Energetics of Nucleic Acid Stability: The Effect of ΔC_P

Anna Tikhomirova, Nicolas Taulier, and Tigran V. Chalikian*

Contribution from the Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ontario M5S 2S2, Canada

Received June 18, 2004; E-mail: chalikan@phm.utoronto.ca

Abstract: We report high-resolution differential scanning calorimetric data on the poly(dAdT)poly(dAdT), poly(dA)poly(dT), poly(dIdC)poly(dIdC), poly(dGdC)poly(dGdC), poly(rA)poly(rU), and poly(rI)poly(rC) nucleic acid duplexes. We use these data to evaluate the melting temperatures, T_M, enthalpy changes, ΔH_M, and heat capacity changes, ΔC_P, accompanying helix-to-coil transitions of each polymeric duplex studied in this work at different NaCl concentrations. In agreement with previous reports, we have found that ΔC_P exhibits a positive, nonzero value, which, on average, equals 268 ± 33 J mol^{-1} K^{-1}. With ΔC_P, we have calculated the transition free energies, ΔG, enthalpies, ΔH, and entropies, ΔS, for the duplexes as a function of temperature. Since, ΔG, ΔH, and ΔS all strongly depend on temperature, the thermodynamic comparison between DNA and/or RNA duplexes (that may differ from one another with respect to sequence, composition, conformation, etc.) is physically meaningful only if extrapolated to a common temperature. We have performed such comparative analyses to derive differential thermodynamic parameters of formation of GC versus AT, AU, and IC base pairs as well as B′ versus A and B helix conformations. We have proposed some general microscopic interpretations for the observed sequence-specific and conformation-specific thermodynamic differences between the duplexes.

Introduction

A conventional way of elucidating and characterizing the physical forces that govern and direct conformational preferences of nucleic acids relies on temperature scanning experiments monitored by UV light absorption spectroscopy and/or differential scanning calorimetry.[1−5] Until recently, such studies and related analyses of DNA stability were carried out under an assumption that a change in heat capacity, ΔC_P, associated with helix-to-coil transitions of nucleic acid duplexes is negligibly small. However, this approximation was proven imprecise after new generation high-sensitivity differential scanning calorimeters had been employed to characterize helix-to-coil transitions of oligomeric and polymeric duplexes.[6−8] In particular, in a recent work, we determined that polymeric duplexes melt with a positive change in heat capacity, ΔC_P, of 272 ± 88 J mol^{-1} K^{-1} (expressed per mole of base pair).[7] This experimental finding has been subsequently supported by theoretical calculations.[9,10]

A nonzero value of ΔC_P suggests that changes in enthalpy, ΔH, and entropy, ΔS, accompanying helix-to-coil transitions of nucleic acids depend on temperature. Consequently, any thermodynamic comparison between duplexes is valid only if the measured values are extrapolated to a common temperature.[7,11] Determination of free energy, ΔG, also should be affected. This notion affects, in particular, numerous nearest-neighbor thermodynamic parameters for helix initiation and propagation reported in the literature that all have been determined under an assumption of temperature invariance of ΔH and ΔS [refs 12−14 and references therein].

The survey of literature reveals that no systematic analysis of the stability of nucleic acid duplexes has been performed with explicit consideration of the effect of ΔC_P. This deficit is unfortunate and prevents one from better understanding the molecular origins of thermodynamic differences between various nucleic acid structures. Moreover, neglecting the temperature dependences of the enthalpic and entropic characteristics of helix-to-coil transitions of nucleic acids may introduce large and unaccounted temperature-dependent errors in the stability characteristics of polymeric and oligomeric duplexes.

To address this deficiency, we have started a series of systematic thermodynamic investigations of the stability of nucleic acid structures in which we employ a combination of calorimetric, volumetric, and high-pressure measurements to identify and quantify the forces that stabilize/destabilize nucleic acid duplexes as well as high-order structures. In this work, we report calorimetric data (including ΔG, ΔH, ΔS, and ΔC_P) on the poly(dAdT)poly(dAdT), poly(dA)poly(dT), poly(dIdC)poly-

(1) Klump, H. H. *Can. J. Chem.* **1988**, *66*, 804−811.
(2) Filimonov, V. V. In *Thermodynamic Data for Biochemistry and Biotechnology*; Hinz, H.-J., Ed.; Springer-Verlag: Berlin, Heidelberg, New York, Tokyo, 1986; pp 45−128.
(3) Tinoco, I., Jr. *J. Phys. Chem.* **1996**, *100*, 13311−13322.
(4) Breslauer, K. J. *Methods Enzymol.* **1995**, *259*, 221−242.
(5) Plum, G. E.; Breslauer, K. J.; Roberts, R. W. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier Science Ltd.: Oxford, UK, 1999; Vol. 7, pp 15−33.
(6) Holbrook, J. A.; Capp, M. W.; Saecker, R. M.; Record, M. T., Jr. *Biochemistry* **1999**, *38*, 8409−8422.
(7) Chalikian, T. V.; Völker, J.; Plum, G. E.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7853−7858.
(8) Jelesarov, I.; Crane-Robinson, C.; Privalov, P. L. *J. Mol. Biol.* **1999**, *294*, 981−995.
(9) Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **1999**, *77*, 3242−3251.
(10) Rouzina, I.; Bloomfield, V. A. *Biophys. J.* **1999**, *77*, 3252−3255.

(11) Lane, A. N.; Jenkins, T. C. *Q. Rev. Biophys.* **2000**, *33*, 255−306.

(dIdC), and poly(dGdC)poly(dGdC) DNA duplexes and the poly(rA)poly(rU) and poly(rI)poly(rC) RNA duplexes. We analyze our resulting data to derive the differential thermodynamic parameters of formation of GC versus AT, AU, and IC base pairs as well as B′ versus A and B helix conformations. We also propose some general microscopic interpretations for the observed thermodynamic differences. To the best of our knowledge, this work represents the first investigation of nucleic acid stability with an explicit consideration of the effect of ΔC_P.

Experimental Section

Materials. The poly(dAdT)poly(dAdT), poly(dA)poly(dT), and poly-(dGdC)poly(dGdC) DNA duplexes and the single-stranded poly(rA) and poly(rU) RNA polymers were purchased from Amersham Pharmacia Biotech, Inc. (Baie d'Urfé, Québec, Canada). The poly(rI)poly-(rC) RNA duplex was obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). The poly(dIdC)poly(dIdC) DNA duplex was obtained from both Amersham Pharmacia Biotech, Inc. and Sigma-Aldrich Canada. The polymers that contained between 3000 and 8000 nucleotides per molecule were of the highest grade commercially available and were used without further purification. The two sets of calorimetric results obtained on the poly(dIdC)poly(dIdC) samples acquired from Sigma-Aldrich and Amersham Pharmacia coincided within experimental uncertainty of our DSC measurements.

All calorimetric measurements were performed in a pH 6.7 buffer consisting of 10 mM cacodylic acid/sodium cacodylate, 1 mM Na2-EDTA, and 20, 50, 100, 200, or 300 mM NaCl. DNA and RNA samples were dissolved in a buffer and exhaustively dialyzed against the same buffer using dialysis tubings with a 1000 molecular weight cutoff (Spectrum, Houston, TX). An additional change of the buffer solutions was made with at least 24 h allowed for the final equilibration.

