Differential Hydration of dA·dT Base Pairing and dA and dT Bulges in Deoxyoligonucleotides†

Krzyztof Zieba,‡ Tram M. Chu,§ Donald W. Kupke,§ and Luis A. Marky*,‡

Department of Chemistry, New York University, New York, New York 10003, and Department of Biochemistry, University of Virginia, Charlottesville, Virginia 22908

Received April 8, 1991; Revised Manuscript Received June 3, 1991

ABSTRACT: The role of water in the formation of stable duplexes of nucleic acids is being studied by determining the concurrent volume change, heats, and counterion uptake that accompany the duplexation process. The variability of the volume contraction that we have observed in the formation of a variety of homoduplexes suggests that sequence and conformation acutely affect the degree of hydration. We have used a combination of densimetric and calorimetric techniques to measure the change in volume and enthalpy resulting from the mixing of two complementary strands to form (a) fully paired duplexes with 10 or 11 base pairs and (b) bulged decameric duplexes with an extra dA or dT unmatched residue. We also monitored absorbance vs temperature profiles as a function of strand and salt concentration for all four duplexes. Relative to the decamer duplex, insertion of an extra dA·dT base pair to form an undecamer duplex results in a favorable enthalpy of −5.6 kcal/mol that is nearly compensated by an unfavorable entropy term of −5.1 kcal/mol. This enthalpy difference correlates with a differential uptake of water molecules, corresponding to an additional hydration of 16 mol of water molecules/mol of base pair. Relative to the fully paired duplexes, both bulged duplexes are 12–16 °C less stable and exhibit marginally larger counterion uptake on forming the duplex. The enthalpy change is slightly lower for the T-bulge duplex and less still for the A-bulge duplex. The volume change results indicate that an unmatched residue increases the amount of coulombic and/or structural hydration. The combined results strongly suggest that the destabilizing forces in bulged duplexes are partially compensated by an increase in hydration levels.

Oligonucleotides of defined sequence have proven to be useful models for studying the structural features found in naturally occurring nucleic acid polymers. Thermodynamic investigations of the helix–coil transition of these oligomeric systems have greatly enhanced our understanding of the conformational transitions of DNA and RNA molecules (Gralla & Crothers, 1973; Uhlenbeck et al., 1973; Breslauer et al., 1986; Freier et al., 1986). In addition to the characteristic heat effects, these transitions are also accompanied by changes in the volume, ΔV (Chapman & Sturtevant, 1969). The volume property reflects more directly upon the degree of hydration, an interaction found very early to be of critical importance for the stability of the double-helical structure of DNA (Falk et al., 1962). However the quantitative role of water in maintaining any DNA conformation as well as its participation in transitions accompanying changing solution conditions remains unknown. Our general efforts, therefore, have been directed toward determining changes in the volume as well as those of the standard thermodynamic functions (ΔG, ΔH, ΔS) upon duplexation of DNA oligomers with known sequence.

The conformation of a DNA duplex containing helical imperfections resulting from the presence of unpaired bases on one strand has provided a model for the mechanism of frameshift mutations that occur with high frequency in homosequences in DNA. Presumably this effect arises from the conformational flexibility of the duplex to slip without loss of base pairing (Lerman, 1963; Streisinger et al., 1966; Ames et al., 1973), resulting in addition or deletion mutations following repair and replication. For this reason, several laboratories have been involved in obtaining the structure of deoxyoligomer duplexes containing bulge bases using NMR and X-ray crystallographic techniques. The solution results of these studies have indicated that the extra base, depending on its nature (purine vs pyrimidine) and/or type of the flanking bases, has the potential of being stacked into the duplex (Patel et al., 1982; Hare et al., 1986; Roy et al., 1987; Woodson & Crothers, 1987, 1988a,b, 1989; Kalnick et al., 1989a,b, 1990) or of looping out into solution (Morden et al., 1983, 1990; van den Hoogen et al., 1988; Nikonowicz et al., 1989). These effects cause perturbations in the phosphodiester backbone extending over several adjacent base pairs (Nikonowicz et al., 1989). The structure of duplexes containing bulges, obtained by crystallographic studies, indicate that the bulge bases loop outside the double helix (Miller et al., 1988; Joshua-Tor et al., 1988). Thermodynamic investigations have been carried out in order to correlate the macroscopic properties with the molecular interactions observed in these duplexes. In general, the presence of a bulge base in a nucleic acid duplex causes a loss of stability relative to the parent duplex (Fink & Crothers, 1972; Morden et al., 1983; Woodson & Crothers, 1987; Groebe & Uhlenbeck, 1989; Longfellow et al., 1990; Morden et al., 1990; LeBlanc & Morden, 1991). This unfavorable free energy has been attributed to a more unfavorable entropy term in the case of a stacked (intrahelical) adenine between a CG/GC base-pair stack (Patel et al., 1982) and to both unfavorable enthalpic and entropic terms for the case of a looped-out (extrahelical) base (Morden et al. 1990; LeBlanc

†This work was supported by Grants GM42223 (L.A.M.) and GM349389 (D.W.K.) from the National Institutes of Health and in part by Grant BRSG SO7 07062 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

*To whom correspondence should be addressed.

‡New York University.

§University of Virginia.

0006-2960/91/0430-8018$02.50/0

& Morden, 1991). In the formation of a bulge duplex, it is important to consider, in addition to the heat changes, the extent of both ion binding and water binding.

In this report we consider the effects of base pairing and bulges during the formation of oligodeoxynucleotide duplexes from two complementary strands. The results of the total thermodynamic description and of the melting behavior of the undecamer duplex, as well as those of the bulge duplexes, are compared with the similar results for the control duplex formed from the corresponding fully complementary decameric strands. Of special interest is the attempt to assess the effect of water molecules and ions in the overall conformational flexibility of these duplexes.

Source of Volume Change upon DNA Duplexation. In biochemical systems at constant temperature and pressure, the volume expansions and contractions attending reactive chemical changes are principally a reflection of the average change in the molar volume of water in the vicinity of the reacting solutes. The solutes themselves ordinarily do not possess the propensity to change the solution volume to the same order of magnitude (a relatively large net difference in the amount of void spaces within globular macromolecules perhaps being an exception). Liquid water by virtue of its quadrupolar nature (and possessing a large dipole moment of 1.85 Debye units, or more in the liquid phase), has the capacity to change its molar volume dramatically. The open, quasi-tetrahedral structure of bulk water is some 50% larger in molar volume than if the individual molecules were packed in a noninteracting hexagonal array (Conway, 1981). Accordingly, ion–water dipole interactions are likely to be the major sources of biochemical volume changes (at constant temperature and pressure). The intense electric field close to an ion induces a local collapse of the bulk-water structure by compressing the adjacent water dipoles at pressures corresponding to several thousand atmospheres—conditions that may even reduce the molar volume of water to less than 12 mL/mol (Conway, 1981). This compression, which depends on the charge and the radius of an ion and on the local dielectric gradient, is known colloquially as electrostriction [first derived by Drude and Nernst (1894) and subsequently refined [cf. Millero (1971) and Conway (1981)]. Hence, neutralization reactions, formation of ion pairs (salt bridges), or the coordination of metal ions result in water decompressions that can give rise to relatively large expansions at millimolar reactant concentrations [cf. Kupke and Shank (1989) and references therein].

