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Thermodynamic Consequences of an Abasic Lesion in Duplex DNA Are Strongly Dependent on Base Sequence†

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ABSTRACT: The abasic site in DNA may arise spontaneously, as a result of nucleotide base damage, or as an intermediate in glycosylase-mediated DNA-repair pathways. It is the most common damage found in DNA. We have examined the consequences of this lesion and its sequence context on DNA duplex structure, as well as the thermal and thermodynamic stability of the duplex, including the energetic origins of that stability. To this end, we incorporated a tetrahydrofuran abasic site analogue into a family of 13-mer DNA duplexes, wherein the base opposite the lesion (A, C, G, or T) and the base pairs neighboring the lesion (C·G or G·C) were systematically varied and characterized by a combination of spectroscopic and calorimetric techniques. The resulting data allowed us to reach the following conclusions: (i) the presence of the lesion in all sequence contexts studied does not alter the global B-form conformation characteristic of the parent undamaged duplex; (ii) the presence of the lesion induces a significant enthalpic destabilization of the duplex, with the magnitude of this effect being dependent on the sequence context; (iii) the thermodynamic impact of the lesion is dominated by the identity of the neighboring base pairs, with the cross strand partner base exerting only a secondary thermodynamic effect on duplex properties. In the aggregate, our data reveal that even in the absence of lesion-induced alterations in global structure, the abasic lesion can significantly alter the thermodynamic properties of the host duplex, with the magntiude of this impact being strongly dependent on sequence context.

In a nucleic acid duplex, loss of a nucleotide base by cleavage of the glycosidic bond results in an abasic lesion [either an apurinic or apyrimidinic (AP) lesion]. Abasic sites in DNA arise in vivo from spontaneous hydrolysis of N-glycosyl bonds (*1*) and as intermediates in DNA glycosylase-mediated repair of damaged bases (*2*−*5*). Nascent abasic lesions often are found with cytosine on the opposite strand, although any of the nucleotide bases may be found opposite the lesion. Abasic sites are repaired in vivo by various enzymatic repair pathways (*6*, *7*), thereby avoiding mutations (*3*, *8*−*10*) that could arise due to the intrinsic lack of coding information at abasic sites. If template-directed nucleic acid synthesis, either in vitro or in vivo, proceeds in the presence of (is not blocked by) an abasic site, the newly synthesized strand may contain an inserted nucleotide, often adenosine, or a deletion (*9*, *11*−*15*).

Structural studies of DNA duplexes containing abasic sites show these duplexes to be in the B-form, with only localized perturbations at the lesion site. There are several intrahelical and extrahelical conformations which have been observed for the natural or model abasic site moieties, as well as for cross-strand nucleotides (*16*−*24*). Because many of the molecular forces which stabilize nucleic acid structures operate over very short distances, subtle differences between NMR-derived duplex structures with and without abasic sites may correspond to large differences in the observed thermodynamic parameters.

The thermodynamic consequences of abasic sites have been examined both calorimetrically (*25*, *26*) and noncalorimetrically (*25*, *27*, *28*) by monitoring thermally induced dissociation of oligomeric duplexes which contain single model abasic sites. These studies reveal that, relative to undamaged control duplexes, incorporation of an abasic site results in significant lesion-induced reductions in thermal (T_m) and thermodynamic ($\Delta G°$) stability, transition enthalpy ($\Delta H°$), and transition entropy ($\Delta S°$). On the basis of the small number of studies to date, it seems that interphosphate distance, rather than the exact structure of an abasic site analogue, is the primary determinant of an abasic lesion's influence on duplex thermodynamic properties (*25*, *27*, *28*). The efficiency of several enzymatic lesion repair processes also is influenced strongly by interphosphate distance (*9*, *29*, *30*). Currently, however, there is little information available on the influence of the base opposite and the base pairs adjacent to the abasic site on the thermodynamic impact of the lesion. It is not known whether or how the thermodynamic and biological effects noted above depend on the base opposite and the base pairs neighboring the lesion. Such knowledge is needed to understand the mechanisms of recognition and repair of abasic lesions.

