

Induced Circular Dichroism Characteristics as Conformational Probes for Carcinogenic Aminofluorene–DNA Adducts

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We report novel induced circular dichroism (ICD) characteristics for probing the conformational heterogeneity induced by the arylamine carcinogen 2-aminofluorene, namely, B type (B), stacked (S), and wedged (W) conformers. CD experiments were conducted with five different aminofluorene-modified DNA duplexes (I–V). An intense positive ICD was observed for the W conformer I in the 290–360 nm range (ICD_{290–360nm}). This was in contrast to the negative ICD_{290–360nm} exhibited by the mostly B conformer V (17% S/83% B). Duplex IV, which adopts an approximately equal mixture of S (53%) and B (47%), exhibited low ellipticities along the baseline. The magnitude of the positive ICD for I was significantly greater than that observed for II (70% S/30% B). While the ICD_{290–360nm} of the W conformer III showed no changes in intensity with increasing temperature from 10 to 35 °C, dramatic changes were observed for I across the same temperature range. Dynamic ¹⁹F NMR results revealed that I exists in an 85:15 mixture of W and S/B conformers. The dramatic intensity changes observed for I are consistent with the presence of a W/B heterogeneity because of its susceptibility to result in a large difference on the magnitude of the ICD_{290–360nm}. In conclusion, the sign and magnitude of the ICD_{290–360nm} are sensitive conformational markers for studying arylamine-induced conformational heterogeneity. The temperature-dependent ICD_{290–360nm} data, coupled with ¹⁹F NMR spectroscopy, provide valuable information about conformational distribution and dynamics, which are important factors that affect mutational outcomes.

Introduction

DNA adduct formation is a signature hallmark of mutation, leading to the initiation of mutagenesis and carcinogenesis (1). Elucidating the mechanism by which DNA adducts lead to mutations has long been a major challenge. Progress has encumbered the complexity of sequence-induced conformational heterogeneities (2), which occur frequently with adducts derived from bulky carcinogens such as arylamines (3) and polycyclic aromatic hydrocarbons (4–6). Upon activation in vivo, the arylamine carcinogen 2-aminofluorene (AF)¹ and its nitro and *N*-acetyl derivatives react with cellular DNA to produce two C8-substituted adducts: *N*-(2'-deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF) and *N*-(2'-deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-C8-AAF) (Figure 1a), along with a small amount of *N*²-substituted adduct (7). The *N*-deacetylated dG-C8-AF is the major and the most persistent adduct, which adopts three distinct conformational motifs depending upon the location of the bulky AF moiety; these are the major groove binding “B type” conformation (B), the based-displaced “stacked” (S) conformation, and the minor groove binding “wedged” (W) conformation (Figure 1b) (2, 3).

A sequence-dependent S/B equilibrium is commonly maintained among fully paired DNA duplexes (8–10). In contrast, the W conformer has been observed only in duplexes, where the lesion was mismatched with purine bases (11–13). As in

S, the modified dG in the W conformer adopts a syn glycosidyl configuration; however, the steric clash at the lesion site places the carcinogenic AF moiety in the minor groove of the double helix. While ¹H NMR (3, 4) and crystallographic (14, 15) studies proved to be powerful tools for obtaining detailed adduct structures, they are neither sensitive nor suitable for dealing with multiple conformers. Herein, we report novel induced circular dichroism (ICD) characteristics that are useful in studying the AF-induced B–S–W conformational heterogeneities.

Experimental Procedures

Caution: AF derivatives are mutagens and a suspected human carcinogen and must be handled with caution.

Crude desalted oligodeoxynucleotides in 10 μmol scale were obtained from Sigma-Genosys (The Woodlands, TX). All HPLC solvents were purchased from Fisher Inc. (Pittsburgh, PA).

