Effects of the Presence of an Aldehydic Abasic Site on the Thermal Stability and Rates of Helix Opening and Closing of Duplex DNA†

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ABSTRACT: The presence of an abasic site in duplex DNA lowers the thermodynamic stability, as monitored by the optical melting temperature, and decreases the rate of imino proton exchange with water, by about an order of magnitude, as monitored by direct measurement of both the exchange lifetimes and the imino proton T1/2. The exchange lifetimes of the imino protons with water as a function of base catalyst concentration were analyzed to determine the origin of the effect of the abasic site on imino exchange lifetimes. Analysis of the results showed that the helix opening rate is not significantly changed by the presence of an abasic site. The differences in exchange lifetimes are attributed to a faster helix closing rate in the presence of an abasic site. The faster rate of helix closing may be an important contribution to the stability of abasic sites in duplex DNA to base-catalyzed elimination reaction. It is noted that duplex DNAs containing analogues of the aldehydic abasic site apparently do not exhibit these exchange lifetime effects.

The in vivo repair of DNA can be initiated by the hydrolysis of the N-glycosyl bond to yield an aldehydic abasic site (Friedberg, 1985; Goldberg, 1987; Lindahl, 1982; Loeb & Preston, 1987). The naturally occurring abasic site is an equilibrium mixture of α- (I) and β- (II) hemiacetals (2-deoxy-D-erythro-pentofuranoses) of aldehyde (III) and of hydrated aldehyde (IV) as depicted in Scheme I. The hemiacetal forms predominate, with about 1% aldehyde being present (Wilde et al., 1989). The strand cleavage at the 3'-side of the abasic side catalyzed by UV endonuclease V of bacteriophage T4 or endonuclease III of Escherichia coli occurs via a syn β-elimination reaction whereas the hydroxide-catalyzed reaction proceeds via a trans β-elimination reaction (Manoharan et al., 1988a,b, 1989). We have recently reported on the characterization of the conformational features of DNA duplexes containing these aldehydic abasic sites and have found that the structures are predominantly in the B-form, with the structural perturbations due to the abasic site being quite localized (Withka et al., 1991). The presence of an abasic site reduces the temperature dependence of the imino proton linewidths of DNA duplexes (Withka et al., 1991). This was a surprising result, indicating that the presence of an abasic site increases the effective exchange lifetimes of the imino protons. Therefore, it was of interest to more fully explore the effects of abasic sites on the thermal stability and imino proton exchange lifetimes of DNA duplexes.

Experiments were performed on d(ClG2C3A4G5-D6C7A8G9C10-C11) paired with d(G2G3C4G5T9C1A1G6T1-C14G13G12), with D indicating the deoxyribose aldehydic abasic site and numbering from 5' to 3'. This sample is referred to as AD1. Also investigated was the "control" sample of d(C1G2C3A4G5-U6C7A8G9C10-C11) paired with d(G2G3C21-G20T19C1A17G16T15C14G13G12), which is referred to as UA1.

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This is a sample for which we have previously reported structural information (Withka et al., 1991). To characterize the effects of abasic sites on the rates of helix opening, the exchange rates of the imino protons with water were obtained as a function of base catalyst concentration. The thermal stability was monitored by the optical melting temperatures.

The exchange rates of the imino protons with water can offer information about helix opening and closing rates (Leroy et al., 1987; Gueron et al., 1987, 1990; Pardi et al., 1982; Pardi & Tinoco, 1982; Benight et al., 1988; Braunlin & Bloomfield, 1988). Imino proton exchange with water can be described as a two-step process. The first step is the opening up to the helix to expose the imino proton. In the second step there is the diffusion-controlled base-catalyzed exchange of the proton with water. The measured exchange rates are dependent on the helix opening rate and the helix closing rate as well as the concentration of the base catalyst.

The exchange lifetimes were directly monitored by two-dimensional NMR methods. In these experiments the cross-peaks between the imino protons and water arise from chemical exchange, and the exchange rates can be determined from the intensities of the diagonal and cross-peaks. Previous studies have examined the T1/2 of the imino protons to investigate the exchange rates of imino protons. The imino proton T1/2s are...
EXPERIMENTAL PROCEDURES

Sample Preparation. DNA single strands were obtained from Pharmacia, and the purity of the samples was checked by HPLC and NMR as described previously (Manoharan et al., 1988a,b; Mazumder et al., 1989; Wilde et al., 1989; Withka et al., 1991). The single-stranded DNAs were dialyzed and the extinction coefficients determined via the total phosphorus method. The single strands were lyophilized and reconstituted in pH 7.0 buffer containing 10 mM sodium phosphate, 100 mM sodium chloride, and 0.05 mM EDTA.

