Effect of Base Pair A/C and G/T Mismatches on the Thermal Stabilities of DNA Oligomers That Form B−Z Junctions†

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ABSTRACT: The thermal stabilities and structures of B−Z junction forming DNA duplexes possessing A/C or G/T base pair mismatches were compared to those of corresponding duplexes possessing perfect matched base pairs. The upper strands of the duplexes have a generalized sequence 5′-(*5meC*G)-LMN-GACTG-3′, where L stands for A or G while M and N are permutations of pyrimidines. The lower strands were either complementary or were such as to create an A/C or G/T mismatch at the position corresponding to L, M, or N. Optical melting and circular dichroism studies were used to investigate the thermal stabilities and structures of both the mismatched base pair and the perfect matched base pair duplexes. Incorporating mismatched A/C or G/T base pairs did not noticeably affect the conformations of the duplexes in 115 mM Na^+ but resulted in perturbed B−Z conformations at 4.5 M Na^+. For any mismatched base pair duplex, the B-DNA domain of the hybrid B−Z structure formed at 4.5 M Na^+ is significantly perturbed while the Z-DNA domain is less perturbed by the presence of the mismatched base pairs. The presence of a mismatch destabilizes a duplex relative to the perfect matched base pair duplex by 1.7−10.0 kcal/mol depending upon position of the mismatch, type of mismatch base pair involved, and Na^+ concentration. The thermodynamic destabilization of a mismatched base pair duplex relative to the perfect matched base pair duplex arises from perturbations in nearest neighbor interactions and hydrogen bonding. In general, we observed that the incorporation of an A/C or G/T base pair mismatch in place of a perfect matched base pair at or near a B−Z junction results in a relatively large change in enthalpy and entropy to produce a significant change in the free energy of the duplex to single strand transition. At 4.5 M Na^+, where the duplexes possess perturbed B−Z junctions, the farther away from the junction that the mismatch is, the greater the extent of the destabilization.

The presence of mismatched base pairs in natural DNA is known to cause genetic mutations (*1*) and to profoundly affect the thermal stabilities of oligomeric DNA duplexes. During DNA replication there are proofreading enzymes such as DNA polymerase that detect and excise mismatch base pairs. Occasionally, some mismatches are left undetected and unexcised; as a result, they propagate during replication or genetic recombination. This process results in erroneous copies of the genome that may cause an irreversible mutation (*2*). The inability for some mismatched base pairs to be unrecognized and unexcised by proofreading enzymes is probably due to structural and thermodynamic stability of the base mispairs. Hence, it is important to study the thermodynamic consequences of having mismatched base pairs in DNA. The use of DNA oligomers to this end is preferred since one has the ability to synthesize large quantities of oligomers with predetermined sequences.

In recent years, several studies of DNA duplexes possessing perfect matched and mismatched base pairs have been reported which focus on the structure and/or thermodynamic stability in terms of base sequence dependence, base stacking, hydrogen bonding, and solvent hydration effects. Most information of the above effects has been obtained by various methods such as X-ray crystallography (*3*−*7*), NMR (*8*, *9*), Raman spectroscopy (*10*, *11*) and UV spectroscopy (*12*−*21*). From a thermodynamic point of view, the stability of a particular duplex sequence can be predicted using nearest neighbor approaches (*22*−*24*). However, such approaches have not yet been developed for predicting the stabilities of oligomeric DNA duplexes possessing mismatched base pairs.

A pseudo *C*2 fold symmetry about the glycosidic bond exists for a perfect matched base pair but is absent for base pair mismatches (*7*). There is a degree of correlation between asymmetry of the mismatch and the relative efficiencies of rates of repair or excisions of mismatches by DNA polymerases (*25*). The G/T mismatch is highly asymmetric and is proofread most efficiently, followed by the A/C mismatch; the least efficiently repaired is the less asymmetric G/A mismatch.

A factor that influences base pair mismatch stability is the hydrogen bonding contribution to the total free energy of duplex formation. Two different hydrogen bonding schemes, the wobble base pairing and tautomeric base pairing, were proposed by Watson and Crick (*26*) and Crick (*27*). In the tautomeric base pairing, one of the bases is in the minor tautomer form while the other base is in the major tautomer form. Such resultant tautomeric base pairing is stereochemically indistinguishable from the Watson−Crick base pairing. The wobble base pair involves both bases in

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Perfect BZ Duplexes

BZ-I	5'-(*5meC*G)$_4$ACTGACTG-3' 3'-(G*5meC*)$_4$TGACTGAC-5'	**BZ-VIII**	5'-(*5meC*G)$_4$GTCGACTG-3' 3'-(G*5meC*)$_4$CAGCTGAC-5'
BZ-II	5'-(*5meC*G)$_4$ATCGACTG-3' 3'-(G*5meC*)$_4$TAGCTGAC-5'	**BZ-VII**	5'-(*5meC*G)$_4$GCTGACTG-3' 3'-(G*5meC*)$_4$CGACTGAC-5'
BZ-VI	5'-(*5meC*G)$_4$ACCGACTG-3' 3'-(G*5meC*)$_4$TGGCTGAC-5'	**BZ-X**	5'-(*5meC*G)$_4$GCCGACTG-3' 3'-(G*5meC*)$_4$CGGCTGAC-5'

