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NMR Studies of the Conformation and Stability of the 4'-Aminomethyl-4,5',8-Trimethylpsoralen (AMT) Cross-Linked DNA Octamer Duplex, d(GGGTACCC)$_2$

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Abstract: The 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT) has been used as intercalating DNA binding drugs in the photo-chemotherapy of skin diseases. The conformation and stability of DNA octamer duplex, d (GGGTACCC)$_2$, cross-linked with AMT has been studied by NMR spectroscopy. All the proton resonances of the psoralen cross-linked octamer were assigned and meting temperature studies were carried out based on the assignment of the proton resonances. The aromatic proton chemical shift data suggest that the conformation of the helix cross-linked with psoralen is destabilized more to furanside of the psoralen, possibly due to the protrusion of the aminomethyl side chain of the psoralen.

Key words: 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT), DNA complex, NMR, melting study, photoadduct.

Psoralens are linear furocoumarins that photochemically alkylate nucleic acids, and have been used as intercalating DNA binding drugs in the photo-chemotherapy of skin diseases (Kanne *et al.*, 1982a; Kanne *et al.*, 1982b; Cimino *et al.*, 1985). The photochemical reactions take place at the 3,4 or 4',5' double bonds of the psoralen with 5.6 double bond in pyrimidines. The planar psoralen first intercalates into a double helical region, and ultraviolet (320~410 nm, UV-A band) irradiation initially induces a single cyclobutane addition with pyrimidine base. The furanside monoadduct still absorbs in this wavelength region, so that a second photoaddition can occur with pyroneside to form the cross-link (Fig. 1) (Isaacs *et al.*, 1977). Psoralen cross-linking occurs at 5'-TpA-3' sites in DNA (Gamper *et al.*, 1984; Zhen *et al.*, 1986) and its photoproducts are primarily in the *cis-syn* conformation (Straub *et al.*, 1981).

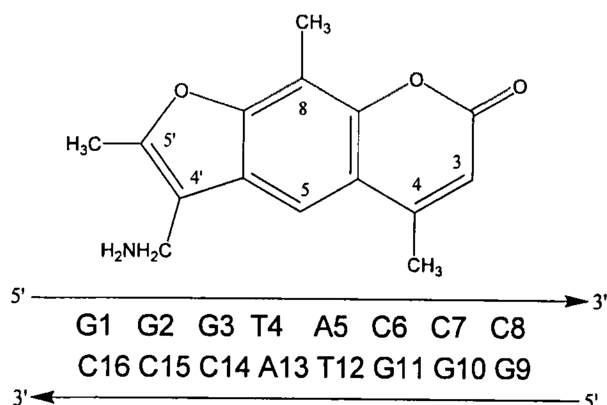

Fig. 1. DNA sequence context and chemical structure of the AMT drug.

The psoralen-adducted DNA interferes with replication and transcription, and is recognized and efficiently removed by repair enzyme systems (Sancar and Sancar, 1988; Shi *et al.*, 1988; Reardon *et al.*, 1991; Sastry and Hearst, 1991a; Sastry and Hearst, 1991b). Therefore, structural studies of the site-specific psoralen adducts of DNA oligomers are required to understand relevant biological process, and in fact, several NMR-derived solution structures of the DNA octamer duplex cross-linked by psoralen have been reported (Tomic *et al.*, 1987; Spielmann *et al.*, 1995, Hwang *et al.*, 1996). In this paper, as an extension of our structural studies, we have studied the conformation and stability of the DNA octamer duplex, d(GGGTACCC)$_2$, which is cross-linked with 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT) using NMR spectroscopy.

Materials and Methods

Preparation of AMT-oligomer cross-link

The DNA oligomer, d(GGGTACCC), was synthesized on an automated Applied Biosystems DNA synthesizer using β-cyanomethyl phosphoramidite chemistry and purified by gel filtration column chromatography. The pu-

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rified oligonucleotides and AMT were dissolved together in 30 mM sodium phosphate buffer. This solution was stirred at room temperature for 1 hour under nitrogen atmosphere, and irradiated with 350-nm UV light at 0℃ for 15 minutes after stirring. This procedure was repeated with one more addition of AMT to the solution to achieve maximum yield. After irradiation, the reaction mixture was diluted with 8 M urea, heated to 50~60℃ in water bath, and maintained at that temperature for 10 minutes. The reaction mixture was lyophilized and resuspended in small volume of water, and subjected to a P-10 gel filtration column chromatography with 4 M urea, 40 mM ammonium acetate buffer, pH 7, at a flow rate of 0.5 ml/min. The fraction containing AMT cross-linked oligomer were pooled, concentrated, and dialyzed to remove urea and other contaminant, and then lyophilized to dryness. Finally, the cross-linked oligomer was dissolved in 1 ml of distilled water and desalted by Sephadex G-25 column. The sample was lyophilized and redissolved in 0.5 ml buffer containing 20 mM sodium phosphate and 150 mM sodium chloride, pH 7.

