Conformational Properties of Z-Forming DNA Oligomers Bearing Terminal Unpaired Bases

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ABSTRACT Three sets of semi-self-complementary deoxyribonucleotide decamers with the sequence XX-(5*me*CG)$_4$, (5*me*CG)$_4$-XX, or Y-(5*me*CG)$_4$-Y, where XX = AA, CC, GG, or TT and Y = A, C, G, or T, were synthesized along with the self-complementary octamer (5*me*CG)$_4$. The 8-mer duplex readily undergoes a B-to-Z conformational conversion upon increasing the NaCl concentration with a transitional midpoint of ~1.1 M NaCl. The 10-mers should form 8-bp duplexes a with core sequence of [(5*me*CG)$_4$]$_2$ with 5′-XX overhangs, 3′-XX overhangs, or 5′,3′-Y/Y mismatches. Circular dichroism was employed to determine the conformations of all oligomers. Salt titrations were performed to measure the effect of overhangs and terminal mismatches on the B-to-Z conversion. In general, the presence of 5′-XX overhangs results in a transition midpoint equal to or slightly higher than the control, whereas the presence of 3′-XX overhangs results in a transition midpoint slightly lower than the control. The 3′-CC and 5′-GG overhangs are exceptions, with transition midpoints much higher than the control. These oligomers apparently form duplexes with 5′,3′-C/C or 5′,3′-G/G mismatches abutting a [(G5*meC*)$_4$]$_2$ duplex core. The presence of terminal mismatches in the third set of oligomers results in transition midpoints higher than the control. Ultraviolet absorbance methods were used to evaluate the effect of the various stacking motifs of the 10-mers on the thermodynamics of melting relative to the 8-mer for both B and Z conformations. We found that in both the B and Z conformations, the presence of an overhang stabilizes the [(5*me*CG)$_4$]$_2$ duplex, with the 5′ overhangs having a greater stabilizing effect relative to the 3′ overhangs. The presence of 5′,3′-Y/Y mismatches also imparts a stabilizing effect on the control 8-mer in both the B and Z conformations. These results are discussed in terms of stacking interactions of the terminal unpaired bases.

INTRODUCTION

The thermodynamic effects of the base stacking of terminal unpaired bases overhanging the ends of double-helical RNA and DNA molecules have been investigated (Petersheim and Turner, 1983; Freier et al., 1985; Senior et al., 1988). In both instances, the presence of overhangs has a stabilizing effect on the free energies of helix formation relative to the same molecules lacking the overhangs. This stabilization was found to be due to favorable enthalpic contributions arising from stacking of the unpaired bases onto the duplexed core. Senior (Senior et al., 1988) investigated placing the dinucleotide TT on either the 5′ ends or the 3′ ends of the (GC)$_3$ and (CG)$_3$ DNA oligomers. For both oligomers, a greater stability was observed when the TT was overhanging the 5′ ends of the duplexed core relative to the 3′ ends. The RNA molecules showed the opposite trend, where a 3′ single overhang residue was more stabilizing relative to a 5′ residue (Petersheim and Turner, 1983; Freier et al., 1985).

A later study investigated the stability of a six-base pair stem-T$_4$ loop DNA hairpin (XYXY-GGATACTTTTG-TATCC, where X, Y = A, T, C, or G) with a series of four base overhangs on the 5′ end of the duplexed stem (Doktycz et al., 1990b). All permutations of the four-base overhang stabilize the hairpin relative to the blunt-ended hairpin. How well a particular four-base overhang stabilizes the hairpin is determined mainly by the first base of the overhang abutting the base-paired region. Stabilization follows the order purine > T > C for the first base.

In this study we set out to determine how a series of 5′ or 3′ overhangs or 5′,3′ mismatches would effect the conformational properties of an oligomer that could adopt both right- and left-handed (B and Z) helical conformations. The 8-base self-complementary DNA oligomer (5*meC*G)$_4$ was synthesized where the cytosine residues C were methylated at the 5 position to facilitate Z-helix formation (Behe and Felsenfeld, 1981). The molecule, which exists as a duplex at ionic strengths greater than 200 mM NaCl at 25°C, is fully right-handed under these conditions and fully left-handed in 3.0 M NaCl (Sheardy, 1991; Sheardy et al., 1993). Twelve additional 10-base semi-self-complementary oligomers were also synthesized with sequences having two additional bases (AA, CC, TT, or GG) on either the 5′ or 3′ end of the (5*meC*G)$_4$ segment or one additional base (A, C, T, or G) on both the 5′ and 3′ ends of the (5*meC*G)$_4$ segment. The first set is referred to as the 5′-XX or 3′-XX overhang oligomers, respectively, and the second set is referred to as the 5′,3′-Y/Y mismatch oligomers. Through optical melting studies, the contribution to the stability of each respective duplex from each type of stacking (i.e., 5′ overhang, 3′ overhang or 5′, 3′ mismatch) for both the B and Z forms of the [(5*meC*G)$_4$]$_2$ core was investigated.

Received for publication 22 July 1996 and in final form 18 September 1996.

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0006-3495/96/12/3361/09 $2.00

The effect that each type of overhang or mismatch has on the ease of B-to-Z conversion is also evaluated in this study. It is known that nucleotide sequence is a major intrinsic factor in determining the ease with which DNA will adopt the Z conformation (Jovin et al., 1983). DNA sequences with alternating pyrimidine-purine repeats, especially $(CG)_n$, are the most conducive to adopting a left-handed helical conformation. Substitution of C by T and G by A in $(CG)_n$ gradually decreases the ease of Z-DNA formation with increasing AT content. The sequence hierarchy for forming Z-DNA follows the order $(CG)_n > (TG)_n = (CA)_n > (TA)_n >$ non(Pyr-Pur). For mixed alternating $(Pyr\text{-}Pur)_n$ repeats the presence of sequential (CG) units stabilizes the Z conformation (Wang et al., 1987; Chen, 1988).