In this study, we examine the influences of sequence context and opposing base of the single abasic site on the structure, energetics, and stability of a DNA duplex. To this

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end, we use circular dichroism spectropolarimetry, temperature-dependent UV absorbance spectroscopy, and differential scanning calorimetry to characterize the properties of a set of 12 non-self-complementary 13-mer duplexes, which are identical except for their central base pair. Four control duplexes contain one of the normal Watson−Crick base pairs in the central position. The remaining eight duplexes contain a model abasic site at the central position of one of the two strands. The terahydrofuranyl abasic site (F), a chemically stable model for the natural abasic site, has been demonstrated to retain the biological properties of the natural abasic site (*8*).

To begin to assess the influence of nucleotide sequence on the impact of the lesion, abasic sites were placed in two sequence contexts, between the two dG residues or between two dC residues. To assess the influence of the partner strand "unpaired" base on the impact of the abasic lesion on duplex properties, each of the four canonical deoxyribonucleotides was placed opposite the abasic site in both sequence contexts. Because the nucleotide sequence beyond the lesion site is maintained, differences between the observed properites of these duplexes can be attributed to differences at and near the site of the lesion.

MATERIALS AND METHODS

Oligonucleotide Synthesis and Purification. Synthesis and purification of oligonucleotides were carried out as described previously (*26*). Ten 13-mer deoxyribonucleotides were synthesized. These oligomers are of two families; within each family, only the central nucleotide was varied. The specific nucleotide sequences are shown below:

Strand 1 5'-CGCATGNGTACGC-3'
Strand 2 5'-GCGTACNCATGCG-3'

where N represents each of the four standard nucleotides, A, C, G, and T, as well as F, the tetrahydrofuranyl abasic site analogue. From these 10 oligonucleotides, 12 duplexes were assembled. Hereafter, we refer to the duplexes by abbreviations of the form Strand 1·Strand 2, where only the central base, N, of each strand is listed. The abbreviations N·F and F·N are used to represent the two families of four duplexes in which N represents each of the four standard bases. Note that the flanking nucleotide sequences of the above-described duplexes are identical to those used in our previous thermodynamic studies of abasic sites (*25*, *26*) and other lesions (*31*, *32*).

A molar extinction coefficient for each oligonucleotide was determined by phosphate analysis using enzyme degradation and colorimetric detection (*33*, *34*; Plum and Breslauer, unpublished results). The molar extinction coefficients (260 nm, 25 °C) so determined are listed in the Supporting Information.

To confirm that duplex formed when pairs of the oligonucleotides were mixed under the experimental conditions, the stoichiometry was evaluated by the method of continuous fractions (*35*, *36*). While monitoring UV absorbance at 260 nm, equimolar solutions of two oligonucleotides were mixed at differing ratios. For each pair of oligonucleotides, an inflection point in the absorbance at 260 nm was observed at a ratio of 1:1 (vol:vol). This observation is consistent with duplex formation.

All experiments were performed in a buffer solution containing 1 M NaCl, 0.1 mM EDTA, and 10 mM sodium phosphate, at pH 7.0.

Circular Dichroism. Circular dichroism spectropolarimetry (CD) was used to evaluate the global structure of each duplex. Spectra were collected between 200 and 350 nm at 0.5 nm resolution and 10 s averaging on an AVIV Model 62DS spectropolarimeter at 25 °C using a 1 mm quartz cuvette with a DNA duplex concentration of 50 μM. CD spectra are presented in molar ellipticity units defined as 100 θ/cl (*37*), where θ is ellipticity (degrees), c is duplex concentration (M), and l is path length (cm).

Duplex Melting. Differential scanning calorimetry (DSC) and temperature-dependent UV absorbance spectrophotometry were used to monitor the thermally induced order−disorder transition of each DNA duplex. Duplex samples at 150−200 μM concentration were subjected to repeated heating and cooling cycles in a Microcal MC2 DSC. Repetative scans were superimposable. Calorimetric enthalpies reported were determined from at least six independently analyzed (both baseline assignment and integration) DSC melting profiles. Analysis of these data to extract the duplex dissociation enthalpy, $\Delta H°$, has been described elsewhere (*38*, *39*).

UV absorbance monitored melting experiments were performed at a minimum of seven concentrations ranging from 200 μM down to 1 μM duplex. Samples in quartz cuvettes, ranging from 0.035 to 1.0 cm in path length, were heated in the thermostated sample chamber of an AVIV 14DS UV−vis-IR spectrophotometer. The absorbance at 260 nm was monitored with 10 s averaging time. Analysis of such data to extract the duplex melting temperature, T_m, has been described elsewhere (*38*, *39*).