Equimolar amounts of the poly(rA) or poly(rU) single strands were mixed in buffer to obtain the poly(rA)poly(rU) duplex. The concentrations of the single- and double-stranded polynucleotides were determined spectrophotometrically using the following molar extinction coefficients for all salts: poly(rA), $\epsilon_{258} = 9800$ M^{-1} cm^{-1}; poly(rU), $\epsilon_{260} = 9350$ M^{-1} cm^{-1}; poly(rA)poly(rU), $\epsilon_{257} = 7000$ M^{-1} cm^{-1}; poly-(rI)poly(rC), $\epsilon_{266} = 5250$ M^{-1} cm^{-1}; poly(dAdT)poly(dAdT), $\epsilon_{260} = 6650$ M^{-1} cm^{-1}; poly(dA)poly(dT), $\epsilon_{260} = 6000$ M^{-1} cm^{-1}; poly-(dGdC)poly(dGdC), $\epsilon_{254} = 8400$ M^{-1} cm^{-1}; and poly(dIdC)poly(dIdC), $\epsilon_{251} = 6900$ M^{-1} cm^{-1}. These values were either provided by the manufacturer or taken from literature.[15−17] All UV absorbance measurements were performed using an Aviv 14 DS UV/vis spectrophotometer (Aviv Associates, Lakewood, NJ).

Differential Scanning Calorimetry. Calorimetric melting profiles of the duplexes were determined at a scan rate of 1 °C/min using a Calorimetry Sciences Corporation model 6100 NanoDSCII differential scanning calorimeter (Calorimetry Sciences Corporation, Provo, UT) with a nominal cell volume of 0.3 mL. Appropriate buffer versus buffer baselines were determined prior to and immediately after the polymer versus buffer scan and averaged. After subtraction of the buffer scan, the polymer scan was normalized for concentration and analyzed as follows. The heat capacity difference, $\Delta C_{P\text{cal}}$, was determined from the difference in the pre- and post-transition baselines at the midpoint of the transition, the calorimetric enthalpy, ΔH_{cal}, was determined by integration of the area enclosed by the transition curve and the pre/post-transition baselines, and the melting temperature, T_M, was deter-
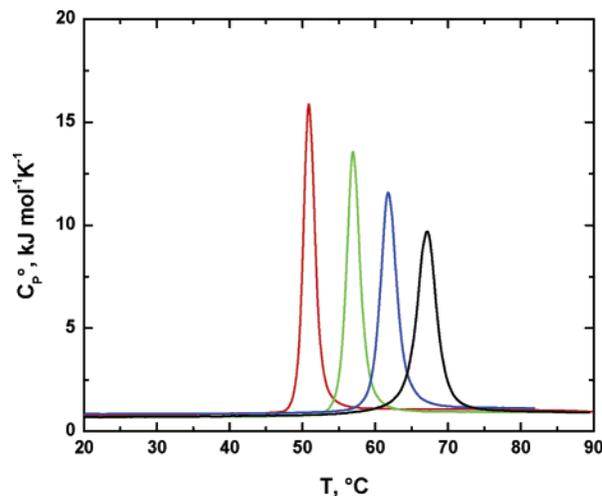
Figure 1. Heat capacity temperature profiles (DSC thermograms) for the poly d(AT)poly d(AT) duplex at 30 (red), 60 (green), 110 (blue), and 210 (black) mM Na$^+$.

mined as the midpoint of the melting transition.[4,5,18,19] Figure 1 presents representative DSC thermograms for the poly(dAdT)poly(dAdT) duplex measured at 30, 60, 110, and 210 mM [Na$^+$].

Isothermal Titration Calorimetry. The isothermal titration calorimetric (ITC) experiments on poly(rA) + poly(rU) were carried out at 20, 25, 30, 35, and 40 °C and 30 and 210 mM NaCl with a Calorimetry Sciences Corporation model 4200 isothermal titration calorimeter (Calorimetry Sciences Corporation, Provo, UT). The calorimeter was routinely calibrated with 500 μJ electrical pulses and by measuring the enthalpy of binding of BaCl$_2$ to 18-crown-6 as indicated in the manual of the instrument. The ITC titration experiments were performed by adding aliquots of poly(rU) to poly(rA).

For all ITC measurements, the concentration of the initial poly(rA) solution in the sample cell was ∼3 mM (per nucleotide), while the concentration of the poly(rU) solution in the syringe (titrant) was typically ∼5 mM (per nucleotide). The initial solution volume in the sample cell was 1.00 mL, and the injection volume was on the order of 10 μL. To take into account the heat of dilution and viscous mixing, the buffer was titrated with the titrant solution following the same injection schedule as employed for the actual titration.[18,19] The resulting heat was subtracted from the raw data.

ITC titration experiments at all temperatures studied in this work have been performed at a large excess of poly(rA), so that each added aliquot of poly(rU) can be confidently considered to fully bind to its complementary strand with subsequent duplex formation. Under these circumstances, the area enclosed by each ITC peak normalized per number of moles of added poly(rU) corresponds with a good approximation to the enthalpy of poly(rA)poly(rU) formation (coil-to-helix transition). Figure 2 presents a representative ITC titration profile for the binding of poly(rU) to poly(rA) at 25 °C and 20 mM NaCl.

Results

Figure 3 plots our DSC-determined melting temperatures, T_M, as a function of Na$^+$ concentration, while Figure 4 shows the transition enthalpies, ΔH_M, plotted versus T_M for the poly-(dAdT)poly(dAdT), poly(dA)poly(dT), poly(dIdC)poly(dIdC), poly(dGdC)poly(dGdC), poly(rA)poly(rU), and poly(rI)poly(rC) duplexes. For the poly(dGdC)poly(dGdC) duplex, the T_M was determined only at the three Na$^+$ concentrations 10, 30, and 50 mM. At higher salt concentration, when the T_M of poly(dGdC)-

(12) Breslauer, K. J.; Frank, R.; Blöcker, H.; Marky, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3476−3750.
(13) Santa Lucia, J., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *95*, 1460−1465.
(14) Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr. *Nucleic Acids: Structures, Properties, and Functions*; University Science Books: Sausalito, CA, 2000.
(15) Chamberlin, M. J. *Fed. Proc.* **1965**, *24*, 1446−1457.
(16) Chamberlin, M. J.; Patterson, D. L. *J. Mol. Biol.* **1965**, *12*, 410−428.
(17) Riley, M.; Maling, B.; Chamberlin, M. J. *J. Mol. Biol.* **1966**, *20*, 359−389.

