

Sequence Effects on the Relative Thermodynamic Stabilities of B-Z Junction-Forming DNA Oligomeric Duplexes

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ABSTRACT Circular dichroism (CD) and ultraviolet absorption techniques were employed in characterizing the sequence-dependent thermodynamic stabilities of B-Z junction-forming DNA duplexes. The Watson strand of the duplexes has the general sequence $(5meC-G)_4-NXYG-ACTG$ (where $N = A$ or G and XY represents all permutations of pyrimidine bases). Duplexes were generated by mixing stoichiometric amounts of the complementary strands. Circular dichroism studies indicate that each duplex is fully right-handed at low salt (e.g., 115 mM Na^+) but undergoes a salt-induced conformational transition to a structure that possesses both left- and right-handed conformations at high salt (4.5 M Na^+), and hence a B-Z junction. Optical melting studies of the DNA duplexes at fixed DNA concentration with total Na^+ concentration ranging from 15 mM to 5.0 M were determined. A nonlinear dependence of the melting temperature (T_m) on $[Na^+]$ was observed. Thermodynamic parameters at Na^+ concentrations of 115 mM and 4.5 M with a wide range of DNA concentrations were determined from UV optical melting studies via construction of van't Hoff plots. A change of a single dinucleotide within these duplexes significantly affected the helix stabilities. The experimentally obtained free energies for the duplex to single-strand transitions were in close agreement with predicted values obtained from two different methods.

INTRODUCTION

The conformational variability of DNA structures has been involved in biological functions such as mutagenesis, control of transcription, and recognition of regulatory proteins. This has motivated many researchers to investigate the dynamics and stability of DNA secondary structures under a variety of conditions. Unusual DNA structures are of interest from a biological point of view, because they may act as recognition sites for DNA binding and modifying proteins, as well as small molecules such as intercalators. Several studies of thermodynamic stability of both usual and unusual DNA structures have been presented (for example, Breslauer et al., 1986; Manzini et al., 1987; Marky et al., 1988; Senoir et al., 1988; Gaffney and Jones, 1989; LeBlanc and Morden, 1991; Li et al., 1991; Doktycz et al., 1992; Paner et al., 1992, 1996; Sheardy et al., 1994). These studies confirm that the conformation of a DNA duplex is strongly dependent on both its base sequence and the conditions under which it is prepared.

Our laboratory has extensively studied B-Z junction-forming DNA duplexes focusing on structural and dynamic properties as well as drug-binding propensities (Sheardy, 1988; Sheardy and Winkle, 1989; Winkle et al., 1991; Guo et al., 1991; Suh et al., 1991; Lu et al., 1992; Sheardy et al., 1993, 1994). The starting point for these investigations was BZ-I (Fig. 1). This 16-bp oligomeric duplex exists as a right-handed (B-form) structure under "low salt" (i.e., <0.5 M Na^+) conditions. However, as the concentration of bulk

Na^+ concentration increases to ~ 4.5 M, the duplex undergoes a conformational transition to a hybrid possessing both a left-handed segment (Z-form) and a right-handed segment, separated by a conformational interface designated as a B-Z junction (Sheardy, 1988).

To evaluate the effects of sequence on junction formation and duplex stability, we have investigated the thermodynamic properties of BZ-I and related duplexes, BZ-II, BZ-V, and BZ-VI (Sheardy et al., 1993, 1994). Together, this set of duplexes represents all pyrimidine permutations of base sequence in the upper strand at positions 10 and 11. These studies evaluated the free energy of junction formation, ΔG_j , as well as the free energy of duplex formation, ΔG° . In the first study, it was demonstrated that the free energy of junction formation was found to be dependent on the dinucleotide sequence abutting the junction (Sheardy et al., 1993). The results indicated that the free energy of junction formation varied in a linear fashion with the calculated stacking free energy of the base pairs abutting the junction. In other words, the more stably stacked the base pairs at positions 9–12, the higher the free energy of junction formation. This correlation arises from the relative difficulty of perturbing the adjacent dinucleotide step to accommodate the junction. In addition, these studies indicated that the B-to-Z transition can best be modeled as a three-step process. The second set of studies demonstrated that the experimentally evaluated free energy of duplex formation was consistent with theoretically determined free energies. More significantly, this study indicated that the presence of the junction destabilized the duplex by only 0.5 kcal/mol (Sheardy et al., 1994).

