Site-Specific Intercalation of an Anthracycline Antitumor Antibiotic into a Y·RY DNA Triplex through Covalent Adduct Formation

Xiaomei Ye,† Ken-ichi Kimura,‡ and Dinshaw J. Patel*,†

Cellular Biochemistry and Biophysics Program
Memorial Sloan-Kettering Cancer Center
1275 York Avenue, New York, New York 10021
Research Institute of Life Science
Snow Brand Milk Products Co. Ltd.
Tochigi 329-05, Japan

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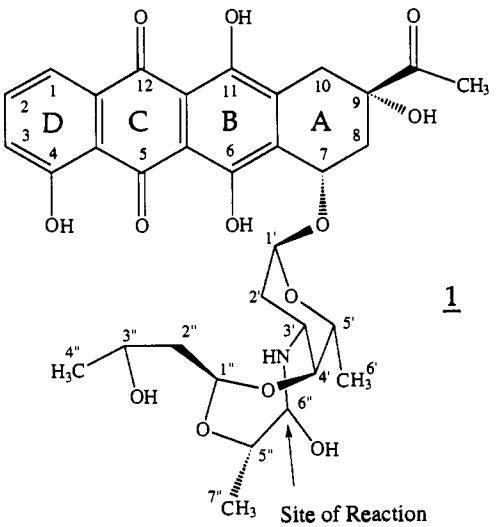
DNA triple helices of the pyrimidine·purine·pyrimidine (Y·RY) class contain a pyrimidine-rich third strand positioned in the major groove and parallel to the purine strand with recognition associated with T·AT and C⁺·GC base triple formation.[1] Such sequence-specific recognition of duplex DNA through triple helix formation has provided new approaches for the modulation of gene regulation as well as the site-specific recognition and cleavage of genomic DNA.[2]

The availability of solution structures of Y·RY DNA triplexes[3] has set the stage for experiments that address the recognition of the triple helix itself by antitumor antibiotics which generally target the unoccupied minor groove. Several groups have studied the noncovalent binding of antibiotics in the minor groove of Y·RY triplexes as well as the noncovalent intercalation of antibiotics into Y·RY triplexes and triplex–duplex junctions and at third strand crossover sites in triplexes.[4] We have recently reported on the duocarmycin–DNA triplex complex where the duocarmycin bound covalently and site-specifically to a single adenine through the N^3 position and aligned the ligand in the minor groove without disruption of the third strand in the major groove.[5]

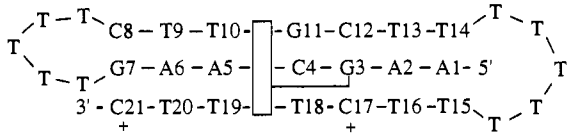
The anthracycline antibiotics, which comprise an aglycone chromophore attached to an amino sugar, have played a key role as important components in combination chemotherapeutic treatments. X-ray[6] and NMR[7] studies have established that the aglycone intercalates at (Y-R) steps and that the amino sugar is positioned in the minor groove of duplex DNA. The structural research on these noncovalent anthracycline–DNA complexes has recently been elegantly extended to their covalent counterparts, where formaldehyde has been used to cross-link the amino sugar to the minor groove exocyclic amino group of guanine in DNA.[8] The X-ray structures of these complexes establish that the aglycone intercalates to the 3′-side of the covalently linked guanine with one base pair separating the intercalation site from the modified guanine.[8]

We have used a natural anthracycline called SN-07 chromophore **1** which contains an eight-membered ring with a carbinolamine functionality fused to the amino sugar residue.[9]



1

We have recently determined the solution structure of the SN-07 chromophore bound to duplex DNA at (*G-C-A)·(T-G-C) sites, where the covalent linkage occurs at *G and the aglycone intercalates at the (C-A)·(T-G) step in the 3′-direction (unpublished results). We now demonstrate the formation of site-specific intercalation of the SN-07 chromophore into a DNA triplex. Our starting point is the intramolecular Y·RY triplex **2** which contains a (G3-C4-A5) step generated by incorporating an unusual T18·C4G11 triple in the center of the triplex. We were able to generate and HPLC purify the SN-07 chromophore covalently bound site-specifically to a single guanine (G3) in this intramolecular DNA triplex.[10]



2

The exchangeable proton NMR spectrum (8.0–16.0 ppm) of the SN-07 chromophore–triplex complex in H_2O buffer, pH 4.8, at 5 °C is plotted in Figure 1. The spectrum is of high quality with well-resolved resonances detected for the hydrogen-bonded

† Memorial Sloan-Kettering Cancer Center.
‡ Snow Brand Milk Products Co. Ltd.

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(10) The adduct was prepared by gradually adding SN-07 chromophore to ~1000 A_{260} units of the DNA triplex in 5 mL of an aqueous buffer containing 10 mM phosphate, 100 mM NaCl, 0.1 mM EDTA, at pH 8.0, room temperature, for 2 weeks. The final concentration of SN-07 chromophore was 1 equiv relative to the triplex. The major adduct (~70% of product) was purified by two successive semipreparative HPLC treatments on a C18 column using a 40 mM phosphate (pH 7)/methanol gradient. The minor adducts were resolved on HPLC but were not characterized further. The pH was then lowered to pH 4.8 to convert the adduct duplex to the adduct triplex.

