Altering the Electrostatic Potential in the Major Groove: Thermodynamic and Structural Characterization of 7-Deaza-2'-deoxyadenosine:dT Base Pairing in DNA

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Supporting Information

ABSTRACT: As part of an ongoing effort to explore the effect of major groove electrostatics on the thermodynamic stability and structure of DNA, a 7-deaza-2'-deoxyadenosine:dT (7-deaza-dA:dT) base pair in the Dickerson–Drew dodecamer (DDD) was studied. The removal of the electronegative N7 atom on dA and the replacement with an electropositive C–H in the major groove was expected to have a significant effect on major groove electrostatics. The structure of the 7-deaza-dA:dT base pair was determined at 1.1 Å resolution in the presence of Mg2++. The 7-deaza-dA, which is isosteric for dA, had minimal effect on the base pairing geometry and the conformation of the DDD in the crystalline state. There was no major groove cation association with the 7-deaza-dA heterocycle. In solution, circular dichroism showed a positive Cotton effect centered at 280 nm and a negative Cotton effect centered at 250 nm that were characteristic of a right-handed helix in the B-conformation. However, temperature-dependent NMR studies showed increased exchange between the thymine N3 imino proton of the 7-deaza-dA:dT base pair and water, suggesting reduced stacking interactions and an increased rate of base pair opening. This correlated with the observed thermodynamic destabilization of the 7-deaza-dA modified duplex relative to the DDD. A combination of UV melting and differential scanning calorimetry experiments were conducted to evaluate the relative contributions of enthalpy and entropy in the thermodynamic destabilization of the DDD. The most significant contribution arose from an unfavorable enthalpy term, which probably results from less favorable stacking interactions in the modified duplex, which was accompanied by a significant reduction in the release of water and cations from the 7-deaza-dA modified DNA.

INTRODUCTION

Nucleoside analogs containing pyrrolopyrimidine bases,1,2 or 7-deazapurines, are used as isosteric analogs of adenine and guanine in biochemical and biophysical studies.3–7 The 7-deazapurines are also used to study the effects of site-specific alteration of the electrostatic potential of the DNA major groove, where it has been shown for 7-deazaguanine that there is a significant alteration in DNA hydration and cation binding.7,8 The 7-deazaguanine base was identified in the antibiotic tubercidin, a ribonucleoside isolated from various species of Streptomyces.9–11 The incorporation of 7-deaza-dA into DNA hinders the processing of the double helix by proteins, e.g., restriction endonucleases.12 It slightly reduces the bending of DNA in oligodeoxynucleotides containing d[(GGCA,C)·d(CCGT,G)] tracts.13,14 The preparation of phosphorimidates containing 7-deaza-dA has been described by Seela et al.15,16

There remains a paucity of quantitative data as to how substitution of adenine with 7-deaza-dA alters the structure and thermodynamics of the DNA double helix. Thermal denaturation of (7-deaza-dA)$_{12}$·dT$_{12}$ as compared to dA$_{12}$·dT$_{12}$ led to the conclusion that destabilization induced by 7-deaza-dA was associated with an unfavorable entropy change.17 Pope et al.18 conducted a high-angle X-ray fiber diffraction study of poly[d(7-deaza-dA-T)]·poly[d(7-deaza-dA-T)] . They suggested that replacement of dA by 7-deaza-dA caused slight alterations to the structure of A-DNA, but greater perturbations to B-DNA. When 7-deaza-dG was incorporated into the Dickerson–Drew dodecamer (DDD)19,20 it had minimal effect on the overall conformation determined by NMR or crystallography.7,21 However, duplex stability was reduced adjacent to the modification site due to a loss of enthalpic stabilization. Moreover, 7-deaza-dG caused a reduction in hydration and cation binding. This was attributed to the elimination of a high affinity major groove cation binding site.21 Clearly, while 7-deaza-dG was an isostere of dG, it altered the ensemble of DNA, water and salts, and thermodynamic stability of the DDD.7

In studies presented herein, an adenine at position A6 in the DDD19,20 has been replaced by 7-deaza-dA15,16 to form the...
Chart 1. (a) Structure of 7-deaza-dA and (b) Sequences and numbering of the nucleotides for unmodified DD, 7-deaza-dA DD, unmodified DDD, 7-deaza-dA DDD (NMR), and 7-deaza-dA DDD (X-ray) duplexes.  