The van't Hoff enthalpies and effective molecularities (*32*, *40*) were determined from the dependence of T_m on the DNA concentration by separate application of the following equation (*38*):

$$\frac{1}{T_m} = \frac{(n-1)R}{\Delta H°} \ln C_t + \text{intercept}$$

where C_t is the total strand concentration. The slope of the $1/T_m$ vs ln C_t curve was measured and set equal to $(n-1)R/\Delta H°$. The van't Hoff enthalpy, ΔH^{vH} is computed by setting the molecularity, n, to a value of 2 and solving for $\Delta H°$. The effective molecularity, n_{eff}, is determined by setting $\Delta H°$ in the equation to the measured calorimetric value and solving for n.

For normal and lesion-containing duplexes, values for the free energy change ($\Delta G°$) associated with DNA duplex dissociation were calculated by combining calorimetrically measured enthalpy changes ($\Delta H°$) with the effective molecularity and T_m data (*32*, *40*) using the relation

$$\Delta G° = \Delta H°\left(1 - \frac{T}{T_m}\right) - RT\,(n_{eff} - 1)\ln\left(\frac{C_t}{2n_{eff}}\right)$$

The error estimates provided are the result of propogation of error through the appropriate equations. As in all such studies, when thermodynamic parameters are designated with

Table 1: Effect of the Abasic Lesion on the Thermal, T_m, and Thermodynamic Stability, $\Delta G^\circ_{25°C}$, of the Duplex

sequence family	duplex	T_m (°C)[a]	ΔT_m (°C)[a,c]	$\Delta G^\circ_{25°C}$ (kcal/mol)	$\Delta\Delta G^\circ_{25°C}$ (kcal/mol)
	A·T	68.2 ± 0.5		19.6 ± 0.6	
-GNG-	C·G	71.4 ± 0.5		20.5 ± 0.5	
-CNC-	G·C	69.2 ± 0.5		20.4 ± 0.6	
	T·A	68.4 ± 0.5		20.4 ± 1.1	
	F·A	56.3 ± 0.5	−12.1 ± 0.7	16.0 ± 0.4	−4.3 ± 0.6
-GFG-	F·C	55.3 ± 0.5	−13.8 ± 0.7	15.4 ± 0.2	−3.0 ± 0.3
-CNC-	F·G	52.2 ± 0.5	−19.2 ± 0.7	14.7 ± 2.0	−5.7 ± 1.0
	F·T	53.0 ± 0.5	−15.2 ± 0.7	15.1 ± 0.6	−4.5 ± 0.4
	A·F	54.6 ± 0.5	−13.6 ± 0.7	12.0 ± 0.5	−7.6 ± 0.4
-GNG-	C·F	50.7 ± 0.5	−20.7 ± 0.7	9.1 ± 0.8	−11.3 ± 0.5
-CFC-	G·F	54.7 ± 0.5	−14.5 ± 0.7	12.1 ± 0.9	−8.3 ± 0.5
	T·F	52.5 ± 0.5	−15.9 ± 0.7	9.1 ± 0.5	−11.3 ± 0.6
-GFG- -CFC-	F·F	55.8 ± 0.5[b]	−13.6 ± 0.7[d]	16.0 ± 0.2[b]	−4.2 ± 0.9[d]
-GG- -CC-	D·D	67.9 ± 0.5[b]	−1.4 ± 0.7[d]	18.5 ± 0.2[b]	−1.7 ± 0.9[d]

[a] All solutions contain 50 μM duplex DNA. [b] Values taken from ref 26. [c] ΔT_m = T_m (abasic containing duplex) − T_m (parent duplex) and $\Delta\Delta G^\circ_{25°C}$ = $\Delta G^\circ_{25°C}$ (abasic containing duplex) − $\Delta G^\circ_{25°C}$ (parent duplex). [d] An average with respect to each of the four parent duplexes.

superscript °'s, they refer to an operationally defined standard state and not to standard temperature and pressure at infinite dilution.

