, the conformational space available to the ODN 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" had been generated for conformations with the largest and smallest total solvent-accessible surface areas during the 1-ns simulations, which were subsequently employed in the McVol calculations.[28]

Solvent-accessible surface areas (S_A) and molecular volumes (V_M) were estimated using McVol, a Monte Carlo algorithm used to estimate protein volumes.[28] There were two sets of computations we carried out for each structure, the first with a probe radius 1.3 Å (default) and the second with a probe radius of 1.4 Å" Other than the probe radius, the default McVol program parameters were used in the calculations.[28] The VMD program and NACCESS version 2.1.1 were additionally used to estimate solvent-accessible surface areas for all structures (http://www.ks.uiuc.edu/Research/vmd/, accessed May 2010);[29] both gave results consistent with those obtained with McVol.

RESULTS

Circular Dichroism Spectra. NMR studies have shown that the human telomeric sequence, d[A(GGGTTA)$_3$GGG], forms an antiparallel structure in the presence of sodium ions.[13] To monitor the formation of antiparallel G-quadruplex, we measured the CD spectrum of the DNA (initially in the tetrabutylammonium buffer) as a function of sodium ion concentration as shown in Figure 1a. Since the initial solution may contain trace amounts of Na^+ and K^+ ions, some fraction of the ODN may adopt folded G-quadruplex conformations. However, the fraction of such folded or partially folded structures should be negligibly small given the fact that the concentration of Na^+ ions needed for one-half of the molecules to adopt the folded quadruplex conformation is ~5 mM (see below), which is orders of magnitude larger than any trace amount of Na^+ or K^+ ion that may be present in solution prior to titration.

Previous work has shown that the CD spectrum of antiparallel DNA G-quadruplexes displays a positive peak at 295 nm and a negative peak at 260 nm.[5] With no NaCl added, the spectrum of the ODN shows a positive peak at 260 nm and a negative peak at 295 nm, which is consistent with the single-stranded ODN being in a coil conformation in tetrabutylammonium phosphate solutions (see Figure 1a). A positive peak at 295 nm and a negative peak at 260 nm arise in the spectrum upon addition of NaCl, consistent with formation of a G-quadruplex structure. Note that the CD spectra in Figure 1a exhibit two isoelliptic points at

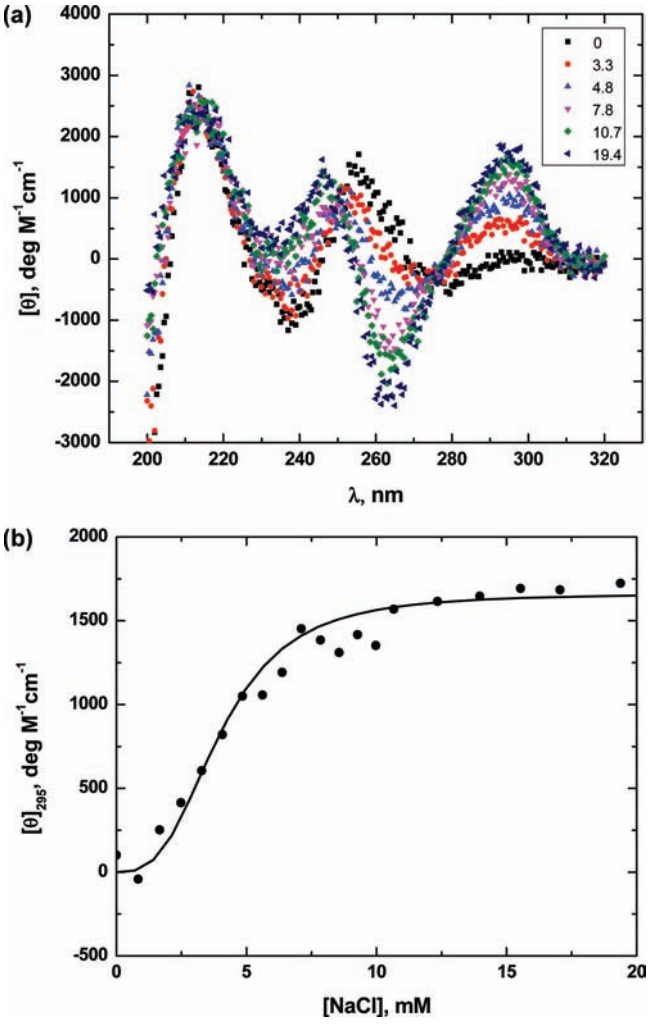


Figure 1. (a) Circular dichroism spectra of the ODN at 25 °C in the presence and absence of NaCl. The DNA was initially in a nonfolding buffer consisting of 10 mM tetrabutylammonium phosphate titrated to pH 7.0, 1 mM EDTA, and 0.1 mM NaN_3. The NaCl concentration (in mM) for each spectrum is given in the inset. (b) Dependence of the molar ellipticity of the ODN at 295 nm on the NaCl concentration. Experimental data were approximated with eq 5 (solid line).