(a)  

(b)  

DD  

5’-G1 C2 G3 A4 T5 T6 C7 G8 C9 T10-3’,  
3’-C10 G9 C8 T7 T6 A5 G4 T3-5’  
7-deaza-dA DD (ODD-1)  

5’-G1 C2 G3 A4 T5 T6 C7 G8 C9 T10-3’,  
3’-C10 G9 C8 T7 T6 A5 G4 T3-5’  

DDD  

5’-C1 G2 C3 G4 T5 T6 C7 G8 C9 T10-3’,  
3’-G10 C9 C8 T7 T6 A5 G4 C3-5’  
7-deaza-dA DDD (NMR) (DDD-1)  

5’-C1 G2 C3 G4 T5 T6 C7 G8 C9 T10-3’,  
3’-G10 C9 C8 T7 T6 A5 G4 C3-5’  
7-deaza-dA DDD (X-ray)  

5’-C1 G2 C3 A4 T5 T6 C7 G8 C9 T10-3’,  
3’-G10 C9 T8 A7 G6 C5 T4-5’  

In solution, the two strands exhibit pseudo-dyad symmetry. In the crystal structure, the two strands were not symmetry related and the nucleotides were individually numbered.

DJD-1 duplex [5’-d(C1G2C3G4A5T6T7C8G9T10)-3’,  
Y=7-deaza-dA] (Chart 1). Crystallography has been used to determine the structure of the DJD-1 duplex. A combination of thermal melting studies monitored by UV absorbance, differential scanning calorimetry (DSC), and NMR studies have been performed. The corresponding decamer DD-1, [5’-d(G1C2G3G4A5A6T7T8C9G10)-3’,  
Y=7-deaza-dA], which does not form an intramolecular hairpin at low salt concentrations, was also used in thermodynamic studies. We demonstrate that 7-deaza-dA has minimal effect upon base pairing geometry and conformation of the DDD. However, the 7-deaza-dA:dT base pair is thermodynamically destabilized, which is primarily attributed to unfavorable enthalpy terms dominated by less favorable stacking interactions, resulting from changes in the base electrostatics and electronic dipole–dipole interactions. There is also a net release of electrostricted waters from the duplex.

**MATERIALS AND METHODS**

Sample Preparation. The oligodeoxynucleotides S’-CGCG AYTTCCGCG-3’ (DDD-1) and S’-CGCATTCGCG-3’, (DD-1), Y = 7-deaza-dA, were synthesized by the University of Nebraska Medical Center Eppeley Institute Molecular Biology Shared Resource. The 7-deaza-dA phosphoramidite was obtained commercially (Glen Research, Sterling, VA, U.S.A.). The oligodeoxynucleotides were purified using semipreparative reverse-phase HPLC (Phenomenex, Phenyl-Hexyl, 5 μm, 250 mm x 10.0 mm) equilibrated with 0.1 M triethylammonium acetate (pH 7.0). The unmodified oligodeoxynucleotides, S’-CGCGAATTCGCG-3’ (DDD) and S’-CGCATTCGCG-3’ (DD), were synthesized by the Midland Reagent Company (Midland, TX) and purified by anion-exchange HPLC. The oligodeoxynucleotides were desalted using Sephadex G-25, lyophilized, and characterized by MALDI-TOF-MS. The oligodeoxynucleotides were dissolved in the appropriate buffers. The concentrations of single-stranded oligodeoxynucleotides were determined by UV absorbance at 260 nm using extinction coefficients of 1.11 x 10^4 M^-1 cm^-1 (dodecamers) and 9.5 x 10^4 M^-1 cm^-1 (decamers) and assuming similar extinction coefficients for 7-deaza-dA and dA. The oligodeoxynucleotides were annealed by heating to 80 °C for 15 min and then cooling to room temperature.

**Temperature–Unfolding Profiles (Melting Curves).** The thermodynamic parameters for the temperature-induced unfolding reactions of the duplexes were measured using a VP-DSC differential scanning calorimeter (Microcal, Inc., Northampton, MA, U.S.A.). The heat capacity profile for each DNA solution was measured against a buffer solution. The experimental curves were normalized for the heating rate, and a buffer vs buffer scan was subtracted using the program Origin (v. 5.0; Microcal, Inc.). The resulting monophasic or biphasic curves were analyzed by deconvolution with the Microcal software; their integration (fΔCp/dT) yielded the molar unfolding enthalpy (ΔHunf), which was independent of the nature of the transition. The molar entropy (ΔS) was obtained similarly, using fΔCp/T dT. The free energy change at any temperature T was obtained with the Gibbs equation: ΔG(T) = ΔH - TΔS.

Absorption versus temperature profiles (UV melts) for each duplex were measured at either 260 or 275 nm using a thermoelectrically controlled UV–vis Avis 14DS (Avis Biomedical, Inc., Lakewood, NJ) or Lambda 40-Perkin-Elmer (Perkin-Elmer, Inc., Waltham, MA) spectrophotometers. The temperature was scanned at heating rates of 0.75–1.00 °C/min. Melting curves as a function of strand concentration (7–70 μM) were obtained to check the molecularity of each oligodeoxynucleotide (i.e., hairpin vs duplex). Additional melting curves were obtained for salt-sensitive and osmolyte concentrations to determine the differential binding of counterions (ΔHosm) and waters (ΔHw), which accompanied the helix-to-coil transitions. For duplexes that melted via biphasic transitions only the TM of the duplex → random coil transition was used for the calculations.