The changes in molar volume of water molecules interacting over much shorter effective distances (dipole–dipole, hydrophobic–dipole, partial charge–dipole, etc.) are less well understood. According to electrostriction theory, we would expect these compressions to be comparatively small. Also, it is not clear whether hydrophobic–water interactions can in some cases cause a small expansion relative to bulk water (Stillinger, 1980). The volume effect of hydrophobic moieties on charged solutes is known to augment the contractions [e.g., King (1969)]; however, unless these moieties undergo chemical changes during mixing of *aqueous* phases, the difference in volume from this source should contribute only a marginal effect. In the case of hydrogen bonding between the partially charged nitrogen and oxygen donor/acceptor atoms of the nucleic acid bases, the electrostriction effect is predicted to be small because the contraction is approximately proportional to the square of the charge density [cf. Millero (1971)]. Moreover, the volume change attending the loss of hydrogen-bonding water to these sites prior to duplexation would tend to compensate for the putative contraction accompanying hydrogen-bond formation between the bases. It appears, therefore, that, during duplexation of complementary oligomeric strands, the volume contractions which we have observed thus far must reflect principally the electrostriction resulting from the regularly spaced phosphate charges that flank the minor groove of the rigid, double-stranded cylinder. Why the duplexation process should generate an enhanced level of electrostriction when the same number of charged phosphates exists as in the single strands prior to the duplexation is not known. What is known, however, is that geometric constraints can markedly increase the amount of electrostriction, as shown by Kauzmann et al. (1962). In those experiments, charged groups that were molecularly constrained to cause overlap of their electric fields correlated with the observed excess volume contractions as would be expected from electrostriction theory. More recently, from electrolyte-tank experiments with clay models in B. Zimm's laboratory, it was concluded that the dielectric discontinuity between the high-permittivity solvent and the low-permittivity DNA molecule created a concentration of the phosphate fields at the surface of the double helix (Troll et al., 1986; Conrad et al., 1988). The focusing effect was most pronounced in the minor groove, which is where a highly organized spine of hydration has been reported [Prive et al. (1991) and references therein]. It is not unreasonable at this point to suppose that the tightly packed water molecules in this spine of hydration within the minor groove are highly compressed relative to that expected from simple application of Coulomb's law. Some volume-change and compressibility data exist that support this hypothesis (Marky & Kupke, 1989; Buckin et al., 1989).

In the experiments reported here, inclusion of an A·T base pair in the center of the TAAT/ATTA core of a decamer duplex to form an undecamer duplex creates an additional amount of compressed water that correlates well with the unfavorable entropy calculated from our calorimetric experiments. The introduction of a bulge base (dT or dA) presents an additional phosphate and also modifies the structure from that of a fully complementary oligomeric duplex. We show here, however, that although the standard thermodynamic parameters describe a loss in stability for the duplexes containing a bulge, these duplexations generate a larger volume contraction than do their fully complementary counterparts. This observation, within our currently unsatisfactory state of volume-change knowledge, may be considered as an additional coulombic hydration (electrostriction) and/or hydrophobic hydration.

Materials and Methods

Materials. The deoxyoligonucleotides d(CGCCTAATCG), d(CGATTAGGCG), d(CGCCTATATCG), and d(CGATATAGGCG) 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. Extinction coefficients of the oligomers in single strands were calculated at 25 °C by using the tabulated values of the dimers and monomer bases (Cantor et al., 1970) and were estimated at high temperatures by extrapolation to 25 °C of the upper portions of the melting curves (Marky et al., 1981), which corresponds to the UV–temperature dependence of the single strands. The concentration of the oligomers were determined in water by using the following extinction coefficients of strands at 260 nm and 80 °C, all values being given in $M^{-1}\cdot cm^{-1}$: d(CGCCTAATCG), 9.31×10^4; d(CGATTAGGCG), 9.94×10^4; d(CGCCTATATCG), 1.12×10^5; d(CGATATAGGCG), 1.03×10^5. Stock oligomer solutions were pre-

pared by dissolving the dry and desalted oligomers in the appropiate buffer and then dialyzing against the same buffer. All other chemicals were reagent grade. The buffer solutions consisted of 10 mM Na_2HPO_4 and 0.1 mM Na_2EDTA, pH 7.0, adjusted to the desired ionic strength with NaCl.

Magnetic Suspension Densimetry. The volume change, ΔV, that accompanies the formation of a given duplex was determined by measuring the density on weighed samples in a magnetic suspension densimeter. The instrument used in this study is an improved version of the earlier design for biochemical purposes by Senter (1969) and has been previously described (Gillies & Kupke, 1988). This instrument requires only 80 μL per measurement, and its sensitivity is such that it can deliver volume differences with a precision of less than half a nanoliter. The calculation of ΔV is done by measuring the mass and the equilibrium density of solutions before and after mixing; the observed change in volume, Δv, upon adding reactant A to reactant B is given by

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

where m is the mass in grams and ρ is the density of the solution in grams per milliliter. The sum of the two terms within parentheses gives the initial volume before mixing. The value of Δv in milliliters is then reduced to that per mole of the limiting reagent to give ΔV. According to eq 1, small weighing errors have no appreciable effect on ΔV. The three density values, while independent, need not be of high absolute accuracy since it is their differences that are required for ΔV. The densimeter is calibrated with aqueous KCl solutions of known density. The density of each sample is obtained by relating the measured voltage to the straight-line calibration equation of voltage versus density since the electrical current required to stably support the tiny permanent magnet (jacketed) at a present height below the meniscus is directly proportional to the density of the surrounding fluid (Kupke & Beams, 1972). To make sure 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. With repetitive samples the density is measured with a precision of better than 5×10^{-6} g/mL. The temperature was kept at 20 ± 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 the mean value was taken as the one reported for ΔV. Three different preparations of each oligomer were studied. In these experiments the concentrations of the individual strands ranged from 0.1 to 0.3 mM. Thus, the effect of solute–solute interactions upon the volume property was deemed to be negligible.

Differential Scanning Calorimetry. The total heat of the duplex–coil transition of each duplex and of each single strand was measured directly with a Microcal MC-2 differential scanning calorimeter (DSC). Typically an oligomer solution with a concentration of 0.9 mM (in strands) versus buffer was scanned from 0 to 90 °C at a heating rate of 45 °C/h. A buffer versus buffer scan was subtracted from the sample scan and normalized for the heating rate; i.e., each data point is divided by the corresponding heating rate. The area of the resulting curve is proportional to the transition heat, which when normalized for the number of moles is equal to the transition enthalpy (ΔH_{cal}). The instrument was calibrated with a standard electrical pulse. Shape analyses of the heat capacity curves are included in the software of the instrument, which allowed us to calculate two-state enthalpies (ΔH_{vH}). Direct comparisons of ΔH_{vH} with ΔH_{cal} allows us to predict the nature of the transition.

Titration Calorimetry. The measurement of the heats of mixing a single strand with its corresponding complementary strand at three different temperatures were carried out with 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 the fully paired duplex or the bulge duplex. A 100-μL syringe was used for the titrant; mixing was effected by stirring this syringe at 400 rpm. The concentration (in strands) of the oligomer in the syringe was generally 25 times higher than the concentration of the complementary strand in the reaction cell. Calorimetric titrations were designed only to obtain the enthalpy of duplex formation as a function of temperature. Typically 10 injections of 5 μL each were performed in a single titration at temperatures wherein duplexes are formed, that is, temperatures approaching that where the duplexes started to melt. Since the reference cell of the calorimeter acts only as a thermal reference to the sample cell, this cell was filled with water. The instrument was calibrated by means of a known standard electrical pulse.