RESULTS AND DISCUSSION

I. Effects of Abasic Lesions on Thermal and Thermodynamic Stability

Formation of an Abasic Lesion Thermally Destabilizes the Duplex. Examination of Table 1 indicates that, relative to the corresponding undamaged parent duplexes (the first four listings), the thermal stability of the abasic lesion containing duplexes always is significantly reduced. The observed reductions in T_m (−12.1 ± 0.7 to −20.7 ± 0.7 °C) are consistent in magnitude with those observed for duplexes of similar length containing other single-base lesions (*41*). On average, removal of a pyrimidine base (producing an abasic site with an opposing purine) results in a slightly smaller reduction in T_m ($\langle\Delta T_m\rangle$ = −13.4 ± 0.7 °C) relative to removal of a purine base ($\langle\Delta T_m\rangle$ = −15.0 ± 0.7 °C).

Sequence Context Modulates the Impact of the Abasic Lesion on Duplex Thermal Stability. The dependence of ΔT_m on the nearest neighbor base pairs is very strong for damage to the G·C and C·G base pairs, with ΔT_m values ranging from −13.8 ± 0.7 to −20.7 ± 0.7 °C. In contrast, relatively small sequence-dependent thermal destabilization effects are observed when A or T residues are removed to form the abasic site, ranging from −12.1 ± 0.7 to −15.9 ± 0.7 °C. On average, the reduction in thermal stability for the two sequence context families is indistinguishable, $\langle\Delta T_m\rangle$ = 15.1 ± 0.7 °C for the F·N family and $\langle\Delta T_m\rangle$ = 16.2 ± 0.7 °C for the N·F family of duplexes.

Abasic Lesion-Induced Changes in Duplex Free Energy are Destabilizing and Depend on Sequence Context and the Identity of the Opposing Base. In Table 1, the free energy changes, ΔG° at 25 °C, associated with lesion formation are presented. Examination of these data reveals that abasic site formation results in a destabilization of the duplex from about −4 to −13 kcal/mol. These $\Delta\Delta G^\circ$ values correspond to factors of 10^3−10^9 in the equilibrium constant. The impact of the lesion on duplex stability is far greater than one would expect for the removal of 1 out of 26 bases. This suggests that the energetic impact of lesion formation propagates into the adjacent helix domains.

Removal of both bases of the central pair to form the F·F lesion (*26*) results on average in a significant reduction of favorable free energy, $\langle\Delta\Delta G^\circ\rangle$ = −4.2 ± 0.9 kcal/mol. This "double lesion"-induced destabilization is less than or equal to that of most of the single base removals listed in Table 1. Extending the excision to complete removal of the central nucleotide base pair to form the 12-mer duplex D·D results in only a modest reduction of favorable free energy relative to the parent duplexes, $\langle\Delta\Delta G^\circ\rangle$ = −1.7 ± 0.9 kcal/mol.

The magnitude of lesion-induced destabilization depends strongly on the identity of the bases which neighbor the abasic site, with a secondary effect of the identity of the base across from the lesion site. When the abasic site is flanked by G residues (F·N family), reductions in favorable free energy are observed $\langle\Delta\Delta G^\circ\rangle$ = −4.8 ± 0.6 kcal/mol. Within experimental error, this destabilization is independent of the identity of the base opposite the lesion. Duplexes in which C residues flank the abasic site (N·F family) are severely destabilized, $\langle\Delta\Delta G^\circ\rangle$ = −9.6 ± 0.5 kcal/mol. In this sequence context, duplexes containing purine bases opposite the lesion are greatly destabilized ($\langle\Delta\Delta G^\circ\rangle$ = −8.0 ± 0.5). However, pyrimidine bases opposite the lesion are particularly deleterious to duplex stability ($\langle\Delta\Delta G^\circ\rangle$ = −11.3 ±0.6).

From these data, we conclude that sequence context is the primary determinate of the magnitude of abasic lesion-induced destabilization. The influence of the base opposite the lesion is secondary and depends strongly on the sequence context. Significantly, differences in thermodynamic stability are not reliably reported by differences in T_m values. It is important to note that while the free energy data in Table 1 are at 25 °C, ΔG° and $\langle\Delta\Delta G^\circ\rangle$ values calculated at different temperatures display the same patterns described above. In the following section, we examine the thermodynamic origins of the abasic lesion-induced duplex destabilizations.