Chen studied the effect of placing extra base pairs on the ends of a $(CG)_4$ repeat (1988). We have determined the midpoint of the B-Z transition of $[(CG)_4]_2$ to be at 2.5 M NaCl (Marotta and Sheardy, unpublished results). Placement of extra A/T base pairs on the ends of $[(CG)_4]_2$ to form $[A(CG)_4T]_2$ increases the midpoint of B-Z transition to 3.3 M NaCl, whereas placement of extra G/C base pairs at the ends of $[(CG)_4]_2$ to form $[G(CG)_4C]_2$ results in a transition midpoint of 4.6 M NaCl. In both modifications, the overall sequence repeat changes from (5′ Pyr-Pur 3′) to (5′ Pur-Pyr 3′). Although both modifications contain four continuous CG repeats, an extra G/C base pair at the ends is more destabilizing to the Z conformation than an extra A/T base-pair. Placement of additional T/A base pairs on the ends of $[G(CG)_4C]_2$ to form $[TG(CG)_4CA]_2$ restores the alternating (5′ Pyr-Pur 3′) repeat and lowers the transition midpoint to 4.0 M NaCl.

Studies on BZ junction-forming molecules have shown that the sequence of the region near the potential BZ junction influences the ease with which the junction will form (Sheardy et al., 1993). Duplexes of $(5meCG)_4$-AMN-GACTG (where MN is a Pyr-Pyr step of sequence -CC-, -CT-, -TC-, or -TT-) and their complements undergo salt-induced transitions whereby the 5′ end (possessing the (*5meC*G) repeat) of the molecule assumes the Z conformation and the 3′ end of the molecule (with the (Pur-Pyr-Pyr Pur)$_2$ repeat) remains in the B conformation. Thus, at 4.5 M NaCl, these oligomers contain B-Z conformational junctions at the middle *5meC*GA step (Sheardy and Winkle, 1989). A correlation was observed between the overall stacking energies (ΔG_{stack}) in the AMNG segment and the corresponding free energy of junction formation (ΔG_j): the more favorable ΔG_{stack} for the sequence near the junction site, the more unfavorable the ΔG_j. This correlation arises from the relative difficulty of perturbing the adjacent dinucleotide step to accommodate the junction.

Although the biological relevance of Z-DNA and B-Z junctions has yet to be established, these conformations are still interesting from a biophysical point of view. The DNA oligomers designed for this study enable us to observe the sequence and stacking effects of 5′ and/or 3′ unpaired bases on both the helix stability for the B and Z forms of the $[(5meCG)_4]_2$ core duplex as well as the ease of its B-to-Z transition. Circular dichroism is used to monitor the conformational state of each oligomer at various NaCl concentrations. The structural data along with optical melting data for 13 oligomers are reported here. The studies here illustrate that even subtle changes in sequence or environment can have a dramatic effect on the structures available to oligomeric DNA.

MATERIALS AND METHODS

DNA oligomers

The DNA oligomers (Fig. 1) were synthesized on an Applied Biosystems 380B DNA (Perkins-Elmer, Foster City, CA) synthesizer using phosphoramidite chemistry (Caruthers, 1991) and purified as previously reported (Sheardy, 1988). After a second preparative high-performance liquid chromatography purification, the desired fractions were pooled and dialyzed using a Spectra/Por 1000 mwco dialysis membrane. The purified DNA was then lyophilized using a Savant SpeedVac Concentrator for storage. The concentrations of the oligonucleotides were determined from the extinction coefficients obtained using an enzyme digestion method (Henderson et al., 1992). For the CD studies and the UV melting studies, the lyophilized DNA was reconstituted in 10 mM phosphate buffer, pH 7.0, with 0.1 mM EDTA. NaCl was added to the buffer to give the desired final sodium concentration. DNA solutions were denatured by heating to 90°C and then reannealed by slow cooling to room temperature. The annealed strands were allowed to equilibrate at 4°C for 12 h before analysis.

Circular dichroism studies

The CD spectra were recorded with an AVIV model 62A DS CD (Aviv Associates, Lakewood, NJ) spectropolarimeter at 25°C from 320 to 220 nm. Spectra of each oligomer ([DNA] = 1×10^{-4} M in single-strand bases) were recorded in phosphate buffer at various NaCl concentrations.

Parent Oligomer

(*5meC*-G)$_4$

5′-Overhang Oligomers

AA-(*5meC*-G)$_4$
CC-(*5meC*-G)$_4$
GG-(*5meC*-G)$_4$
TT-(*5meC*-G)$_4$

3′-Overhang Oligomers

(*5meC*-G)$_4$-AA
(*5meC*-G)$_4$-CC
(*5meC*-G)$_4$-GG
(*5meC*-G)$_4$-TT

5′,3′-Mismatch Oligomers

A-(*5meC*-G)$_4$-A
C-(*5meC*-G)$_4$-*5meC*
G-(*5meC*-G)$_4$-G
T-(*5meC*-G)$_4$-T

FIGURE 1 The DNA oligomers considered in this report. All oligomers were synthesized and purified as noted in Materials and Methods.

A sample of each strand was prepared in 15 mM NaCl buffer and titrated with the same concentration of that strand in 5.0 M NaCl to monitor the B-Z transition. The CD spectrum of Z-DNA is characterized by a trough at 295 nm and a peak at 275 nm (Behe and Felsenfeld, 1981). The [NaCl] at the midpoint of the B-to-Z transition can be semiquantitatively determined by monitoring the formation of the 295-nm trough as the [NaCl] is gradually increased. The percentage transition to Z-DNA is given by (Sheardy, 1991)

$$\% \text{ transition} = \delta\theta_{295}(\text{NaCl})/\delta\theta_{295}(\text{total}), \quad (1)$$

where $\delta\theta_{295}(\text{NaCl}) = \theta_{295}(15 \text{ mM NaCl}) - \theta_{295}([\text{NaCl}])$ for a particular [NaCl] and $\delta\theta_{295}(\text{total}) = \theta_{295}(15 \text{ mM NaCl}) - \theta_{295}(5.0 \text{ M NaCl})$. The [NaCl] at the transition midpoint was taken at 50% transition.