The objective of this investigation is to further examine the sequence dependence of the helix stability of B-Z junction-forming duplexes. The sequences in Fig. 1 are designed in such a manner that the contiguous $(5meC-G)_n$ repeat

Received for publication 17 March 1997 and in final form 15 August 1997.

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0006-3495/97/12/3135/07 \$2.00

A-Series

BZ-I: (5meCG)₄-**ACTG**-ACTG
 BZ-II: (5meCG)₄-**ATCG**-ACTG
 BZ-V: (5meCG)₄-**ATTG**-ACTG
 BZ-VI: (5meCG)₄-**ACCG**-ACTG

G-Series

BZ-VII: (5meCG)₄-**GCTG**-ACTG
 BZ-VIII: (5meCG)₄-**GTCG**-ACTG
 BZ-IX: (5meCG)₄-**GTTG**-ACTG
 BZ-X: (5meCG)₄-**GCCG**-ACTG

FIGURE 1 The DNA duplexes discussed in this report. The permutations in the sequences at positions 9–12 are highlighted in bold. Only the Watson strands are shown; each duplex was generated by combining stoichiometric equivalents of each strand with its Crick complement. 5meC is 5-methylcytosine.

assumes left-handed conformation under high salt conditions, whereas abutted sequences remain right-handed under all conditions. 5-Methylcytosine (5meC) is used to facilitate the B-to-Z transition for this segment (Behe and Felsenfeld, 1981). The A-series duplexes are those already studied, but are included here for total comparison. The G-series duplexes are analogous to the A-series, and the A:T base pair at position 9 has been replaced by a G:C base pair. In addition, the availability of 16 total component single-strand oligomers allows for mixing noncomplementary strands to generate mismatches at or near potential B-Z junctions for additional studies.

According to the NMR studies of the BZ-I, the junction itself spans only 3 bp at positions 7, 8, and 9 (Sheardy and Winkle, 1989). One may imply that the putative B-Z junction of these duplexes would span 3 bp, and at the same positions as BZ-I. The sequences shown in Fig. 1 represent all permutations of the upper strand of BZ-I at positions 9, 10, and 11 while maintaining a *pur-pyr-pyr* motif at those positions. The sequence-dependent effects on the relative thermal stability and the resultant thermodynamic parameters of B-Z junction-forming duplexes are hereby reported.

Although the biological relevance of Z DNA and B-Z junctions has yet to be established, these conformations are still interesting from a structural point of view. The DNA duplexes designed for this study enable us to further investigate the effects of sequence on duplex stability and ease of junction formation for B-Z junction-forming DNA duplexes.

MATERIALS AND METHODS**Synthesis and purification of oligonucleotides**

Individual component strands were synthesized with an applied Biosystems 380B DNA synthesizer (Perkin-Elmer, Foster City, CA), using phosphoramidite chemistry (Caruthers, 1991). Each strand synthesized was purified by C₁₈ reverse-phase high-performance liquid chromatography

(HPLC) as previously described (Sheardy, 1988; Sheardy et al., 1994), and purity of the strands was checked by polyacrylamide gel electrophoresis and analytical HPLC. The duplexes were formed by mixing equal amounts of complementary strands and temperature annealing them by heating at 80°C for 2 min, followed by slow cooling. The annealed duplexes were allowed to equilibrate at 4°C for 24 h before analysis. The extinction coefficient ϵ (L mol⁻¹ cm⁻¹ in base pairs) of the DNA duplexes is estimated to be, on the average, 13,000 (Fasman, 1975).

Circular dichroism studies

The CD spectra of the duplexes ([DNA] = 5.4 × 10⁻⁵ M in base pairs) in a phosphate buffer (10 mM phosphate, 0.1 mM EDTA, pH 7.0) at various total Na⁺ concentrations were recorded at 25°C with an AVIV 62A DS circular dichroism spectropolarimeter (AVIV Associates, Lakewood, NJ).