NMR experiment

All NMR spectra were obtained on Bruker AMX-500 MHz and DMX-600 MHz spectrometers, and the data were processed on Silicon Graphics workstation using the program *Felix* 2.30. One-dimensional nuclear Overhauser effects (NOEs) in H_2O solution were measured by alternatively off-resonance and on-resonance irradiated spectra to minimize the effects of spectrometer drift. The decoupler power used was 20~28 dB and the 500-scanning data were accumulated for any one irradiated frequency. The off-resonance irradiated spectrum was subtracted from the on resonance spectrum to yield a NOE spectroscopy. One-dimensional spectra were obtained at several temperatures to investigate the melting study of DNA base proton and AMT proton in D_2O buffer solution. The phase-sensitive nuclear Overhauser effect spectroscopy (NOESY) spectra were acquired using time proportional phase incrementation. The spectra were acquired with 2 K points in the t_2 direction and 512 points in the t_1 direction. The time-domain data were zero filled in both directions and multiplied with $\pi/3$ shifted squared sine function before Fourier transformation.

Results and Discussion

Assignments of nonexchangeable DNA protons

The one-dimensional NMR spectra of the nonexchangeable protons of the unmodified and AMT cross-linked octamers in D_2O buffer solution are shown in Fig. 2.

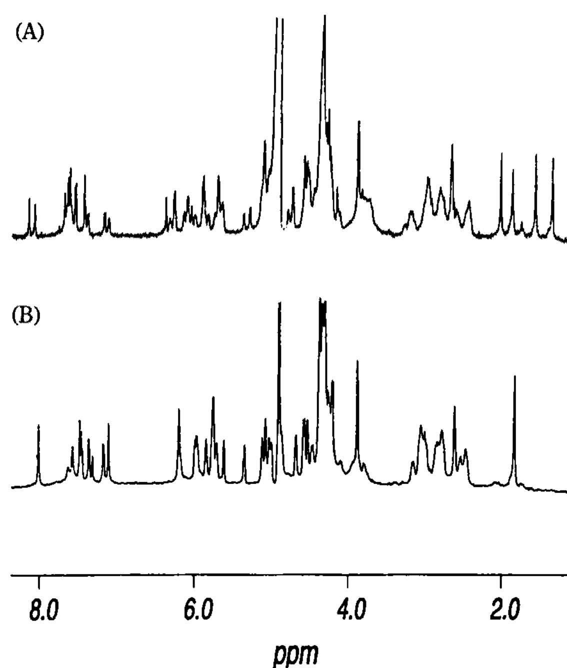

Fig. 2. (A) Nonexchangeable proton NMR spectra of the AMT cross-linked octamer and (B) unmodified octamer in 20 mM sodium phosphate buffer containing 200 mM sodium chloride, pH 7.0, at 25℃.

For unmodified octamer, two equivalent protons of the two strands were resonated in one peak, as was typical for the self-complementary DNA duplex. For cross-linked octamer, the covalent-bound psoralen breaks the symmetry of DNA duplex so that previously equivalent protons on two strands have two separated resonances, which complicates the spectra.

An expanded portion of a 250-ms NOESY spectrum of the cross-linked octamer duplex in D_2O buffer solution, at 10℃, is shown in Fig. 3A. This contour plot outlines the sequential intrastrand NOE connectivities between the base H8/H6 protons and the sugar H1' and cytosine H5 protons. The AMT in cross-linked octamer was attached to the DNA oligomer on the pyroneside by a cyclobutane ring with T4 residue and furanside with T12 residue. Sequential NOEs of the pyroneside-adducted strand were regular from G1 to G3 residue and from A5 to C8 residue, but were not observed at psoralen-adducted T4 residue. Similar results were also observed in the complementary strand. The change in atomic hybridization of the C5 and C6 carbons of the thymine residues upon the formation of the covalent bond between thymine residue and psoralen leads to the large upfield shifts of the H6 protons of T4 and T12 residues (Table 1). Therefore, the covalent photoaddition of the AMT shifts the thymine H6 resonances into the same region as the sugar H3' and H4' resonances (Fig. 3B). The assignments of other nonexchangeable

