Differential Hydration of dA·dT Base Pairs in Parallel-Stranded DNA Relative to Antiparallel DNA†

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ABSTRACT: Parallel-stranded DNA is a novel double-stranded helical form of DNA. Its secondary structure is established by *reverse* Watson–Crick base pairing between the bases of the complementary strands forming a double helix with equivalent grooves. We have used a combination of magnetic suspension densimetry and isothermal titration calorimetry to obtain complete thermodynamic profiles for the formation of two DNA 25mer duplexes. The duplexes contain exclusively dA·dT base pairs in either parallel (ps-d1·D2) or antiparallel (aps-D1·D3) orientation. At 15 °C, the formation of each duplex is accompanied by favorable free-energy terms resulting from the partial compensation of favorable exothermic enthalpies and unfavorable entropies and by an uptake of both counterions and water molecules. By taking into account the contribution of single-strand base-stacking interactions and using the formation of the aps-D1·D3 duplex as a reference state to establish a thermodynamic cycle in which the similar single strands cancel out, we obtained a $\Delta\Delta G°$ term of +10 kcal mol^{-1} duplex formed that results from a partial differential enthalpy–entropy compensation of +32 kcal mol^{-1} and a $\Delta\Delta V$ of 257 mL mol^{-1}. The positive sign of this enthalpy–entropy compensation together with the marginal differential counterion uptake of 0.2 mol of Na^+/mol of duplex is characteristic of processes driven by differential hydration and strongly suggests that the parallel duplex is much less hydrated than its antiparallel counterpart by ~4 mol of water/mol of base pair.

Water plays a fundamental role in the conformational stability of double helical nucleic acids (Saenger, 1984). Experimental evidence indicates that helical nucleic acids are heavily hydrated depending on DNA composition (Tunis & Hearst, 1968; Buckin et al., 1989a). The particular conformation of a DNA duplex (A, B, or Z) depends directly on the degree of hydration, and changing the water activity can shift the equilibrium to favor one duplex conformation over alternatives (Ivanov et al., 1973; Saenger, 1984). In solution, both the detection of water associated with DNA and the determination of its physical properties are difficult; however, recent developments of high-sensitivity density and acoustical techniques have made it possible to determine the change in volume, apparent molar volume, and adiabatic compressibilities on samples containing 1 mg or less of solute (Buckin et al., 1989b; Gillies & Kupke, 1988). Also, the presence of a spine of hydration in the minor groove of dA·dT base pairs has been detected in solution by NMR techniques (Kubinec & Wemmer, 1992) as well as in crystals (Drew & Dickerson, 1981; Kopka et al., 1985).

The negative volume change accompanying the formation of a DNA duplex from two complementary strands is interpreted as reflecting primarily net changes in the electrostriction of water dipoles (Zieba et al., 1991). Previous calorimetric and density measurements that yielded complete differential thermodynamic profiles for the insertion of an extra dA·dT base pair in the middle of a stretch of 4 dA·dT base pairs of a decamer duplex resulted in a differential enthalpy–entropy compensation of −5.1 kcal mol^{-1}, which correlated with a differential uptake of water corresponding to an additional hydration of ~17 mol of water molecules/mol of dA·dT base pair (Zieba et al., 1991). It was also estimated that the molar volume of water is ~15.5 mL mol^{-1} in the vicinity of five dA·dT base pairs in the B conformation, which is due to the effect of the local electrostatic field that compresses the volume by 14% (Marky & Kupke, 1994).