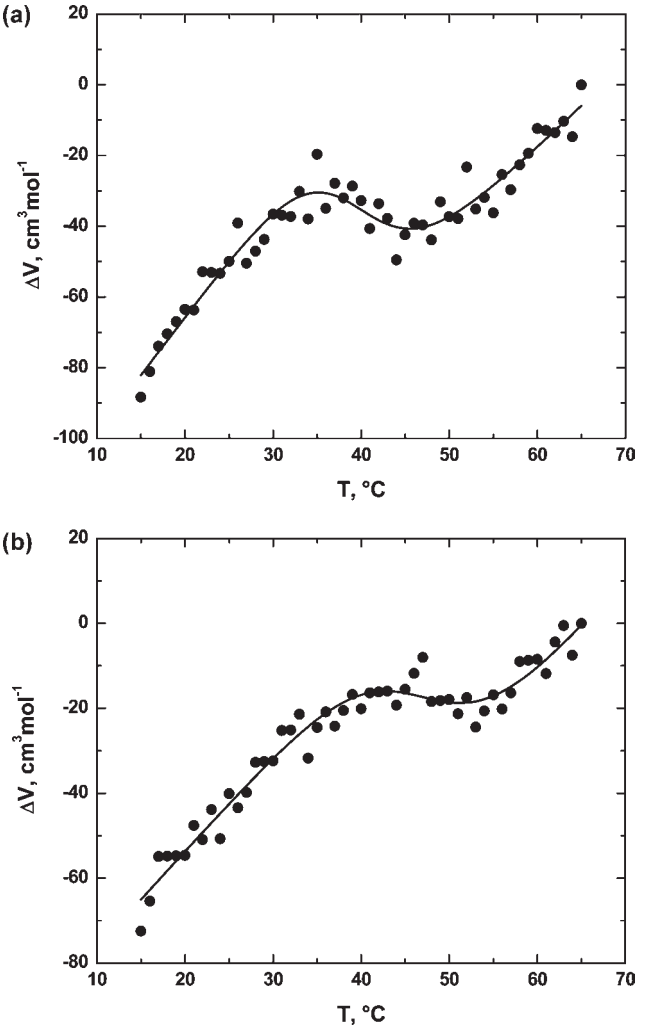


Figure 2. Temperature-dependent changes in the partial molar volume of the ODN at 20 (A) and 50 mM (B) NaCl. Temperature was varied from high to low. Experimental data were fitted by the two-state model of thermal denaturation.

250 and 275 nm, an observation consistent with the two-state nature of the sodium-induced G-quadruplex folding transition.

To ensure that a unimolecular G-quadruplex was formed, thermal UV spectroscopic melting at 295 nm was performed on DNA samples diluted 4-fold (data not shown). The transition temperature, T_M, remained identical to that of the original sample, confirming that the process is unimolecular with respect to the DNA concentration. Examination of the ellipticity profile at 295 nm (see Figure 1b) reveals that one-half of the molecules have adopted the folded quadruplex conformation at approximately ~5 mM NaCl.

The data in Figure 1b were analyzed and approximated within the framework of the following reaction

$$C + nNa^+ \rightleftarrows Q \qquad \text{(Reaction 1)}$$

where C collectively signifies the entire spectrum of unfolded, more or less coil-like conformations; Q signifies the quadruplex conformation; and n is the number of coordinated sodium ions buried inside the central cavity of the quadruplex.

The binding constant of Reaction 1 is given by the expression

$$K_b = \frac{[Q]}{[C][Na^+]^n} = \frac{\alpha}{(1-\alpha)([Na^+]-\alpha[DNA])^n} \qquad (5)$$

where $[Na^+]$ and $[DNA] = [C] + [Q]$ are the total concentrations of NaCl and ODN in solution, $\alpha = [Q]/[DNA]$ is the fraction of DNA in the quadruplex conformation, while $(1 - \alpha) = [C]/[DNA]$ is the fraction of DNA in the coil conformation.

The fraction of the ODN in the quadruplex conformation, α, can be determined for each experimental point of the titration profile shown in Figure 1b as the ratio $\alpha = \Delta X/\Delta X_{max}$, where ΔX is the change in the experimental observable (here, molar ellipticity) relative to its initial value at $[NaCl] = 0$ and ΔX_{max} is the asymptotic maximum change in X corresponding the fully folded (quadruplex) conformation of the ODN. We fitted the titration profile presented in Figure 1b with eq 5. In our analysis, we used $n = 3$, which corresponds to the theoretical maximum of the number of sodium ions coordinated by the quadruplex. The fitting was performed numerically based on the direct search optimization technique. The resulting binding constant, K_b, equals $(7.8 \pm 0.6) \times 10^6\ M^{-3}$.

dx.doi.org/10.1021/ja110495c | J. Am. Chem. Soc. 2011, 133, 4518–4526

Table 1. Coil-to-Quadruplex Transition Temperatures, T_M, Volumes, ΔV_{tr}, and Expansibilities, ΔE_{tr}, Determined from Pressure-Perturbation Calorimetric (PPC), Vibrating Tube Densimetry (VTD), and High-Pressure (HP) Measurements at Different NaCl Concentrations

	[NaCl], mM								
	20			50			100		
method	PPC	VTD	HP	PPC	VTD	HP	PPC	VTD	HP
T_M, °C	40.0 ± 0.6	39.4 ± 0.8	39.4 ± 0.5	49.0 ± 0.7	47.5 ± 0.9	47.4 ± 0.5	55.0 ± 0.7		54.9 ± 0.5
ΔV_{tr}, cm^3 mol^{-1}	66 ± 3	65 ± 9	68 ± 2	56 ± 3	53 ± 9	60 ± 2	56 ± 3		56 ± 2
ΔE_{tr}, cm^3 mol^{-1}K^{-1}	0.84 ± 0.08			0.85 ± 0.08			0.84 ± 0.08		

High-Precision Densimetry and Ultrasonic Velocimetry. The densities of the telomeric oligonucleotide solutions in 20 and 50 mM NaCl were measured as a function of temperature in order to determine the transition volume, ΔV_{tr}; the data are shown in Figure 2A and 2B. The experimental points have been approximated by a two-state transition model $\Delta V_{tr} = V_N(T) + [V_D(T) - V_N(T)]/[1 + \exp(\Delta H_M(T^{-1} - T_M{}^{-1})/R)]$, where $V_N(T)$ and $V_D(T)$ are linear functions that approximate the pre- and postdenaturation baselines, respectively. At 20 and 50 mM NaCl, the coil-to-quadruplex transition volumes, ΔV_{tr}, equal 65 ± 11 and 53 ± 12 cm^3 mol^{-1}, respectively (see Table 1). The transition temperatures, T_M, determined by this method are also shown in Table 1.