Preparation of AF- or 7-Fluoro-2-aminofluorene (FAF)-Modified Oligodeoxynucleotides. The synthesis of the AF- and FAF-modified oligos was carried out by the procedures described previously (8, 10, 20). This involved treating the oligo with the *N*-trifluoroacetyl derivatives of AF and FAF. Each modified oligo was purified by reverse phase HPLC and characterized by UV/enzyme digest/electrospray mass spectrometry as described previously (8). The HPLC system consisted of a Hitachi EZChrom Elite system with a L2450 diode array as a detector and a Luna column (10 mm × 150 mm, 5 μm) (Phenomenex, Torrance, CA). The mobile phase system involved a 30 min linear gradient profile of 5–15% acetonitrile/0.1 M ammonium acetate buffer (pH 7.0) with a flow rate of 2.0 mL/min.

CD and UV Melting Experiments. CD measurements were conducted on a Jasco J-810 spectropolarimeter equipped with a variable Peltier temperature controller. Typically, a duplex sample (~52 μM) was dissolved in 400 μL of a buffer containing 0.2 M NaCl, 10 mM sodium phosphate, and 0.2 mM EDTA, pH 7.0, and

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¹ Abbreviations: AF, 2-aminofluorene; dG-C8-AAF, *N*-(2'-deoxyguanosin-8-yl)-2-acetylaminofluorene; dG-C8-AF, *N*-(2'-deoxyguanosin-8-yl)-2-aminofluorene; dG-C8-FAF, *N*-(2'-deoxyguanosin-8-yl)-7-fluoro-2-aminofluorene; FAF, 7-fluoro-2-aminofluorene; ICD, induced circular dichroism.

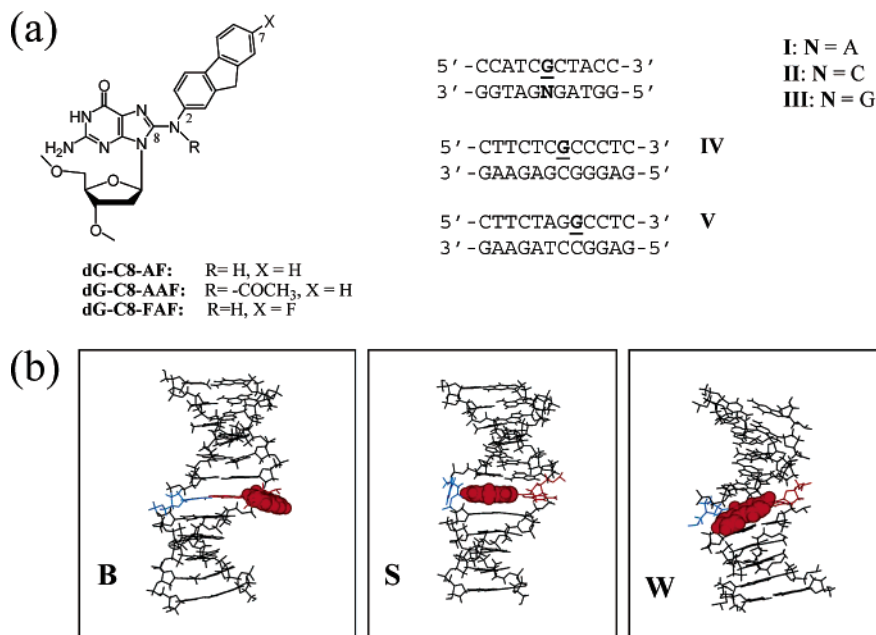


Figure 1. (a) Structures of dG-C8-AF, dG-C8-AAF, and dG-C8-FAF adducts and the sequences used in the present work. **G** = dG-C8-AF or dG-C8-FAF. (b) Views from the minor groove of a duplex for the three conformational motifs of the AF-modified DNA: B, S, and W conformers. The modified dG and the complementary base (dC for B and S; dA for W) residues are shown in red and blue lines, respectively, and the AF moiety is highlighted with red CPK.

placed in a 1 mm path-length cell. The sample was heated at 85 °C for 5 min and then cooled to 15 °C over a 10 min period to ensure duplex formation. Spectra were measured from 200 to 400 nm at a rate of 50 nm/min with an average of 10 accumulations. Data points were acquired every 0.2 nm with a 2 s response time. UV melting experiments were conducted on a Beckman DU 800 UV/vis spectrophotometer equipped with a built-in Peltier temperature controller. The duplex CD samples were also used to obtain melting curves by varying the temperature of the sample cell (1 °C/min) at 260 nm (8).