(18) Breslauer, K. J.; Freire, E.; Straume, M. *Methods Enzymol.* **1992**, *211*, 533−567.
(19) Privalov, G. P.; Privalov, P. L. *Methods Enzymol.* **2000**, *323*, 31−62.
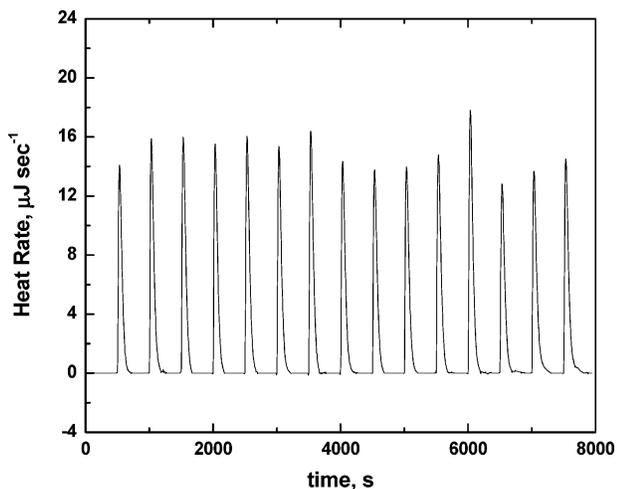
Figure 2. Representative ITC titration profiles at 25 °C for titration of poly(rA) (3 mM per nucleotide) with poly(rU) (5 mM per nucleotide). The initial volume of poly(rA) is 1 mL. The volume of each added aliquot of poly(rU) is 10 μL.
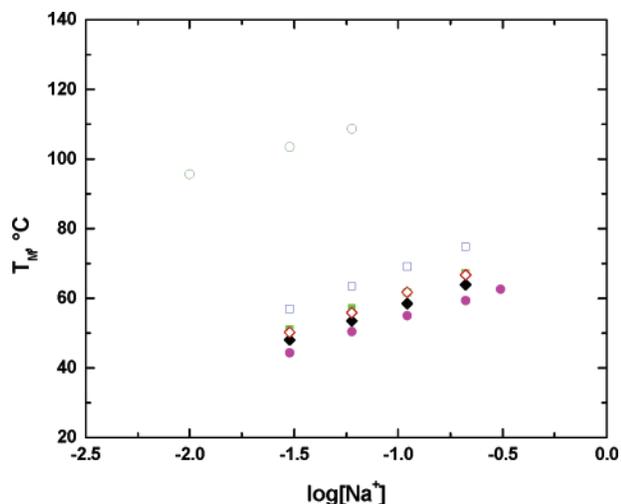
Figure 3. Melting temperatures of poly(dAdT)poly(dAdT), (■), poly(dA)-poly(dT), (□), poly(dIdC)poly(dIdC) (●), poly(dGdC)poly(dGdC) (○), poly-(rA)poly(rU), (◆), and poly(rI)poly(rC) (◇) plotted versus log[Na$^+$].

Figure 4. Transition enthalpies, ΔH_M, for poly(dAdT)poly(dAdT), (■), poly(dA)poly(dT), (□), poly(dIdC)poly(dIdC) (●), poly(dGdC)poly(dGdC) (○), poly(rA)poly(rU), (◆), and poly(rI)poly(rC) (◇) plotted versus melting temperatures, T_M.
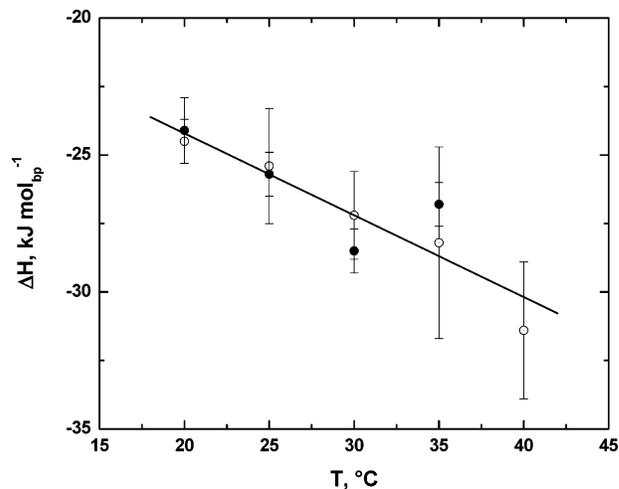
Figure 5. Temperature dependences of the enthalpy of poly(rA)poly(rU) duplex formation determined by ITC at 30 (●) and 210 (○) mM Na$^+$.

poly(dGdC) increases well above 100 °C, our DSC measurements became less reliable. It should be noted, however, that we were able to reliably measure the transition enthalpy, ΔH_M, of poly(dGdC)poly(dGdC) only at 10 mM Na$^+$ (with the T_M 95.6 °C).

The values of $\partial T_M/\partial \log[\text{Na}^+]$ for poly(dAdT)poly(dAdT), poly(dA)poly(dT), poly(dIdC)poly(dIdC), poly(dGdC)poly-(dGdC), poly(rA)poly(rU), and poly(rI)poly(rC) are 19.0 ± 0.3, 21.2 ± 0.2, 17.6 ± 0.5, 16.7 ± 0.3, 19.0 ± 0.2, and 19.3 ± 0.9, respectively. Comparison of these results with literature data reveals a good agreement for the [Na$^+$] dependences of T_M.[1,2,20]

Our measured values of ΔH_M for the polymeric duplexes studied in this work are within the range of the literature values, although data from different literature sources may vary significantly.[1,2,7,20] Changes in heat capacity, $\Delta C_{P\text{cal}}$, directly measured as increments of pre- and post-transitional baselines exhibit large uncertainties. The average values of $\Delta C_{P\text{cal}}$

(20) Klump, H. H. In *Biochemical Thermodynamics*; Jones, M. N., Ed.; Elsevier: Amsterdam, 1988; pp 100−144.

(averaged over various ionic strenghts) for poly(dAdT)poly-(dAdT), poly(dA)poly(dT), poly(dIdC)poly(dIdC), poly(dGdC)-poly(dGdC), poly(rA)poly(rU), and poly(rI)poly(rC) are 176 ± 100, 163 ± 179, 238 ± 238, 184 ± 104, 130 ± 46, and 167 ± 63 J mol^{-1} K^{-1}, respectively. Such large errors prevent their use in any rigorous thermodynamic analysis of the stability of the nucleic acid duplexes as a function of temperature.

In analogy with proteins, a more accurate way of determining ΔC_P would be to calculate it as the slope of the T_M dependence of ΔH_M. In protein studies, T_M is generally shifted by varying pH, while, in DNA studies, it is more common to alter T_M by modulating the solution ionic strength. An important question related to this procedure is how strongly the transition enthalpy, ΔH_M, depends on salt. Only, provided that ΔH_M is salt-independent, the apparent ΔC_P calculated as the slope $\Delta\Delta H_M/\Delta T_M$ will represent the true value of the transition heat capacity.

To address this issue, we have carried out ITC determination of the enthalpy of poly(rA)poly(rU) duplex formation at 30 and 210 mM Na$^+$. These measurements have been performed at 20, 25, 30, 35, and 40 °C. Figure 5 presents the enthalpy of poly-(rA)poly(rU) formation at 30 (●) and 210 (○) mM Na$^+$ plotted

against temperature. Inspection of Figure 5 reveals that, at all temperatures studied, the enthalpies of duplex formation at 30 and 210 mM Na$^+$ are indistinguishable within experimental uncertainty (± 1.7 kJ mol^{-1}). By extension, we assume that enthalpies of duplex formation/disruption for other polymers under question are also salt-independent (more strictly, Na$^+$-independent).

Given the observed insensitivity of ΔH_M to salt, the apparent $\Delta C_P = \Delta\Delta H_M/\Delta T_M$ is the true value of the transition heat capacity. Our calculated values of ΔC_P for poly(dAdT)poly-(dAdT), poly(dA)poly(dT), poly(dIdC)poly(dIdC), poly(rA)-poly(rU), and poly(rI)poly(rC) equal 213 $\pm$ 63, 297 $\pm$ 88, 293 $\pm$ 58, 247 $\pm$ 54, and 280 $\pm$ 37 J mol^{-1} K^{-1}, respectively [we have not included in this list the ΔC_P value on poly(dGdC)-poly(dGdC), since its ΔH was measured at a single T_M of 95.6 °C that corresponds to 10 mM Na$^+$]. The average of these values is 268 $\pm$ 33 J mol^{-1} K^{-1}. This value is in excellent agreement with 272 $\pm$ 92 J mol^{-1} K^{-1}, the average value of ΔC_P we have previously determined for a number of polymeric DNA duplexes.[7] It is gratifying that the value of ΔC_P for the coil-to-helix transition of poly(rA)poly(rU) measured by ITC (-314 ± 33 J mol^{-1} K^{-1}) is in reasonable agreement with the average DSC-determined value of ΔC_P for helix-to-coil duplex transitions of 268 $\pm$ 33 J mol^{-1} K^{-1}.