II. Thermodynamic Origins of Abasic Site Induced Destabilization

Duplexes Containing Abasic Sites Are Enthalpically Destabilized. In Table 2, we present duplex dissociation enthalpy change data (ΔH°) determined by differential scanning calorimetry. The transition enthalpy change measured for each of the four parent duplexes is consistent with expectation based on nearest neighbor data (*42*). For every duplex, introduction of an abasic site results in a significant enthalpic destabilization. The magnitude of this enthalpic destabilization depends both on the neighboring base pairs and the identity of the base opposite the lesion.

The calorimetric data indicate that duplex transition enthalpy depends strongly on the identity of the bases neighboring the abasic site. Regardless of the base opposite the lesion, the duplexes in which two guanine bases flank the abasic site (F·N) display a significant ($\langle\Delta\Delta H^\circ\rangle$ = −9.9 ± 5.0 kcal/mol) enthalpic destabilization relative to the corresponding undamaged parent duplex. When two cy-

Table 2: Effect of the Abasic Lesion on the Dissociation Enthalpy Change, ΔH, and Effective Molecularity of the Duplex

sequence family	duplex	$\Delta H°$ (kcal/mol)	$\Delta\Delta H°$ [b] (kcal/mol)	ΔH^{vH} [c] (kcal/"mol")	n_{eff}
	A·T	101.6 ± 2.4		100.8 ± 2.3	2.01 ± 0.06
-GNG-	C·G	98.1 ± 1.8		89.8 ± 1.7	2.09 ± 0.05
-CNC-	G·C	104.0 ± 1.9		100.0 ± 3.0	2.04 ± 0.07
	T·A	104.1 ± 4.2		98.2 ± 4.4	2.06 ± 0.12
	F·A	95.9 ± 1.7	−8.2 ± 4.6	92.8 ± 1.1	2.03 ± 0.04
-GFG-	F·C	91.0 ± 0.8	−13.0 ± 2.1	87.4 ± 1.6	2.04 ± 0.04
-CNC-	F·G	87.5 ± 9.6	−10.7 ± 9.7	78.4 ± 2.3	2.12 ± 0.24
	F·T	94.0 ± 2.7	−7.6 ± 3.5	89.5 ± 1.8	2.05 ± 0.07
	A·F	72.9 ± 0.7	−28.7 ± 2.4	89.7 ± 3.3	1.81 ± 0.07
-GNG-	C·F	56.5 ± 1.4	−41.6 ± 2.3	80.7 ± 4.3	1.70 ± 0.10
-CFC-	G·F	66.1 ± 1.0	−37.9 ± 2.2	72.6 ± 4.2	1.91 ± 0.11
	T·F	58.2 ± 1.4	−46.0 ± 4.5	92.0 ± 3.4	1.63 ± 0.07
-GFG- -CFC-	F·F	96.9 ± 2.7[a]	5.1 ± 5.3	88.5 ± 2.6[a]	2.05 ± 0.06
-GG- -CC-	D·D	94.8 ± 1.1[a]	7.2 ± 3.7	90.5 ± 2.7[a]	2.09 ± 0.08

[a] Values taken from ref 26. [b] $\Delta\Delta H° = \Delta H°$ (abasic containing duplex) − $\Delta H°$ (parent duplex). [c] From the concentration dependence of T_m determined from UV absorbance vs T curves.

tosines flank the abasic site (N·F), the enthalpic destabilization is extremely large ($\langle\Delta\Delta H°\rangle = -38.6 \pm 2.9$ kcal/mol). This degree of enthalpic destabilization is remarkable in that removal of a single base costs about 40% of the favorable enthalpy stabilizing the 13-mer duplexes. In contrast, removal of the two central bases or nucleotides results in relatively modest enthalpic destabilization of the duplexes ($\langle\Delta\Delta H°\rangle = -5.1 \pm 5.3$ and -7.2 ± 3.7 kcal/mol respectively) (*26*).