The abasic site containing single strands were prepared by treating DNA single strands containing a single U residue with N-uracil glycosylase as described previously (Manoharan et al., 1988a,b; Mazumder et al., 1989; Wilde et al., 1989; Withka et al., 1991). The extent of reaction was monitored during the reaction by reverse-phase HPLC that separates free uracil; the DNA single strand containing U, and the DNA single strand containing the abasic site. The single strand containing the abasic site was purified by gel filtration column and eluted with 25 mM sodium phosphate buffer containing the abasic site. The presence of an abasic site lowers the melting temperature of a DNA duplex, as monitored by optical melting. These observations suggest that there is a significant change in the entropy associated with helix closing in the presence of an abasic site.

Base Catalyst Titration. The duplex samples were in pH 7.6 buffer containing 10 mM sodium phosphate, 100 mM sodium chloride, and 0.05 mM EDTA in 90% H2O/10% 2H2O. The samples were titrated with ammonium chloride in two steps, the first addition bringing the total effective NH3 concentration to 10 mM and the second addition bringing the concentration to 20 mM. The samples were titrated with ammonium chloride in two steps, the first addition bringing the total effective NH3 concentration to 10 mM and the second addition bringing the concentration to 20 mM. The procedures described elsewhere were used to calculate that 10 mM phosphate is equivalent to 3.5 mM NH3 (Gueron et al., 1987, 1990; Leroy et al., 1988). Analytical-scale experiments showed that at higher pHs or at elevated temperatures ammonia induces the base-catalyzed elimination reaction of the abasic site. Therefore, the imino proton exchange experiments on the AD1 heteroduplex were constrained to relatively low NH3 concentration and temperatures below 20 °C.

NMR Procedures. The 400-MHz NMR spectra were obtained using a Varian VXR-400 spectrometer. All data were analyzed by Varian VNMR software on a Sun Microsystems machine. The two-dimensional NOE spectra were obtained as described elsewhere (Ernst et al., 1987) using the States–Haberkorn hypercomplex method (States et al., 1982). Data processing was optimized for each experiment and included Gaussian apodization and Gaussian shift constant in t1 and t2 for resolution enhancement and was base line corrected in both the F1 and F2 dimensions.

Determination of Exchange Rates. The exchange rates of imino protons with water used the results of a phase-sensitive two-dimensional NOESY experiments in which suppression of the water signal was accomplished by use of a 1–1 jump and return detection pulse (Hore, 1983). During the mixing period a field gradient pulse was applied to enhance suppression of the water signal. The spectra were obtained with a 10-kHz spectral width and an acquisition time of 0.067 s. The F2 spectral width was 10 kHz, and 256 increments of t1 were used with 64 accumulations for each increment. Spectra were base line corrected along both dimensions.

The exchange lifetimes were determined from the intensities of the diagonal signals of the imino protons and their cross-peaks with water (Ernst et al., 1987). The chemical exchange between the imino protons and water can be described as a simple process involving only the water to imino pathway for two reasons (Dobson et al., 1986; Schwartz & Cнутell, 1983). First, the concentration of water is about 5 orders of magnitude greater than that of the imino protons. This allows neglect of the imino–water–imino exchange pathway during the mixing time and approximation of the mole fraction of water as unity. Second, the use of the 1–1 jump and return detection pulse, to suppress excitation of the water protons, effectively eliminates observation of the imino to water transfer pathway.

Since the chemical exchange rates of the imino protons are fast compared to other relaxation processes, the exchange rate, kex, is given by kex = (−1/τmix) ln [I0/(I0 + ICP)], with τmix the mixing time of the two-dimensional experiment, I0 the intensity of the diagonal signal, and ICP the intensity of the imino proton–water cross-peak (Dobson et al., 1986; Schwartz & Cнутell, 1983). Since the exchange lifetimes for the different imino proton results are over a wide range, data were obtained for more than one mixing time at each base catalyst concentration. NMR data were collected for the AD1 sample at 3.5 mM catalyst with mixing times of 40, 50, and 100 ms; at 10 mM base catalyst with mixing times of 50 and 100 ms; and at 20 mM base catalyst with mixing times of 50 and 80 ms. NMR data were collected for the AU1 sample at 3.5 mM catalyst with mixing times of 20, 50, 80, and 100 ms; at 10 mM base catalyst with mixing times of 20 and 50 ms; and at 20 mM base catalyst with mixing times of 50, 80, and 100 ms. The exchange lifetimes determined from NMR data collected at different mixing times were within 10% of each other.