BZ Duplexes with Mismatched Base Pairs A/C or G/T

BZ-II 9AC	5'-(*5meC*G)$_4$***A***TCGACTG-3' 3'-(G*5meC*)$_4$***C***AGCTGAC-5'	**BZ-VII 9GT**	5'-(*5meC*G)$_4$***G***CTGACTG-3' 3'-(G*5meC*)$_4$***T***GACTGAC-5'
BZ-I 10CA	5'-(*5meC*G)$_4$A***C***TGACTG-3' 3'-(G*5meC*)$_4$T***A***ACTGAC-5'	**BZ-II 10TG**	5'-(*5meC*G)$_4$A***T***CGACTG-3' 3'-(G*5meC*)$_4$T***G***GCTGAC-5'
BZ-VII 10CA	5'-(*5meC*G)$_4$G***C***TGACTG-3' 3'-(G*5meC*)$_4$C***A***ACTGAC-5'	**BZ-VIII 10TG**	5'-(*5meC*G)$_4$G***T***CGACTG-3' 3'-(G*5meC*)$_4$C***G***GCTGAC-5'
BZ-X 10CA	5'-(*5meC*G)$_4$GC***C***GACTG-3' 3'-(G*5meC*)$_4$C***A***GCTGAC-5'	**BZ-I 11TG**	5'-(*5meC*G)$_4$AC***T***GACTG-3' 3'-(G*5meC*)$_4$TG***G***CTGAC-5'
BZ-VI 11CA	5'-(*5meC*G)$_4$AC***C***GACTG-3' 3'-(G*5meC*)$_4$TG***A***CTGAC-5'	**BZ-VII 11TG**	5'-(*5meC*G)$_4$GC***T***GACTG-3' 3'-(G*5meC*)$_4$CG***G***CTGAC-5'

FIGURE 1: The DNA duplexes discussed in this report: *5meC* is 5-methylcytidine. The upper panel shows the perfect base pair duplexes while the lower panel shows the duplexes containing either an A/C or G/T mismatched base pair (highlighted in bold italics). The mismatched oligomers were generated by mixing the upper strand of one of the perfect duplexes with the lower strand of one of the other perfect duplexes. Hence, **BZ-II 9AC** was generated by mixing the upper strand of **BZ-II** with the lower strand of **BZ-VIII**. The nomenclature for the mismatched duplexes is based upon the origin of the upper strand and the position and types of mismatch. Thus, **BZ-VII 11TG** is an analogue of **BZ-VII** with a T:G mismatch at position 11.

the major tautomer form. However, the wobble base pair is different from Watson−Crick base pair by forming hydrogen bonds between functional groups different from the normal Watson−Crick base pairs. Tautomeric base pairing may coexist with wobble base pairing in solution, but they are unstable compared to wobble base pairs. The G/T wobble base pair has two hydrogen bonds (*7*, *28*) while A/C forms one hydrogen bond in neutral solution (*28*−*31*).

Since the Z-DNA has a zigzag sugar phosphate backbone, it should easily accommodate a wobble base pair mismatch with negligible distortion to the double helix. The B-DNA conformation, with right-handed helical sugar phosphate backbone, should be easily distorted by base pair mismatches. This phenomenon has been observed in the Raman spectroscopic study of d(CGCGTG) and d(CGCGCG) by Benevides et al. (*11*). In their studies, the Z-DNA crystal structures of d(CGCGTG) and d(CGCGCG) gave similar Raman bands from the phosphodiester groups. This suggests that no significant perturbation of Z-DNA backbone occurred as a result of the incorporation of a G/T base pair mismatch. In aqueous solution, both d(CGCGTG) and d(CGCGCG) in B-DNA conformations displayed Raman spectra that were significantly different from each other. The usual B-DNA Raman band at 832 cm^{-1} is about 40% less intense for d(CGCGTG) compared to d(CGCGCG). Further, d(CGCGTG) exhibits a Raman band at 864 cm^{-1} not found in d(CGCGCG).

Our lab has extensively studied B−Z junction forming oligomeric DNA duplexes focusing on structural and dynamic properties as well as drug binding propensities (*20*, *32*−*39*). The starting point for these investigations was **BZ-I** (Figure 1), a 16 base pair duplex that exists as a right-handed (B-form) structure under "low salt" (i.e., <0.5 M NaCl) conditions. However, as the concentration of bulk NaCl increases to around 4.5 M NaCl, the oligomer 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 (*32*).

In order to evaluate the effects of sequence on junction formation and duplex stability, we have investigated the thermodynamic properties of **BZ-I** and seven other related duplexes (*20*, *38*, *39*). These studies evaluated the free energy of junction formation, ΔG_j, as well as the free energy of duplex formation, $\Delta G°$. 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 (*38*). 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 8−11, 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. The second set of studies demonstrated that the experimentally evaluated free energies of duplex formation for the eight duplexes were 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 (*20*, *39*).

According to an NMR study of the perfect base pair duplex **BZ-I** (*33*), the putative B−Z junction spans three base pairs at positions 7, 8, and 9. With the stock of sixteen individual oligomers that comprise the duplexes studied to date, we are now in a position to mix noncomplementary strands in order to generate mismatched base pairs at or near the potential B−Z junction (i.e., at positions 9, 10, and 11). These sequence context variations will disrupt hydrogen bonding and nearest neighbor contributions to the total free energy of duplex formation. The issues to be addressed focus on how the presence of the mismatched base pair influences the overall structure and stability of the duplex under both low and high salt conditions and how the free energy of junction formation is affected. Here, we report studies aimed at determining the thermodynamic stabilities of B−Z junction forming duplexes possessing mismatched base pairs at or near the B−Z junction.

Although the biological implications of B−Z junctions have not yet been ascertained, studying conformational junctions in model DNA oligomers will lead to a better understanding of similar structural perturbations in natural DNAs. Since structural perturbations in natural nucleic acids may serve as recognition sites for protein binding, a basic understanding of their stabilities is a first step in delineating protein−DNA interactions.

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 (*40*). Each strand synthesized was purified by C18 reverse phase HPLC as previously described (*20*, *32*), and purity of the strands was checked by polyacrylamide gel electrophoresis and analytical HPLC. The duplexes were formed by mixing equal amounts of component strands and temperature annealing 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 perfect matched base pair duplexes are shown in the upper panel of Figure 1 and mismatched base pair duplexes are in shown in the lower panel. Both the perfect matched and mismatched duplexes were designed so that the segment containing the contiguous (*5me*CG) repeats assumes a left-handed Z-DNA conformation at high salt (4.50 M Na^+). The incorporation of *5meC* in the Z forming segment of the duplex is to facilitate the salt induced B to Z transition (*41*). The segment abutting the $(5me\text{CG})_4$ should remain as a right-handed B-like conformation at all salt conditions. The boundary interface between the two conformations coexisting in high salt is designated as the B−Z junction. The term perturbed B−Z conformation is used to describe the mismatched base pair duplex conformation at high salt.