Given the relatively large errors of individual ΔC_P determinations, we use in our analyses below the average value of ΔC_P of 268 $\pm$ 33 J mol^{-1} K^{-1} (uncertainty was calculated as standard deviation of the mean for the combined ΔC_P data for all individual duplexes). It should be noted, however, that the differential values of $\Delta\Delta G$, $\Delta\Delta H$, and $\Delta\Delta S$ (when comparing two duplexes) strongly depend on ΔC_P. Consequently, the use of the average value of ΔC_P, while providing important new insights into the stability characteristics of nucleic acids, may compromise our ensuing conclusions. Future studies are required to determine with a higher accuracy the individual values of ΔC_P for each duplex, so that more detailed analyses can be performed to further elaborate on the conclusions and estimates drawn in this investigation.

Discussion

Duplex Stabilities. With the values of T_M, ΔH_M, and ΔC_P, we now proceed to calculate changes in free energy, ΔG, associated with helix-to-coil transitions of nucleic acid duplexes at different ionic strengths. The relationship between ΔG and the values of ΔH_M, T_M, and ΔC_P is given by the following equation:

$$\Delta G(T) = \Delta H_M(1 - T/T_M) + \Delta C_P[T - T_M - T \ln(T/T_M)] \quad (1)$$

where T is the absolute temperature.

Figure 6a shows the calculated temperature dependences of ΔG for the polymeric duplexes studied in this work at 30 mM [Na$^+$]. The calculations have been performed using eq 1 under an assumption of temperature independence of ΔC_P. At other salts, these dependences, while exhibiting similar shapes, are shifted to higher values (not shown). For poly(dGdC)poly-(dGdC), the transition enthalpy, ΔH_M, at 30 mM Na$^+$ (with T_M of 103.4 $\pm$ 0.5 °C) required for calculating $\Delta G(T)$ was evaluated from the experimental value of ΔH_M measured at 10 mM Na$^+$ (with a T_M of 95.6 $\pm$ 0.3 °C) using an average ΔC_P of 268 $\pm$
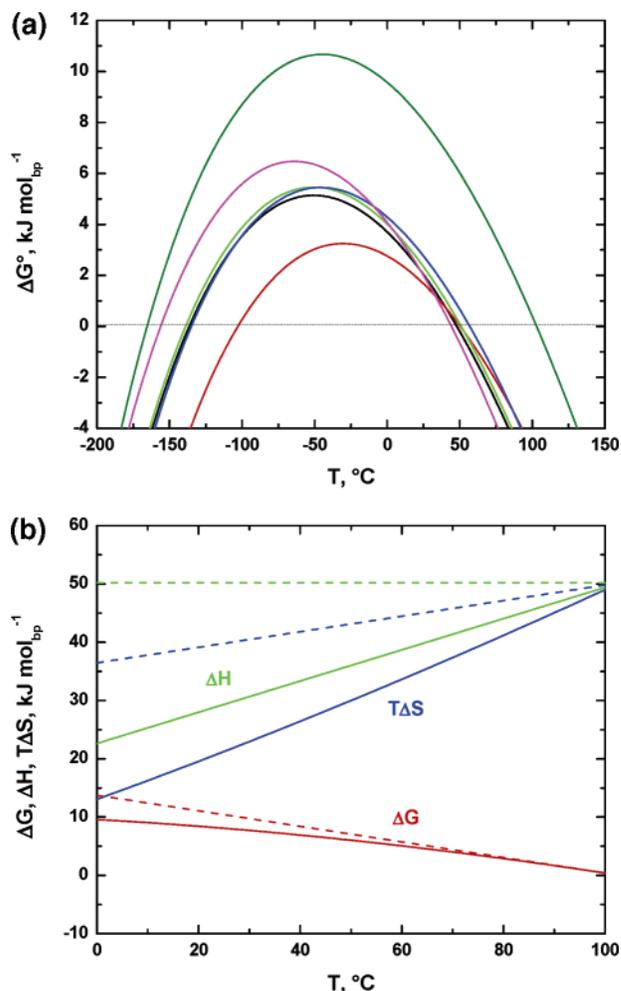
Figure 6. (a) Temperature dependences of free energy changes, ΔG, accompanying helix-to-coil transitions of poly(dAdT)poly(dAdT), (green), poly(dA)poly(dT), (blue), poly(dIdC)poly(dIdC) (magenta), poly(dGdC)-poly(dGdC) (olive), poly(rA)poly(rU), (black), and poly(rI)poly(rC) (red) at 30 mM Na$^+$. (b) Temperature dependences of the transition free energy, ΔG (red), enthalpy, ΔH (green), and entropy, $T\Delta S$ (blue), of poly(dGdC)-poly(dGdC) calculated with ΔC_P equal to 0 (dashed lines) and 268 J mol^{-1}K^{-1} (solid lines).

33 J mol^{-1} K^{-1}. To the best of our knowledge, the plots shown in Figure 6a represent the first analysis of nucleic acid stability, which explicitly takes into account temperature dependences of ΔH and ΔS as represented by ΔC_P.

In previous analyses, the second term of eq 1 was ignored under an assumption of $\Delta C_P = 0$. As readily seen from eq 1, such an assumption drastically changes the profile of the ΔG versus T functions (the plots become linear instead of parabolic-like) thereby introducing significant temperature-dependent errors in determining the stability (ΔG) and/or differential stability ($\Delta\Delta G$) of nucleic acid duplexes. To further clarify this point, it is instructive to compare the stability characteristics of DNA calculated with and without taking into account a change in heat capacity, ΔC_P. Figure 6b presents such a comparison for poly(dGdC)poly(dGdC) within a biologically relevant temperature range of 0 to 100 °C. Specifically, Figure 6b plots a free energy difference between the double-stranded and single-stranded states of poly(dGdC)poly(dGdC), $\Delta G(T)$, as well as its enthalpic, $\Delta H(T) = \Delta H_M + \Delta C_P(T - T_M)$, and entropic components, $T\Delta S(T) = \Delta H_M(T/T_M) + \Delta C_P T \ln(T/T_M)$, evaluated

under assumptions of ΔC_P equal to 0 (dashed lines) and 268 J mol^{-1} K^{-1} (solid lines). As is readily seen from Figure 6b, the two calculation modes yield significantly distinct results for each of the ΔG, ΔH, and ΔS parameters by far exceeding experimental uncertainties. For example, at 0 °C, the values of ΔH, $T\Delta S$, and ΔG calculated with $\Delta C_P = 0$ and 268 J mol^{-1}K^{-1} are equal to 50.2 and 21.7 kJ mol^{-1}, 36.4 and 12.5 kJ mol^{-1}, and 13.8 and 9.6 kJ mol^{-1}, respectively. These discrepancies are significant and emphasize the need for careful consideration of the effect of ΔC_P in temperature-dependent thermodynamic analyses of DNA stability.