Optical melting studies

The thermal denaturation of each oligomer was monitored using a Gilford Response II UV/VIS spectrophotometer set at 275 nm to determine the melting temperature T_m. T_m is defined as the temperature at which the fraction of single strands, α, is equal to 0.5. The temperature was ramped from 20°C to 95°C at a rate of 0.3°C/min by using the temperature programming software of the spectrophotometer. The DNA oligomers were melted in low salt (200 mM NaCl) and high salt (3.0 M NaCl), where they adopt the B and Z conformations, respectively. At each salt concentration, each DNA oligomer was melted at various concentrations to determine the van't Hoff enthalpies and entropies. The DNA concentrations ranged from 1×10^{-3} M to 2×10^{-5} M in single-strand bases. Although thermodynamic parameters can be directly obtained via melting curve analysis, many of the melting curves did not have a sufficient upper baseline to accurately calculate T_m. Furthermore, determination of these parameters via analyses of the T_m versus ln C_T plots may be more reliable (Marky and Breslauer, 1987). Thus the highest point on the first-derivative curve (T_{max}) was used for construction of the van't Hoff plots. We have determined that for those oligomers with sufficient upper baseline, the difference between T_m (determined via curve analysis) and T_{max} (determined from the inflection point of the first-derivative plot) was no more than 1°. It has been shown that at T_{max}, $\alpha = 0.41$. To correct for this deviation for self-complementary duplexes, the relationship between the total concentration of single-strand bases C_T and T_{max} is given by (Gaffney and Jones, 1989, and references therein)

$$1/T_{max} = (R/\Delta H^\circ)\ln C_T + (\Delta S^\circ - 1.8)/\Delta H^\circ. \quad (2)$$

Linear regression analysis of the data plotted according to Eq. 2 provides a slope of $R/\Delta H^\circ$ and a y intercept of $(\Delta S^\circ - 1.8)/\Delta H^\circ$, from which ΔH°, ΔS°, and ΔG° of double-helix formation can be calculated. It should be noted that this analysis is predicated upon a two-state transition. Slight deviations from two-state behavior should have minimal effects on the enthalpies and entropies determined via $1/T_{max}$ versus ln C_T plots (Gaffney and Jones, 1989).

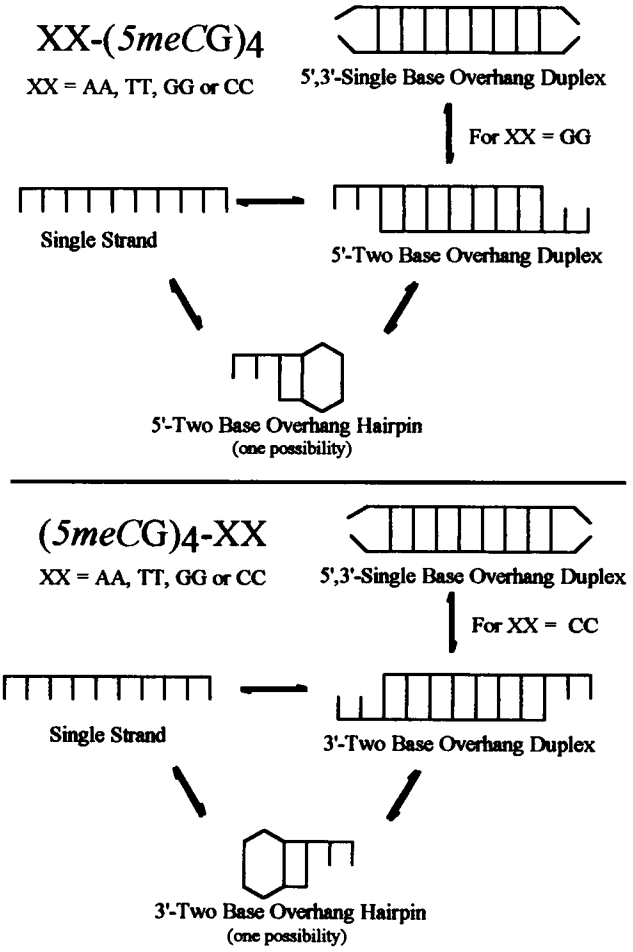

FIGURE 2 Some possible conformational states for the 5′-overhang and 3′-overhang oligomers. It was predicted that the major conformational states for these oligomers under base pairing conditions would be intermolecular duplexes with two unpaired bases on either the 5′ or 3′ termini, schematically drawn as the 5′-two-base overhang duplex and 3′-two-base overhang duplex, respectively. Because of the self-complementary nature of the sequences and their lengths, intramolecular hairpins with two unpaired bases on the termini are also possible. Possible structures for these species are drawn as the 5′-two-base overhang hairpin and 3′-two-base overhang hairpin, respectively. For the 5′-GG overhang and 3′-CC overhang oligomers, an intermolecular duplex could also form with single unpaired bases on both the 5′ and 3′ termini and are drawn as the 5′,3′-single-base overhang duplex. The nature of the equilibrium between the various species will depend upon DNA concentration, NaCl concentration, and temperature. One goal of this project was to determine environmental effects on the conformational states available to these oligomers.

RESULTS

DNA oligomers

Fig. 1 shows the initial set of DNA oligomers synthesized for this study. Both 5′ and 3′ overhang oligomers ((XX-(*5meC*G)$_4$ and (*5meC*G)$_4$-XX, respectively) should form core duplexes of [(*5meC*G)$_4$]$_2$ with two identical unpaired bases on either the 5′ termini or 3′ termini, respectively. However, additional structures may be possible, such as the 5′ or 3′ two-base overhang hairpin or, for certain sequences, the 5′,3′ single-base overhang duplex. Fig. 2 shows the predicted base-paired structures formed having AA, CC, GG, or TT overhangs on either the 3′ or 5′ ends of the molecules. The 5′,3′ mismatch oligomers (Y-(*5meC*G)$_4$-Y) should also form core duplexes of [(*5meC*G)$_4$]$_2$, but with single identical unpaired bases on both the 5′ and 3′ termini. However, hairpin formation may also be possible. Finally, because of the contiguous repeats of *5meC*G, these oligomers may also form "slipped" duplexed structures with cores of 6 *5me*C:G base pairs possessing two base overhangs. However, it is very unlikely that the stabilizing effect arising from the presence of the overhangs would compen

sate the destabilizing effect arising from the the loss of six hydrogen bonds. (The duplexed 8-mer would have a total of 24 hydrogen bonds, whereas the duplexed 6-mer would have 18 hydrogen bonds). One of the goals of this study was to determine the effects of both oligomer and NaCl concentrations on the conformational states of these oligomers.