Circular Dichroism Studies

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

Optical Melting Studies

The DNA UV/vis spectra were recorded with 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) with NaCl added to obtain total concentrations of Na^+ of either 115 mM (low salt) or 4.5 M (high salt). The effect of DNA concentration on the duplex to single strand transition temperatures (i.e., the T_m) of the duplexes were determined at these two Na^+ concentrations. The DNA concentration was varied from 1.0×10^{-5} to 8.0×10^{-4} M (in base pairs). At 115 mM Na^+, all the duplexes assume the right-handed B-DNA conformation; however, all duplexes form complete hybrid conformations at 4.5 M Na^+. In many DNA optical melting studies, the prevalent salt concentration of 1.0 M Na^+ has been used (*16*, *17*, *22*). However, our oligomers show slight conformational transitions (*38*) at this salt concentration.

The thermal denaturation of each oligomer sequence was monitored at 268 nm. The temperature was ramped from 20 to 95 °C at ca. 0.3 °C/min using the Gilford response II spectrophotometer equipped with 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 (20−95 °C) and after the reannealing (95−20 °C) of each oligomer duplex should not exceed 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 vs 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 $1/T_m$ vs $\ln C_T/4$ (where C_T is the total strand concentration ranging from about 1.0×10^{-5} to 8.0×10^{-4} M) were constructed in order to determine the van't Hoff enthalpies ($\Delta H°$) and entropies ($\Delta S°$). For non-self-complementary strands, the relationship of the duplex melting T_m and DNA concentration (*42*) is expressed as

$$1/T_m = (R/\Delta H°)\ln C_T/4 + \Delta S°/\Delta H° \qquad (1)$$

The free energies of the double helix denaturation are calculated by the standard Gibb's equation expressed as

$$\Delta G° = \Delta H° - T\Delta S° \qquad (2)$$

RESULTS

CD Studies

The CD spectra of the perfect matched base pair duplexes at low salt are characteristic of B-DNA conformations (*20*, *38*, *39*). The CD spectrum of a typical DNA oligomer in the B-DNA conformations possesses a deep trough at 255 nm and a peak at 280 nm. At high salt, the CD spectra of the perfect matched base pair duplexes have a shallow trough at 255 nm, a peak at 280 nm and another shallow trough at 295 nm (*20*, *38*, *39*). Low and high salt spectra of a perfect matched base pair duplex **BZ-VII** are shown in the upper left-hand panel of Figure 2. In the panel adjacent to that of

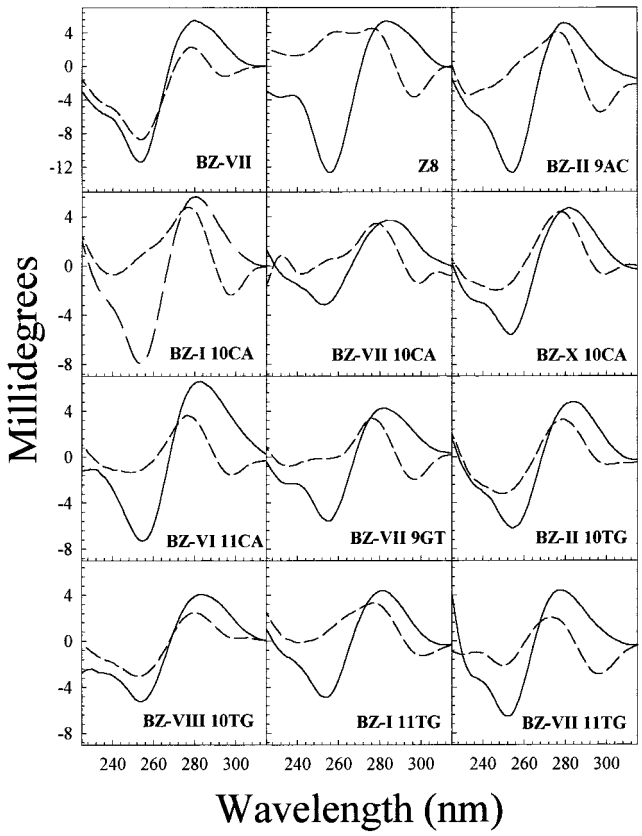

FIGURE 2: Circular dichroism (CD) spectra at 25 °C of the mismatched duplexes as well as for a perfect B−Z junction-forming duplex (**BZ-VII**) and Z-DNA-forming duplex (**Z8**: $[(5meCG)_4]_2$) at 115 mM Na^+ (solid line) and 4.5 M Na^+ (dashed line). Note: The CD spectrum for **Z8** under high salt conditions was recorded in 3.0 M Na^+. The buffer used was standard phosphate buffer (10 mM phosphate, 0.1 mM EDTA, pH 7.0) with NaCl added to give the final concentration of Na^+.

BZ-VII, the low and high salt spectra of **Z8** (i.e., $[(5meCG)_4]_2$ are shown for comparison. The **Z8** oligomer is also fully right-handed at low salt but is fully left-handed at high salt (*20*, *38*). As can be seen, the CD spectrum obtained at high salt (here, the high salt spectrum was obtained at 3.0 M Na^+) is characterized by a trough at 295 nm, a peak at 270 nm, and another peak at 250 nm.

In general, the CD spectra of the mismatched base pair duplexes in low salt are also characteristic of B-like conformations (Figure 2). On the other hand, the high salt spectra are quite different from the high salt spectra of either **BZ-VII** or **Z8**. Each high salt spectrum has a significantly diminished shallow trough at 255 nm, a peak at 280 nm and a shallow trough at 295 nm. The diminished B-DNA characteristic troughs at 255 nm suggest severely perturbed B-DNA segments in the oligomers. However, the presence of the base pair mismatches only marginally perturb the Z-DNA segments of these oligomers in high salt as indicated by the similarities in the troughs at 295 nm of these oligomers to that of **BZ-VII**. It should be noted that the salt induced conformational transition from the fully right-handed form to the perturbed B−Z conformation is essentially complete at 4.5 M Na^+ as indicated by CD titration studies (manuscript in preparation).