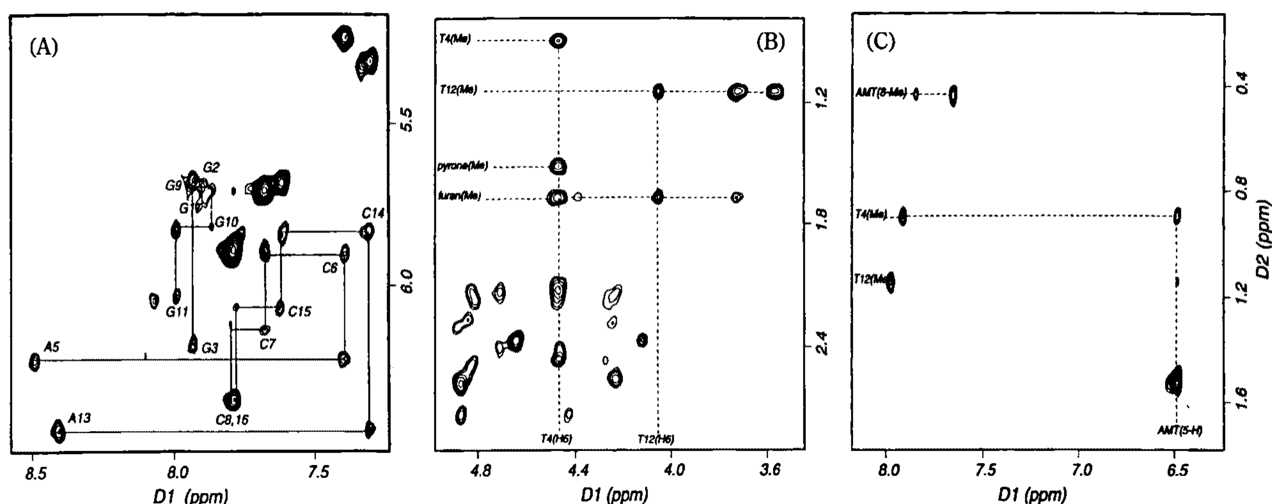

Fig. 3. Expanded NOESY contour plots of the AMT cross-linked octamer in 20 mM sodium phosphate buffer containing 200 mM sodium chloride, pH 7.0, at 10°C. (A) A typical region (base proton to H1' sugar proton) in the sequential NOE connectivity. (B) The crosspeaks between the H3'/H4' and H2'/H2'' sugar protons. The H6 base protons of T4 and T12 residues upfield shifted to 4.47 and 4.06 ppm, respectively, show the intermolecular NOEs of the methyl protons of the AMT drug. (C) The crosspeaks between the 5-Me protons of thymine residues and their base protons. The AMT 5-H proton shows NOEs with 5-Me protons of two thymine residues and the AMT 8-Me protons shows NOEs with the H2 protons of two adenine residues.

Table 1. The chemical shifts of the proton resonances of the AMT cross-linked octamer

1. DNA proton resonances

Residue	Imino		H2/H5		H6/H8		H1'		H2'		H2''		H3'		H4'	
G1/G9	-	12.65			7.91	7.93	5.73	5.68	2.58	2.57	2.73	2.71	4.89	4.88	4.22	4.42
G2/G10	13.08	13.08			7.88	4.47	5.68	5.81	2.71	2.74	2.71	2.74	4.88	4.88	4.21	4.22
G3/G11	12.43	12.52			7.93	7.99	6.22	6.02	2.57	2.64	2.74	2.91	5.07	5.10	4.45	4.47
T4/T12	10.73	11.30	0.90	1.15	4.47	4.06	5.93	5.56	2.12	2.09	2.40	2.18	4.72	4.71	4.17	4.22
A5/A13			8.05	7.84	8.49	8.41	6.23	6.45	2.74	2.96	2.96	3.06	4.48	5.05	4.28	4.24
C6/C14			4.19	5.33	7.39	7.30	5.89	5.83	2.17	2.10	2.46	2.48	4.82	4.83	4.26	4.20
C7/C15			5.62	5.69	7.67	7.62	6.14	6.07	2.29	2.25	2.53	2.55	4.88	4.86	4.24	4.23
C8/C16			5.90	5.90	7.78	7.78	6.35	6.35	2.35	2.35	2.35	2.35	4.66	4.66	4.12	4.12

2. AMT proton resonances

3-H	4-Me	5-H	8-Me	4'-aminomethyl		5'-Me
2.47	1.52	6.51	0.44	3.58	3.75	1.67

protons in the AMT cross-linked octamer were obtained from the NOEs with known resonances.

