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Structures of 2-Acetylaminofluorene Modified DNA Revisited: Insight into Conformational Heterogeneity

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Abstract

Despite the extensive data on dG-AAF, the major DNA adduct derived from the model carcinogen 2-acetylaminofluorene, little is known with respect to its solution structures. Here we provide NMR/CD evidence for three conformers of dG-AAF in duplex DNA: major groove (B), base-displaced stacked (S), and minor groove wedge (W). The S/B/W-conformational heterogeneities were found to be sensitive to the nature of the flanking DNA sequence contexts and pH.

Arylamines and their nitro derivatives are a major group of environmental mutagens, and have been implicated in the etiology of human cancers (1). *In vivo* metabolic activation of the prototype carcinogens 2-acetylaminofluorene (2) and 2-nitrofluorene (3) results in production of dG-AF1 and dG-AAF as major stable adducts (Fig. 1a) (4). Despite their structural similarities, the two DNA adducts exhibit distinct mutation and repair activities. dG-AF has been shown to adopt multiple conformers, giving rise to unique sequence-dependent mutation and repair outcomes (5,6). However, very little is known about the structure-function relationships of dG-AAF, which is remarkable given the extensive *in vivo* and *in vitro* data that have accumulated related to AAF (3,277 items on PubMed Search for “acetylaminofluorene”).

In 1993, O’Handley *et al*. (7) reported that ~70% of an AAF-modified 9-mer duplex in the CG[AAF]C sequence context adopts a base-displaced S-conformer with stacking of the fluorene moiety into a double helix (Fig. 1c). The structure of the remaining 30% of the AAF-modified duplex has not been elucidated. Using an ^{19}F NMR approach (8), we showed that a 12-mer duplex in the TG*A sequence context (Duplex **II**, G*=FAAF, Fig. 1b) exhibits ~40:60% ^{19}F signals with an exclusive presence of the S-conformer, differing only in the *N*-acetyl group’s *cis* and *trans* conformation (9). This result contrasts with data from AF and FAF (Fig. 1a), which consistently adopt an S/B conformational heterogeneity (6,10).

We here present new data that provides further insights on the conformers that contribute to the conformational mix of dG-AAF in solution. Specifically, our results are consistent with adoption by dG-AAF of a major groove B-type (B) and a minor groove “wedge” (W) conformer, in addition to the S-conformer elucidated by O’Handley *et al*. (7) (Fig. 1c), and that sequence context determines the population balance between the states. The B-type conformer has a glycosyl bond conformation that is *anti*, while S and W are *syn*. The

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Supporting Information Available. Experimental details of synthesis and spectroscopic measurements; imino proton spectra, UV-melting curves. This material is available free of charge via the Internet at http://pubs.acs.org.

[1]ABBREVIATIONS: dG-AAF, [*N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene]; dG-AF, [*N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene]; dG-FAAF, [*N*-(2′-deoxyguanosin-8-yl)-7-fluoro-2-acetyl-aminofluorene]; dG-FAF, [*N*-(2′-deoxyguanosin-8-yl)-7-fluoro-2-aminofluorene]; EXSY, exchange correlation spectroscopy; ICD, induced circular dichroism.

representative torsion angles of the S-, B-, and W-conformers are summarized in Supplementary Table 1. Similar to dG-AF (6), the B-type dG-AAF maintains Watson-Crick hydrogen bonds, thereby placing the fluorenyl and the acetyl moieties in the major groove (Fig. 1c). Molecular dynamics simulations have shown that the B-type dG-AAF conformer can readily be accommodated in the active sites of bypass polymerases Dpo4 (11) and Pol iota (12). As observed in the S-conformer, the AAF-modified dG adopts the *syn* conformation; however, instead of having disrupted hydrogen bonding, the modified dG can pair with the complementary dC utilizing its Hoogsteen edge; this places the acetylamino moiety in the narrow minor groove (13)(Fig. 1c). Although this “wedge” W-conformer led to severe distortion of the DNA binding area in the active site of Pol iota (12), it is a distinct possibility in polymerase-free duplex DNA (13).