Optical Melting

The melting profiles of both the mismatched and the perfect matched base pair duplexes at low salt exhibit monophasic behavior as well as high degrees of cooperativity in the transitions (data not shown). At high salt, the thermal melting profiles of the perfect matched base pair duplexes also indicate monophasic cooperative transitions; however, the mismatched base pair duplexes display broad noncooperative, although monophasic, transitions (data not shown). The DNA concentration dependent thermal denaturation temperatures (i.e., T_m values) were obtained over a wide range of DNA concentrations. The resultant data were cast into van't Hoff plots (i.e., $1/T_m$ vs ln $C_T/4$ where C_T is the total strand concentration in base pairs) for analysis. The resultant plots for the A/C mismatch oligomers are shown in Figure 3, and those for the G/T mismatch oligomers are shown in Figure 4. As can be seen, there is a linear relationship of high correlation between $1/T_m$ and ln $C_T/4$ for all duplexes.

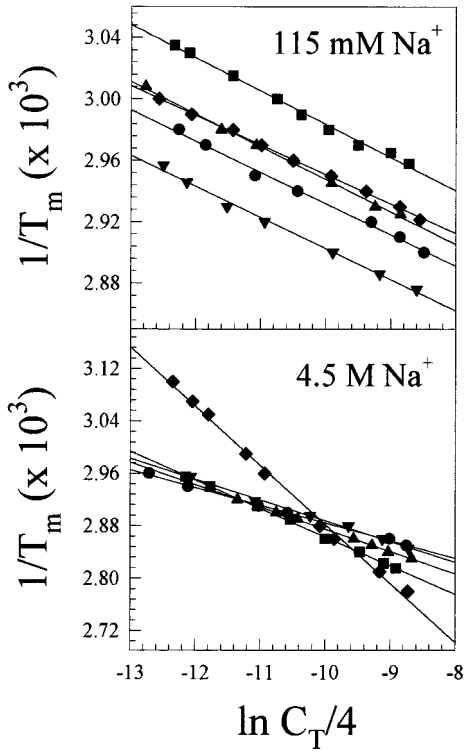

FIGURE 3: The van't Hoff plots ($1/T_m$ vs ln $C_T/4$) for the DNA duplexes possessing A/C mismatches in 115 mM Na^+ (upper panel) and 4.5 M Na^+ (lower panel): **BZ-II 9AC** (circles); **BZ-I 10CA** (squares); **BZ-VII 10CA** (triangles); **BZ-X 10CA** (upside down triangles); and **BZ-VI 11CA** (diamonds). The lines drawn represent the least-squares fits.

The thermodynamic properties determined using eqs 1 and 2 for the duplex to single strand transition are reported in Tables 1 and 2. Table 1 compares the thermodynamic values of A/C mismatched base pair duplexes relative to those of the perfect matched base pair duplexes at both 115 mM Na^+ and 4.5 M Na^+. For **BZ-II 9AC**, where an A:C base pair replaces an A:T base pair at position 9 of **BZ-II**, a decrease in free energy of 3.5 kcal/mol relative to **BZ-II** results due to the mismatch. Replacing a C:G base pair by a C:A base pair at either position 10 or 11 causes a 5.1−5.2 kcal/mol decrease in free energy relative to the perfectly matched base pair parent. These decreased thermodynamic values of the A/C mismatched base pair duplexes relative to the perfect matched base pair duplexes at 115 mM NaCl, are consistent with those reported by Gaffney and Jones (*16*).

In 4.5 M Na^+, the degree of destabilization imparted by replacing an A:T base pair by an A:C base pair is similar to

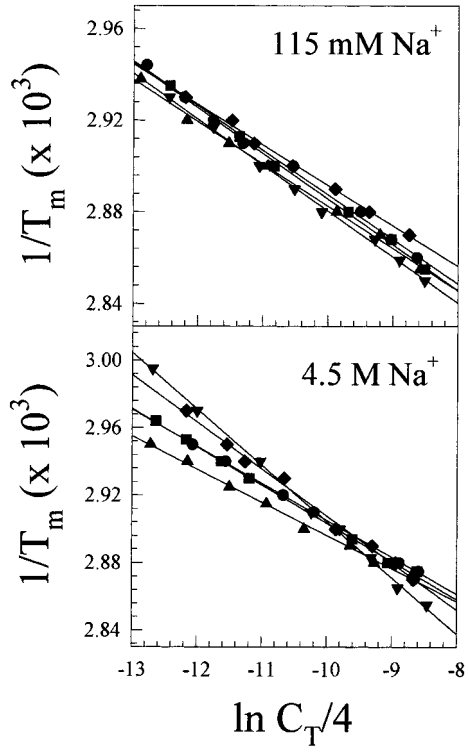

FIGURE 4: The van't Hoff plots ($1/T_m$ vs ln $C_T/4$) for the DNA duplexes possessing G/T mismatches in 115 mM Na^+ (upper panel) and 4.5 M Na^+ (lower panel): **BZ-VI 9GT** (circles); **BZ-II 10TG** (squares); **BZ-VIII 10GT** (triangles); **BZ-I 11GT** (upside down triangles); and **BZ-VII 11GT** (diamonds). The lines drawn represent the least squares fits.

Table 1: Thermodynamic Parameters for the Duplex to Single Strand Transitions for Perfect Matched Base Pair Duplexes vs A/C Base Mismatch Duplexes