CD studies

The CD spectra for the NaCl titration of $[(5meCG)_4]_2$ are shown in Fig. 3 *A*, and the individual 0.1 M, 1.0 M, and 3.0 M NaCl spectra are shown in Fig. 3 *B*. The low-salt spectrum indicates the B conformation with a deep trough at 255 nm and a peak at 280 nm, and the high-salt spectrum indicates the Z conformation with a trough at 295 nm and a peak at 275 nm. The 1.0 M NaCl spectrum shows $[(5meCG)_4]_2$ intermediate between the B and Z conformations. The presence of the isoelliptic point at 279 nm suggests a two-state transition. Previous studies from our laboratory, however, have shown that the B-to-Z conversion can best be modeled as a three-state transition (Sheardy et al., 1993). The 5′ overhang, the 3′ overhang, and 5′,3′ mismatch oligomer CD spectra are shown in Fig. 4. The low-salt spectra demonstrate that all of the respective duplexes assume the B conformation. The 1.0 M NaCl CD spectra indicate that these duplexes are in varying degrees of the B-to-Z conversion. The spectra obtained in 3.0 M NaCl indicate that the transition to the Z conformation is, in general, complete for each duplex. The transition midpoint,

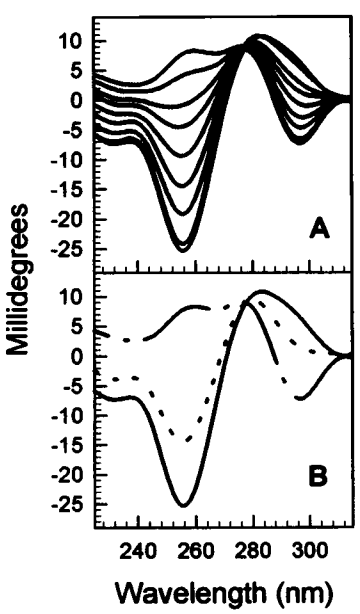

FIGURE 3 (*A*) The CD spectra for the NaCl titration of (5*me*C-G)$_4$, showing the B-to-Z transition. NaCl concentrations ranged from 15 mM to 3.0 M. (*B*) Individual CD spectra for this titration at 200 mM NaCl (——), 1.0 M NaCl (– – –) and 3.0 M NaCl (– · –). CD spectra were obtained at 25°C in 10 mM phosphate buffer, pH 7.0, with NaCl added to various concentrations. The DNA oligomer concentration was 1×10^{-4} M in single-strand bases.

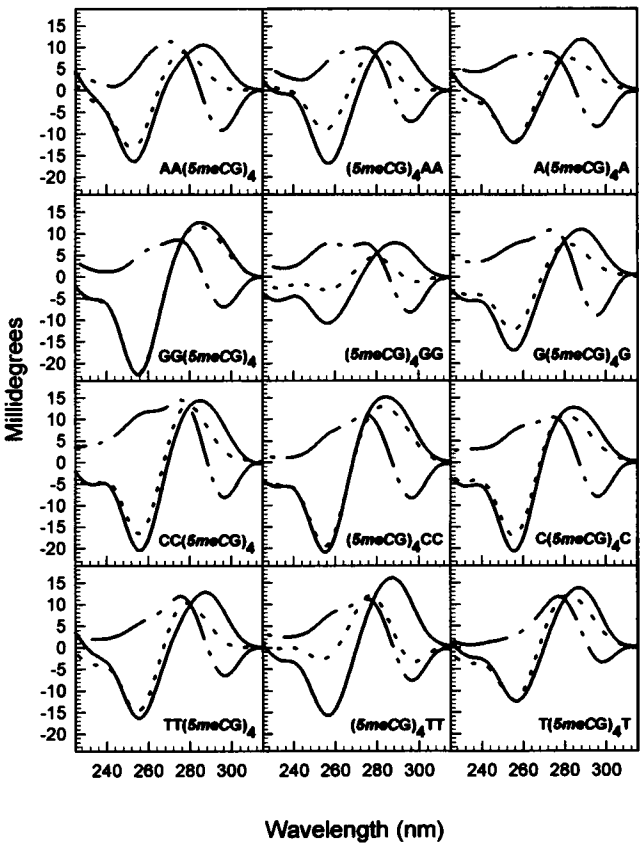

FIGURE 4 CD spectra at 25°C for the DNA oligomers at 200 mM NaCl (——), 1.0 M NaCl (– – –) and 3.0 M NaCl (– · –). CD spectra were obtained at 25°C in 10 mM phosphate buffer, pH 7.0, with NaCl added to various concentrations. DNA oligomer concentration was 1×10^{-4} M in single-strand bases.

calculated using Eq. 1, for each duplex is listed in Table 1. These data show that the overhang duplexes have, in general, transition midpoints similar to the parent $[(5meCG)_4]_2$

TABLE 1 Transition midpoints and T_{max} values for the oligomers of Fig. 1

Oligomer	Transition midpoint [NaCl]	T_{max} (°C) 200 mM NaCl	T_{MAX} (°C) 3.0 M NaCl
$(5meCG)_4$	1.1	70.9	72.6
$(5meCG)_4$AA	1.0	73.0	77.1
$(5meCG)_4$CC	1.9	73.1	76.4
$(5meCG)_4$GG	0.9	71.3	77.8
$(5meCG)_4$TT	0.6	71.3	77.1
AA$(5meCG)_4$	1.2	77.0	79.0
CC$(5meCG)_4$	1.2	74.1	77.3
GG$(5meCG)_4$	2.0	76.2	77.6
TT$(5meCG)_4$	1.1	74.8	78.9
A$(5meCG)_4$A	1.3	78.7	80.1
C$(5meCG)_4$5*meC*	1.5	74.4	78.6
G$(5meCG)_4$G	1.2	75.8	79.1
T$(5meCG)_4$T	1.5	73.3	77.4

Transition midpoints are reported to within ±0.1 M NaCl and T_{max} are within ±0.5°C.

duplex. The notable exceptions are the 3′-CC, the 3′-TT, and the 5′-GG overhang duplexes: the 3′-CC and 5′-GG overhangs dramatically raise the midpoint, whereas the 3′-TT dramatically decreases the midpoint. It is also interesting to note that the 5′,3′ mismatch duplexes, in general, have slightly to moderately higher transition midpoints than the parent $[(5meCG)_4]_2$ duplex, with the pyrimidine mismatches having the more pronounced effect.

