Formation and Structural Features of a Sterigmatocystin–Formamidopyrimidine Adduct at the DNA Duplex Level

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The N^7 position of guanine is the most nucleophilic in DNA and represents a reactive site for covalent adduct formation by mutagens and carcinogens.[1] These adducts result following exposure to food mycotoxins,[2] nitrogen mustards, haloalkyl nitrosoureas, haloalkyl glutathiones, and chemicals such as ethylene oxide and vinyl chloride. Covalent adduct formation at N^7 introduces a positive charge into the five-membered imidazole ring of guanine, which in turn facilitates cleavage chemistry to formamidopyrimidine (FAPY) imidazole ring-opened adducts at basic pH. The opening of the imidazole ring of guanine generates new single bonds, the likely consequence of which is rotameric isomerization resulting in conformational heterogeneity among interconverting FAPY adducts.[3,4] We were interested in the structural characterization of a single FAPY adduct conformation, and we outline below our approach toward achieving this goal for food mycotoxin–FAPY adducts at the duplex level in aqueous solution.

Harris and co-workers were able to generate aflatoxin–N^7-guanine adducts positioned opposite cytosine in DNA duplexes and to characterize them using NMR methods in aqueous solution.[5] These structural studies have established that the aflatoxin intercalates to the 5′-side of the modified guanine without disruption of the modified base pair.[5] We have reported previously on the synthesis and structural characterization of the self-complementary d(A1-A2-T3-[ST]G4-C5-A6-T7-T8) duplex where adjacent [ST]G4 adducts on partner strands are positioned opposite C5 in the center of the duplex.[6] This adduct duplex was left for 2 h at pH 9.8 and 25 °C to quanititatively convert the sterigmatocystin–N^7-guanine adduct, [ST]G4, to its sterigmatocystin-FAPY counterpart, [ST]F4, **1**. The proton NMR spectrum of the self-complementary d(A1-A2-T3-[ST]F4-C5-A6-T7-T8) duplex **2** exhibited resonances corresponding to several isomers of the ring-opened [ST]FAPY adduct in slow equilibrium. In the absence of added salt over a period of 4 days at pH 6.5, this isomer mixture shifted to one major species which will be the focus of our structural efforts.[7]

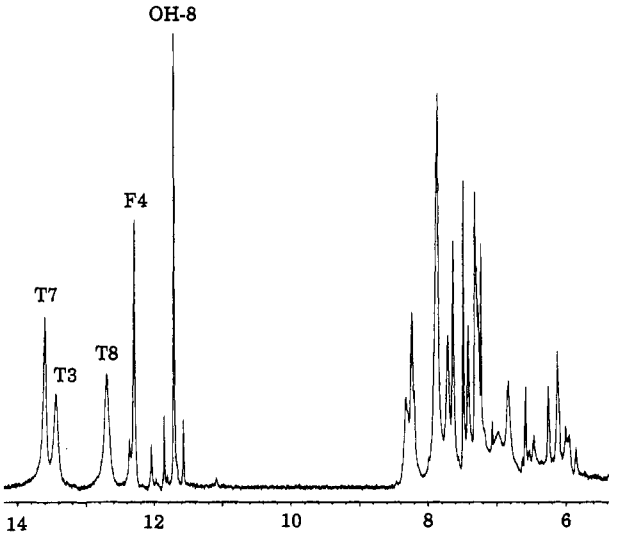
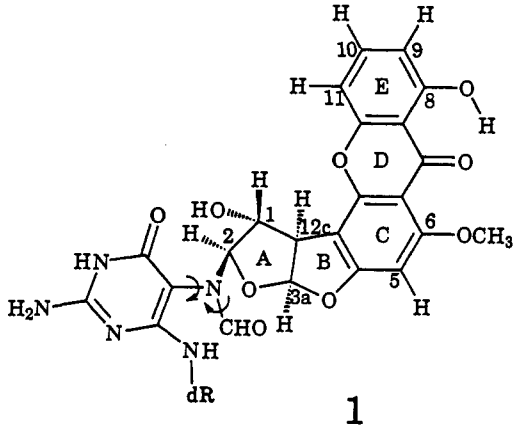
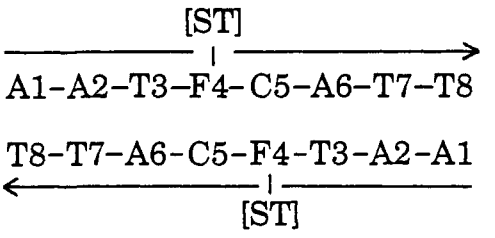


Figure 1. The proton spectrum (6.0–14.0 ppm) of the d(A1-A2-T3-[ST]F4-C5-A6-T7-T8) duplex (two adducts per duplex) **3** in H_2O buffer, pH 6.5, at 5 °C. The nucleic acid imino protons and sterigmatocystin hydroxyl proton are assigned over the figure. Note that the 12.30-ppm imino proton of [ST]F4 is the narrowest imino proton in the spectrum.



1



2

The exchangeable proton NMR spectrum (6–14 ppm) of the [ST]FAPY adduct containing duplex **2** after equilibration in H_2O buffer, pH 6.5, at 5 °C is plotted in Figure 1. The spectral pattern establishes the formation of one major conformation, as well as two minor conformations. We detect all four imino protons in the self-complementary [ST]FAPY adduct containing duplex **2**,and the proton assignments shown over the spectrum follow from an analysis of the NOESY spectrum in H_2O solution at 5 °C (Figure S1, supplementary materials). The cross-strand NOEs

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(6) Gopalakrishnan, S.; Liu, X.; Patel, D. J. *Biochemistry* **1992**, *31*, 10790-10801.