Assignment of Imino Proton Resonances. The assignments of the imino protons of AU1 and AD1 were based on two-dimensional NOESY experiments using procedures described elsewhere (Wüthrich, 1986; Clore & Gronenborn, 1989; Chazin et al., 1986; Wemmer & Reid, 1985). The assignments are listed in Table I.
Imino Proton $T_1$ Measurements. The pulse sequence used for the $T_1$ experiments is $(180^\circ)$selective–delay–$(90^\circ)$x–$\tau$–$(90^\circ)$–x–acquisition, with the $\tau$ delay being 156 $\mu$s. The $(180^\circ)$selective was 20 ms in duration, with a $B_1$ field amplitude of 8 Hz. The decoupler channel was used for the selective pulses. A semiselective method was used for the shorter $T_1$s, less than about 50 ms, and the selective method for longer $T_1$s, greater than about 50 ms. The semiselective $(180^\circ)$ pulse was 3 ms in duration with a $B_1$ field amplitude of 167 Hz. The two methods gave the same results, within experimental error, for intermediate values of $T_1$. Between 10 and 12 values of the delay were used for each $T_1$ determination, and the supplementary material contains typical data. The precision of the $T_1$ values is estimated to be ±15% for signals with a $T_1$ longer than 100 ms and ±50% when the $T_1$ is on the order of 10 ms. The spectra for $T_1$ determinations were not base line corrected. The NMR samples for the $T_1$ experiments were in pH 7.0 buffer containing 10 mM sodium phosphate, 100 mM sodium chloride, and 0.05 mM EDTA in 90% H$_2$O/10% D$_2$O.

Optical Melting Temperature Determination. The UV absorbance measurements were performed using a Varian Cary 219 UV/vis spectrophotometer. Samples of AU1 and AD1 at 0.8 OD$_{260}$ were dissolved in the same buffer, at pH 7.0, as used in the NMR experiments. The samples were placed in standard 10-mm optical cells and the OD$_{260}$ values measured in double-beam mode using a buffer-only control sample. The absorbance values were determined at 15 temperatures within the range of 15 to 85 °C. The samples were equilibrated for 10 min at each temperature. Absorbance at 260 nm was graphically evaluated for determination of melting points. The melting curves are included in supplementary material.

RESULTS AND DISCUSSION

In a previous study the linewidths of the imino protons of AU1 and AD1 were determined as a function of temperature. It was found that the imino proton linewidths of AU1 increase with temperature more than do those of AD1 (Withka et al., 1991). This surprising result showed that the presence of an abasic site increases the exchange lifetimes of the imino protons. Therefore, it was of interest to more fully explore the effects of abasic sites on the thermal stability and imino proton exchange rates of DNA duplexes.

The thermodynamic stabilities of AU1 and AD1 were investigated by monitoring the optical melting at 260 nm, using the same buffer and pH as for the NMR experiments. The melting temperature of AU1 was found to be 60 °C. The melting of AD1 starts at 32 °C and continues until 60 °C. The melting temperature of AD1 could not be precisely determined due to partial, 5–10%, degradation of the sample during a heating cycle. However, the optical melting results, included in the supplementary material, clearly show that AD1 is thermally less stable than AU1 as monitored by optical absorbance.

The imino proton spectra of AU1 and AD1 are shown in Figure 1. The imino proton resonances have been assigned by means of imino–imino NOEs, and the assignments are given in Table I. An estimate of the imino proton exchange rate differences between the AU1 and AD1 duplexes can be made from the $T_1$s of the imino protons. The imino proton $T_1$s of AU1 and AD1 were determined over the temperature range of 10–25 °C with the results given in Table II. These results show that the exchange lifetimes of the imino protons of AD1, at low base catalyst concentrations, are longer than those of AU1 over this temperature range in agreement with the prior observations of the linewidths as a function of temperature (Withka et al., 1991). The $T_1$s could not be compared at higher temperatures due to the sensitivity of the abasic site to degradation. These $T_1$ results clearly demonstrate that the exchange lifetimes of AD1 are longer than those of AU1 but cannot be used to determine the origin of the difference. Therefore, we examined the exchange lifetimes as a function of base catalyst concentration.