UV Melting Curves. Absorbance versus temperature profiles (melting curves) for the oligomeric duplexes and for each strand, at various strand concentrations and in buffer solutions containing 10–100 mM of NaCl, were measured at 260 nm with a thermoelectrically controlled Perkin-Elmer 552 spectrophotometer interfaced to a PC-XT computer for acquisition and analysis of experimental data. The temperature was scanned at a heating rate of 1 °C/min. These melting curves allow us to measure the transition temperatures, T_m, which are the midpoints of the order–disorder transition of these oligomers, as well as the relevant thermodynamic parameters. These parameters were calculated by using standard procedures reported elsewhere (Marky et al., 1981; Marky & Breslauer, 1987) and correspond to a two-state approximation of the helix–coil transition of each molecule. From the T_m salt dependence (T_m vs log Na^+ plots) together with the T_m's and enthalpies obtained from DSC experiments, the amount of counterion release was estimated for each duplex.

Results

By a combination of spectroscopic, densimetric, and calorimetric techniques we have studied the formation (and disruption) of the following DNA duplexes (5′ to 3′):

```
C-G-C-C-T-A-A-T-C-G        C-G-C-C-T-A-T-A-T-C-G
 . . . . . . . . . .        . . . . . . . . . . .
G-C-G-G-A-T-T-A-G-C        G-C-G-G-A-T-A-T-A-G-C
```

(decamer) (undecamer)

```
C-G-C-C-T-A-T-A-T-C-G      C-G-C-C-T-A---A-T-C-G
 . . . . . . . . . .        . . . . . . . . . .
G-C-G-G-A-T---T-A-G-C      G-C-G-G-A-T-A-T-A-G-C
```

(T-bulge) (A-bulge)

Volume Changes. Using a magnetic suspension densimeter, we have measured at 20 °C and atmospheric pressure the equilibrium volume change, ΔV, associated with the formation of each duplex from its respective complementary strands. The results are listed in Table I for four different duplexes. In these experiments the molar ratio (in phosphate) of one strand relative to the complementary strand was 1:1.2. Each entry represents an average of at least four determinations. The results of Table I indicate that, in the 0.1 M NaCl buffer, duplex formation is accompanied by an uptake (or compres-

Table I: ΔV of Duplex Formation at 20 °C[a]

duplex	ΔV [mL·(mol of duplex)$^{-1}$]
decamer	−167 ● 11
undecamer	−208 ● 3
T-bulge	−285 ± 16
A-bulge	−312 ● 12

[a] Values were taken in 10 mM NaP_i buffer containing 0.1 mM Na_2EDTA and 100 mM NaCl at pH 7.0.

Table II: ΔH of Duplex Formation at Several Temperatures[a]

duplex	ΔH (kcal·mol^{-1})		
	20 °C	25 °C	30 °C
decamer	−55.5	−57.2	−57.7
undecamer	−61.1	−62.3	−72.1
T-bulge	−53.2		
A-bulge	−36.0		

[a] Values were taken in 10 mM NaP_i buffer containing 0.1 mM Na_2EDTA and 100 mM NaCl at pH 7.0. The ΔH values are within ±4%.

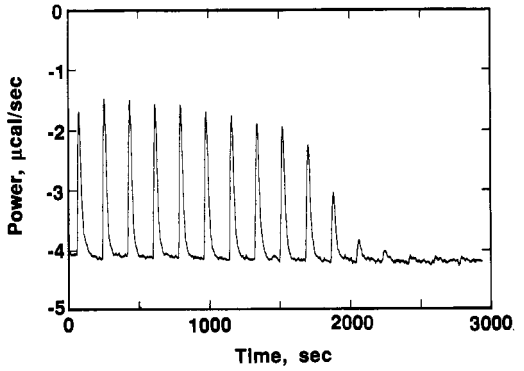

FIGURE 1: Typical calorimetric titration curve; 1.4 mL of d-(CGATTAGGCG) solution with C_T = 9.94 μM was titrated with d(CGCCTAATACG) solution (C_T = 257 μM). Both reagents were in a 10 mM sodium phosphate buffer containnig 0.1 mM Na_2EDTA and 0.1 M NaCl at pH 7. Each peak corresponds to 5-μL injections of the titrant and average heat of 71 μcal for the six initial injections.

sion) of water molecules for all duplexes studied. For the case of the bulge duplexes the volume contraction (or putative water uptake) is larger than that for any of the fully paired duplexes. These values do not discriminate for water uptake upon release of sodium ions (see later).

Titration Calorimetry. In order to help with the interpretation of our densimetric results, we carried out titration calorimetric experiments on the same solutions as used in densimetry for equivalence of states. A typical titration curve is shown in Figure 1. The heats obtained for each injection are dependent on the temperature. After a small correction for dilution heats of the strands, molar binding enthalpies for duplex formation were calculated. Duplex formation enthalpies at several temperatures are summarized in Table II. The significant observation is that we measured exothermic enthalpies for the formation of each of the duplexes under study. The magnitude of these enthalpies depends on the nature of the duplex being formed. The significance of the results will be discussed presently.

UV Melting Curves. Typical melting curves are presented in Figure 2. Melting curves provide us with the transition temperature and model-dependent enthalpies that characterize these duplexes. In addition, melting curves of the single strands (data not shown) can measure the degree of self-stacking of these strands, especially at low temperatures. We have observed single-strand stacking in all of the strands; it was most pronounced for the d(CGATATACCCG) oligomer strand.

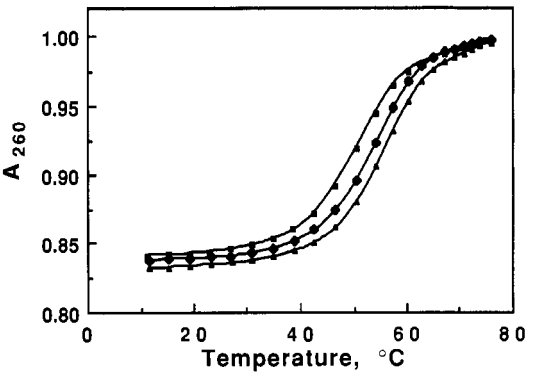

FIGURE 2: Typical melting curves of d(CGCCTAATCG)/d-(CGATTAGGCG) in 10 mM sodium phosphate buffer containing 0.1 mM Na_2EDTA and 0.1 M NaCl at pH 7 and 20 °C at the following strand concentrations: 6.68 μM (■), 32.1 μM (◆), and 58.9 μM (▲).

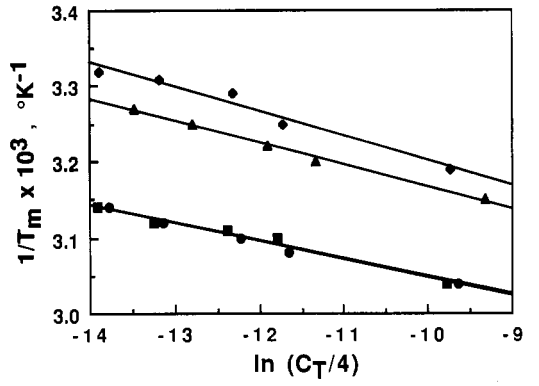

FIGURE 3: Dependence of the transition temperature on strand concentration in 10 mM sodium phosphate buffer containing 0.1 mM Na_2EDTA and 0.1 M NaCl at pH 7: decamer (●), undecamer (■), A-bulge (◆), and T-bulge (▲).