Optical melting studies

The DNA UV/VIS spectra were recorded with a Gilford Response II UV/VIS spectrophotometer equipped with a thermoset cuvette holder. DNA samples were prepared in standard phosphate buffer (10 mM phosphate, 0.1 mM EDTA, pH 7.0 buffer) at various total Na⁺ concentrations. For the first set of studies, the thermal denaturation of the DNA duplex as a function of [Na⁺] was carried out. For these experiments, the total Na⁺ concentration ranged from 15 mM to 5.0 M, and the DNA concentration was constant at 5.4 × 10⁻⁵ M (in base pairs). In a second set of experiments, the effects of DNA concentration on the thermal stability of the duplexes were examined at two fixed Na⁺ concentrations. Here the DNA concentration was varied from 1.0 × 10⁻⁵ M to 8.0 × 10⁻⁴ M (in base pairs), and the Na⁺ concentration was kept constant at 115 mM or 4.5 M. At 115 mM Na⁺ (low salt), all of the duplexes assume the right-handed B-DNA conformation; however, all of the duplexes form a complete hybrid B-Z conformation at 4.5 M Na⁺ (high salt). In many DNA optical melting studies, the prevalent salt concentration of 1.0 M NaCl has been used (Breslauer et al., 1986; Gaffney and Jones, 1989; LeBlanc and Morden, 1991). However, our duplexes show a slight conformational transition for the both the G-series duplexes and the analogous A-series duplexes (Sheardy et al., 1993) at this salt concentration.

The thermal denaturation of each duplex was monitored at 268 nm. The temperature was ramped from 20°C to 95°C at ~0.3°C/min, using the Gilford response II spectrophotometer equipped with a temperature programming. The DNA absorbance was recorded every 0.1°C after 10 consecutive identical readings of the temperature. The difference in absorbance of the DNA sample before the denaturation (20°C to 95°C) and after the reannealing (95°C to 20°C) of each oligomer duplex should not have exceeded 2%. In that case, the particular experiment was discarded and repeated.

A two-state (all-or-none) model was assumed for all of the DNA thermal denaturation experiments. The melting temperature (T_m) of the thermal denaturation was obtained by taking the first derivative of the sigmoidal curve of absorbance at 268 nm versus the temperature plot. The T_m is defined as the midpoint of transition (i.e., at $\alpha = 0.5$, where α is the fraction of single strands) of the sigmoidal curve, and it is equivalent to the T_{max} of the first derivative of the sigmoidal curve. Plots of $1/T_m$ versus $\ln C_T/4$ (where C_T is the total strand concentration, ranging from ~1.0 × 10⁻⁵ M to 8.0 × 10⁻⁴ M) were constructed to determine the van't Hoff enthalpies (ΔH°) and entropies (ΔS°). For non-self-complementary strands, the relationship of the duplex melting T_m and DNA concentration (Marky and Breslauer, 1987) is expressed as

$$1/T_m = (R/\Delta H^\circ) \ln C_T/4 + \Delta S^\circ/\Delta H^\circ \quad (1)$$

The free energies of the double-helix denaturation are calculated by the standard thermodynamic equation, expressed as

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

The experimental free energies reflect the effect of hydrogen bonding and base stacking of the oligomer sequences. It is assumed that the total theoretical free energy of the DNA duplex is a summation of the contributing nucleation, hydrogen bonding, and base stacking free energies. Using methods described earlier (Breslauer et al., 1986; Doktycz et al., 1992; SantaLucia et al., 1996), we also evaluated the theoretical free energies of duplex formation for comparison to the experimentally evaluated free energies. The comparison was made by consideration of only the base permutations in the middle of the oligomer (base pairs 8–11), thereby neglecting end effects. This comparison is thus predicated on the assumption that the nucleation free energy is about the same for each of the duplexes studied. Because of the similarities in length, sequence, and base content, this assumption is valid.

RESULTS

CD studies

The CD spectra of each of the G-series duplexes were determined in both low salt (115 mM Na⁺) and high salt (4.5 M Na⁺). A typical set of spectra is shown in Fig. 2 and is similar to those reported for the A-series duplexes (Sheardy et al., 1994). The low-salt spectra of all duplexes indicate right-handed B-DNA conformations, each with a characteristic deep trough at 255 nm and a peak at 280 nm. The high-salt spectra are indicative of hybrid B-Z DNA conformations, each with a shallow trough at 255 nm, a peak at 278 nm, and another very shallow trough at 295 nm (Sheardy, 1988; Sheardy et al., 1993). NaCl titrations indicate that the salt-induced conformational transition is complete at 4.5 M Na⁺ for all duplexes. Thus the duplexes of the G-series, like their A-series analogs, all possess B-Z conformational junctions at this Na⁺ concentration.