A recent addition to the family of DNA conformations is that of parallel-stranded DNA (ps-DNA) (van de Sande et al., 1988; Jovin, 1991; Rippe & Jovin, 1992), in which both complementary strands have the same 5′ → 3′ orientation held together by *reverse* Watson–Crick base pairs, forming a double helix with two equivalent grooves in depth and width (Ramsing & Jovin, 1988). Previous calorimetric (Rentzeperis et al., 1992a) and optical investigations (Rippe et al., 1989; Rippe & Jovin, 1992) of the helix–coil transition of two duplexes, shown in Figure 1, containing exclusively dA·dT base pairs in the parallel (ps-D1·D2) and antiparallel (aps-D1·D3) orientation reveal a two-state melting behavior for both duplexes with a similar release of counterions (Rentzeperis et al., 1992a). It was postulated that the lower stability and lower enthalpic contribution of parallel DNA, relative to antiparallel DNA, was due to differences in base-pair stacking, hydrogen bonding, and overall hydration. In this paper, we further investigate the source of their stability differences by using a combination of magnetic suspension densimetry and isothermal titration calorimetry to measure at 15 °C the enthalpy and volume change for the formation of each of these two DNA 25mer duplexes by mixing their corresponding complementary strands (see Figure 1). The formation of each duplex is accompanied by favorable free-energy terms that result from the characteristic partial compensation of exothermic enthalpies and unfavorable entropies and the uptake of both sodium ions and water molecules. By setting a

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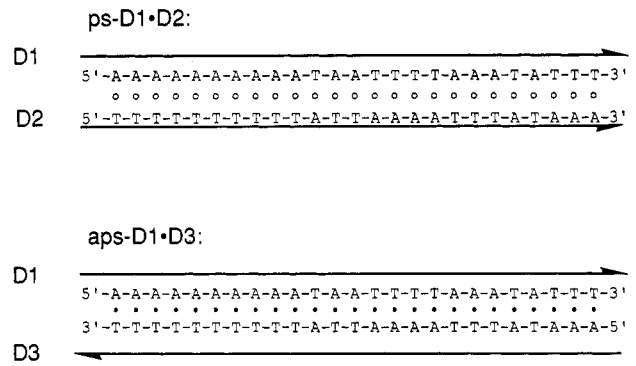

FIGURE 1: Sequences of synthetic oligonucleotides and their duplexes. The Watson–Crick base pairs of the antiparallel duplexes are denoted by the closed symbols and the *reverse* Watson–Crick of the parallel-stranded duplexes by the open symbols.

thermodynamic cycle in which we use the formation of the aps-D1·D3 duplex as a reference state, we obtained a $\Delta\Delta G°$ term of +10 kcal mol^{-1} resulting from a partial differential enthalpy–entropy compensation of +32 kcal mol^{-1} and a marginal differential counterion uptake of 0.2 mol of Na^+/mol of duplex that correlates with a $\Delta\Delta V$ of 257 mL mol^{-1}. The positive sign of this enthalpy–entropy compensation is characteristic of processes driven by differences in hydration and strongly suggests that the parallel duplex is much less hydrated.

EXPERIMENTAL PROCEDURES

Materials. All three deoxyoligonucleotides shown in Figure 1 were synthesized on an ABI PCR-Mate Model 391 automatic DNA synthesizer, using standard phosphoramidite chemistry, purified by HPLC, and desalted on a Sephadex G-10 exclusion chromatography column. The concentration of each oligomer in solution was determined spectrophotometrically at 260 nm and 80 °C in H_2O using the extinction coefficients reported previously (Rentzeperis et al., 1992a). Stock oligomer solutions were prepared by dissolving the dry and desalted oligomers in the appropriate buffer. All other chemicals were reagent grade. The buffer solution consisted of 10 mM sodium cacodylate, 0.1 mM Na_2EDTA, and 0.1 M NaCl at pH 7.

