state or a Hoogsteen base pair by their NMR results. Figure 2(B) illustrates a cross-sectional representation of the NOE connectivities in the imino and aromatic region of the two-dimensional NOESY spectrum of the [d(ACGTTAACGT)]$_2$-echinomycin complex. The strong intra-base pair NOE was observed between T imino and AH2 proton resonances. If the central A·T base pairs are standard Watson-Crick A·T base pairs which have an adenosine H-2 proton adjacent to the imino proton, a strong intra-basepair NOE should be observed. Therefore, in contrast to the results obtained with [d(ACGTATACGT)]$_2$ or [d(CGTACG)]$_2$, these data clearly confirm that the central A·T base pairs in the DNA oligomer with 'TTAA' sequences maintain Watson-Crick base pairs even when echinomycin binds.

In summary, we have demonstrated that the Hoogsteen base pairs are not propagated one base pair away from the binding site and structural changes induced when echinomycins bind are sequence specific. More detailed structural studies of the two decamers are under progress.

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References

1. Ward, D. C.; Reich, E.; Goldberg, I. H. *Science* **1965**, *149*, 1259.
2. Waring, M. J.; Waklein, L. P. G.; *Nature* **1974**, *252*, 653.
3. Katagiri, K.; Yoshida, T.; Sato, K. in *Antibiotics III* Corcoran, J. W.; Hahn, F. E., Eds.; Springer: New York, 1975; p 234-251.
4. Wang, A. H. J.; Ughetto, G.; Quigley, G. J.; Hakoshima, T.; van Marel, G. A.; van Boom, J. H.; Rich, A. *Science* **1984**, *225*, 1115.
5. Gilbert, D. E.; van der Marel, G. A.; van Boom, J. H.; Feigon, J. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 3006.
6. Gilbert, D. E.; Feigon, J. *Biochem.* **1991**, *30*, 2483.
7. Park, J. Y.; Choi, K. H.; Lee, B. L.; Choi, B. S. *Bull. Korean Chem. Soc.*, **1992**, *13*, 223.
8. Choi, K. H.; Park, J. Y.; Lee, B. L.; Choi, B. S. *Bull. Korean Chem. Soc.* **1992**, *13*, 345.
9. Gilbert, D. E.; Feigon, J. *Nuclei Acids Res.*, **1992**, *20*, 2411.
10. Plateau, P.; Gueron, M. *J. Am. Chem. Soc.*, **1982**, *14*, 7310.

Convenient Method for the Preparation of Psoralen Cross-Linked DNA Oligomer

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Psoralens are linear furocoumarines that photochemically alkylate nucleic acids and have been used as intercalating DNA binding drugs in the photochemotherapy of skin diseases.[1] They have also been used as probes for nucleic acid structure and function.[2] Model studies for psoralen cross-linking with DNA have been suggested that the photochemical reactions take place at the 3, 4 or 4', 5' double bonds of the psoralen with the 5, 6 double bond in pyrimidines.[3] Chart 1 shows the structure of cross-linked adduct between thymidine and 4'-aminomethyl-3,5',8-trimethylpsoralen (AMT).[4] Thymidine is the preferred site for monoadduct formation, and psoralen cross-linking occurs at 5'-TpA-3' sites in DNA.[5] In spite of all the work on the monoadduct structure, the solution-state structures and properties of psoralen cross-linked DNA as a function of sequence are still not known. This has been due in large part to the lack of general methods for the preparation of psoralen crosslinked DNA in sufficient purity and quantity for detailed NMR studies. Generally, the denaturating polyacrylamide gel electrophoresis (PAGE), followed by the elution of the DNA from gel slices, is the most common method for the separation of photoadducted DNA oligomers. However, this method is obviously insuitable for the production of large quantities of pure psoralen cross-linked DNA molecules required for NMR studies. Recently, the method for the large-scale synthesis of the photoadducted DNA using laser and HPLC has been reported.[6,7] This method is also not easily used because a laser equipment as a light source should be prepared. To this end, we would like to report a convenient methodology which involves a traditional light source and simple column chromatography for the complete separation of the pure psoralen-adducted DNA oligomers.