oligomer	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/K mol)	$\Delta G°$ (kcal/mol)[a]	$\Delta\Delta G°$ (kcal/mol)[b]
		115 mM Na^+		
BZ-II	121 ± 2	330 ± 5	18.7 ± 0.3	3.5
BZ-II 9AC	98 ± 2	267 ± 3	15.2 ± 0.2	
BZ-I	126 ± 2	347 ± 7	18.4 ± 0.3	5.1
BZ-I 10CA	92 ± 1	254 ± 3	13.3 ± 0.1	
BZ-VII	119 ± 2	320 ± 4	19.8 ± 0.3	5.2
BZ-VII 10CA	94 ± 1	256 ± 5	14.6 ± 0.2	
BZ-X	123 ± 3	330 ± 8	20.7 ± 0.4	5.2
BZ-X 10CA	98 ± 2	266 ± 6	15.5 ± 0.2	
BZ-VI	119 ± 2	321 ± 6	19.5 ± 0.3	5.2
BZ-VI 11CA	103 ± 2	286 ± 5	14.3 ± 0.2	
		4.5 M Na^+		
BZ-II	116 ± 2	318 ± 5	17.4 ± 0.2	3.2
BZ-II 9AC	74 ± 1	193 ± 3	14.2 ± 0.1	
BZ-I	118 ± 3	325 ± 7	17.3 ± 0.3	6.4
BZ-I 10CA	45 ± 2	110 ± 3	10.9 ± 0.1	
BZ-VII	112 ± 3	301 ± 3	18.7 ± 0.3	6.6
BZ-VII 10CA	58 ± 3	148 ± 4	12.1 ± 0.3	
BZ-X	114 ± 2	305 ± 7	19.5 ± 0.4	6.4
BZ-X 10CA	63 ± 3	161 ± 5	13.1 ± 0.2	
BZ-VI	112 ± 1	302 ± 4	18.4 ± 0.2	10.0
BZ-VI 11CA	22 ± 1	44 ± 3	8.4 ± 0.1	

[a] Calculated at 310 K. [b] The $\Delta\Delta G°$ values are obtained by subtracting the $\Delta G°$ for the mismatched duplex from that of the perfect duplex: a positive value indicates the perfect duplex is more stable.

that at low salt for **BZ-II 9AC**. However, replacement of the G:C base pair by a C:A base pair at position 10, as in **BZ-I 10CA**, **BZ-VII 10CA**, and **BZ-X 10CA**, results in an additional destabilization of 1.2−1.3 kcal/mol in high salt. The additional destabilization is even more pronounced when the replacement is at position 11 as observed for **BZ-VI 11CA**. Here, the oligomer is destabilized by an additional 4.8 kcal/mol. These $\delta\Delta\Delta G°$ values are tabulated in Table 3.

Table 2: Thermodynamic Parameters for the Duplex to Single Strand Transitions for Perfect Matched Base Pair Duplexes vs G/T Base Mismatch Duplexes

oligomer	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/K mol)	$\Delta G°$ (kcal/mol)[a]	$\Delta\Delta G°$ (kcal/mol)[b]
		115 mM Na^+		
BZ-VII	119 ± 2	320 ± 4	19.8 ± 0.3	3.4
BZ-VII 9GT	102 ± 2	276 ± 5	16.4 ± 0.2	
BZ-II	121 ± 2	330 ± 5	18.7 ± 0.3	1.8
BZ-I 10TG	100 ± 3	268 ± 6	16.9 ± .02	
BZ-VIII	118 ± 2	317 ± 6	19.7 ± 0.4	1.9
BZ-VIII 10TG	108 ± 2	291 ± 5	17.8 ± 0.3	
BZ-I	126 ± 2	347 ± 7	18.4 ± 0.3	1.9
BZ-I 11TG	99 ± 2	266 ± 6	16.5 ± 0.2	
BZ-VII	119 ± 2	320 ± 4	19.8 ± 0.3	1.7
BZ-VII 11TG	113 ± 3	306 ± 5	18.1 ± 0.3	
		4.5 M Na^+		
BZ-VII	112 ± 3	301 ± 3	18.7 ± 0.3	3.3
BZ-VII 9GT	91 ± 3	244 ± 4	15.4 ± 0.3	
BZ-II	116 ± 2	318 ± 5	17.4 ± 0.2	2.2
BZ-II 10TG	88 ± 2	235 ± 4	15.2 ± 0.1	
BZ-VIII	111 ± 2	297 ± 4	18.9 ± 0.2	2.5
BZ-VIII 10TG	101 ± 3	273 ± 3	16.4 ± 0.2	
BZ-I	118 ± 3	325 ± 7	17.3 ± 0.3	4.7
BZ-I 11TG	60 ± 2	153 ± 3	12.6 ± 0.1	
BZ-VII	112 ± 3	301 ± 3	18.7 ± 0.3	4.4
BZ-VII 11TG	80 ± 2	212 ± 4	14.3 ± 0.2	

[a] Calculated at 310 K. [b] The $\Delta\Delta G°$ values are obtained by subtracting the $\Delta G°$ for the mismatched duplex from that of the perfect duplex: a positive value indicates the perfect duplex is more stable.

Table 3: Comparisons of the Destabilizations Presented by Mismatches as a Function of Type and Position

alteration	position	oligomer	$\Delta\Delta G°$ (kcal/mol) 115 mM Na^+	$\Delta\Delta G°$ (kcal/mol) 4.5 M Na^+	$\delta\Delta\Delta G°$ (kcal/mol)
A:T → A:C	9	**BZ-II 9AC**	3.5	3.2	+0.3
G:C → G:T	9	**BZ-VII 9GT**	3.4	3.3	+0.1
C:G → C:A	10	**BZ-I 10CA**	5.1	6.4	−1.3
	10	**BZ-VII 10CA**	5.2	6.6	−1.4
	10	**BZ-X 10CA**	5.2	6.4	−1.2
T:A → T:G	10	**BZ-II 10TG**	1.8	2.2	−0.4
	10	**BZ-VII 10TG**	1.9	2.5	−0.6
C:G → C:A	11	**BZ-VI 11CA**	5.2	10.0	−4.8
T:A → T:G	11	**BZ-I 11TG**	1.9	4.7	−2.8
	11	**BZ-VII 11TG**	1.7	4.4	−2.7

The data in Table 2 compare the thermodynamic properties of G/T mismatched base pair duplexes to the perfect matched base pair analogues. The data also suggest that G/T mismatched base pair duplexes are less thermally stable compared to the perfect matched base pair duplexes and that the magnitude of the destabilization in low salt depends upon the sequence context and, in high salt, depends upon the type of base pair mismatch as well as its position relative to the B−Z junction. Specifically, replacement of a G:C base pair by a G:T base pair, as observed for **BZ-VII 9GT**, decreases the free energy of duplex formation for the mismatched base pair duplex by 3.4 kcal/mol relative to perfect **BZ-VII**. As observed for the duplex with an A/C mismatch at position 9, the G/T mismatch at position 10 does not impart additional destabilization when the oligomer is prepared in high salt. Replacement of a T:A base pair by a T:G base pair at position