Figure 3 shows the changes in partial molar volume (Figure 3A) and partial molar adiabatic compressibility (Figure 3B) as a function of NaCl concentration at 25 °C. The titration profiles shown in Figures 3A and 3B were analyzed by eq 5 with the resulting binding constants, K_b, of (7.4 ± 1.2) × 10^6 M^{-3} (Figure 3A) and (7.7 ± 0.9) × 10^6 M^{-3} (Figure 3B). These values are in close agreement with each other and the binding constant determined from the analysis of our molar ellipticity data. This analysis explicitly takes into account only those Na^+ ions which penetrate the central cavity and become coordinated to the quadruplex. However, Na^+ ions will, in addition, replace condensed tetrabutylammonium in the vicinity of the ODN as its counterions. We assume that replacement of tetrabutylammonium with Na^+ in the vicinity of DNA causes only slight and monotonic changes in volume and compressibility that do not strongly affect the determined values of K_b. This is a valid assumption since the affinities of the DNA for the monovalent tetrabutylammonium and Na^+ counterions are of the same order. Hence, replacement of tetrabutylammonium with Na^+ should proceed proportionally to the tetrabutylammonium-to-Na^+ ratio in solution. Besides, for most sequences, monovalent counterions retain their hydration shells when condensed around DNA.[30] Consequently, the transfer of Na^+ and tetrabutylammonium ions between the solvation shell of the ODN and the bulk should not cause any significant changes in volume or compressibility.

The folding of the ODN to form an antiparallel G-quadruplex is accompanied by increases in volume, ΔV_{tr}, and adiabatic compressibility, ΔK_{Str}, of 67 ± 5 cm^3 mol^{-1} and (236 ± 20) × 10^{-4} cm^3 mol^{-1} bar^{-1}, respectively. It is of interest to note that the NaCl dependences of the partial molar volume, adiabatic compressibility, and molar ellipticity all exhibit their half-maximum changes at ~5 mM NaCl. Two conclusions can be drawn from this observation. First, formation of G-quadruplex is nearly complete at 12 mM Na^+; second, the three techniques provide consistent results in quantifying the progress of G-quadruplex formation from a comparatively unstructured single-stranded ODN.

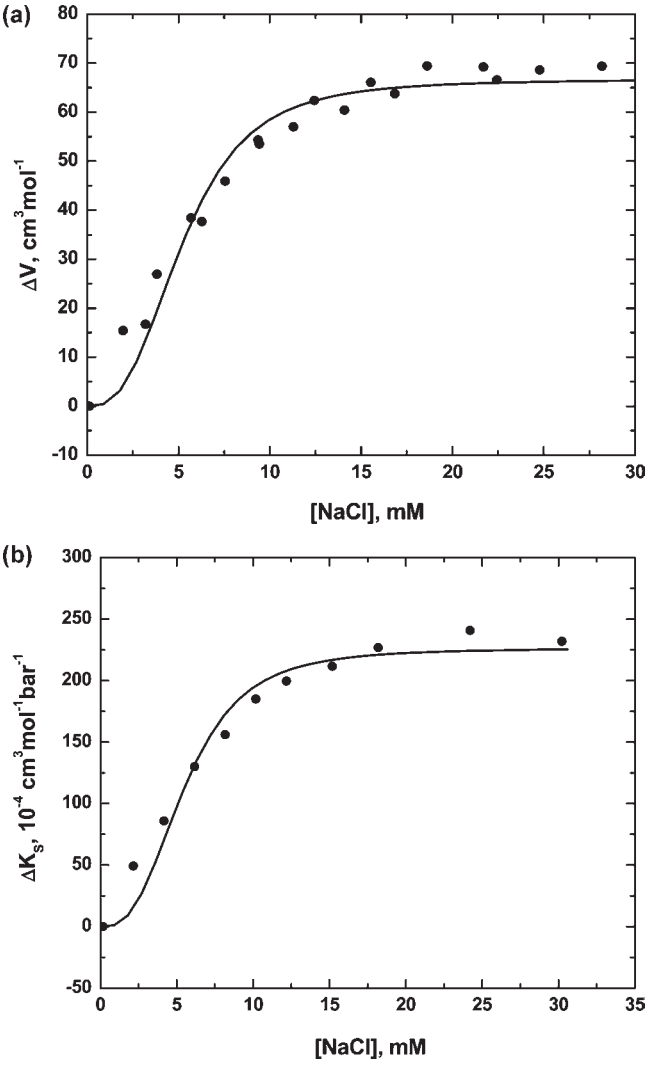


Figure 3. Changes in the partial molar volume (A) and adiabatic compressibility (B) of the ODN plotted against the NaCl concentration at 25 °C. The DNA was initially in a nonfolding buffer consisting of 10 mM tetrabutylammonium phosphate titrated to pH 7.0, 1 mM EDTA, and 0.1 mM NaN_3. Experimental data were approximated with eq 5 (solid lines).