Inspection of Figures 6a reveals two important observations. First, the ΔG-versus-T dependences exhibit maxima which correspond to the maximum stabilities, ΔG_{max}, of the duplexes at temperatures $T_{max} = T_M \exp(-\Delta H_M/\Delta C_P T_M)$. The values of ΔG_{max} vary between 2.9 and 10.5 kJ mol^{-1} and increase with increasing salt, with the values of T_{max} ranging from -70 to -20 °C. Second, all duplexes exhibit cold denaturation at temperatures, T_C, between -100 and -200 °C. We have previously outlined a possibility of cold denaturation of nucleic acid duplexes, which results from positive values of ΔC_P.[21] Cold denaturation has been observed experimentally for proteins and some nucleic acid structures.[22,23]

Both of these observations (temperatures of maximum stability, T_{max}, and cold denaturation, T_C) are related to duplex stability at highly negative, experimentally unachievable temperatures. One might, therefore, become reasonably concerned about the physical meaning and validity of these observations and ensuing conclusions. It is our opinion, however, that the extrapolation to negative temperatures is physically valid, at least, from the qualitative point of view. This extrapolation represents an extension of experimentally determined properties of nucleic acids to subfreezing temperatures. From the quantitative viewpoint, the values of T_{max} and T_C may not be accurate, since they have been obtained under an assumption that ΔC_P is constant (that may not be true at low temperatures). However, the phenomena of maximum stability and cold denaturation are real and represent direct consequences of a positive value of ΔC_P.[22]

The plots presented in Figure 6a permit one to carry out instructive comparisons of the stability profiles of poly(dAdT)-poly(dAdT) versus poly(dA)poly(dT) (B versus B′ conformation), poly(dA)poly(dT) versus poly(rA)poly(rU) (B′ versus A conformation), poly(dAdT)poly(dAdT) versus poly(dIdC)poly-(dIdC) (AT versus IC base pairs), poly(dAdT)poly(dAdT) versus poly(dGdC)poly(dGdC) (AT versus GC base pairs), poly(dIdC)-poly(dIdC) versus poly(dGdC)poly(dGdC) (IC versus GC base pairs), and poly(rA)poly(rU) versus poly(rI)poly(rC) (AU versus IC base pairs). Figure 7 presents the temperature dependences of the differential free energies, $\Delta\Delta G$, for each of the above-mentioned pairs of nucleic acid duplexes at 30 mM [Na$^+$] within the physiologically relevant range 0 to 100 °C. The $\Delta\Delta G$ plots determined at other salts (data are not shown) are not very different from those presented in Figure 7.

Heteropolymeric versus Homopolymeric All-AT Duplexes. Structurally, the heteropolymeric poly(dAdT)poly(dAdT) duplex, being in the classical B conformation, is significantly

(21) Dubins, D. N.; Lee, A.; Macgregor, R. B., Jr.; Chalikian, T. V. *J. Am. Chem. Soc.* **2001**, *123*, 9254−9259.
(22) Privalov, P. L. *Crit. Rev. Biochem. Mol. Biol.* **1990**, *25*, 281−305.
(23) Mikulecky, P. J.; Feig, A. *J. Am. Chem. Soc.* **2002**, *124*, 890−891.
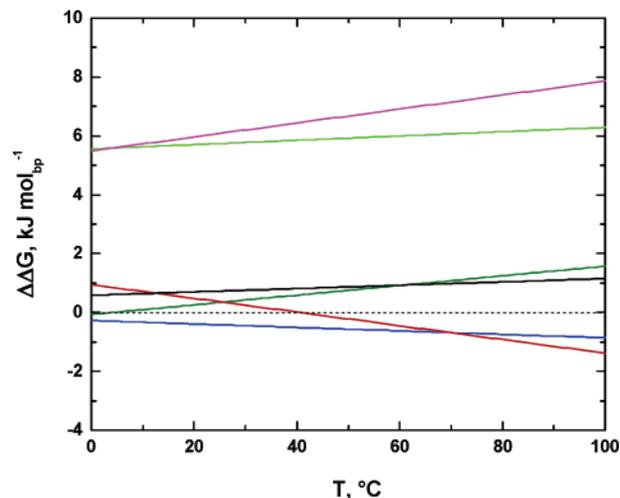
Figure 7. Temperature dependences of the differential free energy, $\Delta\Delta G$, of helix-to-coil transitions of the poly(dAdT)poly(dAdT)/poly(dA)poly(dT) (blue), poly(dA)poly(dT)/poly(rA)poly(rU) (black), poly(dAdT)poly(dAdT)/poly(dIdC)poly(dIdC) (olive), poly(rA)poly(rU)/poly(rI)poly(rC) (red), poly-(dGdC)poly(dGdC)/poly(dAdT)poly(dAdT) (green), and poly(dGdC)poly-(dGdC)/poly(dIdC)poly(dIdC) (magenta) pairs.

Table 1. Differential Enthalpy, $\Delta\Delta H$, and Entropy, $\Delta\Delta S$, of Helix-to-Coil Transitions of Various Pairs of Nucleic Acid Duplexes

duplex pair	$\Delta\Delta H$, kJ mol^{-1}	$\Delta\Delta S$, J mol^{-1}K^{-1}
poly(dAdT)poly(dAdT)/poly(dA)poly(dT)	0.4 ± 1.2	5.8 ± 4.2
poly(dA)poly(dT)/poly(rA)poly(rU)	-0.4 ± 1.2	-5.4 ± 4.2
poly(dAdT)poly(dAdT)/poly(ddIC)poly(dIdC)	-5.8 ± 1.2	-16.3 ± 4.2
poly(rA)poly(rU)/poly(rI)poly(rC)	7.1 ± 1.2	23.0 ± 4.2
poly(dGdC)poly(dGdC)/poly(dAdT)poly(dAdT)	5.8 ± 1.7	-6.7 ± 5.4
poly(dGdC)poly(dGdC)/poly(dIdC)poly(dIdC)	0 ± 1.7	-23.0 ± 5.4

distinct from the homopolymeric poly(dA)poly(dT) duplex, which adopts the B′ conformation.[24,25] The structural difference is further reflected in the differential thermodynamics of helix-to-coil transitions and drug binding of the poly(dAdT)poly-(dAdT) and poly(dA)poly(dT) duplexes.[26−29] Inspection of Figure 7 reveals that the differential free energy, $\Delta\Delta G$, of the helix-to-coil transitions of poly(dAdT)poly(dAdT) and poly-(dA)poly(dT) (blue) is a slightly negative function (decreases from -0.4 to -0.8 kJ mol^{-1} between 0 and 100 °C). The lower thermodynamic stability of poly(dAdT)poly(dAdT) relative to poly(dA)poly(dT) correlates with the melting temperature, T_M, of the latter being 5.9 ± 0.3 °C higher than that of the former (see Figure 3).