Optical melting studies

Thermal denaturation studies of all oligomers at different NaCl concentrations were carried out. As noted above, many of the melting curvess did not have sufficient upper baselines to permit direct curve analysis. Hence T_{max} values were obtained from the inflection points of the first derivative plots of the melting curves. Examination of these first derivative plots indicated that all melting transitions were monophasic (data not shown). The T_{max} of each duplex in 200 mM and 3.0 M NaCl buffer solutions ([DNA] = 1.5×10^{-5} M in single strands) is shown in Table 1. At both salt concentrations, all duplexes have higher T_{max} values than the parent $[(5meCG)_4]_2$ duplex. In addition, a particular duplex has a higher T_{max} with a 5′-XX overhang or 5′,3′-Y/Y mismatch than the corresponding duplex with a 3′-XX overhang. Typical plots constructed according to Eq. 2 for the TT overhang duplexes in 200 mM NaCl are shown in Fig. 5. None of the duplexes have a linear $1/T_{max}$ versus ln[DNA] dependence over the full range of DNA concentrations; most duplexes show linearity at the higher DNA concentrations. This suggests an equilibrium between double-stranded duplex and hairpin duplex at low oligomer and salt (200 mM NaCl) concentrations. Because of the presence of two (or more) species under these conditions, van't Hoff analyses cannot be carried out. Hence the transition enthalpies and entropies were not determined for these oligomers in 200 mM NaCl. Nondenaturing polyacrylamide gel electrophoresis (10 mM Tris-borate, 0.1 mM EDTA, pH 8.0) in 50 mM NaCl revealed that each species was homogeneous, as evidenced by a single band (Marotta and Sheardy, unpublished data). Unfortunately, electrophoresis of these oligomers in 200 mM NaCl is not possible. Hence direct evidence of two or more species cannot be obtained.

Typical plots constructed according to Eq. 2 for select duplexes in 3.0 M NaCl are also shown in Fig. 5. As can be seen, the data are quite linear and can thus be subjected to van't Hoff analysis. Least-squares linear regression analyses of these data resulted in high correlation factors ($r^2 > 0.993$). The slopes and y intercepts calculated from these plots were used to determine the thermodynamic parameters listed in Table 2. All overhang and mismatch duplexes show slight entropic destabilizations relative to the 8-base pair control duplex. These destabilizations are more than compensated for by enthalpic stabilizations resulting in more favorable $\Delta G°$ values when an overhang or mismatch is present.

For the duplexes where XX = AA and Y = A, similar trends in T_{max} values occurred in both 200 mM and 3.0 M NaCl. The T_{max} values decreased in the order $A(5meCG)_4A > AA(5meCG)_4 > (5meCG)_4AA > (5meCG)_4$. The melting temperatures suggest that the presence of AA overhangs or terminal A/A mismatches stabilizes the core duplex at both salt concentrations. The free energies of helix formation in 3.0 M NaCl obtained from the van't Hoff plots indicate that the 5′-AA overhang and the 5′,3′-A/A mismatch stabilize the core

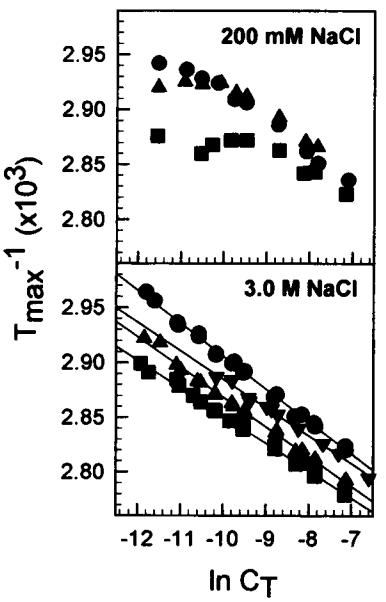

FIGURE 5 Typical plots of T_{max}^{-1} versus ln C_T for $(5meC\text{-}G)_4$ (●), the 5′-TT overhang oligomer (■), the 3′-TT overhang oligomer (▲), and the 3′,5′-T/T mismatch oligomer (▼) in 200 mM NaCl and 3.0 M NaCl. The solid lines drawn at 3.0 M NaCl represent the results of the linear regression of the plotted data.

TABLE 2 Duplex formation in 3.0 M NaCl for the oligomers of Fig. 1 derived from the concentration dependence of optical melting temperatures*,#

Oligomer	$\Delta H°$	$\Delta S°$	$\Delta G°$	$\Delta\Delta G°$
$(5meCG)_4$	−66.7	−173	−15.0	0
$(5meCG)_4AA$	−78.0	−203	−17.5	2.5
$(5meCG)_4CC$	−70.8	−183	−16.3	1.3
$(5meCG)_4GG$	−72.4	−187	−16.6	1.6
$(5meCG)_4TT$	−72.8	−188	−16.7	1.7
$AA(5meCG)_4$	−79.9	−207	−18.2	3.2
$CC(5meCG)_4$	−74.3	−192	−16.9	1.9
$GG(5meCG)_4$	−72.2	−186	−16.7	1.7
$TT(5meCG)_4$	−78.8	−204	−17.9	2.9
$A(5meCG)_4A$	−79.9	−206	−18.3	3.3
$C(5meCG)_45meC$	−73.9	−191	−17.0	2.0
$G(5meCG)_4G$	−78.3	−203	−17.9	2.9
$T(5meCG)_4T$	−77.1	−201	−17.2	2.2

*Enthalpies are in kcal/mol, entropies are in cal/mol-K, and free energies, calculated at 298K, are in kcal/mol. $\Delta\Delta G°$ values are calculated by subtracting the $\Delta G°$ for a particular oligomer from that of the parent oligomer, $(5meCG)_4$. A positive $\Delta\Delta G°$ indicates that that oligomer is more stable than the parent oligomer.

#The errors in determining the enthalpy and entropy values form the van't Hoff plots of are typically ±5%.

duplex equally at high salt and more so than the 3′-AA overhang.

For the oligomers in which XX = CC and Y = C, the melting temperatures follow the order C(*5meCG*)C > CC(*5meCG*)$_4$ > (*5meCG*)$_4$CC > (*5meCG*)$_4$ in both low and high salt. It should be noted that the CD titrations and low salt van't Hoff plots suggest that the (*5meCG*)$_4$CC oligomer may be forming equilibria between the [C(G*5meC*)$_4$C]$_n$ and [(*5meCG*)$_4$CC]$_n$ type helices at low salt where n = 1 (i.e., Watson-Crick hairpin) or 2 (Watson-Crick duplex) as discussed below. The free energy values obtained at 3.0 M NaCl indicate that the 5′-CC overhang and 5′,3′-C/C mismatch stabilize the core to about the same extent as and slightly more than the 3′-CC overhang.