In the determination of ΔHosm, UV melts were measured in the salt range of 10–200 mM NaCl at pH 7.0, whereas in the determination of ΔHw, UV melts were measured in the ethylene glycol concentration range of 0.5–4.0 M at pH 7.0 and 10 mM NaCl. The osmolarities of the solutions were obtained with a UIC vapor pressure osmometer, Model 830 (Jolliet, IL, U.S.A.). These osmolarities were then converted into water activities, aw, using the relationship ln aw = −(Osm/ω0), where Osm is the solution osmolarity and ω0 is the molality of H2O, 55.5 mol/kg. For duplexes that melted via biphasic transitions only the TM of the duplex → random coil transition was used for the calculations.

**Circular Dichroism.** Circular dichroism (CD) measurements were conducted on an Avis model 202SF CD spectropolarimeter (Avis Biomedical, Inc., Lakewood, NJ). To approach 100% duplex formation the spectrum of each sample was obtained using a strain-free 1 cm quartz cell at low temperatures. Typically, 1 OD of a duplex DNA was dissolved in 1 mL of 10 mM sodium phosphate buffer (pH 7.0). The reported spectra correspond to an average of three scans from 220 to 350 nm at a wavelength step of 1 nm.
NMR Spectroscopy. Modified and unmodified duplexes were prepared at 0.3 mM and 1.8 mM concentrations, respectively. The samples were prepared in 10 mM NaH2PO4, 0.1 M NaCl, and 50 μM Na2EDTA (pH 7.0). The samples were exchanged with D2O and dissolved in 0.5 mL of 99.99% D2O to observe nonexchangeable protons. For the observation of exchangeable protons, the samples were dissolved in 0.5 mL of 9:1 H2O/D2O. 1H NMR spectra for unmodified and modified oligodeoxynucleotides were recorded at 600 and 800 MHz. Chemical shifts were referenced to water. Data were processed using TOPSPIN software (Bruker Biospin Inc., Billerica, MA). The NOESY spectra of samples in D2O were collected at 15 °C at 800 MHz; NOESY experiments were conducted at a mixing time of 250 ms. The NOESY spectra of the modified and unmodified sample in H2O were collected at 5 °C at 600 MHz, with a 250 ms mixing time. These experiments were performed with a relaxation delay of 2.0 s. Water suppression was performed using the WATERGATE pulse sequence.

Crystallizations and Data Collection. Crystallization trials were performed with the Nucleic Acid Mini-screen (Hampton Research, Aliso Viejo, CA). The hanging drop vapor diffusion technique was used. Droplets, with a volume of 2 μL, of a 1:1 mixture of sample and mini-screen buffer were equilibrated against 0.75 mL of 35% 2-methyl-2,4-pentanediol (MPD) at 18 °C. The crystal used for data collection was grown in 10% MPD, 40 mM sodium cacodylate (pH 6.0), 12 mM spermine tetra-HCl, and 80 mM NaCl. The single crystal was mounted in a nylon loop and frozen in liquid nitrogen. Diffraction data were collected at low temperature in a cold nitrogen stream on beamline 21-ID-F at LS-CAT, APS (Argonne National Laboratory, Argonne, IL). Separate data sets for high and low resolution reflections were collected. All data were processed with the program HKL2000.

Crystal Structure Determination and Refinement. The diffraction data were processed in space group P212121 (orthorhombic). Phasing was carried out by the molecular replacement method using the program MOLREP in the CCP4 suite. The DDD sequence with PDB entry 355D was used as the starting model. Initial refinements of the model were performed with the CNS program, setting aside 5% randomly selected reflections for calculating the Rfree. Rigid body refinement and simulated annealing were performed. Multiple rounds of coordinate refinements and simulated annealing led to an improved model for which some (2Fo-Fc) and difference (Fo-Fc) Fourier electron density maps were generated. At a later stage solvent water molecules were added on the basis of Fourier 2Fo-Fc sum and Fo-Fc difference electron density maps. Water molecules were accepted based on the standard distances and B-factor criteria.

Further structure refinement was performed using the programs SHELXL and REFMAC in CCP4. A Mg2+ ion and four Na+ ions were identified in the electron density maps based on their low B-factors and the characteristic Mg2+ octahedral and Na+ tetrahedral coordination geometries. Geometry and topology files were generated for the 7-deaza-dA-modified DDD-1 duplex and simulated annealing was performed afterward. The program TURBO-FRODO was used to display electron density maps. The helicoidal parameters of the 7-deaza-dA-modified DDD were analyzed using the program CURVES (version 5.3).