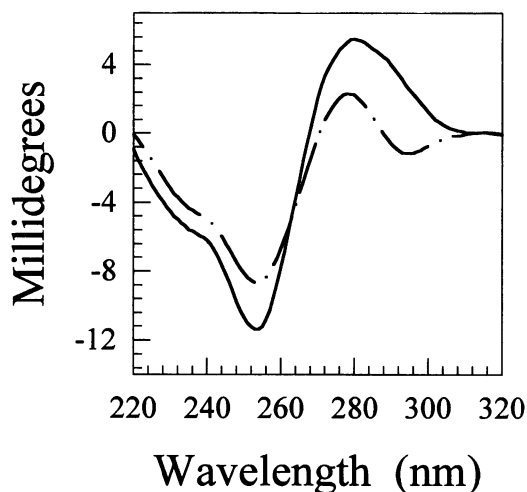


FIGURE 2 Typical CD spectra of a G-series oligomer (BZ-VII) with DNA concentration of 5.4×10^{-5} M in base pairs. CD spectra were obtained in 10 mM phosphate (pH 7.0) buffer at 25°C in 115 mM Na⁺ (—) or in 4.5 M Na⁺ (- - -). Similar spectra were obtained for the other duplexes in both low and high salt. The low-salt CD spectrum for each oligomer is consistent with a right-handed conformation (B-form). At high salt, the spectrum of each oligomer is consistent with a hybrid structure possessing both left-handed (Z-form) and right-handed conformations, indicative of the presence of a B-Z junction.

Optical melting studies

Melting profiles of the G-series duplexes at constant DNA concentration of 5.4×10^{-5} M in base pairs in phosphate buffer with pH maintained at 7.0 and Na⁺ concentrations ranging from 15 mM to 5.0 M are sigmoidal and appear to obey the two-state (all-or-none) model (data not shown). Selected T_m values for the G-series duplexes are shown in Table 1. These data reveal that the relative stabilities of the duplexes are base content dependent and sequence dependent.

Plots of T_m versus $\log[\text{Na}^+]$ for the G-series duplexes with salt concentration ranging from 15 mM to 5.0 M are shown in Fig. 3. The shapes of the plots are similar to those reported for the A series duplexes (Sheardy et al., 1994). In addition, each plot is strikingly similar to that predicted by Soumpasis et al. (1990), who used a potential of mean force (PMF) approach for treating ionic effects on DNA conformation and conformational transitions. The linear region of the low salt range (15–250 mM Na⁺) is expected in light of the counterion condensation theory (Manning, 1978; Record et al., 1978, 1981, 1990). The linearity observed in the high salt range (2.0 M to 5.0 M Na⁺) is similar to that of the A series duplexes (Sheardy et al., 1994). The linear least-squares fits of the data at the low salt range and at the high salt range give straight lines that intercept at an extrapolated point at a [Na⁺] of ~ 0.9 M. The slopes for the fitted lines of the low and high salt regions are, on average, 16.5 deg/log[Na⁺] and -17.5 deg/log[Na⁺], respectively. For comparison, the extrapolated intercepts of the low and high salt range linear fitted lines for the A-series duplexes were reported as ~ 1.04 M Na⁺, and the respective slopes were, on average, 16.9 deg/log[Na⁺] and -27.5 deg/log[Na⁺] (Sheardy et al., 1994).

The thermodynamic characteristics of the duplex-to-single-strand transitions were determined by plotting the reciprocal of T_m versus the natural logarithm of the total strand concentration, based on Eq. 1, for both the low salt and high salt conditions, as shown in Fig. 4. The linear least-squares fits of the data yield straight lines with slopes equal to $R/\Delta H^\circ$ and y intercepts equal to $\Delta S^\circ/\Delta H^\circ$ (Marky and Breslauer, 1987). The thermodynamic parameters for the duplex-to-single-strand transitions are listed in Table 2. For comparison, the previously published parameters for the A-series duplexes are also listed (Sheardy et al., 1994). As can be seen, the enthalpic and entropic contributions lead to

TABLE 1 Influence of [Na⁺] on the T_m of B-Z junction-forming DNA duplexes BZ-VII through BZ-X*

Oligomer	T_m (°C) at specified [Na ⁺]				$\delta T_m/\delta \log[\text{Na}^+]$ [#]
	15 mM	115 mM	815 mM	4.5 M	
BZ-VII	59.2	75.4	83.4	73.3	16.4 ± 0.3
BZ-VIII	59.6	75.4	83.3	74.5	16.3 ± 0.3
BZ-IX	55.1	72.8	80.5	72.3	17.0 ± 0.3
BZ-X	63.1	79.2	85.7	78.6	16.3 ± 0.3

*Experimental T_m values reported are $\pm 0.3^\circ\text{C}$.