Magnetic Suspension Densimetry. The volume change, ΔV, that accompanies the formation of each duplex was determined by measuring the density on weighed samples in a magnetic suspension densimeter that has been described previously (Gillies & Kupke, 1988). The ΔV value is calculated by measuring the mass and the equilibrium density of solutions before and after mixing; the observed change in volume, Δv, upon adding strand A to is complementary strand B to form a DNA duplex AB is given by

$$\Delta v = m_{AB}/\rho_{AB} - (m_A/\rho_A + m_B/\rho_B) \quad (1)$$

where m is the mass in grams and ρ is the density of the solutions in grams per milliliter. The density of each sample is obtained by relating the measured voltage to the straight-line calibration equation of electric current (as voltage) versus density of aqueous KCl solutions of known density. The density values, while independent, need not be of high absolute accuracy since it is their differences that are required for Δv. Since the masses are identical on both sides of the minus sign, small weighing errors do not contribute significantly to the value of Δv. With repetitive samples, the density is measured with a precision of better than 5×10^{-6} g/mL. The value of Δv in milliliters is then reduced to that per mole of the limiting reagent to give ΔV. To make sure that the duplexes are formed completely, weighed duplex samples were heated to 55 °C and cooled to room temperature in tightly closed 0.4-mL polyethylene tubes to prevent evaporation. The temperature was kept at 15 ± 0.001 °C. Usually, equal volumes of solutions of each complementary strand were mixed to give ~350 μL of the final solution to allow for rinsings and duplicate measurements; routinely, two or more such mixtures were prepared, and the mean value was taken as the one reported for ΔV. In these experiments, the concentrations of the individual strands ranged from 4.0 to 4.2 mM in phosphate. Thus, any contributions from solute–solute interactions to the ΔV were assumed to be negligible.

Titration Calorimetry. The measurement of the heats of mixing a single strand with its corresponding complementary strand at 15 °C was carried out using the Omega titration calorimeter from Microcal Inc. (Northampton, MA). A detailed description of this instrument has been presented elsewhere (Wiseman et al., 1989). Solutions of one strand were used to titrate the complementary strand to form each duplex. A 100-μL syringe was used for the titrant; mixing was effected by stirring this syringe at 400 rpm. Typically 10 injections of 10 μL each were performed in a single titration at 15 °C. The concentration (in strands) of the oligomer in the syringe was ~17 times higher than the concentration of the complementary strand in the reaction cell (~9 μM). These concentrations were selected so that the first injection would yield heats that correspond to the complete formation of duplex at this temperature. The reference cell was filled with distilled water, and the instrument was calibrated by means of a known standard electrical pulse. The calorimetric titrations were designed to obtain primarily the enthalpy of forming each duplex and their stoichiometries. The enthalpy is obtained by averaging the resulting heats of the initial 4–5 injections or by deconvolution of the resulting calorimetric isotherms, total heat *vs* total titrant (or strand) concentration using a three-parameter fit, ΔH_{ITC}, stoichiometry, and binding constant (K). These three parameters are obtained iteractively using the standard Marquardt algorithms. The initial fitting procedure, which is part of the software of the calorimeter, is to let all three parameters float or to fix just the enthalpy (obtained from the average of the heats of the initial injections) until the lowest standard deviation is obtained. Both approaches gave similar results. The $\Delta G°$ and $T\Delta S$ terms were derived from the relationships $\Delta G° = -RT \ln K$ and the Gibbs equation, respectively.

RESULTS AND DISCUSSION

Formation of Each Duplex Results in Uptake of Water Molecules. Using a magnetic suspension densimeter, we have measured directly at 15 °C the change in volume associated with the formation of each duplex from mixing their complementary strands. The results are listed in Table 1; in these experiments we used a slight excess of the D2 (or D3) strand over the complementary D1 strand to assume complete formation of each duplex; each entry represents an average of at least two independent determinations. The results of Table 1 indicate that the formation of each duplex is accompanied by an uptake of water molecules; the difference in their ΔV values suggests that the conformation of the duplex formed reflects primarily on the degree of hydration.