In the present study, we conducted spectroscopic studies to investigate the AAF-induced S/B/W-heterogeneity and sequence effects. The utility of ^{19}F over ^{1}H NMR for probing muti-conformeric DNA has been well documented (6,8,9,10). The imino proton spectra of the FAAF-modified 12-mer DNA duplexes **I** (CG*C) and **II** (TG*A)(Supplementary Fig. S2) exhibited at least 14 resolvable imino protons with varying degrees of intensity, indicating the presence of multiple conformations. In line with this interpretation, the ^{19}F NMR spectrum of duplex **I** revealed prominent ^{19}F signals at −115.45, −116.15, and −117.75 ppm at 5°C (Fig. 2a). Their intensity ratio was 5:65:30, which is in close agreement with the ~70%:30% ratios reported in the ^{1}H NMR study cited above in the same CG*C sequence context (7). Accordingly, the major signal at −116.15 ppm was assigned to that arising from the S-conformer. Duplex **II** (Fig. 2b) exhibited three signals at −116.35, −116.95, and −118.35 ppm in a 5:30:65 ratios at 5°C. The conformational similarity of the two duplexes is evident from the temperature dependence of their 1D spectra (Fig. 2). While the three ^{19}F signals in each duplex were in slow exchange at 5°C, they became exchange broadened, giving rise to coalescent signals at 50°C and 42°C for **I** (−115.0 ppm) and **II** (−115.1 ppm), respectively. The strong temperature dependence of off-diagonal peaks in the contour plots of EXSY spectra (Supplementary Fig. S4) confirmed that these signals arise *via* chemical exchange from three slowly exchanging conformations. This contrasts with the lack of chemical exchange reported previously for the same FAAF-duplex, which led to the proposition that the exclusive S-conformer FAAF molecules may differ only in their relative acetyl group orientations, *cis* (γ'~180°) or *trans* (γ'~0°)(9).

Owing to the similarity in flanking sequence and population contexts, we previously assigned the major center signal at −116.15 ppm in the spectrum of duplex **I** as that of the S-conformer (Fig. 2a). Consequently, the similar signal in the spectrum of the TG*A duplex **II** in Figure 2b could likewise be assigned to the S-conformer. It was observed that the chemical shift range of the upfield signals (at −177.8 and −118.3 ppm, respectively for **I** and **II**) falls into the range (−118 ~ −119 ppm) commonly observed for dA-mismatched W-conformer FAF-duplexes (14). As with FAF, the FAAF-induced S/W equilibrium is expected to occur readily since both conformers maintain very close χ, α', and β' torsion angles. The EXSY data in Supplementary Fig. S4 revealed a facile exchange between the S- and W-conformers. Taken together, the upfield signals were assigned as arising from the W-conformation (see below for further elaboration). Finally, the minor, but persistent, downfield signal (at −115.45 and −116.35 ppm, respectively for duplex **I** and **II**) was assigned to the B-conformer. The modest downfield shift (−0.80 ppm) relative to the S-conformer can be attributed to the lack of ring-current effects in the major groove (8). Also noted is the signal’s close proximity in chemical shift to the denatured FAAF-modified single strand 12-mer oligonucleotide (i.e., at 60°C)(6). We were unable to assign other minor conformers (marked as ‘*’, particularly in Duplex **I**,Fig. 2a) detected at 5°C. In principle, the dG-AAF adduct can adopt additional conformations depending on the flexible

rotation of the fluorenyl-nitrogen (β′) and amide (γ′) bonds (13). However, the available data did not allow us to differentiate them into specific β′ and γ′ rotamers.

The W-conformer described above adopts a *syn*-dG[FAAF]:*anti*-dC alignment at the lesion site using the Hoogstein edge of the dG. As shown in Figure 3b, such a scenario might involve hydrogen bonds of the O^6 of guanine with either the N_3-protonated cytosine alone, or jointly with one of the exocyclic amino protons, or the cytosine amino proton alone. If the N_3 protonation of cytosine were required, the solution pH would alter the stability of the W-conformer and thus its relative population. The pK_a of cytosine has been shown to increase to as high as 8 in certain double and triple helices containing Hoogsteen type base pairs (15). Norman *et al*. (16) have proposed a stable *syn*-dG[AF]:*anti*-dA^+ alignment in acidic pHs, which provides a basis for G→T mutations observed *in vivo*.