[#]Determined over the range of 15–250 mM Na⁺.

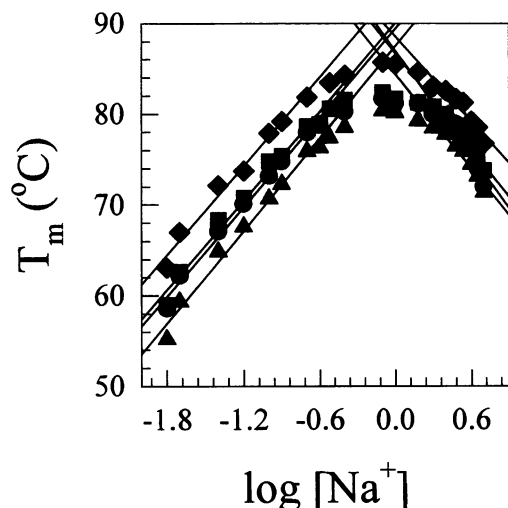


FIGURE 3 The variation in T_m as a function of $\log [Na^+]$, with Na^+ concentration ranging from 15 mM to 5.0 M and $[DNA] = 5.4 \times 10^{-5}$ M in (base pairs) in 10 mM phosphate buffer (pH 7.0) for BZ-VII (●), BZ-VIII (■), BZ-IX (▲), and BZ-X (◆). The least-squares linear regression analysis of the data from 15 mM to 250 mM and from 2.0 M to 5.0 M Na^+ resulted in high correlation fits with the resultant lines drawn.

a destabilization of the free energy of duplex formation by an average of 1.1 kcal/mol for the BZ hybrid conformation in high salt relative to B-DNA conformation at low salt. The observed difference in the free energy values for both low salt and high salt conditions, respectively, reflect base pair sequence dependence and accompanying compensatory changes in enthalpy and entropy. The data in Table 2 reveal the relative stability orders for the duplexes in both low and high salt: BZ-X > BZ-VII \approx BZ-VIII > BZ-VI > BZ-IX \approx BZ-II > BZ-I.

DISCUSSION

The objective of these studies was to ascertain the sequence-dependent stability of B-Z junction-forming DNA duplexes and form a basis for future studies with junction-forming molecules. The data presented indicate that the stabilities of the DNA duplexes are sequence dependent in a predictable fashion. The thermodynamic differences between the various permuted duplexes arise from changes in either hydrogen bonding, nearest-neighbor stacking interactions, or both. Replacing the A:T base pair at any position by a G:C base pair stabilizes the duplex by, on average, 1.0 kcal/mol. Deviations from this value reflect changes in base stacking as a result of the substitution.

The effects of the salt-induced conformational changes from a right-handed B-DNA to a left-handed Z-DNA structure are facilitated by the methylated cytosine of the contiguous $(5meCG)_n$ base pairs (Behe and Felsenfeld, 1981). The CD spectra of these duplexes all indicate the hybrid BZ form at 4.5 M Na^+ . The decreased stabilities of the duplexes at high salt are most likely due to the interplay of several factors, such as differential interaction of the solvent with