Table 4: Comparison of the Thermodynamic Parameters for the Duplex to Single Strand Transitions at Low and High Salt for Base Mismatch Duplexes[a]

oligomer	$\Delta\Delta H°$ (kcal/mol)	$\Delta\Delta S°$ (cal/K mol)	$\Delta\Delta G°$ (kcal/mol)
BZ-II 9AC	24	74	1.0
BZ-I 10CA	47	144	2.4
BZ-VII 10CA	36	108	2.5
BZ-X 10CA	35	105	2.4
BZ-VI 11CA	81	242	5.9
BZ-VII 9GT	11	32	1.0
BZ-II 10TG	12	33	1.7
BZ-VIII 10TG	7	18	1.4
BZ-I 11TG	39	113	3.9
BZ-VII 11TG	33	94	3.8

[a] Values were calculated by subtracting the particular parameter value for the mismatched duplex from that of the perfect duplex using values tabulated in Tables 1 and 2.

10 or 11 results in a destabilization of about 1.8 kcal/mol (as in **BZ-II 10TG**, **BZ-VII 10TG**, **BZ-I 11TG**, and **BZ-VII 11TGT**). As observed with the duplexes possessing C/A mismatches at position 10 or 11, the duplexes possessing G/T mismatches at those positions are additionally destabilized when prepared in high salt. The degree of additional destabilization is slight for the mismatch at position 10 (i.e., *ca*. 0.5 kcal/mol) and moderate for the mismatch at position 11 (i.e., *ca.* 2.8 kcal/mol).

The effects on the stabilities of the mismatched oligomers relative to their parent analogues as a function of position of the mismatch are summarized in Table 3. The $\Delta\Delta G°$ values indicate the destabilization of the mismatched base pair duplex relative to the perfect matched base pair duplex at 115 mM Na^+ where the duplex is fully right-handed or 4.5 M Na^+ where the duplex is in the hybrid BZ conformation. The $\delta\Delta\Delta G°$, hence, indicate the effect of having *both* a B−Z junction and a mismatched base pair nearby. Keeping in mind that the junction in the perfect duplex spans base pairs 7, 8, and 9 (*33*), it is apparent that the farther away the mismatched base pair is from the junction, the more destabilizing effect it has. With the mismatched base pair *in the junction* (i.e, at position 9), little difference is noted.

For a final comparison, it is worthwhile to compare the low and high salt thermodynamic parameters for each individual mismatched base pair duplex (Table 4). For either **BZ-II 9AC** or **BZ-VII 9GT**, the difference in free energy of duplex formation between the low salt form ("B-like") and high salt form ("perturbed BZ conformation") is only 1.0 kcal/mol. This is the same difference observed between the low and high salt forms of the perfect matched base pair duplexes (*38*, *39*). With the base pair mismatches at the other positions, the magnitude of the $\Delta\Delta G°$ is greater at position 11 than at position 10 and is greater for a C:G to C:A permutation than for a T:A to T:G permutation. Finally, both enthalpic and entropic contributions play a role in the magnitudes of the $\Delta\Delta G°$ values.

DISCUSSION

It has been speculated that the B−Z junction is highly flexible. Consequently, B−Z junctions in DNA were thought to be sites that may easily accommodate base pair mismatches with negligible energy cost. Consistent with this speculation is the observation that a mismatched base pair in the junction (i.e., at position 9) destabilizes the duplex to

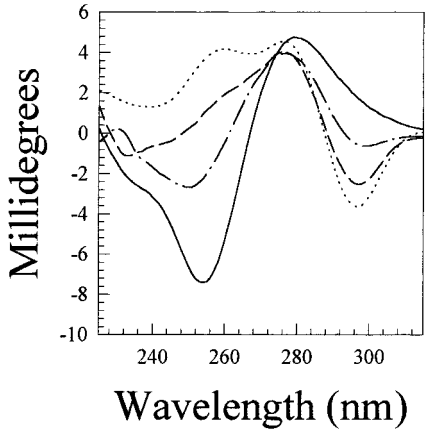

FIGURE 5: A comparison of the CD spectra of **BZ-II 9AC** at 115 mM Na^+ (solid line), 4.5 M Na^+ (dashed line), 200 mM $[Co(NH_3)_6]^{3+}$ (dash-dot line), and **Z8** (dotted line) at 3.0 M Na^+ in standard phosphate buffer at 25 °C.

the same extent under both conformational conditions (i.e., low salt and high salt). However, our studies demonstrate that a base pair mismatch near a B−Z junction results in additional thermodynamic destabilization of the duplexes ranging from 0.5 to 4.8 kcal/mol in 4.5 M Na^+, where the duplexes are in the perturbed B−Z conformation, relative to the perfect matched base pairs duplexes under the same conditions. In addition, the CD spectra of all oligomers are consistent with that of a fully right-handed structure in low salt but a perturbed B−Z conformation under high salt. Hence, the presence of the mismatched base pair strongly influences both the structure and stability of the duplexes under both conformational conditions.

Three possible effects on local structure and stability of a DNA duplex due to base pair mismatches were proposed by Tibanyenda et al. (*13*): (i) loss of free energy (5.0−7.0 kcal/mol) due to interior loop formation by base pair mismatches; (ii) loss of free energy (7.0 kcal/mol) due to formation of two bulged out base pair mismatches accompanied by loss of nearest neighbor stacking interaction; and (iii) integration of the mismatched base pair into double helix, resulting in a significant degree of base stacking with minimal loss of free energy (3.0 kcal/mol). According to these three effects, our UV melting and CD studies of the mismatch base pair duplexes in 115 mM Na^+ indicate that they are essentially intact right-handed double helices.