(7) The desalted sample (pH 6.5) eluted as a single peak at 37 min with a minor component at 35 min on an analytical Waters C18 reverse-phase HPLC column (C18 μ-Bondapak, 3.9 × 300 mm) using an acetonitrile gradient (7.5 to 15% acetonitrile for 30 mins followed by 15 to 50% acetonitrile for 15 min) in 0.01 M triethylammonium acetate, pH 7.0 buffer (flow rate: 1.2 mL/min).

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between thymine imino and adenosine H2 and amino protons establish formation of Watson-Crick A2·T7 and A3·T6 base pairs in adduct duplex **2**. The narrow 12.3-ppm imino proton of the [ST]F4 FAPY adduct **1** exhibits NOEs to its own amino protons and to the amino protons of C5 (Figure S1A), establishing Watson-Crick pairing across the [ST]F4·C5 pair at the modification site. We observe intermolecular NOEs from the 12.35-ppm imino proton of the [ST]F4 FAPY adduct to the sterigmatocystin H9, H10, and OCH_3-6 protons (Table S1, supplementary material), establishing that the sterigmatocystin aromatic chromophore stacks on the [ST]F4·C5 base pair. In addition, the 11.7-ppm sterigmatocystin OH-8 proton exhibits intermolecular NOEs to the H8 and H1′ protons of A6 and the sugar protons of C5 (Table S1), establishing that the E ring of the sterigmatocystin chromophore is stacked between C5 and A6 in the [ST]FAPY adduct containing duplex **2**.

We observe a well-resolved nonexchangeable proton spectrum for the [ST]FAPY adduct containing duplex **2** which has permitted us to trace the NOE connectivities between the base protons and their own and 5′-flanking sugar H1′ and H3′ protons in NOESY spectra in D_2O solution at 15 °C. The NOE connectivities can be readily traced in the expanded NOESY contour plot from A1 to T3 and from A6 to T8 along the length of adduct duplex **2** (Figure S2, supplementary materials). The tracing breaks down at the T3-[ST]F4 step since the H8 proton of [ST]G4 is lost on [ST]F4 FAPY adduct formation. We detect an NOE between the H6 proton of C5 and the 4.80-ppm upfield shifted sugar H1′ proton of the [ST]F4 FAPY adduct for the [ST]F4-C5 step but not between the H6 proton of A6 and the sugar H1′ proton of C5 for the C5-A6 step in adduct duplex **2** (Figure S2). This result establishes formation of intercalation sites at the (T3-[ST]F4)·(C5-A6) steps for the [ST]F4 FAPY adduct, similar to what was reported previously[6] for the (T3-[ST]G4)·(C5-A6) steps for the [ST]G4 adduct within the sequence context of adduct duplex **2**.

The H1, H2, H3a, and H12c protons on the fused bicyclic A/B ring system of sterigmatocystin exhibit NOEs to the major groove protons of the T3-[ST]F4 step (Table S1), consistent with its placement in the major groove and its role in spanning the covalent modification and intercalation sites. The H5 and OCH_3-6 protons on sterigmatocystin aromatic ring C exhibit NOEs to the minor groove protons, while the H9 and H10 protons on sterigmatocystin aromatic ring E exhibit NOEs to the major groove protons (Table S1). This positions the long axis of the intercalating sterigmatocystin chromophore colinear with the long axis of the flanking T3·A6 and [ST]F4·C5 base pairs at the intercalation site with its OCH_3-6 containing edge directed toward the minor groove and its H11 containing edge directed toward the major groove in the [ST]FAPY adduct containing duplex **2**. Thus, at a qualitative level there are striking similarities between structural features of the intercalation sites of [ST]G4[6] and [ST]-F4 FAPY adducts in the sequence context of self-complementary duplex **2**.

In summary, we have generated the [ST]FAPY adduct **1** in the sequence context of the self-complementary d(A1-A2-T3-[ST]F4-C5-A6-T7-T8) duplex **2** and established that a single conformation driven by the intercalating chromophore predominates in aqueous solution with no added salt that was allowed to equilibrate over time under slightly acidic conditions. The alignment of the [ST]FAPY adduct **1** exhibits the same structural features established earlier for its [ST]G adduct counterpart,[6] namely that the sterigmatocystin chromophore intercalates to the 5′-side of the [ST]FAPY modification site without disruption of the Watson-Crick [ST]F4·C5 base pair. We shall next attempt to define a high-resolution structure of the [ST]FAPY adduct containing duplex **2** through a combined NMR–molecular dynamics study.[8]

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Supplementary Material Available: Tables S1 and S2 listing nucleic acid proton chemical shifts and intermolecular NOEs; Figures S1 and S2 showing expanded NOESY contour plots in H_2O and D_2O and a phosphorus–proton correlation contour plot for the [ST]FAPY adduct containing duplex **2** (5 pages). Ordering information is given on any current masthead page.

(8) Our efforts at quantitation of the [ST]FAPY alignment in duplex **2** is hampered somewhat by our inability to identify the NH and CHO protons at the cleavage site. It may be possible to identify these protons by incorporating ^{15}N and ^{13}C at these positions; subsequent NMR–molecular dynamics calculations to define the solution structures at and adjacent to the [ST]-FAPY adduct site in **2** should then be feasible.