High Pressure. Figure 4 shows the transition temperatures, T_M, of the ODN plotted as a function of pressure at 20, 50, and

dx.doi.org/10.1021/ja110495c | J. Am. Chem. Soc. 2011, 133, 4518–4526

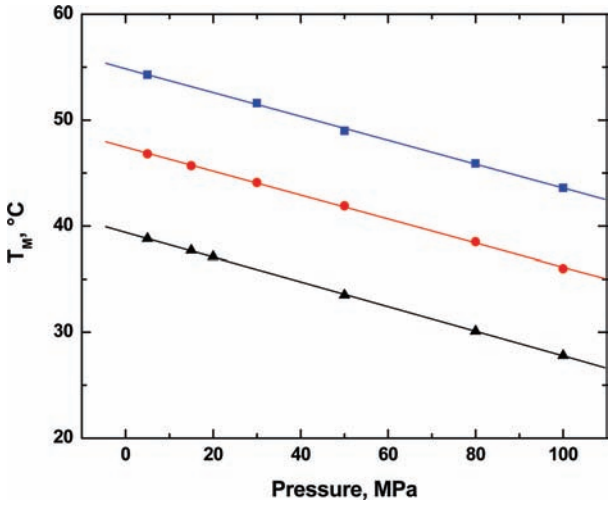


Figure 4. Pressure dependence of the helix−coil transition temperature, T_M, as a function of hydrostatic pressure at 20 mM (▲), 50 mM (●), and 100 mM (■) NaCl. The lines are linear least-squares fits of the data; 0.1 MPa is atmospheric pressure.

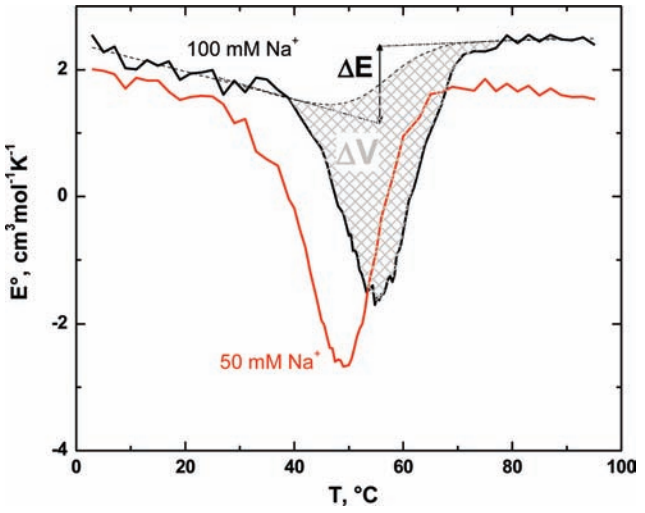


Figure 5. PPC-determined temperature dependences of the apparent molar expansibility of the ODN at 50 and 100 mM NaCl. The value of ΔVtr is obtained as illustrated by integrating the peak from a progress baseline; the difference between extrapolated pre- and post-transition baselines yields the expansibility change, ΔEtr. The position of the maximum of the peak corresponds to T_M.

100 mM NaCl; the atmospheric pressure values of T_M are given in Table 1. The T_M of the unfolding transition decreases linearly with increasing pressure at the three salt concentrations we investigated. Using the T_M-versus-pressure data in conjunction with the Clapeyron equation we calculated the volume change, ΔV_{tr}, for formation of the folded quadruplex structure. As summarized in Table 1, the value of ΔV_{tr} decreases from 68 ± 2 to 56 ± 2 cm^3 mol^{-1} as the concentration of NaCl increases from 20 to 100 mM.

Pressure-Perturbation Calorimetry. Figure 5 presents examples for PPC profiles of the heat-induced quadruplex-to-coil transitions of the ODN obtained at 50 and 100 mM NaCl. We calculated the transition volumes, ΔV_{tr}, at the transition temperatures, T_M, from these PPC profiles by fitting them with an analytical function derived based on the two-state approximation.[21,31] As shown in Table 1, the transition volumes at 20, 50, and 100 mM NaCl equal 66 ± 3, 56 ± 2, and 56 ± 2 cm^3 mol^{-1}, respectively.

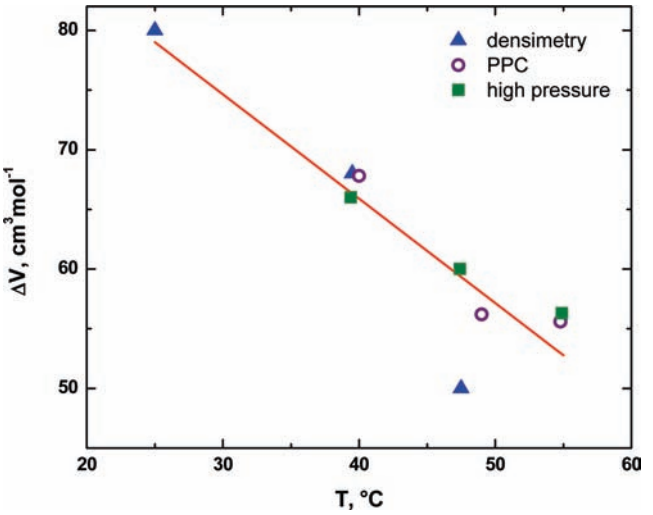


Figure 6. Dependence of the transition volume on the temperature for the three methods employed in this work. The slope of the temperature dependence equals the expansibility change for the reaction. A linear, least-squares fit of the data yields a slope of −0.87 ± 0.16 cm^3 mol^{-1} K^{-1}. The experimental method employed to obtain a particular datum is given in the inset.

PPC enables one to measure a change in expansibility, ΔE_{tr}, accompanying the transition as the difference between the pre- and postdenaturation baselines extrapolated to the transition temperature (see Figure 5). Table 1 presents PPC-determined changes in expansibility, ΔE_{tr}, accompanying the coil-to-quadruplex transitions at 20, 50, and 100 mM NaCl. Note that the value of ΔE_{tr} determined by PPC is equal to −0.84 ± 0.08 cm^3 mol^{-1} K^{-1} and that it does not depend on the solution ionic strength to a first approximation.