NMR Experiments. For NMR experiments, approximately 80 μ mol of the modified oligos were annealed with complementary sequences to produce appropriate duplexes. The duplex samples were ultracentrifuged through a Millipore Centricon YM-3 centrifugal filter (Yellow, molecular weight cutoff = 3000) and redissolved in a Shigemi NMR tube containing 300 μ L of pH 7.0 NMR buffer (10% D₂O/90% H₂O): 100 mM NaCl, 10 mM sodium phosphate, and 100 μ M tetrasodium EDTA. All ¹⁹F NMR data were recorded in the ¹H-decoupled mode using a dedicated 5 mm ¹⁹F/¹H dual probe on a Bruker DPX400 Avance spectrometer (Billerica, MA) operating at 376.5 MHz. ¹⁹F NMR spectra were referenced to CFCl₃ by assigning external hexafluorobenzene in C₆D₆ at -164.90 ppm. Spectra were obtained by collecting 1600 scans with a recycle delay of 1.5 s between acquisitions and were processed by exponential multiplication using line broadening of 20 Hz and Fourier transformation.

Results and Discussion

CD measurements were carried out on an AF-modified single strand and its dA mismatch (**I**, Figure 1a) duplex and the unmodified control duplex. As shown in Figure 2a, upon duplex formation, **I** exhibited a strongly positive ICD in the AF-absorbing 290–360 nm range (hereafter referred to as ICD_{290–360nm}), whereas no such ICD was detected for the control duplex. This was contrasted with the sequence-dependent mostly negative ICD_{290–360nm} observed for fully paired duplexes (8). Previous ¹H NMR studies have shown that **I** adopts W conformer exclusively (11). Thus, the intensely positive ICD_{290–360nm} for **I** must arise from an interaction between the AF and the chiral DNA in the minor groove area, which is

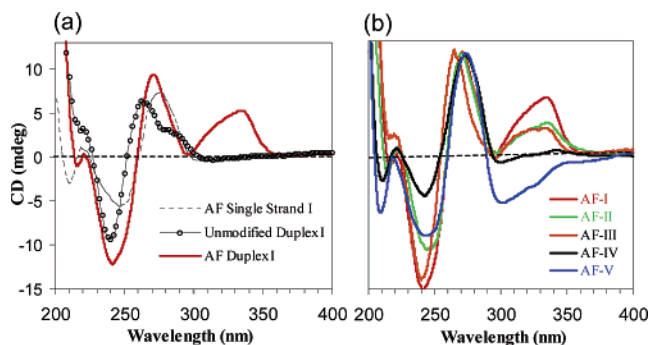


Figure 2. Overlays of CD spectra for (a) an AF-modified single strand and the corresponding dA mismatch and unmodified control dA mismatch duplexes **I**; (b) AF-modified duplexes: **I**, **II**, **III**, **IV**, and **V** (see Figure 1a for sequences).

reminiscent of those interactions observed for minor groove binders (16) and the cis-opened benzo[*a*]pyrene–DNA adducts (17, 18).