Formation of Each Duplex at 15 °C Is Accompanied by Exothermic Heats. In order to help with the interpretation of our densimetric results, we carried out titration calorimetric experiments. A typical titration curve for the addition of D1 to D2 to form the ps-D1·D2 duplex is shown in Figure 2a. The exothermic heats obtained from the initial injections are

Table 1: Thermodynamic Profiles for Formation of 25mer Duplexes at 15 °C[a]

duplex	K (μM^{-1})	$\Delta G°$ (kcal/mol)	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔV (mL/mol)	Δn_{Na^+} (per mol)
			Isothermal Titration Calorimetry (ITC)			
ps-D1·D2	1.6 (±0.8)	−8.2 (±0.2)	−74.5 (±2.5)	−66.3 (±4.2)	−83 (±12)	4.18 (±0.25)
aps-D1·D3	33.0 (±23)	−9.9 (±0.3)	−116.6 (±2.0)	−106.7 (±5.0)	−340 (±12)	4.37 (±0.26)
			Differential Scanning Calorimetry (DSC)			
ps-D1·D2		−9.4 (±0.5)	−116.5 (±3.5)	−107.1 (±3.2)		6.58 (±0.39)
aps-D1·D3		−19.4 (±1.0)	−151.1 (±4.5)	−131.7 (±4.0)		5.66 (±0.34)

[a] All moles are in terms of duplexes formed. [b] The Δn_{Na^+} values, from DSC results of Rentzeperis et al. (1992a), were corrected by an enthalpy factor: $\Delta H_{ITC}/\Delta H_{cal}$ that includes the contribution of single-strand base-stacking interactions at 15 °C.

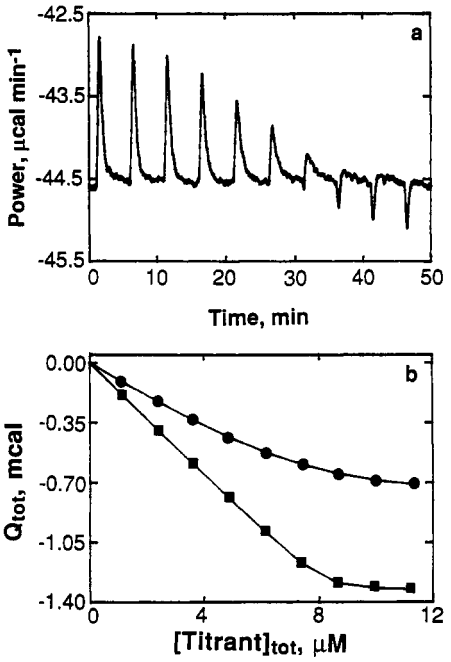

FIGURE 2: (a) Typical calorimetric titration curve performed in 10 mM sodium cacodylate buffer and 0.1 mM Na_2EDTA, at pH 7.0 and 15 °C; 1.4 mL of a 8.5 μM solution of the D1 strand in the sample cell was titrated with a 165 μM solution of the D2 complementary strand by using 10-μL injections from a 100-μL syringe; complete mixing is effected by stirring this syringe at 400 rpm. The instrument is calibrated with a known standard electrical pulse; the precision is about 0.5 μcal in the overall heat of each injection. (b) Derived calorimetric isotherms for the formation of each duplex: ps-D1·D2 (closed circles) and aps-D1·D3 (closed squares). Solid lines correspond to the fitted lines resulting from the three parameter nonlinear fit of experimental data.

independent of the total concentration of added strand and drop as the complete formation of the duplex is approached. After a small correction for the heat of dilution of the titrant (D2 or D3 strands), molar binding enthalpies for base-pair formation are calculated, which are similar to the ones obtained from the calorimetric fits (see Table 1). The significant observation at this temperature of 15 °C is that we measured exothermic enthalpies for the formation of each duplex, which corresponds to the exothermic contributions from both the formation of base pair stacks and the uptake of water molecules that overrides the endothermic contribution from the disruption of base–base stacking interactions of the single strands; uptake of counterions contributes nothing to the enthalpy. The overall magnitude of these enthalpies depend on the nature of the duplex being formed.