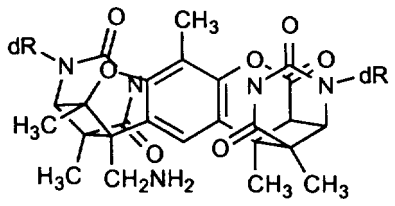
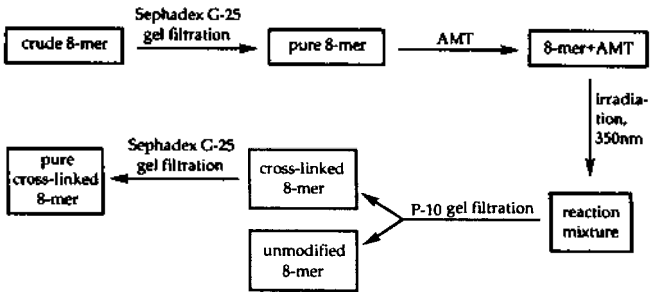


Chart 1.



Scheme 1.

Scheme 1 shows a flow chart of AMT cross-linked oligonucleotide synthetic scheme. The DNA oligomer, d(GGGTACCC), was synthesized on an automated applied biosystems DNA synthesizer using β-cyanoethyl phosphoramidite chemistry on a 10 μmole scale and deprotected using ammonia and 80% acetic acid. The oligonucleotide was purified by Sephadex G-25 gel filtration column chromatography. The purified oligonucleotides (100 mg) and AMT (1.76 mg) toge-

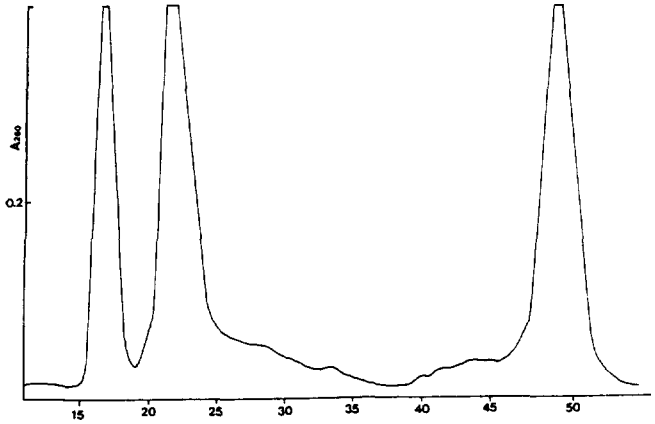


Figure 1. The gel filtration elution profile from a P-10 gel filtration column for the separation of an 8-mer/AMT mixture; 2 m*l* fractions at a flow rate of 0.2 m*l*/min.

ther were dissolved in 4 m*l* of 30 mM Na_2HPO_4 buffer. This solution was stirred at room temperature for 1 hour under nitrogen atmosphere, and was irradiated with 350 nm UV light at 0℃ for 15 minutes after stirring. This procedure was repeated with one more addition of 1.76 mg of AMT to the solution to achieve maximum yield. Irradiations were carried out with 350 nm UV light in a rayonet photochemical reactor equipped with RUL 3500 Å lamps. After irradiation, the reaction mixture was diluted with 4 m*l* of 8 M urea, heated to 50-60℃ in water bath, and maintained at that temperature for 10 minutes. The reaction mixture was lyophilized to the minimum amounts and subjected to a P-10 gel filtration column chromatography (1.6×50 cm) with 4 M urea, 40 mM CH_3COONH_4 buffer, pH 7, at a flow rate of 0.2 m*l*/min. The 60 fractions of each containing 2 m*l* were collected and the elution profile was monitored by measuring UV absorbance at 260 nm (Figure 1). The first peak (fractions, #15-18) contained cross-linked DNA, the second peak (fractions, #19-24) contained mixture of the one-side monoadducted DNA and unmodified 8-mer DNA, and the third peak (fractions, #47-53) contained mostly random coiled, unmodified DNA. The fractions containing psoralen cross-linked DNA were pooled, concentrated and dialyzed against 500 m*l* of water to remove urea and other contaminant, and then lyophilized to dryness. Finally, this cross-linked DNA was dissolved in 1 m*l* of distilled water and desalted by Sephadex G-25 column (1.6×30 cm). The purified products were eluted with distilled water (flow rate, 0.5 m*l*/min). The purity of the psoralen cross-linked DNA was assayed by one-dimensional (1D) and 2D ^{1}H-NMR (Figure 2). Several features of the spectrum were consistent with cross-link formation. The imino proton resonances of the cross-linked thymines were shifted to higher field (~11.5 ppm) compared to those of unadducted thymines (~13.5 ppm) (Figure 2(A)). The NOESY spectrum (Figure 2(B)) was of sufficient quality to enable a standard sequential assignment procedure.

In summary, this method provides a useful alternative to currently available methods in terms of mild conditions, easy operations, and high yields. A full report on the NMR studies of structure determination of the AMT-d(GGGTACCC)$_2$ cross-link will be published in the future.