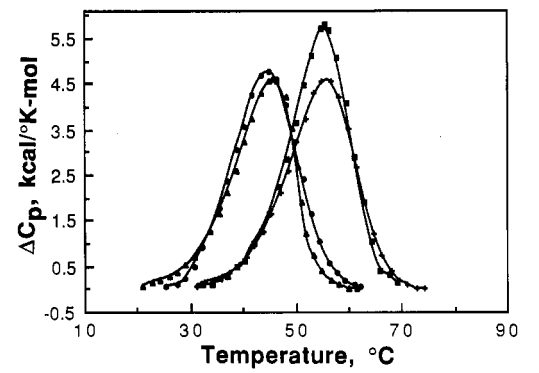

FIGURE 4: Differential scanning calorimetry curves for each oligomer in 10 mM sodium phosphate buffer containing 0.1 mM Na_2EDTA and 0.1 M NaCl at pH 7. The areas under these curves correspond to the transition enthalpy of each oligomer duplex: decamer (+), undecamer (■), A-bulge (▲), and T-bulge (●).

This effect disappeared upon increasing the temperature to 20 °C except for that of the above strand, where the effect disappeared at 35 °C. This phenomenon could affect the values of our thermodynamic parameters at 20 °C. Figure 3 shows the typical linear dependence of $1/T_m$ on ln (C_T/4). The relevant data obtained from these experiments are presented in Table IV.

Differential Scanning Calorimetry and Nature of the Transitions. Typical excess heat capacity versus temperature profiles are presented in Figure 4. The area under these curves is proportional to the total endothermic heat needed to disrupt these duplexes into single strands. These heats when nor-

Table III: Helix–Coil Enthalpies Obtained from Melting Experiments[a]

	UV spectroscopy		calorimetry	
duplex	ΔH_{shape} (kcal·mol^{-1})	ΔH_{vH} (kcal·mol^{-1})	ΔH_{vH} (kcal·mol^{-1})	ΔH_{cal} (kcal·mol^{-1})
decamer	77	79	75	74.4
undecamer	83	84	84	81.6
T-bulge	69	68	71	71.2
A-bulge	60	63	62	59.0

[a] Values were taken in 10 mM NaP_i buffer containing 0.1 mM Na_2EDTA and 100 mM NaCl at pH 7.0. The transition enthalpies were obtained as follows: ΔH_{shape} from the shape of UV melts, ΔH_{vH} from $1/T_m$ vs ln $C_T/4$ plots, the calorimetric ΔH_{vH} from the shape of DSC curves, and ΔH_{cal} from the area of the DSC curves. The ΔH_{cal} values are within ±3%; all other van't Hoff enthalpies are within ±10%.

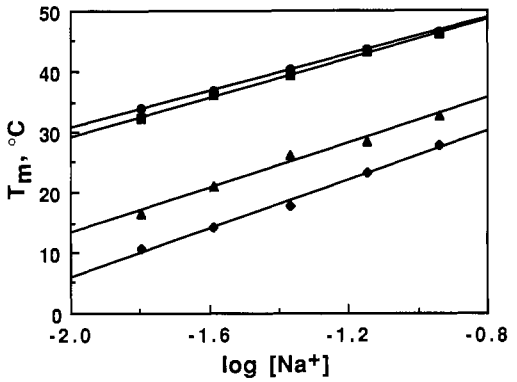

FIGURE 5: Salt dependence of transition temperature for the dissociation of each oligomer duplex in 10 mM sodium phosphate buffer containing 0.1 mM Na_2EDTA at pH 7, adjusted to the required NaCl concentration: decamer (●), undecamer (■), A-bulge (◆), and T-bulge (▲).

malized for the total number of moles of strands are equal to the molar denaturing enthalpies. The van't Hoff and calorimetric enthalpies measured from these curves are compared in Table III. The transition enthalpies of the fully paired duplexes are characteristic of the sum of the nearest-neighbor stacking interactions present in these molecules. In the case of the bulged duplexes relative to the fully paired decameric duplex, we measured a decrease of 3.2 kcal/mol in the enthalpy values for the T-bulge duplex and a decrease of 15.4 kcal/mol for the A-bulge duplex. Comparison of the van't Hoff enthalpies, calculated from the shape of the calorimetric or spectroscopic curves (Marky & Breslauer, 1987), with the transition enthalpies, measured directly by differential scanning calorimetry, allows us to draw conclusions about the nature of these transitions. At this salt concentration, we obtained $\Delta H_{vH}/\Delta H_{cal}$ ratios, of 1.00 and 1.03 for each of the fully paired duplexes and 1.00 and 1.05 for the transitions of the T- and A-bulge duplexes. All duplexes melted in a two-state transition manner. These results are also in quantitative agreement with the enthalpic values from the UV–melting studies, which are independently obtained observables. Comparison of these calorimetric enthalpies of duplex disruption (at $T = T_m$) with the enthalpies of duplex formation obtained from titration calorimetry at 20 °C (see Table II) indicate that the titration enthalpies are 20 kcal/(mol of duplex) smaller than the DSC enthalpies over an average temperature range of 23.5 °C. This difference corresponds to an unfavorable contribution of single-stranded stacking of the strands to the overall enthalpy formation of a duplex. Since all strands are very similar in comparison and also form duplexes that contained at least 7 of the 10 possible nearest-neighbor stacking interactions, we can conclude that the contribution of single-stranded stacking

Table IV: Parameters Used To Calculate Differential Counterion Binding[a]

duplex	dT_m/d log[Na$^+$] (K)	$RT_m^2/\Delta H$ (K)	Δn_{Na^+} [mol·(mol of duplex)$^{-1}$]
decamer	15.1	2.90	0.140
undecamer	16.2	2.64	0.148
T-bulge	18.6	2.81	0.168
A-bulge	19.8	3.30	0.152

[a] Values are taken in 10 mM NaP_i buffer containing 0.1 mM Na_2-EDTA at pH 7.0 and were adjusted to the desired ionic strength with NaCl. The slopes of the T_m vs log Na$^+$ plots were obtained by least-squares analysis with at least a 99% confidence level. The values of $RT_m^2/\Delta H_{cal}$ represent the average of at least five determinations each in buffer solutions containing 100 mM NaCl, with an absolute error of ±3.0%. The values of Δn are ±5%.

Table V: Complete Thermodynamic Profiles of Duplex Formation at 20 °C[a]

duplex	T_m (°C)	ΔG (kcal·mol^{-1})	ΔH (kcal·mol^{-1})	$T\Delta S$ (kcal·mol^{-1})	ΔV (mL·mol^{-1})
decamer	55.6	−6.0	−55.5	−49.5	−167
undecamer	55.0	−6.5	−61.1	−54.6	−208
T-bulge	43.4	−3.9	−53.2	−49.3	−285
A-bulge	39.4	−2.2	−36.0	−33.8	−312

[a] Values were taken in 10 mM NaP_i buffer containing 0.1 mM Na_2EDTA and 100 mM NaCl at pH 7.0. All the thermodynamic parameters are per mole of total duplex T_m values are within ±0.5 °C and correspond to a strand concentration of 200 μM; ΔG and $G\ \Delta S$ values are within 5%.

to the overall heat of forming a duplex at 20 °C corresponds to 0.86 kcal/(K·mol) of duplex or 81 cal/(K·mol) of base pairs. This is in fair agreement with the overall average of 63 cal/(K·mol) of base pairs obtained from the temperature dependence of the enthalpy in the calorimetric titration experiments.