The exchange lifetime of an imino proton, $\tau_{ex}$, in the presence of a base catalyst is given by $\tau_{ex} = 1/k_{ex} = (1/k_{op})(1 + k_d/k_{c7})$, where $k_{ex}$ is the exchange rate of the imino protons, $k_{c7} = k_{collission}[B]/(1 + 10^{\Delta pK})$, $\Delta pK$ is the pK of the imino proton minus that of the base catalyst, $k_{collission}$ is the collision rate constant for the imino proton in the open state with the base catalyst, $k_d$ is the rate constant for the closing of the duplex...
The exchange lifetimes of the imino protons of AU1 and AD1 were determined using two to four mixing times at each of the base catalyst concentrations, and the lifetimes are given in Table I. Due to the sensitivity of AD1 to base-catalyzed elimination reactions, data at higher base catalyst concentrations and at higher temperatures were not obtained.

The slopes of $\tau_{ex}$ versus $1/\vert B \vert$ for the AT base pairs of AD1 are much greater than those for the AT base pairs of AU1, as the plots in Figure 3 demonstrate. The extrapolation of the plots to $1/\vert B \vert = 0$ indicates that the intercepts for the AT base pairs of AU1 and AD1 are quite similar. This implies that $1/k_{op}$ for the AT base pairs in the presence and absence of an abasic site are approximately similar. However, the slopes of $\tau_{ex}$ as a function of $1/\vert B \vert$ are quite different in the two cases. Therefore, the two key results are that the intercepts of AU1 and AD1 are similar while the slopes are very different.

The intercepts of the plots of $\tau_{ex}$ as a function of $1/\vert B \vert$ shown in Figure 3, indicate that $1/k_{op}$ is essentially the same for the AT imino protons of AD1 and AU1. The plots of $\tau_{ex}$ versus $1/\vert B \vert$ indicate that the differences in $1/k_{op}$ for the AT imino protons of AD1 and AU1 differ at most by a factor of 2 or 3. This is too small a difference to account for the difference in the dependence of the exchange lifetimes on $1/\vert B \vert$.

The other possible origins for the differences in the exchange lifetimes on $1/\vert B \vert$ are $\Delta pK$, $k_{\text{collision}}$, and $k_{al}$. To account for the large differences in slopes and observed exchange lifetimes, the $pK$ values of the imino protons in AD1 would need to be about 1 pH unit lower than is the case for AU1. It is unlikely that the $pK$ values of the imino protons in AD1 are anomalous since their resonances are observed at unexceptional chemical shifts, which are essentially the same as those of AU1. In addition, the relevant $pK$ value is of the T imino protons in the open state in which all of the T imino protons should have essentially the same $pK$.

As the collision rate of the imino protons with base catalyst increases, the exchange lifetime decreases. Therefore, to

**Table II: Assignments of AD1 and AU1 Imino Protons, Chemical Shifts, in ppm, and $T_{1s}$, in ms, as a Function of Temperature**

<table>
<thead>
<tr>
<th>base pair</th>
<th>ppm</th>
<th>10 °C</th>
<th>15 °C</th>
<th>20 °C</th>
<th>25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD1 Imino Protons$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A$<em>x$T$</em>{15}$</td>
<td>14.1</td>
<td>240</td>
<td>270</td>
<td>170</td>
<td>30</td>
</tr>
<tr>
<td>A$<em>x$T$</em>{19}$</td>
<td>13.9</td>
<td>250</td>
<td>250</td>
<td>210</td>
<td>160</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{22}$</td>
<td>13.1</td>
<td>190</td>
<td>240</td>
<td>200</td>
<td>120</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{2}$</td>
<td>13.0</td>
<td>190</td>
<td>240</td>
<td>200</td>
<td>120</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{12}$</td>
<td>13.0</td>
<td>190</td>
<td>240</td>
<td>200</td>
<td>120</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{13}$</td>
<td>12.9</td>
<td>290</td>
<td>270</td>
<td>270</td>
<td>120</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{2}$</td>
<td>12.8</td>
<td>290</td>
<td>270</td>
<td>270</td>
<td>120</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{9}$</td>
<td>12.7</td>
<td>290</td>
<td>270</td>
<td>270</td>
<td>120</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{16}$</td>
<td>12.5</td>
<td>160</td>
<td>140</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>AU1 Imino Protons$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A$<em>x$T$</em>{15}$</td>
<td>13.9</td>
<td>25</td>
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<td>&lt;10</td>
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<tr>
<td>A$<em>x$T$</em>{19}$</td>
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<td>25</td>
<td>25</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{22}$</td>
<td>13.0</td>
<td>20</td>
<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{2}$</td>
<td>13.0</td>
<td>20</td>
<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{9}$</td>
<td>12.7</td>
<td>280</td>
<td>200</td>
<td>200</td>
<td>70</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{12}$</td>
<td>12.7</td>
<td>280</td>
<td>200</td>
<td>200</td>
<td>70</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{13}$</td>
<td>12.7</td>
<td>280</td>
<td>200</td>
<td>200</td>
<td>70</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{16}$</td>
<td>12.7</td>
<td>280</td>
<td>200</td>
<td>200</td>
<td>70</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{13}$</td>
<td>12.4</td>
<td>320</td>
<td>270</td>
<td>270</td>
<td>190</td>
</tr>
<tr>
<td>C$<em>{1s}$G$</em>{2}$</td>
<td>12.4</td>
<td>320</td>
<td>270</td>
<td>270</td>
<td>190</td>
</tr>
</tbody>
</table>