Data Deposition. Complete structure factor and final coordinates were deposited in the Protein Data Bank (www.rcsb.org); PDB ID code 3OPI.

RESULTS

Crystallography. The 7-deaza-dA-modified DDD-1 diffracted at a resolution of 1.1 Å. The two strands of the DDD-1 duplex were not symmetry-related in the crystal. Therefore, each of the nucleotides was uniquely numbered (Chart 1). Minimal perturbation of the DNA duplex was observed at the 7-deaza-dA site (Figure 1). The 7-deaza-dA bases were in the anti conformation about the glycosyl bonds and Watson–Crick base pairing was maintained at base pairs Y6·T19 and Y18·T7 along the normal to the base pairs, viewed (a) from the side and (b) from the top approximately named to base pairs, revealing stacking interactions. (c) Watson–Crick base pairing of 7-deaza-dA·dT. Y6 and Y18 bases are shown in blue.
The unfolding of duplexes was studied by temperature-dependent UV spectroscopy. Absorption spectra at low and high temperatures revealed a greater hyperchromic effect at 260 nm for DDD and DD and at 275 nm for DDD-1 and DD-1. At 45 °C, the T′ peak completely broadened. These observations indicated that the T′ imino proton was in enhanced exchange with the solvent and indicated a destabilization of the Y′, T′ base pair.

Figure 3. CD spectra of duplexes in 10 mM sodium phosphate buffer (pH 7.0) at 4 °C, ~10 μM strand concentration: (a) DDD (●) and DDD-1 (○) and (b) DD (●) and DD-1 (○). The spectra without symbols are the spectra of the unmodified DDD and DD at 90 °C.
The overall sequential melting behavior corresponded to duplex → hairpin and hairpin → random coil transitions, while the corresponding decamers, which formed less stable hairpins, melted through a single duplex random coil transition. The \( T_M \) values were determined by taking the first derivative of the melting curves, and shape analysis of these curves are reported in Table 2. Incorporation of 7-deaza-dA was destabilizing for both dodecamer and decamer. The \( T_M \) of the first transition for the dodecamer DDD-1 relative to DDD was unchanged in 16 mM Na\(^+\) (low salt) and 8.2 °C lower in 116 mM Na\(^+\) (high salt) concentrations. At higher salt concentration both melting transitions overlapped and only one transition was observed. The \( T_M \) of the modified DD-1 was lower than that for DD by 3.4 °C in low salt and by 5.5 °C in high salt.

**DSC of the 7-Deaza-dA-Modified Duplexes.** The DSC melting curves for the DDD and DDD-1 dodecamers and the DD and DD-1 decamers are shown in Figure 7, and the thermodynamic profiles are listed in Table 2. At the lower salt concentration (16 mM Na\(^+\)), the helix → coil transition was biphasic for the dodecamers. The DDD unfolded via a broad first transition and a sharper second transition. The biphasic DSC thermogram of DDD-1 revealed a broad peak with a shoulder for the first transition at lower temperature that could not be resolved. At increased salt concentration, the dodecamers unfolded via monophasic transitions. This was attributed to higher screening by salt on the duplex phosphates, relative to the phosphates of the hairpin. This shifts the duplex transition to higher temperatures, confirming the helix → hairpin → random coil transitions of each dodecamer duplex, which was observed in the UV melting studies. For the decamers, the helix → coil transitions were monophasic, confirming their unfolding through a duplex to random coil transition as seen in the UV studies. Enthalpies were determined by deconvolution of the DSC graphs; however, only the model-independent enthalpies of the duplex → random coil transitions are reported in Table 2. The dA to 7-deaza-dA substitution was destabilizing at both low and high salt concentrations.

Figure 4. (a) NOE connectivity for the imino protons for the base pairs G\(^2\)C\(^{11}\) to Y\(^9\)T\(^7\). The experiments were carried out at a mixing time of 250 ms and 600 MHz at 5 °C. (b) Interstrand NOE cross peaks between opposite bases: a1, T\(^N\)N3H → Y\(^Y\)H2; b1, T\(^Y\)N3H → A\(^A\)H2; b2, T\(^Y\)N3H → Y\(^Y\)H2; c1, G\(^2\)N1H → C\(^{11}\)N\(^2\)H2; c2, G\(^3\)N1H → C\(^{11}\)N\(^2\)H1; d1, G\(^{10}\)N1H → C\(^3\)N\(^2\)H2; d2, G\(^{10}\)N1H → C\(^3\)N\(^2\)H1; e1, G\(^4\)N1H → C\(^9\)N\(^2\)H2; e2, G\(^4\)N1H → A\(^5\)H2; e3, G\(^4\)N1H → C\(^9\)N\(^2\)H1.