Assignment of exchangeable DNA protons

Imino protons were assigned from chemical shifts, melting profiles, and 1-D NOE difference spectra and their chemical shifts were listed in Table 1. The AMT binding of two thymine residues caused their imino proton resonances to be shifted upfield to approximately 10.7 and 11.3 ppm for T4 and T12, respectively, from their position at 13.4 ppm in native DNA. Strong NOE connectivity was observed from the T12 imino proton to an H2 proton of A5 residue, indicating stable base pairing of A5·T12 (Fig. 4). The larger upfield shift and line broadening of the T4 imino proton suggest that it is accessible to solvent and weakly hydrogen-bonded. This behavior may be due to the 4'-aminomethyl side chain on furanside of the psoralen which interact with the base of the A13 residue and then distorts the T4·A 13 base pairing (Tomic *et al.*, 1987). In contrast, the T 12 imino proton is the last to exchange with solvent, suggesting that the hydrogen bonding with A5 residue is very stable after cross-linking (Tomic *et al.*, 1987). Large upfield shifts are observed for T residues that lack the deshielding effect of base pairing (Hare and Reid, 1986). The upfield shift of the T4 and T12 imino protons may arise primarily from electronic effects, with an additional contribution for T4 residue from weaker hydrogen bond effects.

Intermolecular AMT-DNA contacts

The psoralen pyroneside ring proton resonances were assigned from NOE crosspeaks between H6 proton of

T4 residue and AMT 4-Me group and 3-H proton (Fig. 3B). The furanside ring proton resonances were also assigned from NOEs between T12 H6 proton and AMT 5'-Me group (Fig. 3B). These crosspeaks indicate the close proximity between DNA thymine residues and AMT drug after the cross-linking. The AMT 5-H and 4'-aminomethyl proton resonances are assigned from NOEs for 4-Me ↔ 5-H and 3'-Me ↔ 4'-aminomethyl proton (data not shown). The assignments of the cross-linked AMT proton resonances are listed in Table 1.

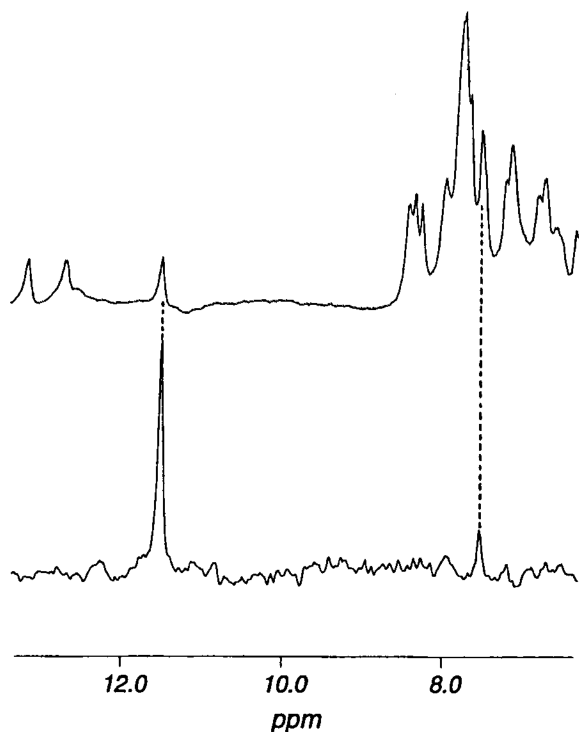

Fig. 4. One-dimensional NOE difference spectrum of AMT cross-linked octamer in 20 mM sodium phosphate buffer containing 200 mM sodium chloride, pH 7.0, at 25℃. Saturation of the T12 imino proton resonance results in an NOE to H2 proton of A5 residue.

The unusual large upfield shift for AMT 8-Me protons is probably due to the ring current effects, resulting from that 8-Me group locates inside of DNA helix. This is confirmed by the fact that the AMT 8-Me group shows strong NOEs with the H2 protons of both A5 and A13 residues (Fig. 3C). NOE between the 4'-aminomethyl proton and H6 proton of T12 residue indicates the adduct formation of AMT with DNA to be the *cis-syn* cyclobutane ring geometry.