Interplay between Sequence Context and the Lesion-Opposing Base. Neighboring base identity alters not only the magnitude of the duplex transition enthalpy but also its dependence on the identity of the cross-strand base. In contrast to the free energy data, where the four duplexes were indistinguishable, there is a 7 kcal/mol range in $\Delta H°$ when the abasic lesion is flanked by G residues (F·N). The range of $\Delta H°$ values observed extends to about 16 kcal/mol when the abasic lesion is flanked by C residues (N·F). This difference in ranges is not simply an amplification of the cross-strand base dependence by the substitution of neighboring bases, because the relative order of transition enthalpies associated with cross-strand partner depends on the bases flanking the abasic lesion.

van't Hoff Enthalpy Measurements Are Blind to the Enormous Neighbor Effects on Duplex Transition Enthalpy. The van't Hoff equation can be applied to estimate enthalpy values from the temperature dependence of the equilibrium constant. The van't Hoff model, as typically formulated for nucleic acids, assumes a process with a molecularity of 2 in which there are no thermodynamically significant intermediate states. That is, a DNA duplex dissociates in a single step to yield two fully disordered single strands. Table 2 contains van't Hoff enthalpy values derived from the concentration dependence of T_m.

The model-dependent van't Hoff enthalpies, determined optically or calorimetrically, do not reflect the dramatic dependence of the enthalpic impact of the lesion on the neighboring base pairs. On the basis of the van't Hoff enthalpy data in Table 2, one would erroneously conclude that the effect of the neighboring bases is secondary to the identity of the opposing base in determining the enthalpic impact of the abasic lesion.

The calorimetric enthalpies are combined with studies of the concentration dependence of thermal melting, to calculate an effective molecularity for the thermally induced order−disorder transitions of nucleic acid complexes. Deviations from the van't Hoff model are absorbed into the effective molecularity parameter. Here, we see that the parent and the G-flanked abasic site (F·N) containing duplexes exhibit behavior which is consistent with a bimolecular melting process. In contrast, three of the four duplexes in the N·F family exhibit an apparent molecularity which is less than 2.

Taken together, these observations of van't Hoff enthalpies and effective molecularities suggest that the mechanism of melting is dependent on the identity of the base pairs neighboring the abasic site. It is important to note, however, that differences in melting mechanism do not necessarily imply differences in thermodynamics.

Enthalpy−Entropy Compensation. The differences in transition free-energy change ($\Delta\Delta G°$) observed upon formation of an abasic site are significantly smaller than the observed differences in transition enthalpy change ($\Delta\Delta H°$). As noted above, for duplexes in which the two cytosines flank the abasic site (N·F) the enthalpic destabilization is on average -38.6 ± 2.9 kcal/mol, while the overall lesion induced destabilization as reflected in $\Delta\Delta$G° values is $\langle\Delta\Delta G°\rangle = -9.6 \pm 0.5$ kcal/mol. Stated somewhat differently, a large part of the energetic penalty of lesion formation is recovered by entropic effects. Such enthalpy−entropy compensation is a common feature of duplex melting, particularly damaged or modified DNA duplexes (*40*, *43*). Interestingly, the enthalpic destabilization of the duplexes in which two guanines flank the abasic site (F·N) is entropically compensated less strongly ($\langle\Delta\Delta H°\rangle = -9.9 \pm 5.0$ kcal/mol vs $\langle\Delta\Delta G°\rangle = -7.0 \pm 0.6$ kcal/mol) than in the N·F series. The observed trends in $\Delta\Delta G°$ values are preserved when n_{eff} is fixed at a value of 2, as in the conventional van't Hoff analysis. The initial "duplex" states to which these $\Delta\Delta G°$ values refer may reflect ensembles of structurally undefined states. This circumstance is acceptable since our arguments are purely thermodynamic in nature and our analysis assumes a common final single-stranded form as the reference state.

III. Abasic Lesion Effects on Duplex Structure

Duplexes Containing Abasic Sites Retain B-Form Global Structure. Circular dichroism spectra of the 12 duplexes are displayed in Figure 1. Independent of the lesion, its cross-strand partner base, and sequence context, each spectrum is consistent with a global B-form DNA structure, while second order differences likely reflect more subtle differences in conformation, base composition, and base sequence (*44*). Comparisons between pairs of duplexes which differ only by the orientation of the central base:abasic site or base pair are straightforward because the two duplexes have identical chromophores. The comparison between the profiles of the C·G and G·C duplexes highlights the strong dependence the CD spectrum of B-form DNA can have on nucleotide sequence. In contrast, the A·T and T·A duplexes exhibit