Figure 5. \(^1\)H NMR of imino proton resonances as a function of temperature. (A) 7-deaza-dA DDD-1 duplex. (B) Unmodified DDD duplex. Modified and unmodified duplexes were prepared at 0.3 mM and 1.8 mM concentration respectively. The samples were prepared in 10 mM NaH\(_2\)PO\(_4\), 0.1 M NaCl, and 50 μM Na\(_2\)EDTA at pH 7.0.
Analysis of thermograms of dodecamers revealed decreased endothermic enthalpies of 40.0 and 35.5 kcal/mol for DDD-1 relative to DDD in 10 and 100 mM NaCl, respectively (Table 3). For decamers, endothermic enthalpies of 80.1 kcal/mol for DD and a reduced unfolding enthalpy of 56.4 kcal/mol for DD-1 (Table 3) were obtained at low salt. At the higher salt concentration, the ∆H was 18.2 kcal/mol for DD vs DD-1.

**Thermodynamic Profiles for the Formation of Each Duplex.** The thermodynamic data is provided in Table 2. The favorable Gibbs free energies, indicating spontaneous formation of each duplex, resulted from compensation of favorable enthalpy and unfavorable entropy contributions. The favorable enthalpies arose from the formation of base pairs and base pair stacks, uptake of electrostricted waters, and release of structural waters, whereas the unfavorable entropy terms included the ordering of two strands to form a duplex, condensation of counterions, and immobilization of waters.

Relative to the unmodified oligodeoxynucleotides, the 7-deaza-dA modified oligodeoxynucleotides were destabilized at low and high salt concentrations. The inclusion of two 7-deaza-dA modifications in DDD-1 yielded a decrease in ∆G of 2.3 and 5.1 kcal/mol in 10 and 100 mM NaCl, respectively, whereas in decamers ∆G decreases of 1.8 and 2.5 kcal/mol in low and high salt, respectively, were observed following two 7-deaza-dA substitutions.

**Differential Association of Water Molecules.** ∆T values on water activity were studied to determine the thermodynamic association of water molecules to DNA duplexes. By increasing concentrations of the osmolyte ethylene glycol from 0.5 to 4.0 M the activity of water was decreased. The UV melting curves showed that the ∆T of the dodecamers (DDD and DDD-1) and decamers (DD and DD-1) decreased linearly with increasing osmolyte concentrations (i.e., decreasing activity of water). The ∆T dependence on water activity of dodecamers and decamers are shown in Figure 8. The slopes of these lines, ∂T/∂ log a_{w}, where a_{w} is the activity of water at a given concentration, were used to obtain the differential association of water molecules. The ∆n_{w} values for the formation of each duplex in 10 mM NaCl are shown in Table 2. Water uptake values, expressed as mol H2O per mol duplex, measured in low salt, were 38 (DDD) and 19 (DDD-1) for dodecamers, and 30 (DD) and 17 (DD-1) for decamers. At the higher salt concentration (116 mM Na+), ∆n_{w} values followed a similar trend. Lower ∆n_{w} values at this salt concentration (Table 2) were due to increased screening of the water dipoles at higher salt concentration. The overall effect, and assuming that the random coil states of all the duplexes behave similarly at higher temperature, was that the substitution of

![Figure 6](image1.png) UV melting curves in 10 mM sodium phosphate buffer (pH 7.0) ~40 μM total strand concentration for (a) DDD (□) at 260 nm and DDD-1 (○) at 275 nm and (b) DD (●) at 260 nm and DD-1 (○) at 275 nm.

![Figure 7](image2.png) DSC curves in 10 mM sodium phosphate buffer (pH 7.0): (a) DDD (□) and DDD-1 (○) at ~200 μM and (b) DD (●) and DD-1 (○) at ~300 μM.

| oligodeoxynucleotide | NaCl | T<sub>M</sub> | ∆G° | ∆H | ∆S | ∆n<sub>Na+</sub> | ∆n<sub>w</sub> |
|----------------------|------|--------------|------|-----|-----|----------------|----------------|
| DDD | 10  | 33.3 | -6.9 | -116.0 | -109.1 | -2.3 ± 0.2 | -38.0 ± 2.0 |
| | 100 | 57.7 | -15.5 | -109.5 | -94.0 | -1.8 ± 0.1 | -30.0 ± 2.0 |
| DDD-1 | 10  | 34.5 | -4.6 | -76.0 | -71.4 | -1.4 ± 0.1 | -19.0 ± 2.0 |
| | 100 | 49.5 | -10.4 | -74.0 | -63.6 | -1.1 ± 0.1 | -15.0 ± 2.0 |
| DD | 10  | 29.5 | -5.6 | -80.1 | -74.5 | -2.2 ± 0.2 | -30.0 ± 4.0 |
| | 100 | 53.0 | -8.2 | -72.3 | -64.1 | -1.7 ± 0.1 | -22.0 ± 3.0 |
| DD-1 | 10  | 26.1 | -3.8 | -56.4 | -52.6 | -1.5 ± 0.2 | -17.0 ± 2.0 |
| | 100 | 47.5 | -5.7 | -54.1 | -48.4 | -1.3 ± 0.1 | -14.0 ± 2.0 |