The low-salt melting temperatures for the XX = GG and Y = G duplexes follow the order GG(*5meCG*)$_4$ > G(*5meCG*)$_4$G > (*5meCG*)$_4$GG ≥ (*5meCG*)$_4$. The GG(*5meCG*)$_4$ and G(*5meCG*)$_4$G duplexes show significant increases in the melting temperatures, whereas the (*5meCG*)$_4$GG duplex shows only a slightly higher melting temperature than (*5meCG*)$_4$. Like the (*5meCG*)$_4$CC oligomer, the GG(*5meCG*)$_4$ oligomer may exist as a mix between [G(G*5meC*)$_4$G]$_n$ and [GG(*5meCG*)$_4$]$_n$ type helices, where n = 1 or 2 at low salt (see below). At high salt the melting temperatures follow the order G(*5meCG*)$_4$G > GG(*5meCG*)$_4$ = (*5meCG*)$_4$GG > (*5meCG*)$_4$. The free-energy values reveal that the 5′-GG and 3′-GG overhang molecules show the same degree of stabilization at high salt and the 5′,3′-G/G mismatch results in the most stabilization.

In 200 mM NaCl the melting temperatures for the duplexes where XX = TT and Y = T decrease in the order TT(*5meCG*)$_4$ > T(*5meCG*)$_4$T > (*5meCG*)$_4$TT ≥ (*5meCG*)$_4$ (within the uncertainty of the T_{max} measurements), suggesting stabilization from the 5′-TT overhangs and 5′,3′-T/T mismatches. At 3.0 M NaCl, the T_{max} values indicate that the 5′,3′-T/T mismatches and 3′-TT overhang increase the T_{max} of the parent oligomer to about the same extent, whereas the 5′-TT overhang results in a larger increase in T_{max}. Inspection of the free energies of helix formation confirms the greater stabilization induced by the 5′-TT overhang.

DISCUSSION

Inspection of the circular dichroism spectra reveals that the presence of terminal unpaired bases can effect the ease with which the base-paired core [(*5meC*-dG)$_4$]$_2$ undergoes the B-to-Z transition. The propensity for a duplex to undergo a B-to-Z transition is dominated by the number of contiguous CG repeats (Chen, 1988). The 3′-XX and 5′-XX overhang duplexes should contain four consecutive *5meCG* repeats. It appears that, in certain cases, nucleotides flanking either the 5′ ends or the 3′ ends of the duplexed core containing the *5meCG* repeats can modulate the ease of the B-to-Z transition even if these nucleotides are not base paired to the opposite strand. The midpoint of the NaCl-induced transition can be used to semiquantitatively assess the free energy of the B-to-Z transition (Doktycz et al., 1990a; Sheardy, 1991). Within experimental error, the 3′-AA, 3′-GG, 5′-AA, 5′-CC, and 5′-TT duplexes form Z-DNA with nearly the same free energy demand as the parent [(*5meCG*)$_4$]$_2$ duplex (i.e., the transition midpoints are all in the range of 0.9–1.2 M NaCl). For this group of oligomers, the 5′-XX oligomers, in general, have slightly higher midpoints than the 3′-XX oligomers, suggesting stabilization of the B form for these oligomers. Consistent with this notion are the observed higher T_m values associated with the 5′-XX oligomers of this group.

Because all of the above duplexes have the same core as the 3′-TT overhang duplex, it is quite surprising that the 3′-TT overhang apparently enhances the B-Z conformational transition (transition midpoint of 0.6 M NaCl). It has also been observed that the presence of 3′-TT overhang on the unmethylated analog lowers the B-Z transition midpoint from 2.5 M NaCl for (CG)$_4$ to 2.1 M NaCl for (CG)$_4$TT (Marotta and Sheardy, unpublished results). The data in Tables 1 and 2 indicate that both the B and Z forms of (*5meCG*)$_4$TT are stabilized relative to the 8-mer lacking the overhang. This stabilization has also been observed for the unmethylated analog (Marotta and Sheardy, unpublished results). Hence the observed enhancement of the B-Z transition imparted by the 3-TT overhang may arise from stabilization of the intermediate formed proposed by Sheardy et al. (1993) in the three-state mechanism (B ⇄ I ⇄ Z).

It is also interesting to note the apparent inhibition of the B-Z transition imparted by the presence of the 5′-GG and 3′-CC overhangs to the core duplex (i.e., the transition midpoints are 1.9–2.0 M NaCl). We believe that the GG(*5meCG*)$_4$ oligomer forms a [G(G*5meC*)$_4$G]$_2$ duplex (designated as the 5′,3′ single-base overhang duplex in the upper panel of Fig. 1), which results in a [(G*5meC*)$_4$]$_2$ base-paired core with G/G mismatches at the 3′ and 5′ ends. The resultant [(G*5meC*)$_4$]$_2$ core contains only three contiguous *5meCG* repeats and would therefore not convert to the Z conformation as easily as [(*5meCG*)$_4$]$_2$, which contains four contiguous *5meCG* repeats. As in the case of the 5′-GG oligomer, the 3′-CC oligomer (*5meCG*)$_4$CC can form a duplex with a [(G*5meC*)$_3$(GC)]$_2$ base-paired core instead of a [(*5meCG*)$_4$]$_2$ core. The resulting duplex [*5meC*(G*5meC*)$_3$(GC)C]$_2$ has C/*5meC* mismatches on the 3′ and 5′ ends of the

TABLE 3 Comparisons of the 5′-TT and 3′TT overhangs on (*5meCG*)$_4$ and (CG)$_3$ duplexes*

Oligomer	ΔΔH°	ΔΔS°	ΔΔG°
(CG)$_3$TT#	8.4	24.8	1.0
(*5meCG*)$_4$TT	6.1	15	1.7
TT(CG)$_3$#	15.8	44.4	2.6
TT(*5meCG*)$_4$	12.1	31	2.9

*Values obtained by subtracting the value for the oligomer reported from that of the blunt-ended analog. Enthalpies and free energies (at 298K) are in kcal/mol and entropies are in eu.
#From Senior et al. (1988).

$[(G5meC)_4(GC)]_2$ core (designated as the 5′,3′ single-base overhang duplex in the lower panel of Fig. 1). This duplexed core also contains only three contiguous base-paired *5meC*G repeats and should not convert to the Z conformation as easily as the originally predicted $[(5meCG)_4CC]_2$ 3′ overhang duplex structure, which would contain four continuous base-paired *5meC*G repeats. Apparently the G/G and *5meC*/C mismatches are more stabilizing to their respective duplexed cores than the 5′-GG and 3′-CC overhangs are to their respective duplexed cores, leading the equilibrium depicted in Fig. 2 to favor the mismatch over the overhang forms under these salt conditions.