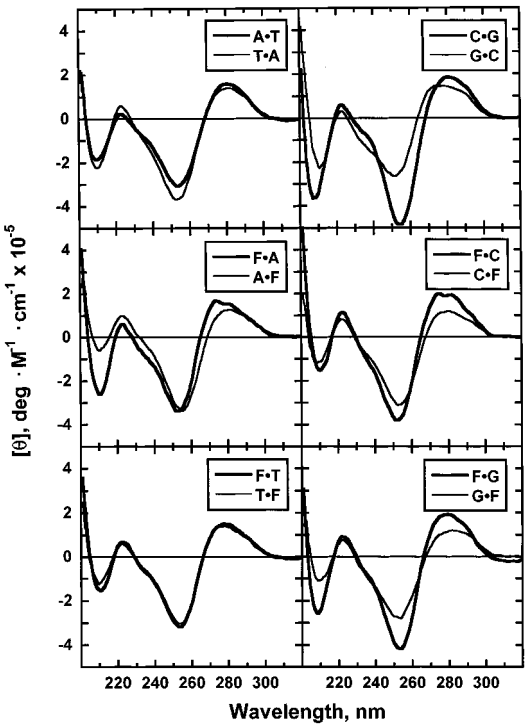

FIGURE 1: Circular dichroism spectra of pairs of duplexes in which the nucleotide base chromophores are identical but the orientation of the central base pair is different. The identity of the duplexes is indicated on each panel. The two top panels display spectra of canonical DNA duplexes and the four lower panels display spectra of abasic lesion-containing duplexes.

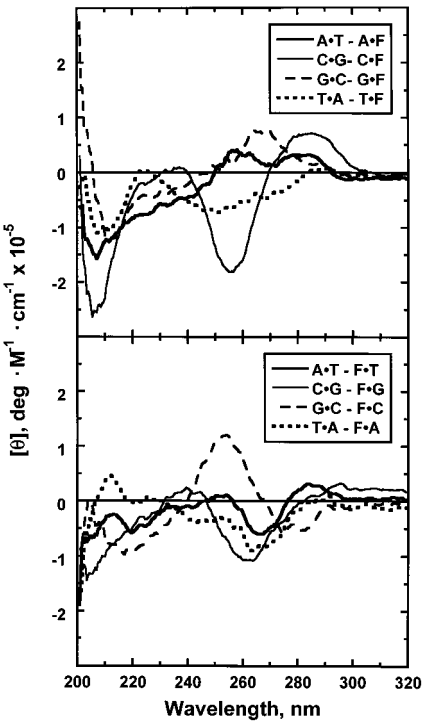

FIGURE 2: Circular dichroism difference spectra of abasic lesion-containing and the appropriate parent duplexes. Spectra of the N·F family of duplexes are displayed in the top panel. Spectra of the F·N family of duplexes are displayed in the bottom panel.

nearly identical CD spectra. These observations are consistent with predictions of B-form duplex CD spectra based on a nearest neighbor model (*45*). One might expect to see spectral differences between F·N and N·F spectra, in the cases where N represents A, C, G, or T. Similarly to the parent duplexes, the CD spectra of the F·T and T·F duplexes are nearly coincident.

Figure 2 displays circular dichroism difference spectra between each lesion containing duplex spectrum and the appropriate parent duplex spectrum. Comparison of these difference spectra reveals that the N·F family, in which the abasic site is flanked by cytosines, share a common feature. A negative band centered between 205 and 210 nm is observed. This feature is missing from the F·N family of duplex difference spectra, with the exception of the F·T duplex. Thus, this differential CD profile may indicate that the N·F family and the F·T duplex belong to a conformational subfamily within B-form duplexes which is distinct from the subfamilies of B form duplexes populated by the parent duplexes and the remaining three of the F·N family of duplexes.

A Variety of Structures Are Accessible to Abasic Lesion Containing DNA. Removal of a base to form an abasic site creates a void where the base had been. One can envision at least three potential local structural accommodations of such a void. The space previously occupied by the base can be filled by solvent. If the solvent acts as a "pseudobase", one might envision little change in the overall stucture of the DNA. A second possible structural accommodation of the lesion would involve the abasic moiety and/or the base opposite the abasic site shifting toward the center of the DNA helix, thereby helping to maintain at least partial base stacking along both DNA strands, while minimizing unfavorable solvation of the base surfaces. A third potential mode of accommodation would involve the abasic site, and/or the base opposite, rotating and projecting out of the helix, thereby allowing the neighboring bases to interact directly. Each of these three potential structural accommodations of the lesion is consistent with a structure in which B-form DNA global structure is maintained, as suggested by the CD spectra, with only local perturbations in the vicinity of the abasic lesion.