An independent measure of ΔE_{tr} can be obtained from the dependence of ΔV_{tr} on the transition temperature, T_M. In Figure 6 the transition volumes, ΔV_{tr}, determined from all experimental techniques employed in this study are plotted against the transition temperatures, T_M. The slope of this dependence, which is equal to the change in expansibility, $\Delta E_{tr} = \Delta\Delta V_{tr}/\Delta T_M$, for the coil-to-quadruplex transition, has a value of −0.87 ± 0.16 cm^3 mol^{-1} K^{-1}. The value of ΔE_{tr} evaluated this way is in agreement with that determined directly from PPC.

PPC, Densimetry, and High-Pressure Studies Yield Consistent Results. To our knowledge, this is the first direct comparison of reaction volumes obtained by three different methods using identical samples. Given the importance of volumetric parameters in ascertaining the role of water in the stabilization of biological structures, our finding that the methods yield similar values and trends is important. As discussed recently, volume changes measured by PPC and high-pressure experiments may show systematic deviations owing to the different conditions studied.[32] In contrast to high-pressure techniques, PPC utilizes minute pressure jumps and measures volume and expansibility changes of the thermotropic transition at ambient pressure. For proteins the pressure-denatured state may differ substantially from the heat-denatured one.[33,34] Our data show a very good agreement of ΔV_{tr} measured

dx.doi.org/10.1021/ja110495c |J. Am. Chem. Soc. 2011, 133, 4518–4526

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

	V_M, Å^3	S_A, Å^2
coil	6039 ± 103	5137 ± 118
quadruplex	6426 ± 26	3907 ± 99

by PPC, high-pressure spectroscopy, and high-precision densimetry implying that this is not the case for the quadruplex studied here. Our results further suggest that the three techniques are sensitive to and reflect the same properties of the transition.

There is one more potential point of concern. The folding/unfolding transitions of the Na^+ form of the human telomeric quadruplex sequence are characterized by the folding kinetics with three relaxation times the longest of which is ~10 s.[5,6,18] This is shorter than the typical time range for all of our experimental techniques. Therefore, our measurements should not be affected by the slow kinetics of folding/unfolding transitions of the ODN. This notion is also supported by the observed agreement between the ΔV_{tr} results produced by the three volumetric techniques employed in our study.

Molecular Dynamics Simulations. The average values for the simulated solvent-accessible surface areas and molecular volumes of the quadruplex and coil forms are summarized in Table 2. These geometric parameters are used further below in our microscopic analyses of the measured changes in volume accompanying formation of the folded structure.

DISCUSSION

Changes in Hydration Accompanying G-Quadruplex Formation. In this section, we present an approximate algorithm for the estimation of the difference in the number of water molecules, Δn_h, that interact with the coil and folded forms of the ODN. The algorithm utilizes a number of assumptions due to the limited amount of volumetric data available for nucleic acids. Despite their approximate nature, the values we obtain for Δn_h suggest that significant dehydration of the ODN accompanies its quadruplex formation. This result underscores the importance of the changes in hydration in the energetics of the coil-to-quadruplex transitions of nucleic acids.

The change in volume associated with G-quadruplex formation, ΔV_{tr}, can be presented as the following sum

$$\Delta V_{tr} = \Delta V_M + \Delta V_T + \Delta V_I - n V_{Na^+} \quad (6)$$

where ΔV_M is the change in the molecular volume of the oligonucleotide (geometric volume impenetrable to water molecules), ΔV_T is the change in thermal volume, ΔV_I is the change in interaction volume, and V_{Na+} is the partial molar volume of a sodium ion. As mentioned above, we use n = 3 corresponding to the theoretical maximum of the number of sodium ions coordinated by the quadruplex.

Thermal volume, V_T, results from mutual vibrations of solute and solvent molecules and steric and structural effects reflecting imperfect packing of solute and solvent molecules and the open structure of water.[35,36] As a first approximation, the thermal volume of a macromolecule is proportional to the number of contacts with solvent molecules and, hence, the solvent-accessible surface area. Therefore, a change in thermal volume can be calculated by multiplying the change in solvent-accessible surface area, ΔS_A, by the effective "thickness" of thermal volume, δ.

To compute the molecular volume, V_M, and solvent-accessible area, S_A, of the ODN in its sodium-induced quadruplex conformation, we used structural data (PDB entry 143D) and the standard calculation procedures.[28,37−39] The values of V_M and S_A calculated as averages for the six NMR-based structural models of the telomeric oligonucleotide d[A(GGGTTA)$_3$GGG][13] are 6426 ± 26 Å^3 (3870 ± 16 cm^3 mol^{-1}) and 3907 ± 99 Å^2, respectively. The structural properties of the ensemble of coil conformations of the ODN are unknown. Therefore, as noted above, we employed molecular dynamics (MD) simulations to generate unfolded structures beginning from the stacked, fully helical conformation. Following a 1-ns MD simulation, we selected the conformations exhibiting the highest and the lowest solvent-accessible surface areas. On the basis of these simulations, we calculated V_M of 6039 ± 103 Å^3 (3637 ± 62 cm^3 mol^{-1}) and S_A of 5137 ± 118 Å^2 as the average values for the three coil conformations we considered (see Table 2). Thus, changes in molecular volume, ΔV_M, and solvent-accessible surface area, ΔS_A, accompanying the coil-to-quadruplex transition equal 387 ± 106 Å^3 (233 ± 64 cm^3 mol^{-1}) and −1230 ± 154 Å^2, respectively.

For small molecules, various theoretical and empirical estimates of the thickness of the thermal volume, δ, have produced similar values ranging between 0.50 and 0.57 Å.[35,36,40] For rigid nucleic acids, the value of δ should be close to that of small molecules.[8] In our analysis, we use the value of δ equal to 0.5 Å.[36] Hence, ΔV_T in eq 6 equals (−1230 ± 154) × 0.5 = −615 ± 77 Å^3 (−370 ± 46 cm^3 mol^{-1}).