To elucidate the thermodynamic origins of the differential stability of polymeric duplexes, it is instructive to examine the enthalpic, $\Delta\Delta H$, and entropic, $\Delta\Delta S$, contributions to $\Delta\Delta G$ ($\Delta\Delta G = \Delta\Delta H - T\Delta\Delta S$). Table 1 presents the differential enthalpy, $\Delta\Delta H$, and entropy, $\Delta\Delta S$, of the heat-induced helix-to-coil transitions of poly(dAdT)poly(dAdT) and poly(dA)poly-(dT). Note that since the $\Delta H = \Delta H_M + \Delta C_P(T - T_M)$ and ΔS

(24) Arnott, S.; Chandrasekaran, R.; Hall, I. H.; Puigjaner, L. C. *Nucleic Acids Res.* **1983**, *11*, 4141−4155.
(25) Alexeev, D. G.; Lipanov, A. A.; Skuratovskii, I. Y. *Nature* **1987**, *325*, 821−823.
(26) Chan, S. S.; Breslauer, K. J.; Hogan, M. E.; Kessler, D. J. *Biochemistry* **1990**, *29*, 6161−6171.
(27) Herrera, J. E.; Chaires, J. B. *Biochemistry* **1989**, *28*, 1993−2000.
(28) Marky, L. A.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4359−4363.
(29) Marky, L. A.; Macgregor, R. B., Jr. *Biochemistry* **1990**, *29*, 4805−4811.

$= \Delta H_M/T_M + \Delta C_P \ln(T/T_M)$ functions of all duplexes have been calculated using the same value of ΔC_P, the $\Delta\Delta H$ and $\Delta\Delta S$ data shown in Table 1 are temperature-independent. Inspection of data in Table 1 reveals that the greater stability of poly(dA)-poly(dT) over poly(dAdT)poly(dAdT) is entropic in nature. Specifically, the values of $\Delta\Delta H$ and $\Delta\Delta S$ are both positive and equal to 0.4 kJ mol^{-1} and 5.8 J mol^{-1}K^{-1}, respectively. The positive value of $\Delta\Delta S$ may reflect a complex interplay between the structural differences between the B and B′ duplex conformations and persisting residual structure of single-stranded poly-(dA). Structural discrepancies between the two all-AT duplexes are reflected, in particular, in unusually high torsional rigidity of poly(dA)poly(dT) compared to other DNA duplexes.[30] This feature should entropically penalize the B′ conformation of poly-(dA)poly(dT). On the other hand, persisting residual structure of single-stranded poly(dA) should render the double-stranded form of poly(dA)poly(dT) entropically less unfavorable. The thermodynamic impact of residual structures of single-stranded nucleic acids has been demonstrated and discussed by Vesnaver and Breslauer.[31] It is difficult to speculate if the differential hydration of poly(dAdT)poly(dAdT) and poly(dA)poly(dT) contributes to $\Delta\Delta S$. If it does, it should predominantly involve the coil state, since, in the duplex state, the poly(dAdT)poly-(dAdT) and poly(dA)poly(dT) duplexes exhibit similar hydration.[32−35]

B′ Duplex Conformation versus A Conformation. Inspection of Figure 7 reveals that the differential stability, $\Delta\Delta G$, of poly(dA)poly(dT) versus poly(rA)poly(rU) (black) increases from ~0.4 kJ mol^{-1} at 0 °C to ~1.2 kJ mol^{-1} at 100 °C. The greater thermodynamic stability, ΔG, of poly(dA)poly(dT) relative to poly(rA)poly(rU) correlates with higher thermal stability of the former, $\Delta T_M = 8.9 \pm 0.9$ °C (see Figure 3).

As is seen from Table 1, the helix-to-coil transition of poly-(dA)poly(dT) exhibits slightly smaller enthalpy ($\Delta\Delta H = -0.4$ kJ mol^{-1}) and entropy ($\Delta\Delta S = -5.4$ J mol^{-1} K^{-1}) than that of poly(rA)poly(rU). The observed enthalpic and entropic disparity may reflect a host of possible microscopic origins, including structural differences between the B′ and A conformations, differential base stacking, differential hydration of the duplex and/or coil states, the presence of the 2′-OH group in ribose, residual structure differences of the coil states of poly(dA) and poly(rA), differential uptake/release of counterion and the state of counterion hydration in the vicinity of the A- and B-form duplexes, etc.. Future studies are required to quantify and discriminate between these possible microscopic origins.

AT and AU Base Pairs versus IC Base Pair. The IC base pair is stabilized by two hydrogen bonds analogous to the AT and AU base pairs. Notwithstanding, the energetics of the stability of the all-IC duplexes is markedly different from that of the all-AT and all-AU duplexes. Inspection of Figure 7 reveals that that the differential stability, $\Delta\Delta G$, of the heteropolymeric poly(dAdT)poly(dAdT) and poly(dIdC)poly(dIdC) DNA duplexes (olive) increases from nearly zero at 0 °C to

~1.7 kJ mol^{-1} at 100 °C. The greater thermodynamic stability of poly(dAdT)poly(dAdT) relative to poly(dIdC)poly(dIdC) correlates with a higher T_M value of the former ($\Delta T_M = 6.7 \pm 0.5$ °C). Inspection of data in Table 1 reveals that the higher stability of poly(dAdT)poly(dAdT) relative to poly(dIdC)poly-(dIdC) reflects enthalpy−entropy compensation and is entirely entropic in origin. The values of ΔH and ΔS of the helix-to-coil transition of the all-AT duplex are significantly smaller than those of the all-IC duplex; $\Delta\Delta H$ is -5.8 kJ mol^{-1} and $\Delta\Delta S$ is -16.3 J mol^{-1} K^{-1}. The negative sign of $\Delta\Delta S$ is consistent with results of our volumetric study in which we found that counterions in the vicinity of poly(dAdT)poly(dAdT) duplexes strongly interact with DNA and retain only $65 \pm 18\%$ of their original hydration shell.[36] In contrast, counterions in the vicinity of poly(dIdC)poly(dIdC) are hydrated independently and essentially retain their full hydration shell.[36] Consequently, the counterion release from the hydration shell of poly(dAdT)poly-(dAdT) upon its helix-to-coil transition should be accompanied by an enhancement in hydration of released counterions, an event that will reduce a net increase in entropy associated with DNA denaturation. Clearly, other factors may also contribute to the differential energetics of the stability of the poly(dAdT)-poly(dAdT) and poly(dIdC)poly(dIdC) duplexes. These factors may include the differential base stacking, hydration in the helix and coil states, configurational entropy, etc.. In fact, judging by our volumetric data, the poly(dIdC)poly(dIdC) duplex is significantly more hydrated than the poly(dAdT)poly(dAdT) duplex.[33−35] In one estimate, the hydration shells of poly(dIdC)-poly(dIdC) and poly(dAdT)poly(dAdT) comprise 91 and 49 waters per base pair, respectively.[35] There are no direct data on the hydration properties of the single-stranded poly(dAdT) and poly(dIdC) polymers. However, based on the partial molar volume and adiabatic compressibility observables, the hypoxanthine plus cytosine pair of heterocyclic bases is significantly more hydrated than the adenine plus thymine base pair.[37] Similarly, the 2′-deoxyinosine plus 2′-deoxycytidine nucleoside pair is significantly more hydrated than the 2-deoxyadenosine plus thymidine pair.[37] Consequently, one may intuitively expect that single-stranded poly(dIdC) is more strongly hydrated than single-stranded poly(dAdT). However, further studies are required to determine the relative hierarchy of single-stranded poly(dIdC) and poly(dAdT) hydration and its impact (if any) on the differential energetics of their stability.