<sup>a</sup> Parameters are measured from UV (∆T<sub>M</sub>) and DSC melting curves in 10 mM sodium phosphate buffer (pH 7.0). The observed standard deviations are ∆T<sub>M</sub> (±0.5), ∆H<sub>cal</sub> (±3%), ∆G°<sub>20</sub> (±5%), and ∆S<sub>cal</sub> (±3%).<sup>b</sup> Salt concentration in mM. <sup>c</sup>Determined at 20 °C. <sup>d</sup>kcal/mol. <sup>e</sup>Per mol DNA. ∆n<sub>Na+</sub> was determined experimentally, using the linking number: ∆n<sub>Na+</sub> = ∂ ln K/∂ ln [Na<sup>+</sup>], where K corresponds to two single strands in equilibrium with a duplex.
Dodecamer and Decamer Duplexes

DD-1 decamer. The DDDD-1 dodecamer, and 2.2 for the DD dodecamer and 1.5 for the DDD-1 dodecamer, respectively, and 2.2 for the DD dodecamer and 1.5 for the DDD-1 dodecamer increased the electronegative N7-dA atom to a carbon atom, which alters the electrostatics of the nucleobase. Consistent with this expectation, the downfield shift of the T7 imino resonance (Figure 5) is attributed to stronger hydrogen bonding with the more electronegative 7-deaza-dA N1 nitrogen. Thus, the observed destabilization of 7-deaza-dA does not result from a decrease in H-bonding but from the increased stability of the random coil states of the different single strand oligodeoxynucleotides.

Table 3. Differential Thermodynamic Profiles for Pairs of Dodecamer and Decamer Duplexes

| NaCl (mM) | ΔHf (kcal/mol) | ΔG°′ (kcal/mol) | ΔTΔS (kcal/mol) | ΔΔHNa+ (kcal/mol) | ΔΔGNa+ (kcal/mol) |
|-----------|----------------|-----------------|-----------------|-------------------|-------------------|
| 10        | 40.0           | 2.3             | 37.7            | 0.9               | 19.0              |
| 100       | 35.5           | 5.1             | 30.4            | 0.7               | 15.0              |

Substitution of dA6 with 7-Deaza-dA in DDD (DDD-1 Minus DDD)

Substitution of dA5 with 7-Deaza-dA in DD (DD-1 Minus DD)

7-deaza-dA into duplex DNA caused a decreased association of water molecules. For instance, there was a ΔΔnw of 19 and 15 between DDD and DD-1 at 10 mM and 100 mM NaCl, respectively, and ΔΔnw of 13 and 8 between the pair of dodecamer duplexes at low and high salt, respectively (Table 3). Parameters used to calculate differential water binding for dodecamers are shown in Table S1 in the Supporting Information.

Differential Association of Counterions. UV melting curves at salt concentrations ranging from 16 to 216 mM [Na+] were measured to examine the thermodynamic association of counterions with the DNA duplexes. The Tm values of the DDD and DDD-1 dodecamers, and DD and DD-1 decamers increased linearly with salt concentration (Figure 9), consistent with the expectation that the duplex states should have higher charge density parameters. The Tm dependence on salt concentration for dodecamers and decamers are shown in Figure 9, panels a and b, respectively. The slopes of these lines, ∂Tm/∂log[Na+], in conjunction with the experimentally determined ΔH/RTM terms, allowed measurement of differential counterion binding. The ΔΔHNa+ values for the formation of each duplex, from the association of two complementary strands, in low and high salt are shown in Table 2. In low salt, the Na+ uptake as measured in mol Na+ per mol duplex was 2.3 for the DDD dodecamer and 1.4 for the DDD-1 dodecamer, and 2.2 for the DD dodecamer and 1.5 for the DD-1 decamer. The ΔΔHNa+ values at the higher salt concentration of 116 mM showed a similar trend; however, the values were lower due to the higher screening of the phosphates by salt (Table 2). The average differential Na+ uptake as measured in mol Na+ per mol phosphate was estimated as 0.094 (DDD and DD) in this range of salt concentration, which was consistent with the fact that these oligodeoxynucleotides were not behaving electrostatically as long polyelectrolytes. However, the main effect, assuming that the random coil states of the different single strand oligodeoxynucleotides were thermodynamically similar at higher temperatures, was that the introduction of 7-deaza-dA into the duplex DNA caused a slightly decreased association of counterions. For instance, there was a ΔΔHNa+ of 0.9 and 0.7 between DDD and DD-1 at 10 and 100 mM NaCl, respectively, and ΔΔGNa+ of 0.7 and 0.4 between the pair of dodecamer duplexes at low and high salt, respectively (Table 3). Parameters used to calculate differential counterion binding for dodecamers are presented in Table S2 in the Supporting Information.