Complete Thermodynamic Profiles of Duplex Formation. Complete thermodynamic profiles obtained from isothermal titration calorimetry and volumetric measurements at 15 °C and the standard thermodynamic profiles that were reported earlier from differential scanning calorimetry experiments (Rentzeperis et al., 1992a) are presented in Table 1. For proper comparisons, these latter parameters have been extrapolated to 15 °C, assuming heat capacity differences equal to zero. In addition, the values for the uptake of counterions, Δn_{Na^+}, have been corrected for the contribution of single-strand base-stacking interactions at 15 °C by multiplying the previously reported Δn_{Na^+} values (Rentzeperis et al., 1992a) by the enthalpy factor: $\Delta H_{ITC}/\Delta H_{cal}$. At 15 °C, very similar favorable free-energy values are obtained for the formation of each duplex, which results from the characteristic partial compensation of exothermic enthalpies and unfavorable entropies. However, differences are seen in the magnitude of the forces (enthalpy versus entropy) that contribute to the overall observed free energy change. The unfavorable entropy values are in good agreement with the volume change measurements that indicate an increase in order (decrease in entropy) due to the compression of water molecules in the formation of a duplex. Other unfavorable contributions to the entropy include both the uptake of counterions, due to the higher charge density of the duplex, and the ordering associated with a bimolecular reaction. Comparison of the two sets of thermodynamic parameters, isothermal at 15 °C, *vs* calorimetric melts, indicates discrepancies in the enthalpy and free-energy differences, yielding also entropy differences. The enthalpy differences of 42.0 kcal mol^{-1} (for psD1·D2) and 34.5 kcal mol^{-1} (for aps-D1·D3) between the two methods can be explained in terms of the additional contribution of base-stacking interactions of the single strands that are enhanced at lower temperatures (Zieba et al., 1991; Vesnaver & Breslauer, 1991). The derived ΔG values for the formation of the ps-D1·D2 duplex are in good agreement ($\Delta\Delta G_{dif}$ = +1.2) while those of the aps-D1·D3 duplex are not ($\Delta\Delta G_{diff}$ = +9.5). This can be explained as follows: The K values that are obtained from the calorimetric titration experiments are lower limiting values. A strand concentration of 9 μM (in the calorimetric cell) was used in both titration experiments; therefore, the best K values that can be obtained are in the range of 10^5–10^6 (Weber, 1992). The shape of the titration curve for the aps-D1·D3 duplex is linear (see Figure 2b) and indicates the stoichiometric association of the two strands. Hence, its associated K has to be much larger than 10^6. Using the extrapolated $\Delta G°$ value of −19.4 kcal mol^{-1} for the formation of the aps-D1·D3 (from calorimetric melts) and by taking into account the unfavorable contribution of single-strand base-stacking interactions of the ps-D1·D2 duplex ($\Delta\Delta G°_{dif}$ = 1.2 kcal mol^{-1}), we obtained a $\Delta G°$ value of −18.2 kcal mol^{-1} for the formation of the aps-D1·D3 duplex at 15 °C.

Differential Thermodynamic Profiles. If each of the thermodynamic parameters for the formation of the ps-D1·D2 duplex are subtracted from the corresponding parameters of forming the aps-D1·D3 duplex, reactions 1 and 2 (the contributions from the D1 single strands) cancel out exactly, and the resulting differential thermodynamic profiles cor-

respond to the substitution reaction 3:

$$\text{aps-D1·D3} \rightarrow \text{D1} + \text{D3} \quad (1)$$

$$\text{D1} + \text{D2} \rightarrow \text{ps-D1·D2} \quad (2)$$

$$\text{aps-D1·D3} + \text{D2} \rightarrow \text{ps-D1·D2} + \text{D3} \quad (3)$$