The presence of 4.5 M Na^+ leads to additional structural and thermodynamic perturbations for the oligomers possessing mismatched base pairs adjacent to the B−Z junctions formed under these conditions. These perturbations arise from a combination of contributions from variances in base stacking and hydrogen bonding as well as solvent dehydration. Consistent with our CD studies, it is likely that at 4.5 M Na^+, dehydration significantly contributes to the distortion of the hybrid BZ conformation of the mismatch base pair duplexes. The B-DNA domain's CD troughs at 255 nm of the perturbed hybrid BZ structures diminish while the Z-DNA domain's CD troughs at 295 nm are minimally perturbed relative to the perfect base pair analogues. It almost appears that the perturbed hybrid B−Z conformation of these duplexes, especially those of **BZ-II 9AC** and **BZ-VII 9GT,** resemble Z-DNA (Figure 2). However, close examination of the CD spectra for **BZ-II 9AC** presented in Figure 5 is consistent a hybrid BZ conformation in 4.5 M

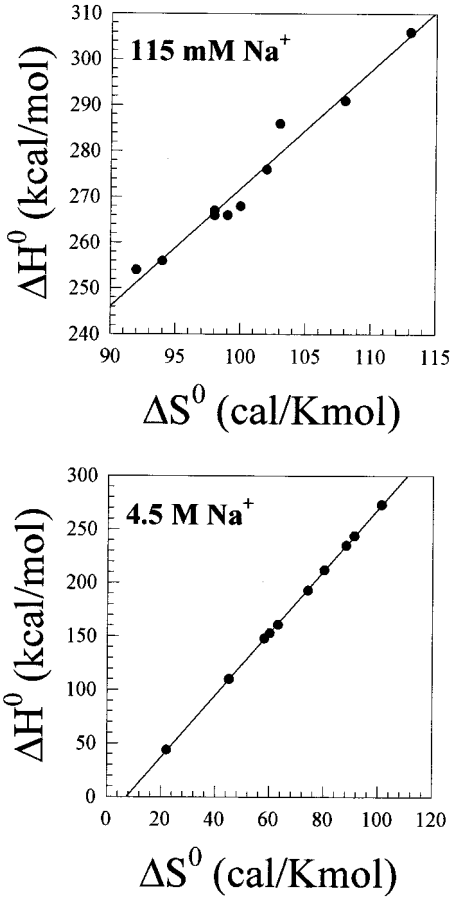

FIGURE 6: Plots of enthalpy vs entropy for the mismatched duplexes at 115 mM Na^+ (upper panel) and 4.5 M Na^+ (lower panel). The lines represent the least-squares fits.

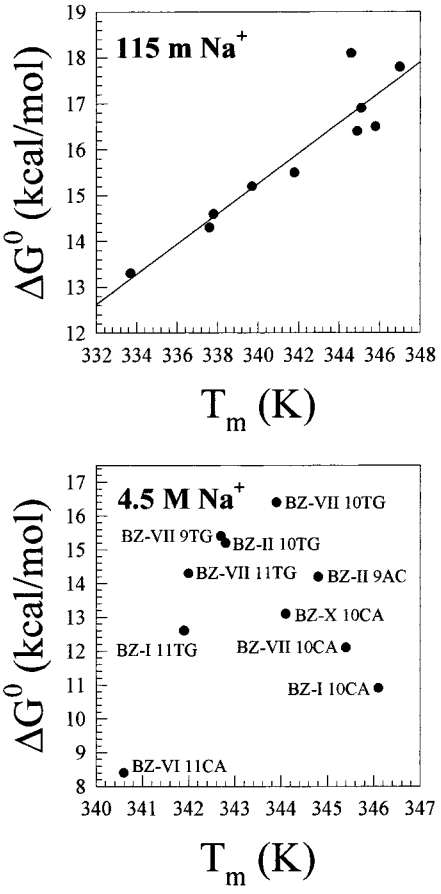

FIGURE 7: Comparison of the experimentally determined free energies of transition to the calculated T_m of the duplex at 100 mM in base pairs. The line drawn for the 115 mM Na^+ plot represents the least squares fit.

Na^+. In particular, the titration of **BZ-II 9AC** with non dehydrating transition metal complex, cobalt hexamine, results in a spectrum more typical for an unperturbed BZ junction duplex. Comparison of the high salt CD spectrum of **BZ-II 9AC** with that of **Z8** indicates the absence of the peak at 250 nm. Together, these data suggest that the conformations of the mismatch base pair duplexes are not pure Z-DNA but rather perturbed B−Z conformations.

In order to assess the validity of our thermodynamic parameters listed in Tables 1 and 2, we carried out the following analyses. Figure 6 shows a plot of enthalpy vs entropy for the mismatch base pair DNA duplexes. As can be seen, there is very good linear correlation at 115 mM Na^+ ($r^2 = 0.967$) and excellent correlation at 4.5 M Na^+ ($r^2 = 0.999$). Excellent linear correlations between enthalpy and entropy were also found for the perfect base paired duplexes (data not shown). These correlations indicate that the resultant $\Delta G°$ values should be quite reasonable. In the case of the perfect duplexes, we have shown very good to excellent agreements between experimentally determined $\Delta\Delta G°$ ($\Delta\Delta G°$ compares the free energy of duplex formation of a particular duplex relative to **BZ-I**) values to theoretical values calculated using three different approaches (*39*). In the case of the mismatched duplexes, there are currently no models available to determine theoretical $\Delta\Delta G°$ values. Certainly, the presence of a mismatched base pair will result in enthalpic destabilization due to alterations in hydrogen bonding. The mismatch would also result in a distorted (i.e., less ordered) duplex giving rise to an entropic change of lower magnitude upon denaturation. Hence, the loss in the enthalpic contribution to the total free energy is compensated by the entropic contribution.

We have also found a very good correlation between T_m (calculated for 100 mM DNA at 1.0 M NaCl) and $\Delta G°$ for the perfect matched base pair duplexes at both 115 mM and 4.5 M Na^+ (Otokiti and Sheardy, unpublished results). Figure 7 illustrates the correlation between the T_m (calculated for [DNA] = 100 mM) and $\Delta G°$. As can be seen, there is good correlation at 115 mM Na^+ ($r^2 = 0.878$) with one deviant point corresponding to **BZ-VII 11TG**. However, there is a complete lack of correlation at 4.5 M Na^+. Examination of the assignments of the data points indicates a complete randomness in terms of type and position of the base pair mismatch variations. This lack of correlation is difficult to rationalize in light of the excellent correlation between $\Delta H°$ and $\Delta S°$ depicted in Figure 6. One source for the apparent lack of correlation at high salt may be due to deviations from two-state behavior for the duplex to single-strand transition. Without calorimetric measurements, one cannot determine the magnitudes of such deviations.