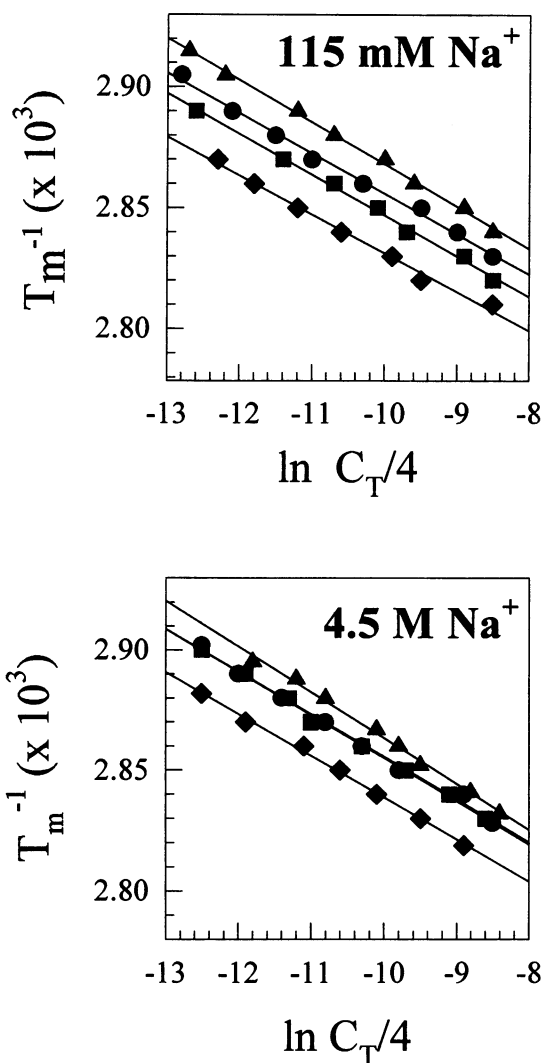


FIGURE 4 The van't Hoff plots ($1/T_m$ versus $\ln C_T/4$) in 10 mM phosphate buffer (pH 7.0) at the Na^+ concentrations indicated for BZ-VII (●), BZ-VIII (■), BZ-IX (▲), and BZ-X (◆). The lines represent the least-squares linear regression fits.

A-T and G-C base pairs, dehydration of the duplex, and the presence of the B-Z junction. Furthermore, destabilization by these factors may be partially counterbalanced by the greater thermal stability of base pairs in the Z conformation. In a previous study, the free energy of duplex formation for BZ-I was compared to that of the unmethylated sequence analog, which does not form a B-Z junction at high salt. The results indicated that the presence of the junction in BZ-I in high salt results in an additional, but slight, destabilization of the duplex relative to the unmethylated control (Sheardy et al., 1994).

The nonlinear dependence of T_m over the entire range of salt concentration in Fig. 3 has been observed by other investigators (Gruenwedel et al., 1971; Gruenwedel, 1975; Soumpasis et al., 1990; Record et al., 1990; Sheardy et al., 1994). The linear region in the low salt range of the plot in Fig. 3 may be interpreted in terms of the counterion con-

TABLE 2 Experimentally determined thermodynamic parameters for the duplex-to-single-strand transitions of B-Z junction-forming DNA duplexes

Oligomer	ΔH° (kcal/mol)	ΔS° (cal/Kmol)	ΔG° (kcal/mol)*
115 mM NaCl			
BZ-I	126 ± 2	347 ± 7	18.4 ± 0.3
BZ-II	121 ± 1	330 ± 5	18.7 ± 0.3
BZ-V	116 ± 1	318 ± 4	17.4 ± 0.2
BZ-VI	119 ± 2	321 ± 6	19.5 ± 0.3
BZ-VII	119 ± 2	320 ± 4	19.8 ± 0.3
BZ-VIII	118 ± 2	317 ± 6	19.7 ± 0.4
BZ-IX	114 ± 2	307 ± 5	18.8 ± 0.3
BZ-X	123 ± 3	330 ± 8	20.7 ± 0.4
4.5 M NaCl			
BZ-I	118 ± 3	325 ± 7	17.3 ± 0.3
BZ-II	116 ± 2	318 ± 5	17.4 ± 0.2
BZ-V	107 ± 1	293 ± 4	16.2 ± 0.2
BZ-VI	112 ± 2	302 ± 4	18.4 ± 0.2
BZ-VII	112 ± 3	301 ± 3	18.7 ± 0.3
BZ-VIII	111 ± 2	297 ± 4	18.9 ± 0.2
BZ-IX	104 ± 3	278 ± 6	17.8 ± 0.3
BZ-X	114 ± 2	305 ± 7	19.5 ± 0.4

* ΔG° values were determined at 37°C. Values reported for BZ-I, BZ-II, BZ-V, and BZ-VI are from Sheardy et al. (1994).

denaturation theories of Manning (1978) and Record and co-workers (1978, 1981, 1990). The average value of the slope $\delta T_m / \delta \log[\text{Na}^+]$ for the low salt region (15 mM to 250 mM Na^+) is 16.5 deg/ $\log[\text{Na}^+]$. This value is similar to that reported for polymeric calf thymus DNA (slope = 17.5 deg/ $\log[\text{Na}^+]$) and *M. lysodeikticus* DNA (slope = 16.8 deg/ $\log[\text{Na}^+]$), as reported for NaCl solutions (Gruenwedel et al., 1971).