Counterion Release. Figure 5 shows plots of the transition temperature versus log [Na$^+$]. An increase in the salt concentration results in an increase in the stability of all duplexes. A linear regression analysis of the T_m–log [Na$^+$] lines allowed us to estimate the slopes, which are proportional to the differential number of bound counterions between the single strand and duplex states. We obtained slope values ranging from 15 to 20 °C; see Table IV. These values are characteristic of ion-binding processes that take place with DNA duplexes of 10 base pairs (Marky, unpublished results). The estimation of the differential number of released counterions, Δn_{Na^+}, for the unfolding of a nucleic acid duplex to single strands was carried out as follows: Using the equation for calculating the average number of bound ions, Δn, where $\Delta n = d \ln K/d \ln (Na^+)$, and applying the chain rule and the van't Hoff equation, $d \ln K/dT = \Delta H/RT_m^2$, we obtain $dT_m/d \ln [Na^+] = -0.9\ (RT_m^2/\Delta H)\Delta n$ (Record et al., 1978); the negative sign indicates a release of counterions and the 0.9 value is a proportionality factor for converting mean ionic activities to ionic concentrations. All the experimental values used to calculate the counterion binding parameters for each transition are listed in Table IV. We obtained Δn_{Na^+} (per phosphate) values ranging from 0.14 to 0.17. These values are very close to the value of 0.17 obtained for polymeric DNA and indicate that oligomers as short as the ones reported here behave electrostatically as long rods, in agreement with a recent theoretical report (Fenley et al., 1991). The significant observation is that relative to the fully paired decamer duplex we obtained a somewhat higher counterion release (~ 0.01–0.03) with the undecamer and the bulge duplexes.

Complete Thermodynamic Profiles of Duplex Formation. In order to compare our thermodynamic results directly, we present in Table V tabulated values of the observed inde-

pendent variables ΔV and ΔH along with the calculated dependent variables ΔG and ΔS. All values refer to a common temperature of 20 °C. The ΔG functions were obtained from the equation $\Delta G = \Delta H(1 - 293.15/T_m)$, which assumes $\Delta Cp = 0$. The ΔH values used in this equation are the ones obtained in the calorimetric titrations at 20 °C that include single-strand base stacking heats if any [a slight improvement is obtained if one uses $\Delta H(T)$; unfortunately we only have one or two enthalpic values, 20 and 25 °C, that reflect the full formation of the duplex]. The T_m values correspond to the transition temperature of the duplexes at a common concentration of 200 μM (in strands), as used in the density measurements. The entropies are calculated from the standard thermodynamic relation $\Delta G = \Delta H - T\Delta S$. Overall, we obtained favorable free energies that result from partial compensation of favorable enthalpies with unfavorable entropies. The unfavorable entropies are in agreement with the uptake of water molecules observed in our density measurements and with the uptake of sodium ions as determined from our salt-dependence studies.

The significant observations are as follows: (1) The free energy change for the formation of the undecamer (fully paired) is 0.5 kcal/(mol of duplex) more favorable than that for the decameric one. This small increase in stability reflects the additional heat of formation (presumably due to the formation of an additional base-pair stack) that is not quite compensated by the observed loss in entropy. This loss in entropy is consistent with an apparent increase in hydration, as indicated by the larger volume contraction shown in the last column of Table V. (2) The free energy values per mole of duplex obtained for the formation of the bulge duplexes are 2.1 kcal/mol (T-bulge) and 3.8 kcal/mol (A-bulge) less favorable than for the same number of base pairings in the decameric duplex. These less favorable free energy changes are primarily enthalpic in origin because the entropy changes for the bulge duplexations were nearly as unfavorable as those for the nonbulge ones (column 5, Table V). Although the entropic contribution is only marginally less negative for the T-bulge duplex, a distinctly more favorable such change is evident in the formation of the A-bulge one. In both cases, however, the larger volume contractions (relative to the nonbulge duplexations) indicate a decrease in disorder owing to the apparent increase in electrostriction or other folding of water molecules. Notwithstanding, however, the greater reduction in unfavorable entropy for the A-bulge case compared to that for the T-bulge is noteworthy because we found a larger volume contraction (i.e., ordering effect) for the former duplexation (see Discussion). Notwithstanding, however, the greater reduction in unfavorable entropy for the A-bulge case compared to that for the T-bulge is noteworthy because we found a larger volume contraction (i.e., ordering effect) for the former duplexation (see Discussion).

Discussion

In order to discuss our observed thermodynamic parameters of the available structural information and molecular events, it is useful to cast the formation of a DNA duplex from two complementary single strands (SS_A and SS_B) in the general form

$$SS_A(a\mathrm{Na}^+, b\mathrm{H_2O}) + SS_B(c\mathrm{Na}^+, d\mathrm{H_2O}) \rightarrow \text{duplex } (x\mathrm{Na}^+, y\mathrm{H_2O}) + m\mathrm{Na}^+(z\mathrm{H_2O}) + n\mathrm{H_2O}$$

Each of the thermodynamic parameters reported in this paper at 20 °C and atmospheric pressure refers to the above reaction. Each single strand and duplex state will have associated sodium ions and bound water molecules. Formation of a duplex will result in the binding of counterions with a change in the overall hydration state of each of the species involved. Thus, the hydrated sodium ions and free water molecules on the right-hand side of the reaction will be negative in sign. Each of the relevant parameters for this reaction has been measured or calculated from experimental data, so that comparisons can be made directly.

Free Energies of Duplex Formation. The overall free energy of forming a DNA duplex will include the following contributions: two unfavorable free energy terms, one of nucleation due to the entropy loss of a bimolecular association and a second due to the symmetry of the sequence (which is a combinatorial factor due to the complementarity of the oligomers); a favorable free energy of propagation, which corresponds to the sum of the nearest-neighbors' base-stacking interactions derived from its primary sequence; and in the cases for the bulge duplexes, a last term that is due to the addition of an extra base, which will depend on the nature of the base as well as on the local environment (i.e., if the base is stacked within nearest-neighbor bases or bulging out). The first two terms are identical for all four duplexes so that the differences in the free energies will depend primarily on the oligomer sequence and on the presence of a bulge base. Proper comparisons can be made if these free energy terms are compared relative to the decamer duplex. The free energy difference between the undecamer duplex and the decamer duplex corresponds to the disruption of 1 AA/TT base-pair stack and the formation of two base pair stacks: 1 AT/TA and 1 TA/AT. This net formation of an extra base-pair stack corresponds to a difference in free energy of −0.5 kcal (Breslauer et al., 1986), in excellent agreement with our free energy difference of −0.5 kcal. The free energy differences with the bulge duplexes are 2.1 kcal/mol (T-bulge) and 3.8 kcal/mol (A-bulge) less favorable than the free energy of the decamer duplex. Thus, inserting an extra base between the bases of an AA/TT base-pair stack clearly reduces the overall stability. This extra base can be stacked between the two helical residues, causing stretching of the opposite phosphodiester backbone, or it can lie outside the helix (completely exposed to solvent or lying in the grooves of the DNA). We can speculate about the conformation with our values of the counterion uptake, which can distinguish between these two possibilities. If the base is intrahelical, we will expect a counterion uptake for these bulge duplexes that is between the values obtained for the fully paired duplexes. The observed counterion releases are larger than both paired duplexes by ~0.01–0.03 mol/(mol of duplex); therefore, we propose that the extra base in both bulges is outside (extrahelical), causing an increase in the local charge density due to the close proximity of the negatively charged phosphate groups. This suggests an increased binding of counterions and water molecules. From enthalpy considerations one can arrive at similar conclusions (see below). This picture is in agreement with the NMR experiments of Morden's group (Morden et al. (1990) and personal communication).