$^a$ $T_{1s}$ of CI-G$_{22}$, CI-G$_{2}$, and CI-G$_{13}$ are reported as one group as are those of CI-G$_{12}$, GI$_{4}$G$_{6}$, CI-G$_{16}$, and CI-G$_{13}$. $^b$ $T_{1s}$ of A$_x$T$_{15}$ and A$_x$T$_{19}$ are reported as one group as are those of CI-G$_{12}$ and CI-G$_{13}$ and those of CI-G$_{9}$, CI-G$_{20}$, CI-G$_{16}$, and CI-G$_{13}$. 

FIGURE 2: Typical NMR results used for the determination of the exchange lifetimes of the imino protons of AD1 and AU1. The spectra shown are traces along the F$_2$ axis of two-dimensional NOESY maps obtained using a 50-ms mixing times with the samples in the presence of 3.5, 10, and 20 mM base catalyst. The AD1 traces are at the F$_2$ frequency of the imino proton of the A$_x$T$_{15}$ base pair. The AU1 traces are for the imino protons of A$_x$T$_{15}$ and A$_x$T$_{19}$ whose resonances overlap. The water proton frequency is at 4.9 ppm. In the presence of 10 mM base catalyst, for example, the exchange lifetime of the AU1 sample is much shorter than that of the AD1 sample since the ratio of the intensity at the imino frequency to the intensity at the water frequency is much smaller for the AU1 case than for the AD1 case.

FIGURE 3: The plot shows the exchange lifetimes of the imino protons of the AT base pairs of AD1 and AU1 as a function of the inverse base catalyst concentration. Each lifetime was determined from the results of experiments performed at two or three mixing times. The exchange lifetimes were determined from the NMR results as described in the text.
The physical basis for these exchange lifetime results may be the entropy associated with the helix closing. All of the experimental results can be explained by having the entropy of activation barrier to helix closing being lower in the presence of an abasic site while the abasic site is less stable to thermal melting due to less favorable thermodynamic enthalpy and/or entropy of helix formation. This is physically reasonable since the entropy difference between the open and closed forms of the helix for an abasic site is likely to be the less unfavorable than that of a residue which forms a base pair in the closed state.

It is noted that duplex DNA containing analogues of the aldehydic abasic site apparently do not exhibit these changes in helix opening rates since no anomalies in the imino proton linewidths, comparable to those observed with AD1 (Withka et al., 1991), have been reported (Cuniasse et al., 1987, 1990; Kalnik et al., 1988, 1989). In addition, an examination of the thermal stabilities of DNA duplexes containing analogues of the aldehydic abasic site showed that these have lower thermal stability than the corresponding undamaged DNA duplexes (Vesnaver et al., 1989). The structures of DNA duplexes containing analogues of the aldehydic base pair apparently have structural consequences different from those of the aldehydic abasic site as well (Withka et al., 1991).

The faster rate of helix closing may be an important contribution to the chemical stability of abasic sites in duplex DNA. A fast helix closing rate will reduce the susceptibility of an abasic site to base-catalyzed elimination reactions. This may be the explanation for the chemical stability of DNA duplexes. The stability of the abasic site in duplex DNA cannot be attributed to a reduced population of the reactive aldehydic form (Wilde et al., 1989). Further investigations to more fully characterize the physical and chemical properties of abasic sites in duplex DNAs are planned. These include studies of the hydration and hydrogen bonding interactions of the abasic site, the sequence context dependence of the melting due to less favorable thermodynamic enthalpy and/or entropy of helix formation. This is physically reasonable since the entropy difference between the open and closed forms of the helix for an abasic site is likely to be the less unfavorable than that of a residue which forms a base pair in the closed state.

SUPPLEMENTARY MATERIAL AVAILABLE

Melting curves of AU1 and AD1 are presented (Figure 1). A typical set of spectra used for the determination of imino proton T$_{1s}$ is included (Figure 2), as is a plot of the signal intensity versus recovery time used to determine the T$_{1s}$ (Figure 3) (3 pages). Ordering information is given on any current masthead page.

REFERENCES


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