To gain further insight into the structure, we performed ^1H and ^{19}F NMR assessments of FAAF-duplex **II** as function of pH. As shown in Supplementary Fig. S5a, there was no change in the imino spectrum between pH 6.7 and 5.8. At pH 8.0, base-catalysis caused fast exchange of the imino protons at or near the lesion site as well as frayed terminal ends. The exchange was almost complete at pH 9.0. However, the original imino protons reappeared after the solution was titrated back to pH 5.8. In line with the imino case, the ^{19}F signal pattern was stable in the pH range of 5.8 to 8.0 (Fig. 3a). However, a new set of signals appeared in the −115 ~ −116 ppm region at the expense of the original B/S/W signals seen at neutral pH. The conformational transition was nearly complete at pH 9.0. As in the imino spectra, the original ^{19}F NMR pattern signals could be titrated back at pH 5.8. Our NMR data seem to argue against the presence of any of the Hoogsteen-type hydrogen bonding scenarios depicted in Figure 3b. First, there were no proton signals above the 15.0 ppm and 9.0–11.0 ppm range, where the N_3-protonated cytosines and the exocyclic amino protons involved in base pairing are supposed to appear (17). Second, we observed neither a significant change in imino spectral patterns nor the S/W population ratios as the pH was shifted from 5.8 to 8.0. Based on these results, we conclude that dG-FAAF may exist in a Hoogsteen type alignment with *anti*-dC, but not involving any hydrogen bonds.

Figure 3c shows the pH dependence of the CD spectra of FAAF-duplex **II**. At neutral pH (7.0), the duplex exhibited major positive and negative ellipticities around 275 and 240 nm, respectively, which is indicative of a typical B-form DNA duplex. The major negative dip in the 280–320 nm range presumably arises from the unique interaction between the acetylaminofluorene chromophore and DNA. We previously reported similarly induced CD data ($ICD_{290–350nm}$) for dG-AF and dG-FAF adducts: positive for an S- or W-type conformation and negative for a B-type conformation (18). At pH 9.0, the negative $ICD_{280–320\ nm}$ pattern disappeared while the S-shape CDs at 240 nm and 275 nm were shifted to longer wavelengths by 11 and 5 nm, respectively. These red shifts indicate that there has been a substantial disruption of the regular B-form double helix and appear to be in good agreement with the appearance of a new set of conformational heterogeneities observed in the ^{19}F NMR spectrum at the same pH (Fig. 3a). Although we do not know the structural details of this new set of conformers, they clearly originated from the FAAF-S/W/B conformers and are stable even with the Watson-Crick imino protons fully exchanged. As in the NMR experiments, the FAAF-induced conformational heterogeneity was fully titratable in CD experiments.

In conclusion, we have provided ^{19}F NMR/ICD evidence showing that the dG-AAF adduct in duplex DNA exists in an equilibrium of S-, B-, and W-conformers with their population ratios varying significantly depending on the flanking sequence context. Our data also revealed that AAF-induced heterogeneity is unaltered from a pH of ~5.8 to a pH of ~8.0, but transforms into a new set of conformations in a highly basic pH (8.5 ~ 9.0). These new