The differential ion binding (Δn) reflects the difference in charge density between the duplex and single-stranded states and indicates how many counterions are released per duplex upon thermal denaturation. The differential binding term is expressed by Eq. 3 and assumes that ΔH_m is independent of $[\text{Na}^+]$ (Record et al., 1978):

$$\delta T_m / \delta \log[M] = \{2.303RT_m^2 / \Delta H_m\} \Delta n \quad (3)$$

T_m is the midpoint transition melting temperature of the duplex to single strands, $[M]$ is the molar concentration of monovalent counterion, ΔH_m is the enthalpy of the melting transition (equivalent to ΔH°), R is the gas constant, and Δn is the differential ion-binding term. Using the slope given above and the data reported in Tables 1 and 2, a value for $\Delta n = 3.5$ is determined. Similar results were reported for the A-series duplexes (Sheardy et al., 1994).

According to Eq. 4, the number of sodium ions released from a 16-bp segment embedded in a polynucleotide should be 5.1 (Record and Lohman, 1978; Olmsted et al., 1991):

$$\Delta n = N_p(\Psi_d - \Psi_c) \quad (4)$$

In Eq. 4, Ψ_d is the charge density of a polymeric duplex and has a reported value of 0.88 (Record et al., 1978; Olmsted et al., 1991); Ψ_c is the charge density of a polymeric random

coil and has a reported value of 0.71 (Record et al., 1978; Olmsted et al., 1991); and N_p is the number of phosphates in the DNA segment under consideration (for a linear 16-mer, $N_p = 30$). (For the calculation using Eq. 4, $N_p = 30$, $\Psi_D = 0.88$, and $\Psi_C = 0.71$, giving $\Delta n = 5.1$.) The value of 5.1 noted above thus represents the upper limiting value for Δn (Olmsted et al., 1991; Paner et al., 1996). For duplexes less than 24 bases in length, deviation from ideal polyelectrolyte behavior should not be surprising (Olmsted et al., 1991). Hence the difference between the experimental and calculated Δn is due to these deviations and may be due in part to a dependence of ΔH_m on sodium ion concentration (for example, Gruenwedel, 1971; Gruenwedel et al., 1975; Paner et al., 1996; Hicks et al., 1997).

The linear region of the T_m versus $\log[\text{Na}^+]$ from 2.0 to 5.0 M Na^+ may simply be due to the changes in water activity as a result of the high ionic strength. Changes in water activity will then influence the hydration of the nucleic acid. In fact, the destabilization of DNA at high salt concentrations has been attributed to dehydration and anion binding (Gruenwedel et al., 1971; Record et al., 1990). The difference between the extrapolated intercepts of the low and high salt linear regression lines for the A-series and G-series duplexes is most likely due to the higher GC content of the G-series and may be reflective of differences in hydration, which is certainly base content dependent (Tunis and Hearst, 1968; Buckin et al., 1989; Rentzeperis et al., 1993, 1994) and may also be due to differential anion uptake.

Table 3 lists the difference in free energies between all other duplexes and BZ-I (i.e., $\Delta \Delta G^\circ$). The differences in free energies of the duplexes result from a combination of differences in both hydrogen-bonding and base-stacking

TABLE 3 A comparison of the calculated and experimental free energies of duplex formation for B-Z junction-forming DNA duplexes*

Oligomer	$\Delta \Delta G^\circ$ experimental	$\Delta \Delta G^\circ$ calculated (Breslauer et al.) [#]	$\Delta \Delta G^\circ$ calculated (Doktycz et al.) [§]	$\Delta \Delta G^\circ$ calculated (SantaLucia et al.) [¶]
BZ-I	0.0	0.0	0.0	0.0
BZ-II	+0.3	+1.9	+0.2	+0.3
BZ-V	-1.0	+0.5	-0.8	-0.8
BZ-VI	+1.1	+3.2	+1.0	+1.3
BZ-VII	+1.4	+3.3	+1.4	+1.2
BZ-VIII	+1.3	+3.2	+1.4	+1.3
BZ-IX	+0.4	+1.8	+0.4	+0.2
BZ-X	+2.3	+6.5	+2.4	+2.5