Figure 8. Tm dependence on osmolyte concentration (as a function of ethylene glycol) for duplexes in 10 mM sodium phosphate buffer (pH 7.0), ∼5 μM strand concentration for (a) DDD () and DDD-1 () and ∼7 μM strand concentration for (b) DD () and DD-1 (○).

Figure 9. Tm dependencies on salt concentration for duplexes in 10 mM sodium phosphate buffer (pH 7.0), ∼5 μM strand concentration for (a) DDD () and DDD-1 () and ∼7 μM strand concentration for (b) DD () and DD-1 (○).

**DISCUSSION**

It has been assumed that 7-deaza-dA, an isostere for dA in duplex DNA, does not substantially perturb the duplex, and thus provides a good model for dA. However, in light of suggestions that 7-deaza-dA introduces a large structural perturbation to the B-form of poly(dA-dT)·poly(dA-dT),18 it was of interest to provide a comprehensive characterization of B-DNA with a 7-deaza-dA modification. The Dickerson—Drew dodecamer19,20 provides a well-characterized system suitable for detailed crystallographic analysis,44 as well as NMR analysis.45,51,52 The present studies provide the first high-resolution crystallographic data for the substitution of adenine with 7-deaza-dA in duplex DNA.

**Structure of the 7-Deaza-dA:dT Base Pair.** The structure of the 7-deaza-dA:dT base pair in the DDD duplex reveals that 7-deaza-dA has minimal effect on duplex conformation (Figure 1) and base pair geometry (Figure 2) as compared to a canonical dA:dT base pair. Substitution of 7-deaza-dA changes the electronegative N7-dA atom to a carbon atom, which alters the electrostatics of the nucleobase. Consistent with this expectation, the downfield shift of the T7 imino resonance (Figure 5) is attributed to stronger hydrogen bonding with the more electropositive 7-deaza-dA N1 nitrogen. Thus, the observed destabilization of 7-deaza-dA does not result from a decrease in H-bonding but from the increased stability of the random coil states of the different single strand oligodeoxynucleotides.
must be due to other changes induced by the perturbation of the electrostatic potential in the major groove. Other NMR chemical shift perturbations are minimal, which indicates that the modification does not affect the structure at the flanking nucleotides. Our results differ from those of Pope et al., who suggested that replacement of dA by 7-deaza-dA caused perturbations to B-DNA for the poly[d(7-deaza-dA-T)]-poly[d(7-deaza-dA-T)] duplex. The physical properties of poly(dA-dT) differ from the DDD, and it may be of interest to look for structural perturbations induced by 7-deaza-dA in other sequences.

7-Deaza-dA Enthallypically Destabilizes the DDD. The 7-deaza-dA substitution thermodynamically destabilizes the DDD-1 and DD-1 duplexes, compared to the unmodified DDD and DD duplexes. This is evidenced by the ΔΔG values (computed as the average of 10 and 100 mM [Na+] solution, Table 3). At 20 °C, ΔΔG is decreased by 3.7 kcal/mol for DDD-1 and by 2.2 kcal/mol for DD-1. In both cases, the major contributor to the reduced ΔΔG values is the enthalpy term, which drops 37.8 kcal/mol for DDD-1 and 20.9 kcal/mol for DD-1 (Table 3). The differential ΔΔH values at different salt concentrations suggest the presence of heat capacity effects. The heat capacity changes were 0.8 kcal/K mol (DDD) and −0.08 kcal/K mol (DDD-1), and −0.5 kcal/K mol (DD) and −0.2 kcal/K mol (DD-1). These may be due to exposures of nonpolar groups to solvent and/or to changes in structural hydration between the random coil and duplex states of DDD-1 and DD-1.53 The present data lead to a different conclusion than did studies of (7-deaza-dA)11A as compared to dA11 as, conducted by Seela and Thomas.52 They concluded that destabilization induced by 7-deaza-dA was minimal and was associated with an unfavorable entropy change.52 It should be noted, however, that the DDD presents a different sequence context than does the A-tract dA12 sequence.53