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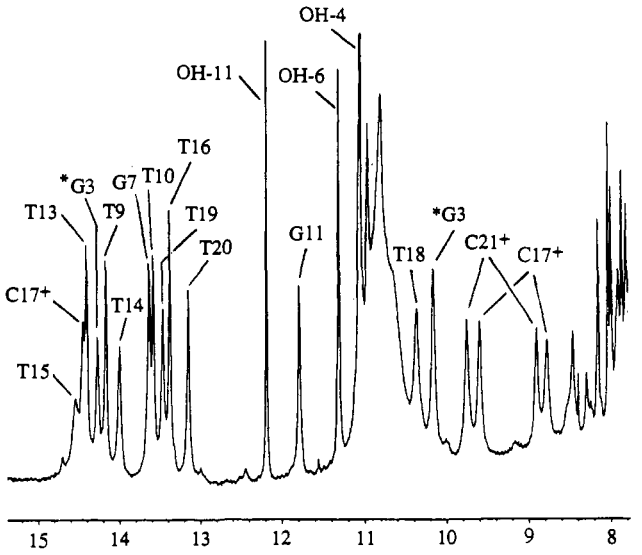

Figure 1. Exchangeable proton spectrum (8.0–16.0 ppm) of the SN-07 chromophore-triplex complex **2** in 100 mM NaCl, 10 mM phosphate, H_2O, pH 4.8 at 5 °C. The exchangeable proton assignments are listed over the spectrum.

(11.5–14.0 ppm) and exposed (10.0–11.5 ppm) imino protons, the protonated cytidine amino protons (8.6–10.0 ppm), and the SN-07 chromophore hydroxyl protons (10.8–12.4 ppm). The resonances have been identified by standard assignment strategies,[1e,f] and these are listed over the spectrum and tabulated for the central ([SN]G3-C4-A5)·(T10-G11-C12)·(C17-T18-T19) segment in the supplementary material, Table S1. The covalent adduct forms as expected at the minor groove amino group of G3 since we can clearly detect a strong NOE between the 14.47-ppm imino proton and the 10.17-ppm single amino proton of this modified base. Further, the covalent adduct forms without disruption of the third strand since we observe downfield-shifted amino protons from protonated cytidines C21 (8.90 and 9.84 ppm) and C17 (8.80 and 9.63 ppm) characteristic of an intact triplex (Figure 1).[1e,f] The imino proton of T18 resonates upfield at 10.75 ppm and exchanges rapidly with solvent water since it does not participate in the hydrogen-bonding alignment of the T·CG triple in either unmodified triplexes (unpublished results) or in the complex reported here. The intercalation of the aglycone of the SN-07 chromophore occurs between intact T19·A5T10 and T18·C4G11 triples as established from an analysis of NOESY spectra of complex **2** in H_2O buffer, pH 4.8, at 5 °C (supplementary material, Figures S1 and S2). We do not detect an NOE between the imino protons of T10 and G11 (Figure S1A), consistent with generation of the intercalation site at the T10-G11 step. The imino proton of G11 exhibits intermolecular NOEs to the OH-6 hydroxyl of the SN-07 chromophore (Figure S1A), while the imino proton of T19 in the third strand exhibits intermolecular NOEs to the OH-4 and OH-6 hydroxyls of the SN-07 chromophore (Figure S1B) in the complex. Further, the SN-07 chromophore OH-6 hydroxyl exhibits an NOE to the minor groove H1′ proton of C4, while the OH-4 hydroxyl exhibits an NOE to the major groove H8 proton of A5 in complex **2** (Figure S2B), thus establishing the alignment of the aglycone at the intercalation site.

Further support for the proposed aglycone intercalation site between the T19·A5T10 and T18·C4G11 triples comes from an analysis of NOESY spectra of complex **2** in D_2O buffer, pH 4.8, at 20 °C (supplementary material Figures S3 and S4). The base and sugar H1′, H2′, 2″, and H3′ protons in complex **2** have been assigned and are listed for the central trinucleotide segment in the supplementary material Table S1. We do not detect NOEs between the base and the 5′-flanking sugar H1′ protons (Figure S3) or between the base and the 5′-flanking sugar H3′ protons (Figure S4) for the T10-G11, C4-A5, and T18-T19 steps in complex **2**, thus defining the intercalation site in the triplex. We detect additional intermolecular NOEs between the SN-07 chromophore and the triplex (listed in the supplementary material Table S2) that confirm that the amino sugar and attached eight-membered ring of the SN-07 chromophore are positioned in the minor groove following covalent adduct formation at the amino group of G3 and that the aglycone intercalates between the T19·A5T10 and T18·C4G11 triples such that ring A is positioned in the minor groove and ring D projects into the major groove in complex **2**.

The above NMR study on the SN-07 chromophore-Y·RY DNA triplex complex **2** establishes the formation of a sequence-specific intercalation complex at a single site in the triplex through covalent adduct formation in the minor groove without disruption of the third strand in the major groove.

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Supplementary Material Available: Expanded NOESY contour plots of complex **2** in H_2O and D_2O solution and tables listing chemical shifts and intermolecular NOEs for the central trinucleotide segment of the complex (9 pages). Ordering information is given on any current masthead page.