findings concerning the conformers that contribute to the structural heterogeneity adopted by dG-AAF should be useful in interpreting nucleotide excision repair data (19) which shows that sequence context plays a role in determining the relative excision susceptibility (20).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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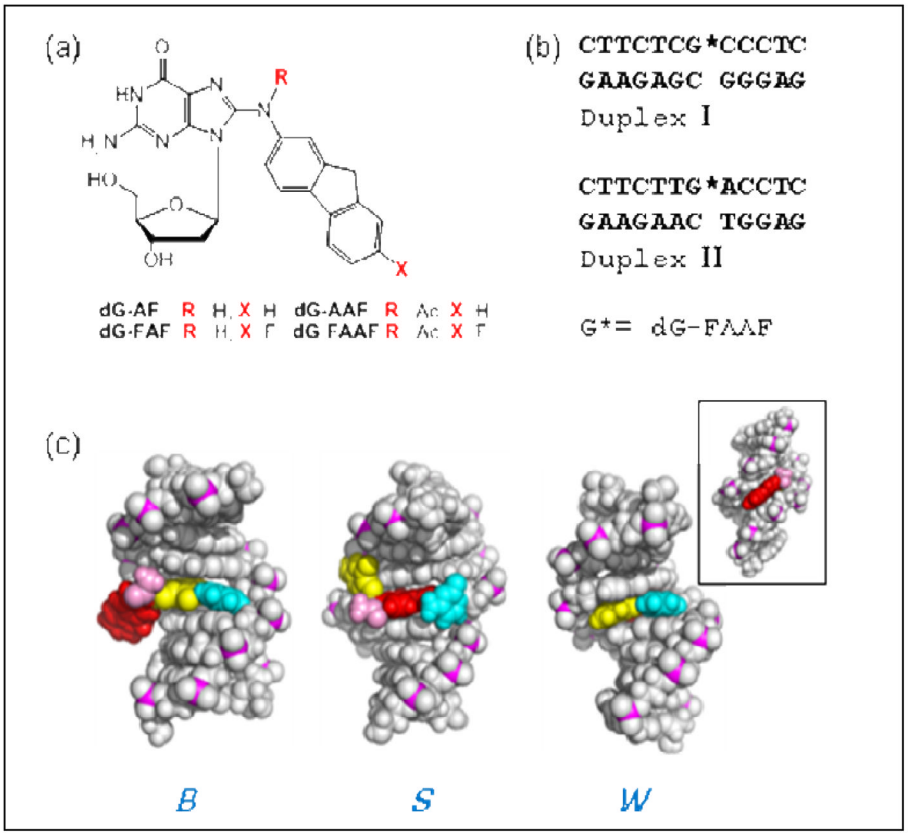

Figure 1.

(a) Structures of dG-AF, dG-AAF and their fluoro models, dG-FAF, dG-FAAF; (b) 12-mer duplexes **I** and **II** used in the present study; (c) Views from the major-grooves of a duplex for three FAAF-induced conformational motifs: B, S, and W-conformers. Color code: modified G (yellow) and complementary C (cyan) at the lesion site; fluorene (red) and *N*-acetyl (pink). Inset shows the minor groove view of W-conformer.