The differential stability, $\Delta\Delta G$, of the homopolymeric poly-(rA)poly(rU) and poly(rI)poly(rC) RNA duplexes (red line in Figure 7) decreases from 0.8 kJ mol^{-1} at 0 °C to -1.2 kJ mol^{-1} at 100 °C passing zero at 41 °C (see Figure 7). Thus, poly-(rA)poly(rU) is thermodynamically more stable than poly(rI)-poly(rC) at low and moderate temperatures. In contrast, above 41 °C, poly(rI)poly(rC) becomes thermodynamically more stable than poly(rA)poly(rU), an observation which is consistent with a slightly higher thermal stability of the former ($\Delta T_M = 2.3 \pm 1.6$ °C). As is seen from data presented in Table 1, the near zero value of $\Delta\Delta G$ results from compensation between the enthalpic and entropic terms. The value of $\Delta\Delta H$ is 7.1 kJ mol^{-1}, while $\Delta\Delta S$ is 23.0 J mol^{-1} K^{-1}. Thus, poly(rA)poly(rU) is enthalpically more and entropically less stable than poly(rI)-pol(rC).

(30) Bhattacharyya, D.; Kundu, S.; Thakur, A. R.; Majumdar, R. *J. Biomol. Struct. Dyn.* **1999**, *17*, 289−300.
(31) Vesnaver, G.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3569−3573.
(32) Chalikian, T. V.; Sarvazyan, A. P.; Plum, G. E.; Breslauer, K. J. *Biochemistry* **1994**, *33*, 2394−2401.
(33) Chalikian, T. V.; Breslauer, K. J. *Biopolymers* **1998**, *48*, 264−280.
(34) Chalikian, T. V.; Völker, J.; Srinivasan, A. R.; Olson, W. K.; Breslauer, K. J. *Biopolymers* **1999**, *50*, 459−471.
(35) Chalikian, T. V. *J. Phys. Chem. B* **2001**, *105*, 12566−12578.

(36) Tikhomirova, A.; Chalikian, T. V. *J. Mol. Biol.* **2004**, *341*, 551−563.
(37) Lee, A.; Chalikian, T. V. *Biophys. Chem.* **2001**, *92*, 209−227.

We have previously shown that counterions in the vicinity of poly(rA)poly(rU) are significantly dehydrated and retain only $34 \pm 21\%$ of their hydration shell.[36] By contrast, in the vicinity of poly(rI)poly(rC), counterions are fully hydrated.[36] Thus, the helix-to-coil transition of poly(rA)poly(rU) will bring about an uptake of water molecules into the hydration shells of released counterions with concomitant decrease in entropy. The fact that the transition entropy of poly(rA)poly(rU) is greater than that of poly(rI)poly(rC) suggests that other factors contribute to the observed value of $\Delta\Delta S$. One possibility may be related to the existence of partially ordered tetraplex structures formed by single-stranded poly(rI). In this scenario, tetraplex structures, which potentially may be formed by poly(rI), are more ordered than the residual double-stranded helical structures that may be formed by poly(rA). Understandably, other factors (such as differential base stacking, hydration, configurational entropy, etc.) may also contribute to the differential stability of the poly(rA)poly(rU) and poly(rI)poly(rC) duplexes.

GC versus AT and IC Base Pairs. In contrast to AT and IC base pairs, GC base pairs are stabilized by three hydrogen bonds which leads to a significantly higher thermal and thermodynamic stability of GC-rich DNA and RNA duplexes. As is seen from Figure 7, the differential values of $\Delta\Delta G$ for the poly(dGdC)poly(dGdC) and poly(dAdT)poly(dAdT) pair (green) and the poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) pair (magenta) are both increasing functions of temperature. Upon an increase in temperature from 0 to 100 °C, the value of $\Delta\Delta G$ for the poly(dGdC)poly(dGdC)/poly(dAdT)poly(dAdT) pair increases from 5.4 to 6.3 kJ mol^{-1}, while that for the poly(dGdC)poly(dGdC)/poly(dIdC)poly(dIdC) pair increases from 4.2 to 7.9 kJ mol^{-1}. Inspection of data in Table 1 reveals marked differences in the thermodynamic origins of $\Delta\Delta G$ values for the poly(dGdC)poly(dGdC)/poly(dAdT)poly(dAdT) and poly(dGdC)poly(dGdC)/poly(dIdC)poly(dIdC) pairs. The differential enthalpy, $\Delta\Delta H$, for the poly(dGdC)poly(dGdC) and poly(dAdT)poly(dAdT) duplexes (green) is substantial and equals 5.8 kJ mol^{-1}. In contrast, $\Delta\Delta H$ for the poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) duplexes is practically zero. Thus, the helix-to-coil transitions of poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) are isoenthalpic if extrapolated to a common temperature. The differential entropy, $\Delta\Delta S$, for the poly(dGdC)poly(dGdC) and poly(dAdT)poly(dAdT) duplexes is -6.7 J mol^{-1} K^{-1}, while $\Delta\Delta S$ for the poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) duplexes is significantly more negative and equals -23.0 J mol^{-1} K^{-1} (see Table 1).

Given the chemical similarity of the guanine and hypoxanthine heterocyclic bases and similar hydration of counterions in the vicinity of these duplexes,[36] we propose that the differential energetics (including the $\Delta\Delta G$, $\Delta\Delta H$, and $\Delta\Delta S$ differential functions) of poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) predominantly reflects an extra hydrogen bond in the GC base pair. By extension, we propose that the influence of other factors on the differential stability of poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) is minor. In other words, we propose that the differential energetics of poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) can be almost entirely assigned to a breaking of an extra hydrogen bond in the GC base pair.

On the other hand, the stabilities of the poly(dGdC)poly(dGdC) and poly(dAdT)poly(dAdT) duplexes may be governed

by significantly different intra- and interstrand interactions that may change in magnitude with temperature. In addition to an extra hydrogen bond in the GC base pair, these differential interactions may include differential base stacking, hydration of the helix and coil states, configurational entropy, residual structure of the coil state, etc. Note that volumetric and computational results suggest that, in the duplex form, poly-(dGdC)poly(dGdC) is more hydrated than poly(dAdT)poly-(dAdT).[32-35,38,39] The hydration shell of a GC base pair in poly-(dGdC)poly(dGdC) involves 69 water molecules versus 49 waters solvating an AT base pair in poly(dAdT)poly(dAdT).[35] In addition, counterions in the vicinity of poly(dAdT)poly-(dAdT) are partially dehydrated and retain only $65 \pm 18\%$ of their original hydration shell, while, in the vicinity of poly-(dGdC)poly(dGdC), counterions are fully hydrated.[36] Clearly, the differential hydration of counterions in the vicinity of the poly(dGdC)poly(dGdC) and polydAdT)poly(dAdT) should be reflected in the value of the differential entropy, $\Delta\Delta S$.

Hydrogen Bond Contribution to the Duplex Stability. The difference in stability between the GC base pair and the AT and IC base pairs has been traditionally interpreted in terms of an extra hydrogen bond that stabilizes the GC pair.[1,3] As noted above, the poly(dGdC)poly(dGdC) duplex shares a greater deal of similarity with the poly(dIdC)poly(dIdC) duplex relative to the poly(dAdT)poly(dAdT) duplex. Therefore, the poly(dGdC)-poly(dGdC) and poly(dIdC)poly(dIdC) duplexes make a more suitable pair for evaluating the hydrogen bond thermodynamics. Judging by the differential thermodynamics of the stability of poly(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC), the free energy, ΔG, enthalpy, ΔH, and entropy, ΔS, of breaking a hydrogen bond are equal to 6.3 kJ mol^{-1} (at 25 °C), $\sim$0 kJ mol^{-1}, and -23.0 J mol^{-1} K^{-1}, respectively. Thus, the stabilizing influence of a hydrogen bond is purely entropic in nature.