Enthalpies of Duplex Formation. The observed binding enthalpies for the formation of each duplex from two complementary strands show some differences: (1) These measured enthalpies will comprise endothermic contributions from the disruption of the single-stranded stacking interactions prior to duplexation, and (2) exothermic contributions are expected upon formation of a duplex, which include base-stacking interactions, specific H-bonding, and van der Waals interactions and probably also an exothermic contribution from an increase in hydration. We can assume that ion-pair formation con-

tributes negligibly because any opposing charges appear to be well separated in these duplexes. The formation of an extra base-pair stack in going from the decamer to the undecamer duplex increases the exothermicity of the enthalpy by 5.6 kcal/mol at 20 °C (7.2 kcal/mol at the T_m if we use the DSC enthalpies) in good agreement with the 5.5 kcal/mol value at 25 °C predicted from the nearest-neighbor stacking enthalpies (Breslauer et al., 1986). For the bulge duplexes relative to the decamer duplex, the enthalpies per mole at 20 °C are less exothermic by 2.3 kcal (T-bulge) and 19.5 kcal (A-bulge) (see Table II), or 3.2 and 15.4 kcal if DSC enthalpies are used (Table III). This unfavorable enthalpic effect is consistent with a net loss of base-stacking interactions, especially if the extra base is adenine. In the case of the A-bulge duplex, if the extra adenine is stacked between the TT/AA base-pair stack, the expectation is to obtain an enthalpic value similar to that of the fully paired decamer duplex because the disruption of one full TT/AA base-pair stack is compensated by the formation of two partial TA and AT stacks. Positioning the large ring of adenine outside of the helix will produce an endothermic local helical perturbation that involves loss of base-stacking interactions, overwhelming any exothermicity from an increase in the state of hydrating water. With the smaller ring of thymine this local perturbation is much less and the AA/TT base pair stack is maintained intact, so perhaps the net result is changes in hydration that are less enthalpic.

Entropy of Duplex Formation. In a similar fashion the derived entropy change is equal to the sum of the following contributions: $\Delta S_{\text{molecularity}}$ (the loss of entropy due to a bimolecular association reaction), $\Delta S_{\text{symmetry}}$ (the loss in entropy due to the complementary nature of the oligomers), $\Delta S_{\text{conformation}}$ (changes in the oligomer configuration in going from single strand to duplex), $\Delta S_{\text{counterions}}$ (uptake or release of counterions), and $\Delta S_{\text{hydration}}$ (uptake of water molecules). Each of the first three contributions is presumed to be identical for both bulge and nonbulge duplexations. The fourth term is marginally larger for the formation of the bulge duplexes. The fifth term is substantially different for the four systems according to our ΔV results, i.e., a putative increase in the hydration state was inferred for the bulge duplexes relative to the fully paired duplexes. These entropic terms will be discussed in the following sections.

Comparison of Our Bulge Free Energies with Previous Works. Our reported free energy values refer to the formation of a duplex at 20 °C and takes into account the contribution of single-strand stacking. In order to make a fair comparison with the reports of other laboratories in which thermodynamic parameters were obtained from optical melting data, we have calculated the free energy for each of the bulges and decamer duplexes using the van't Hoff enthalpies (see Table III) obtained form the slopes of the T_m concentration dependence plots. For the bulge duplexes relative to the decamer duplex, the free energies per mole at 37 °C are less favorable by 3.1 kcal (T-bulge) and 4.0 kcal (A-bulge), in close agreement with the recently reported $\Delta\Delta G$(37 °C) values of 3.6 and 4.5 kcal/mol for the T and A bulges with similar flanking bases and overall environment of A·T base pairs (LeBlanc & Morden, 1991). Several other laboratories have reported this destabilizing effect of a bulge ranging from 2.7 to 4.6 kcal/mol DNA molecules (Patel et al., 1982; Morden et al., 1983, 1990; Woodson & Crothers, 1987; LeBlanc & Morden, 1991), and from 2.8 to 3.7 kcal/mol with RNA molecules (Fink & Crothers, 1972; Longfellow et al., 1990). The actual values depend on the nature of the bulged base and flanking bases and on overall oligomer stability.

Table VI: Complete Thermodynamic Parameters[a] for the Reaction Decamer + Undecamer ↔ A-Bulge + T-Bulge

$\Delta\Delta G^b$	+6.4 ± 0.3 kcal·mol^{-1}
$\Delta\Delta H^c$	+27.4 ± 1.1 kcal·mol^{-1}
$\Delta\Delta H^d$	−25.8 ± 0.8 kcal·mol^{-1}
$\Delta(T\Delta S)$	+21.0 ± 1.0 kcal·mol^{-1}
$\Delta\Delta V$	−222.0 ± 16 mL·mol^{-1}
$\Delta\Delta n_{Na^+}$	0.03 ± 0.08 mol

[a] Values were taken in 10 mM NaP_i buffer containing 0.1 mM Na_2EDTA and 100 mM NaCl at pH 7.0 and are per mole of duplex. [b] This value was normalized by using the enthalpies obtained from titration calorimetry as described in the text. [c] Enthalpy obtained from titration calorimetry. [d] Enthalpy value obtained from DSC experiments (disruption of duplexes) assuming $\Delta Cp = 0$. This value is included to show internal consistency with the enthalpy change obtained from titration calorimetry.

Differential Thermodynamic Profiles of dA·dT Base Pairing. When the observed thermodynamic profiles for the fully paired duplexes are considered in terms of a differential effect, that is, if each thermodynamic parameter of the decamer duplex is subtracted from the corresponding parameter of the undecamer duplex (this assumes that the coil states are similar), we form an extra dA·dT base pair in the middle of an existing TAAT/ATTA core. We obtained a small $\Delta\Delta G$ term of −0.5 kcal/mol, which is the result of a compensation of a favorable $\Delta\Delta H$ of −5.6 kcal/mol and an unfavorable $\Delta(T\Delta S)$ of −5.1 kcal/mol; this last term correlates in sign with the $\Delta\Delta V$ of −41 mL (mol of duplex). Since the overall counterion binding is very similar in the formation of these two duplexes, this result strongly suggests that the addition of an extra dA·dT base pair to an existing AT segment is accompanied by an uptake of water molecules. If we assume further that this water is purely electrostricted with an average molar volume of 15.5 mL/mol (Millero et al., 1974) then this differential entropy corresponds to a net hydration of about 16 mol of water molecules/mol of base pair. Obviously, more sequences must be studied before this result can be generalized.