At 25 °C, the partial molar volume contribution of sodium ion, V_{Na+}, is −5.9 cm^3 mol^{-1}.[41] At 25 °C, the measured change in volume accompanying the coil-to-quadruplex transition, ΔV_{tr}, is 67 ± 5 cm^3 mol^{-1}. With this value, we use eq 6 to calculate a change in the interaction volume; ΔV_I = 67 − 233 + 370 − 3 × 5.9 = 186 ± 79 cm^3 mol^{-1}. The interaction volume, V_I, reflects solvent contraction around polar and charged groups of a solute. A change in interaction volume, $\Delta V_I = -\Delta n_h(V_h - V_0)$, in eq 6 is determined by the number of water molecules released to the bulk, Δn_h, and the average differential partial molar volume of water of solute hydration and bulk water, $(V_h - V_0)$. For proteins and nucleic acids, the partial molar volume of water of hydration, V_h, is ~10% smaller than that of bulk water, V_0.[42] Thus, $(V_h - V_0)$ can be taken to be roughly equal to −1.8 cm^3 mol^{-1}, while the number of water molecules, $\Delta n_h = -\Delta V_I/(V_h - V_0)$, released to the bulk upon quadruplex formation is 103 ± 44 [(199 ± 79)/1.8]. The number of water molecules released to the bulk from the first coordination layer of the oligonucleotide is ~137 as calculated by dividing ΔS_A by 9 Å^2, the effective cross-sectional area of a water molecule. This number is reasonably close to our estimated Δn_h of 103 ± 44. This observation is consistent with a picture in which DNA dehydration associated with quadruplex formation is predominantly limited to water molecules in direct contact with the DNA.

The total number of water molecules, n_h, in direct contact with the telomeric sequence in its unfolded or coil conformation can be estimated as the ratio of the solvent-accessible surface area to 9 Å^2, the effective cross-section of water molecule; n_h = 570 (5137/9). Thus, the number of water molecules released to the bulk upon quadruplex formation corresponds to ~18% (103/570) of the net hydration of the coil conformation.

Expansibility-Based Estimates. The partial molar expansibility of a solute is sensitive to its hydration.[19,43−45] Significant dehydration is suggested by the negative change in expansibility accompanying the coil-to-quadruplex transition, -0.87 ± 0.16 cm^3 mol^{-1} K^{-1}; however, as discussed below, not all of the measured change in expansibility can be attributed to dehydration. A change in expansibility associated with quadruplex formation can be presented as

$$\Delta E_{tr} = \Delta E_M + \Delta E_h + \Delta E_{rel} - nE_{Na^+} \quad (7)$$

where ΔE_M is the change in the intrinsic expansibility of the oligonucleotide, ΔE_h is the term signifying a change in expansibility due to a change in DNA hydration, E_{Na+} is the partial molar expansibility of a sodium ion, and ΔE_{rel} is the change in the relaxation contribution, $E_{rel} = (\langle \Delta H \Delta V \rangle - \langle \Delta H \rangle \langle \Delta V \rangle)/RT^2$, $\langle \Delta H \rangle$ and $\langle \Delta V \rangle$ are, respectively, the ensemble average changes in enthalpy and volume relative to a "ground state" conformation.[46]

Since the value of E_{Na+} is unknown, eq 7 can be rewritten in the following simplified form

$$\Delta E_{tr} = \Delta E_M + \Delta E_h + \Delta E_{rel} \quad (7a)$$

where ΔE_h is the collective term reflecting changes in hydration of both the DNA and the sodium ions participating in Reaction 1.

The term $\Delta E_h = \Delta n_h(E_h - E_0)$ is determined by the number of water molecules released to the bulk, Δn_h, and the differential partial molar expansibilities of water of solute hydration and bulk water, $(E_h - E_0)$. For charged solutes, such as DNA, $(E_h - E_0)$ can be estimated by using zwitterionic glycine as a model. The value of $(E_h - E_0)$ can be obtained by dividing the partial molar expansibility of glycine, 0.068 ± 0.01 cm^3 mol^{-1} K^{-1}, by its hydration number of ∼15; $(E_h - E_0) \approx 0.0045$ cm^3 mol^{-1} K^{-1} (= 0.068/15).[47,48] The total number of water molecules released to the bulk following quadruplex formation is roughly $\sim 133 \pm 44$. This value can be obtained by adding ∼30 water molecules to our volume-based estimate of Δn_h of 103 ± 44; each of the three sodium ions taken up from the bulk upon quadruplex formation has ∼10 water molecules within its first coordination sphere. Thus, the hydration component ΔE_h in eq 7a equals -0.60 ± 0.20 cm^3 mol^{-1} K^{-1} (-140×0.0045), while the sum of the changes in intrinsic and relaxation expansibility, $(\Delta E_M + \Delta E_{rel})$, is -0.27 ± 0.26 cm^3 mol^{-1} K^{-1} ($-0.87 + 0.60$).

The observed decrease in $(\Delta E_M + \Delta E_{rel})$, if statistically significant, is not due to a decrease in E_M; the intrinsic expansibility, E_M, is predominantly determined by the expansibility of water-inaccessible voids inside the oligonucleotide structure. Since the number of such voids is greater in the folded G-quadruplex than in the coil conformations, the change in E_M associated with the coil-to-quadruplex transition should be positive rather than negative. To account for this observation, we propose that the negative value of $(\Delta E_M + \Delta E_{rel})$ suggests a significant decrease in the relaxation component, E_{rel}, which offsets an increase in E_M. In other words, the coil conformations are characterized by a larger relaxation expansibility than the G-quadruplex conformation. This notion implies the existence of a broadly distributed isoenergetic population of unfolded, coil-like conformations differing in their packing and hydration which, in turn, result in differences in enthalpy and volume. An increase in temperature shifts the population of coil-like conformations toward the species with greater enthalpy and volume which results in an additional (relaxation) increase in the partial expansibility of the coil state.