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References

1. Cimino, G. D.; Gamper, H.; Issacs, S. T.; Hearst, J. E. *Annu. Rev. Biochem.* **1985**, *54*, 1151.
2. Takasugi, M.; Guendouz, A.; Chassignol, M.; Decout, J. L.; Lhomme, J.; Thoung, N. T.; Helene, C. *Proc. Natl. Acad. Sci. U. S. A.*, **1991**, *88*, 5602.

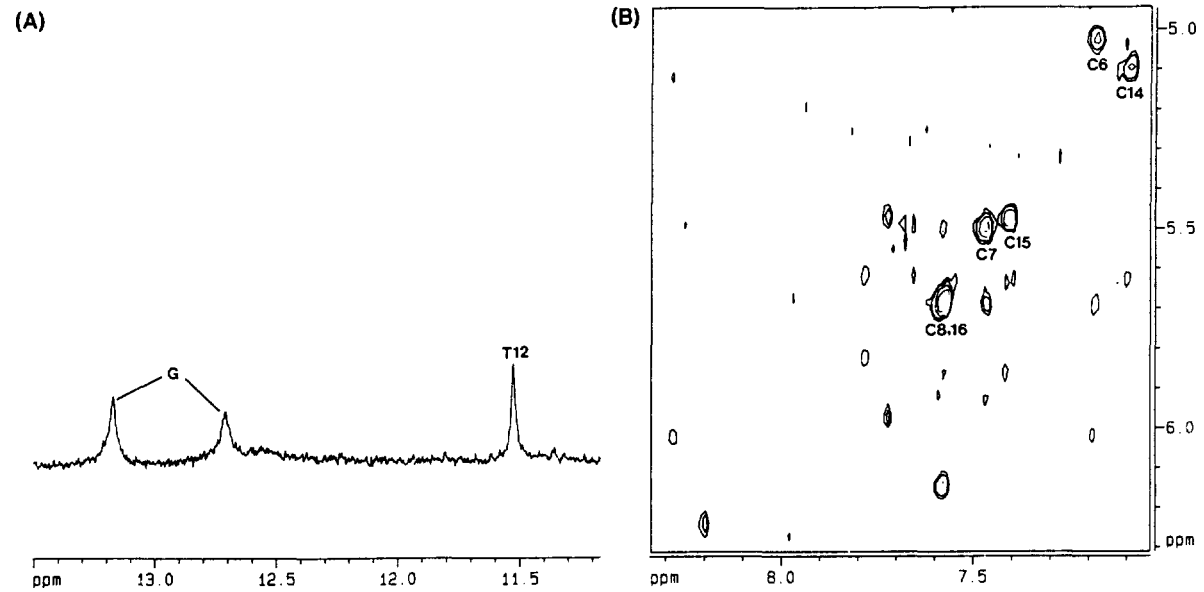

Figure 2. (A) The 500-MHz imino proton spectrum of the psoralen cross-linked DNA in 20 mM phosphate, 200 mM NaCl, H_2O, pH 7 at 25℃; (B) Expanded region of the NOESY spectrum (τ_m=250 ms) of the psoralen cross-linked DNA in 20 mM phosphate, 200 mM NaCl, D_2O, pH 7 at 25℃, showing the base-sugar H1′ region. The spectrum was acquried with a sweep width of 5050 Hz in both dimensions, 512t_1 values of 200 scans, and 2 K complex points.

3. Kanne, D.; Straub, K.; Hearst, J. E.; Rapoport, H. *J. Am. Chem. Soc.* **1982**, *104*, 1754.
4. Tomic, M.; Wemmer, D.; Kim, S. H.; *Science* **1987**, *238*, 1722.
5. Zhen, W.; Buchardt, O.; Nielsin, H.; Nielsin, P. E. *Biochemistry* **1986**, *25*, 6598.
6. Spielmann, H. P.; Sastry, S. S.; Hearst, J. E. *Proc. Natl. Acad. Sci. U. S. A.*, **1992**, *89*, 4514.
7. Sastry, S. S.; Spielmann, H. P.; Dwyer, T. J.; Wemmer, D. E.; Hearst, J. E. *J. Photochem. Photobiol. B. Biol.* **1992**, *14*, 64.

In Situ Electrochemical Quartz Crystal Microbalance Studies of Self-assembled N-Docosyl-N'-Methyl Viologen Films at Gold Electrodes

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Although we[1] and others[2-4] have investigated the electrochemical behaviors of molecular self-assemblies of asymmetric surfactant viologens, no work has been performed regarding the dynamic movements of ions (and solvents) during the charge transfer process of self-assembled viologen films[5] at electrode surfaces. In this communication, we wish to describe some preliminary results of the first *in situ* electrochemical quartz crystal microbalance (EQCM) studies on the first redox reaction of such films at gold electrode surfaces in aqueous solutions.