Figure 2b shows CD spectra of AF-modified duplexes in all five sequence contexts listed in Figure 1a. It has been shown that AF-modified **I** and **III** exist exclusively in a W conformation (11, 12). Mao et al. (19) have shown that the fully paired duplex **II** exists in a 70:30 mixture of the S and B conformations. Also included for comparison are **IV** (53% S/47% B) and **V** (17% S/83% B), whose S/B population ratios have been determined by the ¹⁹F NMR methodology (20, 21) using duplexes containing the “fluorine probe” dG-C8-FAF (Figure 1a) (Supporting Information Figure S1). The intensely positive ICD_{290–360nm} value observed for the W conformeric **I** and **III** was in contrast to the negative ICD_{290–360nm} exhibited by the mostly B conformeric **V** (83% B). Not surprisingly, **IV** (47% B) exhibited low intensity ellipticity along the baseline. The magnitude of the positive ICD for **I** is significantly greater than that observed for **II** (70% S). This difference is probably due to the transition moment of the AF moiety in the W conformer, which forms a skewed angle with the helical bases in the minor groove (Figure 1b) (16). The ICD of such a conformation for various minor groove binders has been shown to be positive

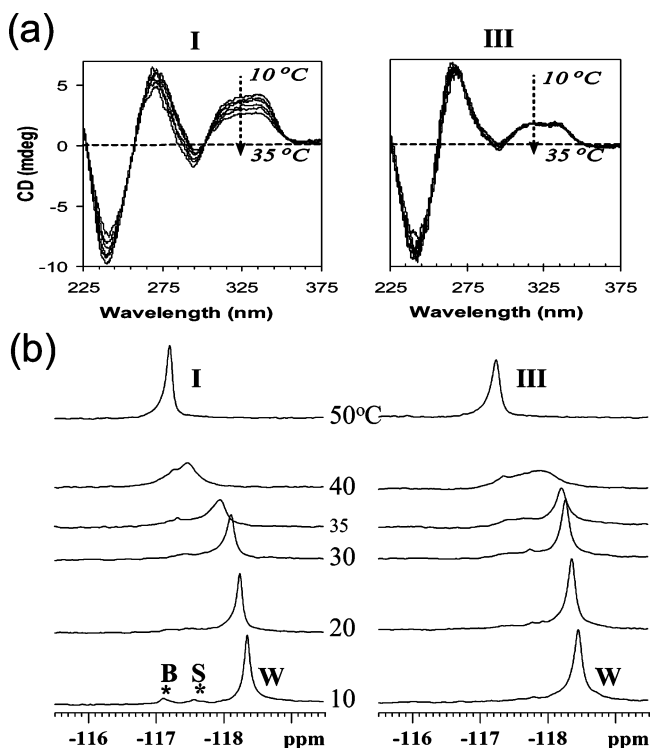


Figure 3. Temperature-dependent (a) CD and (b) ^{19}F NMR spectra of the FAF-modified dA (**I**) and dG mismatch (**III**) duplexes.

with a magnitude that is one or two orders greater than those observed for intercalated ligands (e.g., S-like conformation) (16). It was noted that **I** exhibited greater intensity of $\text{ICD}_{290-360\text{nm}}$ than the dG mismatch duplex **III**, even though both have been shown to adopt the W conformation (11, 12) (also see below). These results indicate that the intensity of $\text{ICD}_{290-360\text{nm}}$ is influenced by not only the flanking sequences but also the nature of the base directly opposite the lesion. Thus, while the $\text{ICD}_{290-360\text{nm}}$ for a B conformer is mostly negative, S and W conformers are characterized by a positive $\text{ICD}_{290-360\text{nm}}$. An important consideration here is that the S/B heterogeneity occurs primarily among fully paired duplexes, whereas the W conformer is observed only for mismatched duplexes in which purine bases are opposite the lesion as in **I** and **III** (3, 8).