We have recently proposed that solvent reorganization around polar groups is an unfavorable process.[40] The overall change in free energy accompanying dissolution of polar groups is favorable because the favorable enthalpic contribution of direct solute−solvent hydrogen bonding prevails over the unfavorable contribution of solvent reorganization. Consequently, polar groups in macromolecular structures will tend to form inter- or intrasolute hydrogen bonds out of contact with water rather than form solute−solvent hydrogen bonds. In this scenario, the polar groups will satisfy their hydrogen bonding propensity while avoiding the penalty of solvent reorganization. As one estimate of thermodynamics of solvent reorganization around polar groups, we have used the differential $\Delta\Delta G$, $\Delta\Delta H$, and $\Delta\Delta S$ values for the helix-to-coil transitions of the poly(dGdC)poly-(dGdC) and poly(dAdT)poly(dAdT) duplexes.[40] As noted above, however, the thermodynamics of breaking a hydrogen bond can be more correctly evaluated by comparing the stability characteristics of the poly(dGdC)poly(dGdC) and poly(dIdC)poly-(dIdC) duplexes. At 25 °C, the free energy, ΔG, enthalpy, ΔH, and entropy, ΔS, of breaking a hydrogen bond are equal to 6.3 kJ mol^{-1}, $\sim$0 kJ mol^{-1}, and -23.0 J mol^{-1} K^{-1}, respectively. Since the enthalpies of solute−solute and solute−solvent hydrogen bond formation are roughly equal, the values of ΔG, ΔH, and ΔS predominantly reflect the effect of solvent

(38) Elcock, A. H.; McCammon, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 10161−10162.
(39) Feig, M.; Pettitt, B. M. *J. Mol. Biol.* **1999**, *286*, 1075−1095.
(40) Chalikian, T. V. *Biopolymers* **2003**, *70*, 492−496.

reorganization around two polar groups that become solvent-accessible upon dissociation of the duplex. Solvent reorganization around each polar group is characterized by the values of ΔG, ΔH, and ΔS of 3.1 kJ mol^{-1} (6.3/2), $\sim$0 kJ mol^{-1}, and -11.5 J mol^{-1} K^{-1} ($-23.0/2$), respectively. The value of ΔG coincides with our previous estimate.[40] In contrast, the values of ΔH and ΔS are distinct from our previous estimates 1.38 $\pm$ 0.12 kJ mol^{-1} and -6.7 ± 1.2 J mol^{-1} K^{-1}, respectively, that were based, in particular, on comparison of the energetics of helix-to-coil transitions of the poly(dGdC)poly(dGdC) and poly-(dAdT)poly(dAdT) duplexes. For the reasons described above, it is our opinion that the estimate based on comparing poly-(dGdC)poly(dGdC) and poly(dIdC)poly(dIdC) provides a more reliable estimate of the thermodynamics of hydrogen bonding and solvent reorganization around polar groups.

Conclusion

We have used differential scanning calorimetric measurements to determine the melting temperatures, T_M, enthalpy changes, ΔH_M, and heat capacity changes, ΔC_P, accompanying helix-to-coil transitions of the poly(dAdT)poly(dAdT), poly-(dA)poly(dT), poly(dIdC)poly(dIdC), poly(dGdC)poly(dGdC), poly(rA)poly(rU), and poly(rI)poly(rC) nucleic acid duplexes at various salt concentrations. In agreement with previous reports, we have found that ΔC_P exhibits positive, nonzero values which, on average, equal 268 $\pm$ 33 J mol^{-1} K^{-1}. We have used this value of ΔC_P to evaluate as a function of temperature the transition free energies, ΔG, enthalpies, ΔH, and entropies, ΔS, for each duplex we studied. We have observed that the ΔG versus T dependences exhibit maxima which correspond to the maximum stabilities, ΔG_{max}, of the duplexes at temperatures $T_{max} = T_M \exp(-\Delta H_M/\Delta C_P T_M)$. The values of ΔG_{max} range from 2.9 to 10.5 kJ mol^{-1}. The T_{max} values for all the duplexes studied are negative being within the range -70 to -20 °C. Furthermore, all duplexes exhibit cold denaturation between -100 and -200 °C.

Since the ΔG, ΔH, and ΔS functions are all temperature dependent, the thermodynamic comparison between the helix-to-coil transitions of the duplexes (that may differ from one another with respect to sequence, composition, conformation, etc.) is physically meaningful only if extrapolated to a common temperature. We have performed such comparative analyses to derive the differential thermodynamics of formation of GC versus AT, AU, and IC base pairs as well as B′ versus A and B helix conformations. For the helix-to-coil transitions of poly-(dAdT)poly(dAdT) (B conformation) and poly(dA)poly(dT) (B′ conformation), the differential enthalpy, $\Delta\Delta H$, equals 0.4 kJ mol^{-1}, the differential entropy, $\Delta\Delta S$, equals 5.8 J mol^{-1} K^{-1}, while the differential free energy, $\Delta\Delta G$, decreases from -0.4 to -0.8 kJ mol^{-1} between 0 and 100 °C. For the poly(dA)-poly(dT) (B′ conformation)/poly(rA)poly(rU) (A conformation) pair, $\Delta\Delta H$ equals -0.4 kJ mol^{-1}, the $\Delta\Delta S$ equals -5.4 J mol^{-1} K^{-1}, while the $\Delta\Delta G$ increases from 0.4 to 1.2 kJ mol^{-1} between 0 and 100 °C. For the poly(dAdT)poly(dAdT)/poly(dIdC)poly-(dIdC) pair, $\Delta\Delta H$ equals -5.8 kJ mol^{-1}, $\Delta\Delta S$ equals -16.3 J mol^{-1} K^{-1}, while $\Delta\Delta G$ increases from 0 to 1.7 kJ mol^{-1} between 0 and 100 °C. For the poly(rA)poly(rU)/poly(rI)poly-(rC) pair, $\Delta\Delta H$ equals 7.1 kJ mol^{-1}, $\Delta\Delta S$ equals 23.0 J mol^{-1} K^{-1}, while $\Delta\Delta G$ of decreases from 0.8 to -1.2 kJ mol^{-1} between 0 and 100 °C. For the poly(dGdC)poly(dGdC)/poly-(dAdT)poly(dAdT) pair, $\Delta\Delta H$ equals 5.8 kJ mol^{-1}, $\Delta\Delta S$ equals -6.7 J mol^{-1} K^{-1}, while $\Delta\Delta G$ increases from 5.4 to 7.9 kJ mol^{-1} between 0 and 100 °C. Finally, for the poly(dGdC)poly-(dGdC)/poly(dIdC)poly(dIdC) pair, $\Delta\Delta H$ is zero, $\Delta\Delta S$ is -23.0 J mol^{-1} K^{-1}, while $\Delta\Delta G$ increases from 5.4 to 6.3 kJ mol^{-1} between 0 and 100 °C.

We have proposed some general microscopic interpretations for the observed thermodynamic differences. However, independent of the veracity of our proposed microscopic interpretations, our results presented in this work are useful and provide new insights into the thermodynamic origins of the stability of nucleic acid duplexes. In particular, our results underscore a notion that any thermodynamic analysis of the stability/structure relationship of nucleic acid duplexes should be performed with explicit consideration of the temperature dependences of the ΔH and ΔS terms as reflected in the values of ΔC_P.