The EQCM instrumentation and the experimental procedures were essentially similar to those reported previously[6]. AT cut quartz crystals of 13 mm diameter (Toyo Kurafuto 5 MHz) were coated on both faces with Au (*ca.* 300 nm) by vacuum deposition using a Cr adhesion layer (2 nm). A Pt wire and a sodium chloride saturated silver-silver chloride were used as the counter and reference electrodes, respectively. An asymmetric keyhole electrode arrangement was used, in which the piezoelectrically active area (0.28 cm^2) was smaller than the area of the working electrode face (0.64 cm^2), to mitigate the effect of compressional stress. The electrochemically active area of the working electrode surface was 0.50 cm^2. Sufficient time was allowed to elapse for adsorption equilibrium to be achieved prior to EQCM measurements, as judged by absence of further changes in the voltammetric response.

Figure 1 shows the EQCM data for a typical scan across the first redox wave of N-docosyl-N'-methyl viologen ($C_{22}VC_1$) at a solution concentration of 10 μM in the presence of 0.1 M sodium para-toluenesulfonate (TS). The surface coverage at this concentration of $C_{22}VC_1$, 3.2×10^{-10} mol/cm^2, obtained from the integration of the voltammogram approximately corresponds to a full monolayer coverage of viologen sites with the anions employed. The resonance frequency of composite resonator increased concomitantly with electroreduction of the viologen dications to monocations at electrode surfaces. From the data in the Figure, a total frequency change of 8.0 (± 0.5) Hz is observed for the redox process, which is equivalent to a reversible mass change at the electrode surface of 441 g/mol of electrons by using the Sauerbrey equation, $\Delta f = -C_f \Delta m$, with a proportionality constant (C_f) of 56.6 Hz·cm^2/μg[6,7]. Satisfactory agreement of the half mass change and half charge potentials (−0.5 V *vs* Ag/AgCl) indicates that the mass transport and charge transfer processes are simultaneous. Therefore the data imply that one anion and *ca.* 10 molecules of H_2O simultaneously exit and enter, assuming the unidirectional transport with permselectivity to anions, the viologen monolayer during reduction and

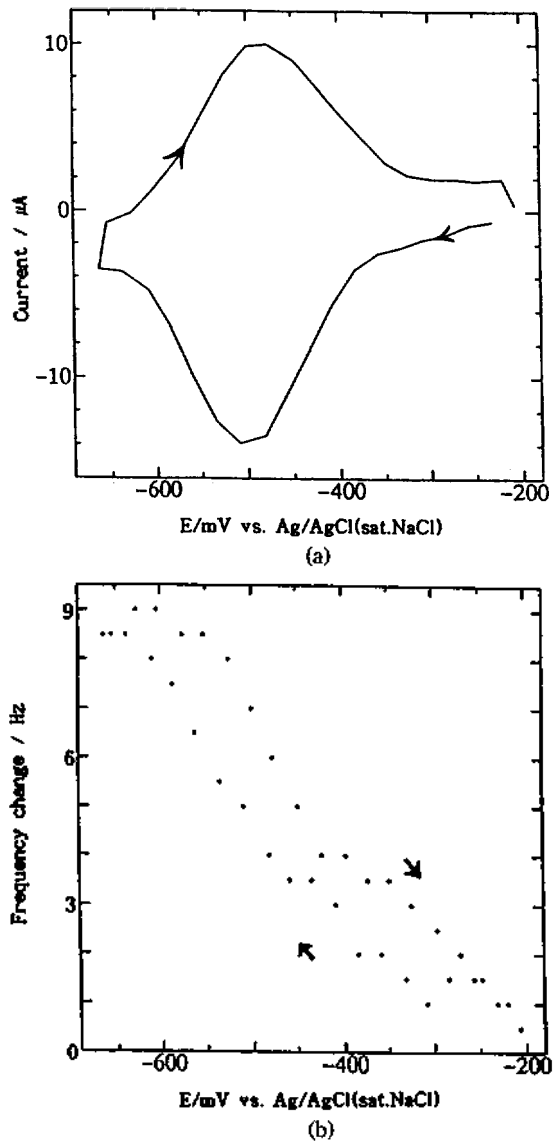

Figure 1. EQCM scan for 10 μM $C_{22}VC_1$ in 0.1 M NaTS. (a) Cyclic voltammogram (100 mV/s) and (b) EQCM frequency response for (a).