Assuming that an A/C wobble base pair has one hydrogen bond (at pH 7.0) and a G/T wobble base has two hydrogen bonds, then A:T → A:C and a G:C → G:T permutations both result in the net loss of one hydrogen bond. The data in Table 3 indicate that this loss results in a decrease in the free energy of duplex formation of about 3.5 kcal/mol in both low and high salt. A C:G → C:A permutation results in a net loss of two hydrogen bonds and subsequent decrease in the free energy of duplex formation of about 5.2 kcal/

Table 5. Comparisons of the $\Delta\Delta G°$ Values Reported in This Work to Previously Reported Values for Different Types of Permutations

alteration	H-bonds lost[a]	$\Delta\Delta G°$ (kcal/mol)					
		b	*c*	*d*	*e*	*f*	*g*
T:A → T:G	0	2.4	3.2, 2.6, 1.8	2.0	1.1	3.3	−
A:T → A:C	1	4.2	3.7	5.2	3.5	3.4	−
G:C → G:T	1	3.9	3.1	3.7	3.0	3.6	2.6
C:G → C:A	2	5.9	−	5.5	3.5	5.5	−

[a] Assuming a T·G mismatch has two hydrogen bonds and an A·C mismatch has one hydrogen bond. Free energy values were calculated at $T = 298$ K. [b] This report, determined at 115 mM Na^+ and recalculated for $T = 298$ K. Values entered for T:A → T:G and C:G → C:A are averaged. [c] Tibanyenda, N., De Bruin, S. H., Hassnoot, C. A. G., van der Marel, G. A., van Boom, J. H., and Hilbers, C. W. (1984) *Eur. J. Biochem. 139*, 19−27; determined at 1.0 M Na^+. The values listed for the T:A → T:G alteration varies as a function of position in the duplex. [d] Aboul-ela, F., Koh, D., and Tinoco, I., Jr. (1985) *Nucleic Acids Res. 13*, 4811−4824; determined at 1.0 M NaCl. [e] Werntges, H., Steger, G., Riesner, D., and Fritz, H.-J. (1986) *Nucleic Acids Res. 14*, 3773−3790; determined in 500 mM NaCl. [f] Gaffney, B. L., and Jones, R. A. (1989) *Biochemistry 28*, 5881−5889; determined at 115 mM Na^+. [g] Plum, G. E., Grollman, A. P., Johnson, F., and Breslauer, K. J. (1995) *Biochemistry 34*, 16148−16160; determined at 1.0 M NaCl.

mol at low salt. However, at high salt, an additional destabilization of about 1.3 kcal/mol is observed when the base pair mismatch occurs at position 10 and 4.8 kcal/mol with the mismatch at position 11. Finally, a T:A → T:G permutation results in a net loss of no hydrogen bonds but still destabilizes the duplex by about 1.8 kcal/mol at low salt. At high salt, an additional 0.5 kcal/mol of destabilization is observed when the base pair mismatch occurs at position 10 and 2.8 kcal/mol with the mismatch at position 11. Hence, as the relative position of the base pair mismatch moves further away from the potential B−Z junction, the duplex becomes more thermally unstable. It should be kept in mind that all of these sequence context variations will affect the contribution to the total free energy of duplex formation from nearest neighbor interactions as well. Table 5 compares the values determined here (recalculated for $T = 298$ K) to those from previous studies. In general, there is good agreement among the various sets of values at least as far as trends are concerned. Comparison of the magnitudes of the $\Delta\Delta G°$ values should be taken with caution due to the variation in concentration of Na^+ that the values were determined at.

Previous NMR studies demonstrated that the B−Z junction is dramatically distorted from either B or Z conformation (*33*). However, this distortion apparently contributes only about 0.5 kcal/mol to the total destabilization of the duplex under high salt conditions (*20*). The additional destabilization in high salt of the duplexes possessing mismatched base pairs at positions 10 or 11 must arise from additional perturbations of the structure. The B−Z junction in **BZ-I** spans positions 7, 8, and 9 and the NMR studies indicated that these base pairs were distorted from either a true B or Z conformation (*33*). The presence of a mismatched base pair at position 10 expands the number of distorted base pairs from three to four; the presence of the mismatch at position 11 may result in a total of five distorted base pairs. NMR studies of the duplex of d(CGCGAATTCACG) indicate that the presence of the two A/C mismatches perturbs base pairing up to three base pairs away from the mismatches (*8*). Hence, the magnitudes of the $\delta\Delta\Delta G°$ values in Table 3 should not be surprising. Although the junction is presumably fully hydrogen bonded (*33*), the presence of the junction may also influence the hydrogen bonding of the nearby mismatches. Certainly, NMR structural studies on these mismatch duplexes would address that question.

SUMMARY

The detailed optical melting studies of the mismatched base pair duplexes reveal that their thermodynamic stabilities vary with the type of base pair mismatch involved and its relative position in duplex. Both hydrogen bonding schemes and geometric asymmetry of base pair mismatches strongly influence the perturbation of B−Z junctions at high salt. The effects of dehydration at high NaCl concentration decrease the propensity of the free polar groups on DNA to form hydrogen bonds with the solvent molecules thereby decreasing the thermal stability of the duplex. The thermodynamic destabilization of the base pair mismatches A/C and G/T range from 1.7 to 5.2 kcal/mol in low salt and from 2.2 to 10.0 kcal/mol in high salt in free energy relative to the perfect matched base pair duplexes. The additional destabilization of the base pair mismatched duplexes at 4.50 M NaCl depends on the relative position of the base pair mismatch, nearest neighbor base pair interactions and dehydration due to high NaCl concentration.

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