Compressibility-Based Estimates. In a manner similar to our treatment of the volume and expansibility results, the change in adiabatic compressibility accompanying quadruplex formation can be parsed into different contributing factors. These contributions include changes in the compressibility arising from intrinsic structural factors, ΔK_M, and changes arising from differential hydration of the two states, ΔK_h

$$\Delta K_{Str} = \Delta K_M + \Delta K_h + K_{rel} - nK_{Na+} \quad (9)$$

where $K_{rel} = (\langle \Delta V^2 \rangle - \langle \Delta V \rangle^2)/RT$ is the relaxation contribution to compressibility; and $K_{Na+} = -33.5 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} is the partial molar adiabatic compressibility of sodium ion.[49]

The hydration contribution, ΔK_h, is given by $\Delta K_h = \Delta n_h(K_h - K_0)$, where $(K_h - K_0)$ is the difference between the partial molar adiabatic compressibilities of water of solute hydration and bulk water. For charged solutes, the partial molar adiabatic compressibility of water of hydration, K_h, is roughly ∼25% smaller than that of bulk water, K_0.[42] Thus, $(K_h - K_0)$ is approximately -2×10^{-4} cm^3 mol^{-1} bar^{-1}. With this estimate, ΔK_h can be calculated to be $(206 \pm 88) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} [$(103 \pm 44) \times 2 \times 10^{-4}$]. The sum $(\Delta K_M + \Delta K_{rel})$ in eq 9 equals $-(67 \pm 90) \times 10^{-4}$ cm^3 mol^{-1}bar^{-1} [$(240 \times 10^{-4}) - (206 \times 10^{-4}) - 3 \times (33.5 \times 10^{-4})$].

The intrinsic compressibility of double stranded DNA is essentially zero.[7,8,50,51] There are no experimental data concerning the intrinsic compressibility of quadruplex DNA. However, the bulkiness of the structure and the presence of the central cavity inside the G-quadruplex lead us to propose that it has a higher intrinsic compressibility than the duplex conformation. A large intrinsic compressibility cannot be expected for a solvent-exposed, coil-like conformation for which the intrinsic compressibility should be close to zero. Consequently, a change in intrinsic compressibility, ΔK_M, accompanying the coil-to-quadruplex formation should be positive. We propose, therefore, that the observed decrease in $(\Delta K_M + \Delta K_{rel})$ reflects a significantly higher relaxation compressibility, K_{rel}, of the coil relative to the quadruplex conformation. The relaxation contribution to compressibility results from the pressure-induced shift in the ensemble population solute molecules toward the species with smaller partial molar volume. Thus, as in the case of expansibility, our results are consistent with the picture in which the ensemble of coil conformations consists of a variety of nonrandom coil-like species differing in volume.

The observed decrease in the relaxation contributions to expansibility and compressibility associated with quadruplex formation reflects the variety of unfolded species with respect to enthalpy and volume. We propose that, in general, all order−disorder transitions should be characterized by large positive relaxation contributions to compressibility, expansibility, and, by extension, heat capacity; $C_{Prel} = (\langle \Delta H^2 \rangle - \langle \Delta H \rangle^2)/RT^2$. The condition for the relaxation contributions to be significant is the presence of sizable populations of isoenergetic, yet structurally and hydrationally distinct, "disordered" species that also differ in enthalpy and volume. On a molecular level, the relaxation component results from the shift in the fractional population of disordered species induced by changes in pressure and temperature.

■ CONCLUSIONS

We used complementary volumetric techniques of PPC, UV melting under high pressure, vibrating tube densimetry, and

ultrasonic velocimetry to determine changes in volume, expansibility, and adiabatic compressibility associated with G-quadruplex formation by the human telomeric oligonucleotide sequence d[A(GGGTTA)$_3$GGG]. Wherever the results of PPC, high-pressure measurements, and densimetry can be compared with each other, they agree well.

Our volume data, combined with structural data, suggest that quadruplex formation is accompanied by significant dehydration of the oligonucleotide with 103 ± 44 water molecules released to the bulk. Comparison of this number with the solvent-accessible surface area buried upon quadruplex formation reveals that the observed dehydration is predominantly limited to water molecules in direct contact with the DNA.

Analysis of our expansibility and compressibility results suggests that the changes in these parameters cannot be accounted for solely within the framework of dehydration of DNA and sodium ions associated with quadruplex formation. Our analysis suggests the presence of large relaxation components to expansibility and compressibility reflecting the variation in enthalpy and volume in population of unfolded species constituting the ensemble of isoenergetic coil-like conformations.

AUTHOR INFORMATION

Corresponding Author
chalikan@phm.utoronto.ca

Author Contributions
†These authors contributed equally.

ACKNOWLEDGMENT

This work was supported by NSERC grants to H.H., T.V.C., and R.B.M. Y.L.S. acknowledges graduate support from the CIHR Protein Folding Training Program.