*For the purpose of comparison, $\Delta \Delta G^\circ$ values are calculated by subtracting the ΔG° for a particular oligomer from that of BZ-I. The experimental values were those obtained at 115 mM Na^+ . For the calculated values, only the base-pair permutations at positions 8-12 were included, because inclusion of the other base pairs, end effects, and nucleation terms will simply cancel out. A positive $\Delta \Delta G^\circ$ value means that the DNA oligomer is more stable than BZ-I.

[#]Breslauer et al. (1986) (determined for 1.0 M NaCl).

[§]Doktycz et al. (1992) (determined for 115 mM Na^+).

[¶]SantaLucia et al. (1996) (determined for 1.0 M NaCl).

components. For example, the difference in the overall free energies between BZ-I, BZ-VII, and BZ-IX as well as between BZ-VI, BZ-VII, and BZ-VIII is solely dependent on the stacking free energy. The calculated $\Delta\Delta G^\circ$ values were determined by using nearest-neighbor free energies from three different methods for comparison to the experimentally determined values. As noted above, by comparing only the nearest-neighbor free energies for the base pair permutations at positions 8–12, one can neglect the nucleation term. As can be seen, comparisons between the difference in the change of the free energies ($\Delta\Delta G^\circ$) of the calculated and experimentally determined values are in excellent agreement with the methods of Doktycz et al. (1992) and SantaLucia et al. (1996). In general, the values calculated according to the method of Breslauer et al. (1986) are higher by 1.5–4 kcal/mol. However, the general trend in stabilities, with the exception of BZ-V, is in agreement with that observed. It should be noted that the published nearest-neighbor free energies of Doktycz et al. (1992) have been calculated at different Na^+ concentrations. Hence the $\Delta\Delta G^\circ$ values calculated by this method and listed in Table 3 were evaluated by using nearest-neighbor free energies at 115 mM Na^+ , the same concentration as our experimental denaturations. The published nearest-neighbor free energies of Breslauer and SantaLucia were calculated at 1 M NaCl. Hence differences between predicted and experimental $\Delta\Delta G^\circ$ values may be due to differences in the NaCl concentration.

The van't Hoff analysis of optical melting profiles is predicted on the assumptions that 1) ΔC_p is zero over the temperature range, and 2) the thermally induced transition can be described by a two-state model (Marky and Breslauer, 1987). In the absence of calorimetric data, these assumptions cannot be tested. In addition, the errors listed in Table 2 are statistical errors, whereas typical van't Hoff analysis results in errors up to 10%. However, the excellent agreement between the experimental $\Delta\Delta G^\circ$ values and the $\Delta\Delta G^\circ$ values calculated by three different models indicates that ΔC_p and deviations from two-state behavior are relatively insignificant. Finally, the reported thermodynamic profiles correspond to the two-state melting of a cooperative unit, not necessarily the whole oligomer.

SUMMARY

The stabilities of the DNA duplexes in Fig. 1 are sequence dependent, as indicated by the free energy values in Table 2, in a predictable fashion (Table 3). The nonlinear dependence of T_m at Na^+ concentrations ranging from 15 mM to 5.0 M has been proposed to be a combinational effect of electrostatic interactions and dehydration. The T_m of the duplexes increases up to a concentration of ~ 1.0 M Na^+ and then decreases at higher Na^+ concentrations. At 4.5 M Na^+ , the free energy of duplex formation for the duplexes decreases, on the average, by 1.1 kcal/mol relative to 115 mM Na^+ . This decrease is due to, among other factors,

destabilization from dehydration and the presence of the B-Z junction formed at high salt. The thermodynamic data indicate that the permutation of a single dinucleotide step within the duplexes significantly alters the energetics of the duplex melting. The changes in free energy of melting are accompanied by changes in both enthalpy and entropy. The experimentally determined $\Delta\Delta G^\circ$ values are in excellent agreement with the calculated $\Delta\Delta G^\circ$ values from two different sets of nearest-neighbor free energies.

Acknowledgment is made of the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. EOO is an American Chemical Society–Petroleum Research Fund Fellow.

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