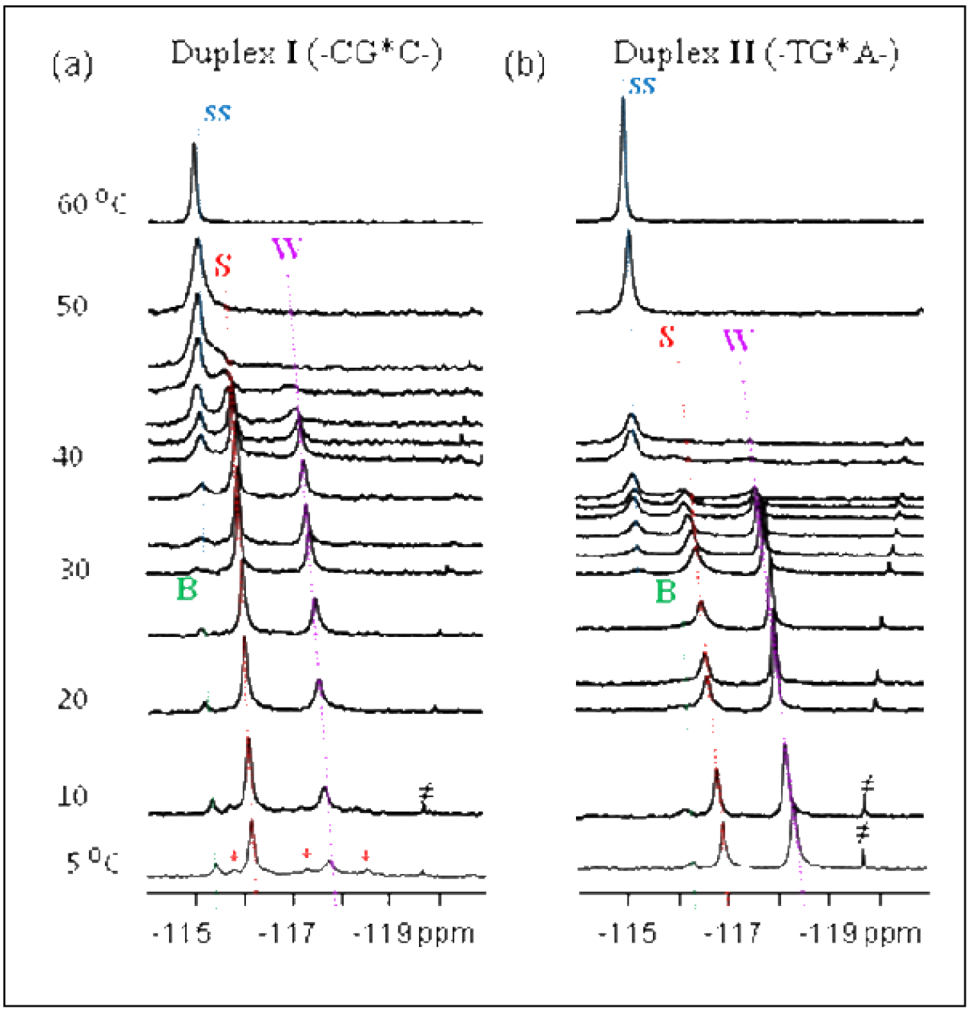

Figure 2.
Temperature dependence of 1D ^{19}F NMR (−114 ~ −121 ppm) spectra of the FAAF-modified duplex (a) **I** and (b) **II** recorded in 10% H_2O buffer at pH 6.8. ss (single strand, blue), B (green), S (red), W (purple) conformers. ≠ impurity.

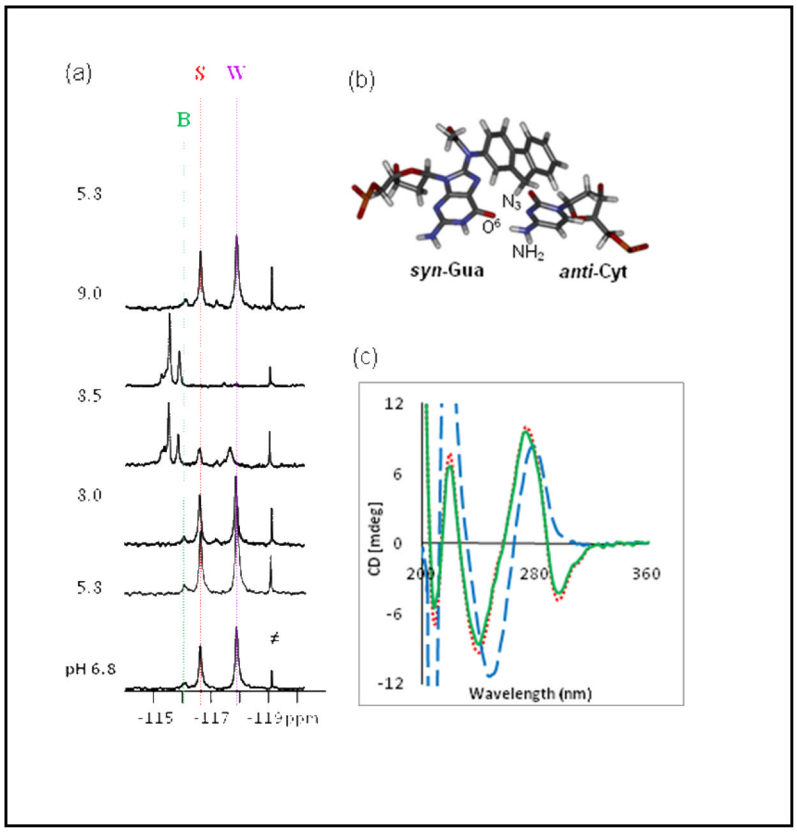

Figure 3.
pH dependence of (a) fluorine NMR (5°C, ≠ impurity) and (b) CD spectra (20°C)[pH 7.0 (red); pH 7.0→9.0 (blue); pH 7.0→9.0 →7.0 (green)] of the FAAF-modified duplex **II**. c) a Hoogsteen-type *syn*-dG[FAAF]:*anti*-dC alignment.