Differential Thermodynamic Profiles of Looped-Out Bases. When the observed thermodynamic parameters are considered in terms of a differential effect, (whereby the decamer and undecamer reference parameters are subtracted from those of the bulge-containing duplexes), all random coil states cancelled out exactly to give the values shown in Table VI. These results show that, in the composite reaction linking all four duplexes (Table VI), those duplexes not containing the bulges would exist in greater concentration at equilibrium. As noted before, the net positive enthalpy attending the formation of the bulge-containing duplexes provides the basis for the reduced free energy of their formation since the net entropy change is also positive. On the other hand, the clearly larger volume contraction observed for the composite reaction in this direction would, by itself, correlate with an entropy decrease, if indeed the contraction represents only the immobilization of water dipoles.

Differential Hydration versus Differential Entropies. As noted above, the larger contractions in volume observed upon duplexation of those complementary strands that give rise to a bulge suggest that an additional ordering of water by electrostriction occurs when compared to the lesser contraction seen upon duplexing fully complementary strands. It is highly premature at this stage in our knowledge on volume–structure relationships to speculate upon the bases for the additional volume contractions observed when a bulge duplex is formed. The observation runs counter to intuition based on the introductory remarks for electrostrictive enhancement by geometric

factors because the bulge phosphates are believed to lie outside the DNA cylinder and may not contribute to the focusing of the electric field along the grooves. We also rule out the possible formation of a void region within duplexes containing an unpaired nucleotide because void inclusions lead to an expansion of the volume (this assumes that no voids can exist within the dissolved single strands prior to the mixing). On the other hand, the hydrophobic moiety of the bulge nucleotide may occupy cavity space present within the structure of normal water. Structural hydration involving a strengthened H-bonded network near nonpolar solutes usually occurs with an economy of space (Desnoyers, 1977). Thus, at our current level of knowledge, the additional volume contraction accompanying the formation of duplexes containing a bulge is consistent with an increased level of coulombic hydration (electrostriction) and/or a structural hydration component whereby the nonpolar moiety of the unmatched base protrudes into the void space of the solvent. The volume decrease in the latter case implies that the single strands prior to mixing adopt an average conformation that shields the bases from the surrounding solvent. The heat transfer data do show a loss of stability for these duplexes, which is concerned with an unfavorable entropy change associated with an increase in hydration. Notwithstanding, however, the overall entropy change of the composite reaction linking all bulge and nonbulge duplexes was found to be positive (Table VI). Thus, if our measurements are accurate, other contributions to the entropy of a positive nature must override that from an increased ordering by electrostriction and structural hydration. Accordingly, we suggest that the destabilization of duplexes containing a bulge is attended by an increase in the hydration, which in itself acts to stabilize any double-stranded structure as determined by others long ago. In effect, the additional hydration here partially compensates for the destabilizing effect of the bulge. As such, this presumed role for water would have biological importance in maintaining the many examples of bulges and mismatches found in native DNA. Obviously, one needs to extend this study to include not only the bulges presented by cytosine and guanine nucleotides but also the positioning of any unpaired nucleotide in the test oligomers.

Acknowledgments

We thank Dr. Arthur Pardi for his generous gift of decameric strands that were used in preliminary measurements of this work, Dr. S. Nancy Marky for valuable discussions, Professor Neville R. Kallenbach for critical reading of the manuscript, and Beverly Shank for technical assistance.

References

Ames, B., Lee, F. D., & Durston, W. E. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 782–786.

Breslauer, K. J., Frank, R., Blocker, H., & Marky, L. A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 84*, 3746–3750.

Buckin, V. A., Kankiya, B. I., Burlichov, N. V., Lebedev, A. V., Gukovsky, I. Ya., Chuprina, V. P., Sarvazyian, A. P., & Williams, A. R. (1989) *Nature (London) 340*, 321–322.

Cantor, C. R., Warshaw, M. M., & Shapiro, H. (1970) *Biopolymers 9*, 1059–1077.

Chapman, R. E., & Sturtevant, J. M. (1969) *Biopolymers 7*, 527–537.

Conrad, J., Troll, M., & Zimm, B. J. (1988) *Biopolymers 27*, 1711–1732.

Conway, B. E. (1981) in *Ionic Hydration in Chemistry and Biophysics*, Elsevier, New York.

Desnoyers, J. E. (1977) *Phys. Chem. Liq. 7*, 63–108.

Drude, P., & Nernst, W. (1894) *Z. Physik Chem. 15*, 79–85.

Falk, M., Hartman, K. A., Jr., & Lord, R. C. (1962) *J. Am. Chem. Soc. 84*, 3843–3846.

Fenley, M. O., Manning, G. S., & Olson, W. K. (1991) *Biopolymers* (in press).

Fink, T. R., & Crothers, D. M. (1972) *J. Mol. Biol. 66*, 1–12.

Freier, S. M., Kierzek, R., Jaeger, J. A., Sugimoto, N., Caruthers, M. H., Neilson, T., & Turner, D. H. (1986) *Proc. Natl Acad Sci U.S.A. 83*, 9373–9377.

Gillies, G. T., & Kupkè, D. W. (1988) *Rev. Sci. Instrum. 59*, 307–313.

Gralla, J., & Crothers, D. M. (1973) *J. Mol. Biol. 73*, 497–511.

Groebe, D. R., & Uhlenbeck, O. C. (1989) *Biochemistry 28*, 742–747.

Hare, D., Shapiro, L., & Patel, D. J. (1986) *Biochemistry 25*, 7456–7464.

Joshua-Tor, L., Rabinovich, D., Hope, H., Frolow, F., Appella, E., & Sussman, J. L. (1988) *Nature 334*, 82–84.

Kalnik, M. W., Norman, D. G., Swann, P. F., & Patel, D. J. (1989a) *J. Biol. Chem. 264*, 3702–3712.

Kalnik, M. W., Norman, D. G., Zagorski, M. G., Swann, P. F., & Patel, D. J. (1989b) *Biochemistry 28*, 294–303.

Kalnik, M. W., Norman, D. G., Li, B. F., Swann, P. F., & Patel, D. J. (1990) *J. Biol. Chem. 265*, 636–647.

Kauzmann, W., Bodanszky, A., & Rasper, J. (1962) *J. Am. Chem. Soc. 84*, 1777–1788.

King, E. J. (1969) *J. Phys. Chem. 73*, 1220–1232.

Kupke, D. W., & Beams, J. W. (1972) *Methods Enzymol. 26*, 74–107.

Kupke, D. W., & Shank, B. S. (1989) *J. Phys. Chem. 93*, 2101–2106.

LeBlanc, D. A., & Morden, K. M. (1991) *Biochemistry 30*, 4042–4047.

Lerman, L. S. (1963) *Proc. Natl Acad Sci U.S.A. 83*, 94–102.

Longfellow, C. E., Kierzek, R., & Turner, D. H. (1990) *Biochemistry 29*, 278–285.

Marky, L. A., & Breslauer, K. J. (1987) *Biopolymers 26*, 1601–1620.

Marky, L. A., & Kupke, D. W. (1989) *Biochemistry 28*, 9982–9988.

Marky, L. A., Canuel, L., Jones, R. A., & Breslauer, K. J. (1981) *Biophys. Chem. 13*, 141–149.

Miller, M., Harrison, R. W., Wlodawer, A., Apella, E., & Sussman, J. L. (1988) *Nature 334*, 85–86.

Millero, F. J. (1971) *Chem. Rev. 71*, 147–176.

Millero, F. J., Wand, G. K., Lepple, F. K., & Hoff, E. V. (1974) *J. Phys. Chem. 78*, 1636–1643.