To test whether the $GG(5meCG)_4$ and the $(5meCG)_4$-CC oligomers form the $[G(G5meC)_4G]_2$ and $[5meC(G5meC)_3(GC)C]_2$ 5′,3′ mismatch duplexes, the oligomer $(G5meC)_4$ was synthesized along with $(G5meC)_4GG$ (which is sequence equivalent to $G(5meCG)_4G$) and $C5meC(G5meC)_4$, which is sequence equivalent to $C(5meCG)_45meC$). The data suggest that the $GG(5meCG)_4$ and the $(5meCG)_4CC$ oligomers preferably form end mismatch-type structures rather than the 5′ or 3′ overhang-type structures, respectively. If the $(G5meC)_4GG$ and $C5meC(G5meC)_4$ oligomers behave similarly, the resulting duplexes will have $(5meCG)_4$ cores, which should increase the ease with which they can adopt the Z conformation. The $(G5meC)_4GG$ oligomer salt titration resulted in a transition midpoint of 1.2 M NaCl, which is considerably lower than the 3.7 M NaCl observed for the transition midpoint for $(G5meC)_4$ and the 2.0 M NaCl observed for the titration midpoint for $GG(5meCG)_4$. In fact, $(G5meC)_4GG$ forms Z-DNA as readily as the parent duplex. Therefore, $(G5meC)_4GG$ is apparently forming a $[G(5meCG)_4G]_2$ 5′,3′ mismatch duplex rather than a $[(G5meC)_4GG]_2$ 3′ overhang duplex, which would have a transition midpoint equal to or higher than 3.7 M NaCl. This supports the suggestion that $GG(5meCG)_4$ is forming a $[G(G5meC)_4G]_2$ 5′,3′ mismatch duplex. Its transition midpoint of 2.0 M NaCl is shifted higher than the parent oligomer, yet it is still lower than the transition midpoint of 3.7 M NaCl for $(G5meC)_4$. We would predict that a $[G(G5meC)_4G]_2$ 5′,3′ mismatch structure would have a transition midpoint equal to or higher than that of $(G5meC)_4$. The fact that $GG(5meCG)_4$ has a transition midpoint of 2.0 M NaCl implies that the equilibrium between $[G(G5meC)_4G]_2$ 5′,3′ mismatch and $[GG(5meCG)_4]_2$ 5′ overhang structures depicted in Fig. 1 may be shifted to the structure that forms the more stable Z conformation as the salt concentration is increased (i.e., toward $[GG(5meCG)_4]_2$ 5′ overhang). The $C5meC(G5meC)_4$ oligomer has a transition midpoint of 1.5 M NaCl, indicating that it forms the $[C(5meCG)_45meC]_2$ 5′,3′ mismatch duplex. The $(5meCG)_4CC$ oligomer has a transition midpoint of 1.9 M NaCl, higher than the 1.5 M midpoint of $C5meC(G_45meC)_4$, yet considerably lower than the 3.7 M midpoint of $(G5meC)_4$. Again for the $(5meCG)_4CC$ oligomer, the equilibrium between the $[5meC(G5meC)_3(GC)C]_2$ 5′,3′ mismatch and $[(5meCG)_4CC]_2$ 3′ overhang duplexes is apparently shifted toward the $[(5meCG)_4CC]_2$ 3′ overhang structure as the salt concentration is increased.

Both the $G(5meCG)_4G$ (sequence equivalent to $(G5meC)_4GG$ above) and the $C(5meCG)_45meC$ (sequence equivalent to $C5meC(G5meC)_4$ above) oligomers have transition midpoints similar to those of the $A(5meCG)_4A$ and $T(5meCG)_4T$ oligomers (Table 1), indicating that they form similar end mismatch duplexes. The oligomers $Y(5meCG)_4Y$ (Y = A, C, G, and T) thus form duplexes of $[Y(5meCG)_4Y]_2$ (i.e., a $(5meCG)_4$ core with an A/A, C/C, G/G, or T/T mismatch on each end) with B-Z transition midpoints of 1.2–1.5 M NaCl. The presence of these mismatches only modestly inhibits the BZ transition of the $[(5meCG)_4]_2$ core relative to the parent duplex.

Optical melting studies were employed to determine the contributions of each type of terminal stacking interaction to the free energy of duplex formation of $[(5meCG)_4]_2$. The changes in the free energies due to the presence of the overhangs were compared for $[(5meCG)_4]_2$ in both the B and the Z conformations. Duplexes were melted over a large DNA concentration range in 100 mM NaCl (data not shown) to construct van't Hoff plots to determine their thermodynamic parameters. These plots were flat and scattered, showing little or no $1/T_{max}$ versus ln C_T dependence. These experiments were repeated in 200 mM NaCl, and van't Hoff plots for the two overhang sets of oligomers were constructed. The plots show $1/T_{max}$ versus ln C_T dependence at high DNA concentrations, with a gradual flattening of the curve as the DNA concentration decreases. The loss of T_{max} dependence on ln C_T as the concentration of DNA decreases suggests that an equilibrium between double-stranded duplex and hairpin duplex exists at these lower salt concentrations. This equilibrium is schematically represented in Fig. 2.

Stable DNA hairpins have been shown to form for oligomers as short as heptamers (Hirao et al., 1994). Previous NMR studies have been performed on the mismatched DNA octamer *5meC*G*5meC*GTG*5meC*G (Orbons et al., 1987). These authors concluded that at low DNA concentrations and low ionic strength the octamer was forming a hairpin with a stem of three base pairs and a loop of two residues. At higher DNA concentrations or ionic strength an exchange between the hairpin and a B-DNA dimer occurred. At high salt conditions a Z-DNA dimer was formed containing two G/T mismatches. Our data are completely consistent with these observations.

Because of the lack of $1/T_{max}$ versus ln C_T dependence at low salt conditions the thermodynamic parameters were not generated via a van't Hoff analysis. The lack of a sufficient upper baseline for many of the melting curves also made obtaining accurate van't Hoff enthalpies via the shape analysis of the curve difficult (Marky and Breslauer, 1987). Only the T_{max} values for a fixed DNA concentration (i.e., [DNA] = 1.5×10^{-5} in single-stranded base pairs) are reported (Table 1). Melting studies in 3.0 M NaCl did yield linear van't Hoff plots over a 1.5 order of magnitude concentration range, suggesting that the oligomers are duplexed in high salt. The thermodynamic parameters in Table 2 were generated from the van't Hoff plots constructed for the three sets of oligomers. These data show that all overhang and terminal mismatch modifications stabilized the $[(5meCG)_4]_2$

core. The greatest stabilizations occur with the 5′ overhangs and the terminal mismatches.