In addition, we can also cancel out the D2 and D3 single strands of reaction 3 because their sequences are identical but in reverse orientation, and their thermodynamic contributions from base-stacking interactions and hydration may be assumed to be identical. For reaction 3, we obtain a $\Delta\Delta G°$ of +10.0 kcal/mol of duplex, which is the result of a compensation of an unfavorable $\Delta\Delta H°$ of +42.1 kcal/mol of duplex and a favorable $\Delta(T\Delta S°)$ of +32.1 kcal/mol duplex and a $\Delta\Delta V$ of +257 mL/mol of duplex. This reaction is accompanied with a marginal differential counterion uptake, $\Delta\Delta n_{Na^+}$, of 0.019 of Na^+/mol of duplex. The positive $\Delta\Delta G$ value in reaction 3 provides evidence for the greater stability of the more native antiparallel (Watson–Crick) duplexes under physiological conditions. The positive sign of the enthalpy–entropy compensation is characteristic of processes that are driven by a differential hydration as have been indicated previously (Lumry & Rajender, 1970; Marky & Kupke, 1989; Rentzeperis et al., 1992b, 1993). Therefore, a Δn_{water} term needs to be included on the right-hand side of eq 3 that corresponds to a change in the release or compression of water molecules. This strongly suggests that the ps-D1·D2 parallel duplex is much less hydrated than the antiparallel counterpart. Furthermore, for the estimation of this differential hydration (Δn_{water}), we use the average value of 0.3 kcal mol^{-1} for the enthalpy of releasing 1 mol of electrostricted water from native DNA (Tunis & Hearst, 1968; Gasan et al., 1990) and $\Delta\Delta H = \Delta(T\Delta S°) = +32.1$ kcal mol^{-1}; we thus obtain 107 ± 24 mol of H_2O/mol of duplex or ~4 ± 1 mol of H_2O/mol of base pair from this heat-exchange experiment. If, however, we use 2.5 mL mol^{-1} for the average compression of electrostricted water around the negatively charged DNA or dA·dT base pairs (Marky & Kupke, 1994) as found for the average total electrostriction in ionized salt solutions (Millero et al., 1974), our $\Delta\Delta V$ of 257 mL/mol of duplex reduces to 103 ± 7 mol of electrostricted H_2O/mol of duplex or ~4 mol of H_2O/mol of base pair from the volume change measurements.

Comparison with Earlier Calorimetric Measurements. Using the thermodynamic parameters obtained from the calorimetric melts (Table 1) and using similar thermodynamic cycles, we obtained $\Delta\Delta G°$ of +10.0 kcal/mol of duplex, $\Delta\Delta H°$ of +34.6 kcal/mol of duplex, and $\Delta(T\Delta S°)$ of 24.6 kcal/mol of duplex. The enthalpy–entropy compensation term of 24.6 kcal/mol of duplex then yields a differential hydration of 82 ± 17 mol of H_2O/mol of duplex (24.6/0.3) or ~3 ± 1 mol of H_2O/mol of base pair. Therefore, the differential hydration numbers reported here are in excellent agreement with the estimates of the differential hydration of the minor grooves of these duplexes obtained in calorimetric melting studies of the unligated duplexes (Rentzeperis et al., 1992a) and with the predicted 3–4 water molecules per base pair from binding studies of netropsin to the minor grooves of these duplexes (Rentzeperis & Marky, 1993).

Lower Hydration of Parallel DNA May Be the Result of Wider Minor Grooves. The resulting differential thermodynamic profiles together with the similarity of the differential hydration numbers strongly indicate that the ps-D1·D2 duplex is less hydrated than the aps-D1·D3 duplex and suggest that the lower hydration state of parallel DNA is contributing significantly for its lower stability. Furthermore, if base-pair stacking contributions are similar in the energetics of the formation of both duplexes, then the widening of the minor groove of parallel DNA may be accompanied by a release or decompression of water molecules to decrease its hydration state. This is in excellent agreement with the increased hydration of the narrower minor groove of AA/TT base pairs stacks in poly(dA)·poly(dT) (Marky & Kupke, 1989; Rentzeperis et al., 1992b) and that of B-DNA relative to the A conformation (Rentzeperis et al., 1993).

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