Melting temperature study of cross-linked octamer

Plots of aromatic base proton chemical shifts of AMT-cross-linked and unmodified octamer in D_2O buffer as a function of temperature are shown in Fig. 5. The unusual melting patterns were observed for the base proton resonances of the cross-linked octamer. Resonances on one side of the DNA duplex began to melt at lower temperature of 10~20℃ than on the other side. The difference must arise from asymmetric perturbation of the DNA structure at the AMT cross-link. The H8 proton resonance of the A13 residue shifts with increasing temperature, starting at 50℃ (Fig. 5A). However, those of the A5 residue on the opposite side of the drug begin to melt at 60℃ (Fig. 5A). Similar

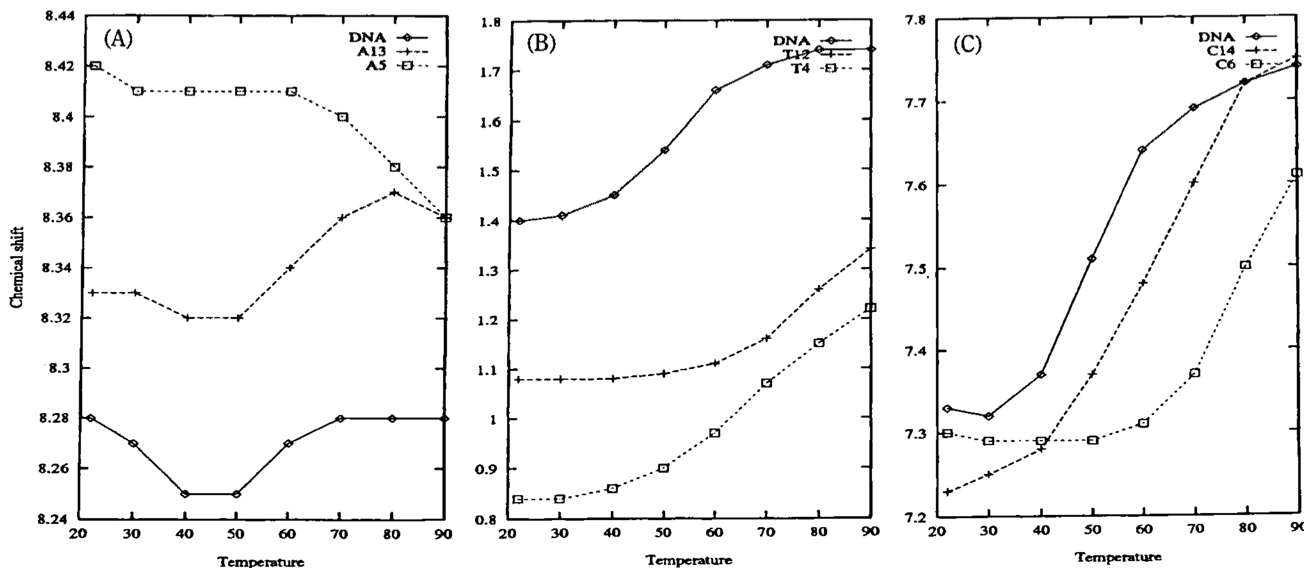

Fig. 5. (A) Temperature dependence of the H8 proton chemical shifts of A5 and A13 residues for cross-linked octamer and A5 residue for unmodified DNA, (B) 5-Me protons chemical shifts of T4 and T12 residues for cross-linked octamer and T4 residue for unmodified DNA, and (C) H6 proton chemical shifts of C6 and C14 residues for cross-linked octamer and C6 residue for unmodified DNA in 20 mM sodium phosphate buffer containing 200 mM sodium chloride, pH 7.0.

results were observed for the 5-Me resonances of their complementary thymine bases, showing that that of T4 began to melt at a lower temperature of 20°C than that of T12 (Fig. 5B), and the H6 resonances of their neighbor bases of C14 versus C6 (Fig. 5C), suggesting that the A5·T12 base pair which is adducted with furanside ring of the drug and its neighbors are more stable than the T4·A13 base pair and its neighbors. This behavior may be due to the AMT 4'-aminomethyl side chain on furanside ring of the psoralen that forces A13 base to propeller twist in a direction opposing the bend in the helix, distorting or disrupting the T4·A13 base pairing (Tomic *et al.*, 1987). The distorting of the T4·A13 base pair causes all base pairs of their side to desabilize more than those of the opposite side.

It is reported that the initial strand incision made by the (A)BC excinuclease complex preferentially cuts the DNA on the furanside of an psoralen (Jones and Yeung, 1988). This has been suggested to be a consequence of presence of the 4'-aminomethyl group in AMT (Cheng *et al.*, 1991). We have observed that the abnormal propellar twisting in T12-A13 base step due to the 4-aminomethyl group results in a severe structural perturbation in this step, indicating that the repair enzymes preferenetially cut the T12 residue adducted with the furanside ring of the drug. We are currently studing the dynamical kinetics of the AMT cross-linked octamer.

Acknowledgements

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