The most revealing way to analyze the data is to consider trends in free energies within families of oligomers. The enhanced stabilities of DNA oligomers possessing unpaired overhang bases has generally been attributed to enthalpic contribution arising from the stacking of the unpaired base on the base-paired duplex coupled to enhanced stacking within the duplex (Senior et al., 1988; Doktycz et al., 1990b). For the oligomers under investigation here, one can consider the type of stacking: 5′-XX overhang versus 3′-XX overhang versus 5′,3′-Y/Y mismatch given the same base (X = Y), or one can compare the different base analogs within the same type of stacking. Many studies have considered thermodynamic analysis of DNA oligomeric duplexes at NaCl concentrations less than 115 mM (for example, Doktycz et al., 1992) or equal to 1.0 M NaCl (for example, Senior et al., 1988). Because of the problems of the hairpin ⇄ duplex at 100 mM NaCl and the incomplete B-Z transition at 1.0 M NaCl, we choose to compare the free energies at 3.0 M NaCl, conditions under which duplexes are conformationally homogeneous (i.e., all Z form).

In all cases, the 5′-XX overhang is more stabilizing than the 3′-XX overhang, as observed for the 5′-TT and 3′-TT overhang oligomers of Senior et al. (1988). As can be seen from Table 3, our results for the TT overhang duplexes compare quite favorably with those of the Breslauer group. For example, the 5′-TT overhang on their $[(CG)_3]_2$ core contributed a $\Delta\Delta H^\circ$ = 15.8 kcal and a $\Delta\Delta S^\circ$ = 44.4 eu to the stabilization of the core duplex, resulting in a free energy increase of $\Delta\Delta G^\circ$ = 2.6 kcal/mol. The 5′-TT overhang on our $[(5meCG)_4]_2$ core contributed a $\Delta\Delta H^\circ$ = 12.1 kcal/mol and a $\Delta\Delta S^\circ$ = 31 eu to the stabilization of the core duplex, resulting in a free energy increase of $\Delta\Delta G^\circ$ = 2.9 kcal/mol. Although their results were obtained in 1.0 M NaCl and ours were obtained in 3.0 M NaCl, the magnitudes of the various thermodynamic parameters are comparable.

In general, the 5′,3′-Y/Y mismatch oligomers are as stable as (for Y = X = A or Y = X = C) or more stable than (X = Y = G) their 5′-XX analogs. The one exception is when X = Y = T, where the 5′-TT overhang is more stable than the 5′,3-T/T mismatch. The stability generally observed for the 5′,3′-Y/Y mismatches most likely arises from the ability of the two unpaired bases to stack on both bases of the terminal basepairs of the duplex. For the Y = G mismatch, there is a larger enthalpic contribution (6.1 kcal/mol) to the overall stability of the core than for the 5′-GG overhang. On the other hand, the difference in free energies between the 5′-TT overhang and the 5′,3′-T/T mismatch is due to a larger enthalpic contribution (1.7 kcal/mol) from the overhang.

For a given type of stacking, the order for decreasing stability for the 5′,3′-Y/Y mismatch series is 5′,3′-A/A > 5′,3′-G/G ≫ 5′,3′-TT > 5′,3′-CC. The observation that the unpaired purine bases stabilize more than unpaired pyrimidines bases is consistent with the data of Doktycz et al. (1990b) for the stabilization by unpaired bases on the 5′ ends of hairpin molecules. For the 5′-XX overhang series the order of decreasing stability is 5′-AA > 5′-TT > 5′-CC > 5′-GG, and the order for the 3′-XX overhang series is 3′-AA > 3′-TT ≥ 3′-GG > 3′-CC. One must be cautious in considering these orders because the 5′-GG and the 3′-CC oligomers may exist in two different structures, even at 3.0 M NaCl. For X = A, T, or C in the 5′-XX overhang series and X = A, T, or G in the 3′-XX overhang series, the duplexed core is $[(5meCG)_4]_2$ with four *5meC*-G and three G-*5meC* dinucleotide stacking interactions.

At low salt, GG$(5meCG)_4$ and $(5meCG)_4$CC apparently give rise to duplexes possessing $[(G5meC)_4]_2$ cores with either G/G or C/C mismatches on both ends, whereas $(G5meC)_4$GG and C*5meC*$(G5meC)_4$ give rise to duplexes possessing a $[(5meCG)_4]_2$ core with either G/G or C/C mismatches on both ends. Even though the stacking interactions are different for the two cores, the added stability imparted by the presence of the unpaired bases on both 3′ and 5′ termini must be the driving force for forming the particular cores. Although it appears that high salt may favor the overhang structures for these oligomers, the observation that these oligomers exist in multiple duplexed states complicates a direct comparison of their free energies to the other oligomers.

CONCLUSIONS

The results presented here indicate that the presence of unpaired bases on the termini of DNA oligomeric duplexes influences the ease of the B-to-Z transition for these duplexes and stabilizes the duplex in both B and Z conformations. These factors are most likely due to stacking of the unpaired bases on the duplexed cores. Both 5′-XX overhangs and 5′,3′-Y/Y mismatches stabilize more than the corresponding 3′-XX overhang. We cannot rule out additional contributions from unusual base pairing in the cases of the 5′,3′-Y/Y mismatches. Within a family of oligomers, the presence of unpaired A bases leads to the greatest amount of stabilization. From a conformational point of view, these results, along with others (Senior et al., 1988; Doktycz et al., 1990b), suggest that 5′ overhangs stabilize B- and Z-form structures more than 3′ overhangs, whereas 3′ overhangs stabilize A-form structures more than 5′ overhangs (Petersheim and Turner, 1983; Freier et al., 1985). Hence the stacking interaction of an unpaired base on a duplexed core is dependent on the DNA conformation. The observation that, in some cases, the presence of the overhangs or mismatches influences the ease of the B-to-Z transition is also intriguing. Thus the unpaired bases not only affect the relative stabilities of the B and Z forms; they may also affect the stability of the intermediate formed in the transition. We are currently carrying out full spectral analyses (Sheardy et al., 1993) of the CD data to evaluate the free energies of the B-Z transition by application of a three-state transition model.

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

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