REFERENCES

(1) Keniry, M. A. *Biopolymers* **2000**, *56*, 123–146.
(2) Shafer, R. H.; Smirnov, I. *Biopolymers* **2000**, *56*, 209–227.
(3) Gellert, M.; Lipsett, M. N.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A* **1962**, *48*, 2013–2018.
(4) Williamson, J. R. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703–730.
(5) Lane, A. N.; Chaires, J. B.; Gray, R. D.; Trent, J. O. *Nucleic Acids Res.* **2008**, *36*, 5482–5515.
(6) Chaires, J. B. *FEBS J.* **2010**, *277*, 1098–1106.
(7) Chalikian, T. V.; Breslauer, K. J. *Biopolymers* **1998**, *48*, 264–280.
(8) Chalikian, T. V.; Macgregor, R. B. *Phys. Life Rev.* **2007**, *4*, 91–115.
(9) Kankia, B. I.; Marky, L. A. *J. Am. Chem. Soc.* **2001**, *123*, 10799–10804.
(10) Olsen, C. M.; Marky, L. A. *J. Phys. Chem. B* **2009**, *113*, 9–11.
(11) Miyoshi, D.; Karimata, H.; Sugimoto, N. *J. Am. Chem. Soc.* **2006**, *128*, 7957–7963.
(12) Miller, M. C.; Buscaglia, R.; Chaires, J. B.; Lane, A. N.; Trent, J. O. *J. Am. Chem. Soc.* **2010**, *132*, 17105–17107.
(13) Wang, Y.; Patel, D. J. *Structure* **1993**, *1*, 263–282.
(14) Parkinson, G. N.; Lee, M. P.; Neidle, S. *Nature* **2002**, *417*, 876–880.
(15) Phan, A. T.; Patel, D. J. *J. Am. Chem. Soc.* **2003**, *125*, 15021–15027.
(16) Li, J.; Correia, J. J.; Wang, L.; Trent, J. O.; Chaires, J. B. *Nucleic Acids Res.* **2005**, *33*, 4649–4659.
(17) Mergny, J. L.; Phan, A. T.; Lacroix, L. *FEBS Lett.* **1998**, *435*, 74–78.
(18) Gray, R. D.; Chaires, J. B. *Nucleic Acids Res.* **2008**, *36*, 4191–4203.
(19) Lin, L. N.; Brandts, J. F.; Brandts, J. M.; Plotnikov, V. *Anal. Biochem.* **2002**, *302*, 144–160.
(20) Heerklotz, H. *Methods Mol. Biol.* **2007**, *400*, 197–206.
(21) Schweiker, K. L.; Makhatadze, G. I. *Methods Enzymol.* **2009**, *466*, 527–547.
(22) Wu, J. Q.; Macgregor, R. B., Jr. *Biochemistry* **1993**, *32*, 12531–12537.
(23) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601–1620.
(24) Sarvazyan, A. P. *Ultrasonics* **1982**, *20*, 151–154.
(25) Eggers, F.; Funck, T. *Rev. Sci. Instrum.* **1973**, *44*, 969–977.
(26) Sarvazyan, A. P.; Chalikian, T. V. *Ultrasonics* **1991**, *29*, 119–124.
(27) Filfil, R.; Chalikian, T. V. *J. Mol. Biol.* **2003**, *326*, 1271–1288.
(28) Till, M. S.; Ullmann, G. M. *J. Mol. Model.* **2010**, *16*, 419–429.
(29) Humphrey, W.; Dalke, A.; Schulten, K. *J. Mol. Graphics* **1996**, *14*, 33–38.
(30) Tikhomirova, A.; Chalikian, T. V. *J. Mol. Biol.* **2004**, *341*, 551–563.
(31) Lee, S.; Heerklotz, H.; Chalikian, T. V. *Biophys. Chem.* **2010**, *148*, 144–147.
(32) Rayan, G.; Tsamaloukas, A. D.; Macgregor, R. B.; Heerklotz, H. *J. Phys. Chem. B* **2009**, *113*, 1738–1742.
(33) Winter, R.; Lopes, D.; Grudzielanek, S.; Vogtt, K. *J. Non-Equilib. Thermodyn.* **2007**, *32* (1), 41–97.
(34) Panick, G.; Herberhold, H.; Sun, Z.; Winter, R. *Spectroscopy* **2003**, *17*, 367–376.
(35) Edward, J. T.; Farrell, P. G. *Can. J. Chem.* **1975**, *53*, 2965–2970.
(36) Kharakoz, D. P. *J. Solution Chem.* **1992**, *21*, 569–595.
(37) Connolly, M. L. *Science* **1983**, *221*, 709–713.
(38) Richards, F. M. *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 151–176.
(39) Richards, F. M. *Methods Enzymol.* **1985**, *115*, 440–464.
(40) Likhodi, O.; Chalikian, T. V. *J. Am. Chem. Soc.* **1999**, *121*, 1156–1163.
(41) Conway, B. E. *J. Solution Chem.* **1978**, *7*, 721–770.
(42) Chalikian, T. V. *J. Phys. Chem. B* **2001**, *105*, 12566–12578.
(43) Mitra, L.; Smolin, N.; Ravindra, R.; Royer, C.; Winter, R. *Phys. Chem. Chem. Phys.* **2006**, *8*, 1249–1265.
(44) Batchelor, J. D.; Olteanu, A.; Tripathy, A.; Pielak, G. J. *J. Am. Chem. Soc.* **2004**, *126*, 1958–1961.
(45) Chalikian, T. V. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 207–235.
(46) Chalikian, T. V. *J. Phys. Chem. B* **2008**, *112*, 911–917.
(47) Kharakoz, D. P. *Biophys. Chem.* **1989**, *34*, 115–125.
(48) Chalikian, T. V.; Sarvazyan, A. P.; Breslauer, K. J. *J. Phys. Chem.* **1993**, *97*, 13017–13026.
(49) Mathieson, J. G.; Conway, B. E. *J. Solution Chem.* **1974**, *3*, 455–477.
(50) Buckin, V. A.; Kankiya, B. I.; Sarvazyan, A. P.; Uedaira, H. *Nucleic Acids Res.* **1989**, *17*, 4189–4203.
(51) Wilton, D. J.; Ghosh, M.; Chary, K. V. A.; Akasaka, K.; Williamson, M. P. *Nucleic Acids Res.* **2008**, *36*, 4032–4037.