Base Stacking Effects. The most significant contribution to the unfavorable ΔΔH term (Table 3) of 32.7 kcal/mol for DDD-1 (17.6 kcal/mol for DD-1) results from a reduction of stacking enthalpy in the modified duplexes, attributed to less favorable π–π interactions involving the pyrrolopyrimidine ring of 7-deaza-dA and the neighboring base pairs vs adenine. In the CD spectra, the intensities of the negative bands near 250 nm are thought to track base stacking contributions. The band intensities at 250 nm are consistent with reduced base stacking in DDD-1 and DD-1 at low temperature (Figure 3). There is an 18% decrease in the intensity of the 250 nm band for DDD-1 relative to DDD. The decreased intensity of the 250 nm band for DD-1 relative to DD is 10%. However, changes in the electronic structure of 7-deaza-dA may modulate the relative optical dipole orientations responsible for the CD bands. Exchange-mediated line broadening of DNA amino protons is often associated with the rate-limiting formation of an open state of the base pair in which the imino proton is freed from its hydrogen bond and is accessible to the base that catalyzes the proton exchange.55–59

The increased broadening of the Y6°-T° base pair thymine N3 imino resonance (Figure 5) is consistent with this model, which correlates with reduced stacking enthalpy of the DDD-1 duplex relative to the DDD duplex. However, the possibility that base pair opening is not rate-limiting cannot be ruled out, with the line broadening reflecting a more rapid hydrogen exchange catalysis for the substituted duplex.60 In this regard, the C7–H on the 7-deaza-dA (as compared to the: N7 on the natural dA) would be anticipated to exhibit a reduced electrostatic repulsion with hydroxide or phosphate base catalyst.

Duplex Hydration. The unfavorable ΔΔH term observed upon incorporation of 7-deaza-dA is partially attributed to reduced hydration of the modified duplexes. This may, in part, be due to the more hydrophobic major groove edge of 7-deaza-dA as compared to dA. Thus, 7-deaza-dA substitution results in a ΔΔG of 17 H2O per mol DNA for DDD-1 and 11 H2O per mol DNA for DD-1 (obtained by averaging the data obtained in 10 and 100 mM NaCl, Table 3). This “translates” into a reduction of approximately 9 H2O per mol DNA per 7-deaza-dA nucleotide for the DDD-1 duplex and 6 H2O per mol DNA per 7-deaza-dA nucleotide for the DD-1 duplex, assuming localized effects. To evaluate the release of water from the DDD-1 duplex (11 water molecules from the DDD-1 duplex near 7-deaza-dA nucleotide. Another way to interpret the data is that the displacement of water by ethylene glycol, used in the osmotic stress experiments, near 7-deaza-dA will be more facile than at dA because of the reduced electrostatic interaction with solvent. In any case, similar reductions in hydration were observed for DNA modified with 7-deaza-dG nucleotides.

Cation Binding. The introduction of the 7-deaza-dA:dT pair into the DDD causes a decrease in the differential association of cations. This is reflected in the ΔΔG of 0.9 and 0.7 between DDD and DD-1 at 10 and 100 mM NaCl, respectively, and in the sequence-dependent distribution of waters and counterions in B-DNA.39,44,63 When the DDD was crystallized in the presence of T1+, no high-occupancy cation binding sites were observed in the major groove near A6.44 Likewise, Tershiko and Egli44 did not observe a high affinity cation site near A6. In the present crystallographic unit cell two Mg2+ ions interact with the DNA, but they are not associated with the major groove edge of either Y6 or Y18 (Figure 1; Figure S2 in the Supporting Information). This is consistent with the notion that cation binding in A-T tracts occurs in the minor groove.68 It seems possible that the thermodynamically measured decrease in the association of cations could be due to the disruption of non-specific cation binding, particularly in the minor groove. In any case, the contribution to the large ΔΔH term for the release of counterions is anticipated to be negligible since counterion release contributes predominantly to the Δ(TAS) term.71

In contrast, the major groove high affinity cation sites in the DDD were associated with the major groove edge of dG nucleotides.69,70 Indeed, the incorporation of 7-deaza-dG into the DDD was accompanied by changes in hydration and major groove cation organization.

Summary. Introduction of the 7-deaza-dA:dT base pair into the DDD has minimal effect upon base pairing geometry and DNA conformation, as evidenced by a combination of crystallographic and NMR studies. The 7-deaza-dA retains Watson–Crick hydrogen bonding, but the 7-deaza-dA:dT base pair is thermodynamically destabilized. A detailed analysis reveals that this is due to primarily to unfavorable enthalpy terms, which are dominated by less favorable

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Supporting Information. The Supporting Information includes Tables S1, parameters used to calculate differential counterion binding for the dodecamers; S2, parameters used to calculate differential water binding for dodecamers; and Figures S1, stick model and electron density of the crystal structure of 7-deaza-dA:dT pair into the DDD causes a decreased association of cations, which is reflected in the TAD term.

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