Morden, K. M., Chu, Y. G., Martin, F. H., & Tinoco, I., Jr. (1983) *Biochemistry 22*, 5557–5563.

Morden, K. M., Gunn, B., & Maskos, K. (1990) *Biochemistry 29*, 8835–8845.

Nikonowicz, E., Roongta, V., Jones, C. R., & Gorenstein, D. G. (1989) *Biochemistry 28*, 8714–8725.

Patel, D. J., Kozlowski, S. A., Marky, L. A., Rice, J. A., Broka, C., Itakura, K., & Breslauer, K. J. (1982) *Biochemistry 21*, 445–451.

Prive, G. G., Yanagi, K., & Dickerson, R. E. (1991) *J. Mol. Biol. 217*, 177–199.

Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.

Roy, S., Sklenar, V., Appella, E., & Cohen, D. S. (1987) *Biopolymers 26*, 2041–2052.

Senter, J. P. (1969) *Rev. Sci. Instrum. 40*, 334–338.

Stillinger, F. H. (1980) *Science 209*, 451–457.

Streisinger, G., Okada, Y., Emrich, J. N., Tsugita, E., & Inouye, M. (1966) *Cold Spring Harbor Symp. Quant. Biol. 31*, 77–84.
Troll, M., Roitman, D., Conrad, J., & Zimm, B. H. (1986) *Macromolecules 19*, 1186–1194.
Uhlenbeck, O. C., Borer, P. N., Dengler, B., & Tinoco, I. (1973) *J. Mol. Biol. 73*, 483–496.
van den Hoogen, Y., Th., van Beuzekom, A. A., van den Elst, H., van der Marcel, G. A., van Boom, J. H., & Altona, C. (1988) *Eur. J. Biochem. 178*, 1–38.
Wiseman, T., Williston, S., Brandts, J. F., & Lin, L.-N. (1989) *Anal. Biochem. 179*, 131–137.
Woodson, S. A., & Crothers, D. M. (1987) *Biochemistry 26*, 904–912.
Woodson, S. A., & Crothers, D. M. (1988a) *Biochemistry 27*, 436–445.
Woodson, S. A., & Crothers, D. M. (1988b) *Biochemistry 27*, 3130–3141.
Woodson, S. A., & Crothers, D. M. (1989) *Biopolymers 28*, 1149–1177.

DNA Unwinding Produced by Site-Specific Intrastrand Cross-Links of the Antitumor Drug *cis*-Diamminedichloroplatinum(II)†

Steven F. Bellon,‡ Julia H. Coleman, and Stephen J. Lippard*
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Received April 9, 1991; Revised Manuscript Received June 6, 1991

ABSTRACT: The DNA unwinding produced by specific adducts of the antitumor drug *cis*-diamminedichloroplatinum(II) has been quantitatively determined. Synthetic DNA duplex oligonucleotides of varying lengths with two base pair cohesive ends were synthesized and characterized that contained site-specific intrastrand N7-purine/N7-purine cross-links. Included are *cis*-[$Pt(NH_3)_2${d(GpG)}], *cis*-[$Pt(NH_3)_2${d(ApG)}], and *cis*-[$Pt(NH_3)_2${d(GpTpG)}] adducts, respectively referred to as *cis*-GG, *cis*-AG, and *cis*-GTG. Local DNA distortions at the site of platination were amplified by polymerization of these monomers and quantitatively evaluated by using polyacrylamide gel electrophoresis. The extent of DNA unwinding was determined by systematically varying the interplatinum distance, or phasing, in polymers containing the adducts. The multimer that migrates most slowly gives the optimal phasing for cooperative bending, from which the degree of unwinding can be obtained. We find that the *cis*-GG and *cis*-AG adducts both unwind DNA by 13°, while the *cis*-GTG adduct unwinds DNA by 23°. In addition, experiments are presented that support previous studies revealing that a hinge joint forms at the sites of platination in DNA molecules containing *trans*-GTG adducts. On the basis of an analysis of the present and other published studies of site-specifically modified DNA, we propose that local duplex unwinding is a major determinant in the recognition of DNA damage by the *Escherichia coli* (A)BC excinuclease. In addition, local duplex unwinding of 13° and bending by 35° are shown to correlate well with the recognition of platinated DNA by a previously identified damage recognition protein (DRP) in human cells.

cis-Diamminedichloroplatinum (*cis*-DDP[1] or cisplatin) is one of the most effective anticancer drugs. Its probable mode of action involves formation of platinum–DNA adducts capable of blocking DNA replication (Bruhn et al., 1990; Heiger-Bernays et al., 1990; Pinto & Lippard, 1985). The interaction with and processing of cisplatin–DNA adducts by cellular proteins, referred to as damage recognition proteins or DRPs, are important aspects of the molecular mechanism. These proteins may be repair enzymes that excise platinum damage from DNA, or they may bind to the sites of platination, rendering them inaccessible to other cellular components. Both possibilities are potentially relevant to the mechanism of action (Donahue et al., 1990; Ciccarelli et al., 1985; Eastman & Schulte, 1988; Gibbons et al., 1990; Hoeijmakers et al., 1990; Sheibani et al., 1989). It is therefore of considerable importance to understand the structures of the major cisplatin–DNA adducts and to learn how these structures modulate their interaction with DRPs. From such knowledge it may be possible to explain why *cis*-DDP is active while its trans isomer is not and why the drug is effective against only certain types of cancers and eventually to facilitate the rational design of more effective chemotherapeutic agents.

cis-DDP binds bifunctionally to DNA with a high affinity for the purine N7 positions. As a result, a variety of adducts form readily when the platinum complex binds to random sequence DNA (Eastman, 1986; Fichtinger-Schepman et al., 1985, 1987). It is not yet clear which are responsible for the anticancer activity. The relative amounts of the different

† This work was supported by U.S. Public Health Service Grant CA 34992.
‡ Whitaker Health Sciences Predoctoral Fellow.

[1] Abbreviations: DDP, diamminedichloroplatinum(II); *cis*-GG, *cis*-[$Pt(NH_3)_2${d(GpG)}] intrastrand cross-link; *cis*-AG, *cis*-[$Pt(NH_3)_2${d(ApG)}] intrastrand cross-link; *cis*-GTG, *cis*-[$Pt(NH_3)_2${d(GpTpG)}] intrastrand cross-link; *cis*-GXG, *cis*-[$Pt(NH_3)_2${d(GpXpG)}] intrastrand cross-link (where X is any nucleoside); *trans*-GXG, *trans*-[$Pt(NH_3)_2${d(GpXpG)}] intrastrand cross-link (where X is any nucleoside); DRP, damage recognition protein; HPLC, high-performance liquid chromatography; en, ethylenediamine (when used in conjunction with any of the abbrevations for *cis*-DDP adducts, the corresponding [$Pt(en)Cl_2$] adduct is implied); dach, 1,2-diaminocyclohexane; AAF, *N*-acetylaminofluorene. GG20, GG21, AG21, GTG20, etc. are terms used to identify duplex oligonucleotide monomers, as defined in Figure 1; *cis*-GTG21, *cis*-AG, etc. are terms used to denote these oligonucleotides containing *cis*-[$Pt(NH_3)_2${d(GpTpG)}], *cis*-[$Pt(NH_3)_2${d(ApG)}], etc. intrastrand cross-links.

0006-2960/91/0430-8026$02.50/0