While confirmation of the above structural hypothesis must await detailed NMR or X-ray crystallographic studies of this family of duplexes, there are some studies in the literature which address the structural accommodation of abasic lesions. To date, published structures of single abasic site-containing oligonucleotides fall into two catergories, based on whether the abasic site is intrahelical or extrahelical. Extrahelical conformations are observed only when purines flank the abasic site on both sides (*17*, *18*, *23*, *24*), while intrahelical conformations arise independent of sequence context (*16*−*19*, *21*, *23*, *24*, *46*). Several of the reports indicate that extrahelical and intrahelical conformations appear to exist in equilibrium with both conformational states significantly populated. While flanking by purines is necessary but not sufficient for observable extrahelical abasic lesion conformations, all but one of the reported structures (*17*) (corresponding to F·G) are consistent with the hypothesis of structural collapse facilitating translesional purine residue-stacking continuity.

IV. Biochemical Implications

Taken together, the NMR studies emphasize the range of possible structural consequences to abasic lesion formation.

Coupled with the wide range of energetic consequences of lesion formation we report here, these data indicate that the cellular enzymatic abasic lesion repair system potentially is challenged with an energetically and structurally diverse family of possible substrates. Independent of structure, for every case of abasic lesion-containing DNA so far examined thermodynamically (this work; *25*−*28*), significant enthalpically driven reductions in duplex stability are observed. This feature of abasic lesions is consistent with the hypothesis that defects in DNA are located by repair enzymes due to lesion induced alterations in duplex energetics (*40*).

Cross-strand base effects on AP nuclease activity have been examined (*29*), with negligible influence on nuclease activity observed. However, the only sequence context examined corresponds to the family of duplexes where Gs flank the lesion which is less perturbed thermodynamically. This coincidence may mask a larger effect present in other sequence contexts.

A study of the ability of HIV-1 reverse transcriptase (RT) to polymerize past an abasic site-terminated template (*14*) concluded that purines were preferentially inserted as the next base 3′ to a "pair" containing an abasic site in the template strand. Sequence dependence of nucleotide insertion at this site was studied extensively by varying the template bases following the abasic site. The observed preference for purines is consistent with the thermodynamic data reported here, as explained below.

The preference for purines as the next base added by RT requires that a pyrimidine be in the template strand, thus a pyrimidine is one of the neighbors of the abasic site (and an adenine is, in this study, always the other neighbor). This sequence is most consistent with the less thermodynamically perturbed sequence in our current studies. When RT skipped the expected next base on the template, this base was always a purine, which would have resulted in pyrimidine as the next extended base on the primer. This sequence is consistent with the more perturbed orientation in our thermodynamic studies, where two purines are flanking the abasic site. These correlations suggest that the thermodynamic consequences of abasic sites might correlate with alterations in an enzymatic process such as RT activity.

CONCLUSIONS

The thermal and thermodynamic stability of duplex DNA are reduced by formation of an abasic lesion. The thermodynamic origins of that destabilization are enthalpic. A significant enthalpy−entropy compensation results in the masking of very large sequence context dependent differences in the enthalpic contribution to duplex stability. As part of its influence on DNA duplex thermodynamics, the sequence context of the lesion also appears to influence the mechanism of duplex dissociation. The identity of the lesion's cross-strand partner base has only a secondary effect of duplex stability and the origins of that stability.

Most investigations of the impact of single-base alterations on biochemical processes include the effect of each of the four possible cross-strand bases. However, as the data reported here show, the sequence context seems to be the primary determinant in the effect of the abasic site upon DNA thermodynamics and possibly local duplex structure. Single-base damage should be studied as a function of cross-strand partner base and sequence context, thereby avoiding the unintentional masking of biological, biochemical, and biophysical properties of damaged DNA.

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SUPPORTING INFORMATION AVAILABLE

One table lists molar extinction coefficients at 260 nm determined by phosphate analysis for the 10 oligodeoxyribonucleotides used in this study (1 page). Ordering information is given on any current masthead page.

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