The aforementioned $\text{ICD}_{290-360\text{nm}}$ is also a valuable tool for probing the dynamics of B–S–W conformational heterogeneities. Figure 3 shows the temperature-dependent ICD and ^{19}F NMR spectra of FAF-modified **I** and **III** duplexes (dA and dG mismatch, respectively). While the $\text{ICD}_{290-360\text{nm}}$ of **I** decreased substantially with increasing temperature from 10 to 35 °C, **III** showed no changes in intensity across the same temperature range (Figure 3a). In both cases, the characteristic AF-induced ICD quickly disappeared as the duplexes approached their optical melting points ($T_m = 38$ and 41 °C, respectively, for **I** and **III**), which are comparable or slightly higher than those of the respective unmodified control duplexes. The observed thermal stability of the W conformer presumably arises from the van der Waals interactions between the hydrophobic AF moiety and the surfaces in the minor groove.

The ^{19}F NMR spectrum of **III** (Figure 3b) revealed a single major resonance at -118.5 ppm, in good agreement with previous ^1H NMR results that showed the exclusive presence of a W conformer. A similar ^{19}F profile was obtained for **I**; however, in this case, the main signal at -118.4 ppm was accompanied by two minor resonances (asterisked) of approximately equal intensity, which accounted for $\sim 15\%$ of the

total conformer population at 10 °C (Figure 3b). The major signals in the spectra of **I** and **III** exhibited a substantial H/D shielding effect (~ 0.15 ppm) when the deuterium content of the solvent was increased from 10 to 100%, confirming the partially exposed nature of the fluorine atom in the minor groove of the W conformer (20). Each of the two minor resonances observed for **I** represents a unique fluorine environment and eventually coalesces with the W conformeric signal at 30 °C. The two minor conformers at -117.1 and -117.6 ppm could be assigned to a B conformer and an S conformer, respectively, both of which interconvert slowly to the major W conformer via chemical exchange. The stacked S conformer has been shown to be more susceptible to a ring current effect, which enables it to be shielded relative to the B conformer (20). It should be noted that an envelope of broad signals near the melting temperature (~ 40 °C) observed for both duplexes represents a complex mixture of B–S–W conformers and denatured FAF-modified single strands. The sharp resonance at 50 °C primarily indicates a fast rotating FAF residue of the single-strand FAF-modified oligomer.

The inert nature of the $\text{ICD}_{290-360\text{nm}}$ for **III** (Figure 3a) in the 10–35 °C temperature range reflects the exclusive existence of the W conformer as evidenced by the ^{19}F NMR data shown in Figure 3b. Thus, the dramatic intensity changes observed in the CD spectrum of **I** in the same temperature range must arise from the unique conformational heterogeneity discussed above, which could be either a W/S or a W/B equilibrium. We believe it could be the latter, since the impact on the magnitude of the $\text{ICD}_{290-360\text{nm}}$ would be expected to be much greater for a W/B equilibrium; i.e., from the most positive (W) to the most negative (B) (Figure 2b). A $W_{\text{syn}}/B_{\text{anti}}$ conformational switch is theoretically plausible (22) through an initial $W_{\text{syn}}/S_{\text{syn}}$ transition followed by an $S_{\text{syn}}/B_{\text{anti}}$ one. The interconversion energy required for the $S_{\text{syn}}/B_{\text{anti}}$ transition is not known in the mismatch sequence context, although a similar transition in the fully paired duplex requires ~ 14 kcal/mol (20). The $W_{\text{syn}}/S_{\text{syn}}$ equilibrium is considered to be a low energy process since it does not require a glycosidyl conformational switch.

In conclusion, we have demonstrated the utility of the $\text{ICD}_{290-360\text{nm}}$ characteristics as simple and sensitive conformational markers for probing the AF-induced B–S–W heterogeneities. When combined with ^{19}F NMR spectroscopy, the temperature-dependent $\text{ICD}_{290-360\text{nm}}$ data provide extremely valuable information about conformational distribution and exchange dynamics, which are important factors affecting the mutational outcomes of AF and other arylamine adducts.

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Supporting Information Available: ^{19}F NMR and experimental data for **IV** and **V**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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