Interaction of C-1027 Chromophore with d(GTATAC)$_2$: A Binding Model Based on NMR Experiments

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A new antitumor antibiotic C-1027 consists of a novel chromophore and a noncovalently bound apoprotein.[1] The chromophore of C-1027 is a member of the enediyne family, such as neocarzinostatin chromophore,[2] calicheamicin,[3] esperamicin,[4] dynemicin,[5] and kedarcidin chromophore.[6] This nine-membered enediyne chromophore produces DNA damage without reductant following rearrangement to form a diradical species which abstracts hydrogen atoms from deoxyribose.[7] Although C-1027 chromophore is known to cause sequence-specific double-strand DNA cleavage,[8] the chromophore−DNA interaction mode has never been clarified. Very recently, DNA intercalation by the benzoxazolinate group of C-1027 has been proposed from increasing viscosity of DNA solution and DNA-induced quenching of UV absorption.[9] Herein, we first demonstrate the nature of C-1027 chromophore binding with DNA on the basis of NMR experimental results.

In this study, a stable aromatized form of C-1027 chromophore (Chr), which structurally resembles reactive diradical intermediate, was used,[10] because the enediyne chromophore cleaves DNA and undergoes cycloaromatization even in the absence of a reducing agent.[8] A self-complementary hexamer, d**(G$_1$T$_2$A$_3$T$_4$A$_5$C$_6$)$_2$**, was chosen as the DNA substrate, and indeed the DNA oligomer contains a (5′-ATA/3′-TAT) recognition site of the antibiotic.[8a] A Bruker DMX-500 spectrometer was used in these NMR experiments. The 1:1 C-1027 chromophore−d(GTATAC)$_2$ complex (2 mM) was studied at 5−55 °C in D_2O, pD 7.0, containing 100 mM NaCl. In order to improve extensive line broadening of the one-dimensional ^{1}H NMR spectra of the complex, NMR investigation was usually performed at relatively high temperature (40 °C).[11] Proton assignments[12] for the complex of the DNA oligomer with C-1027 chromophore were carried out by standard DQF-COSY,[13] NOESY,[14] and TOCSY[15] methods.

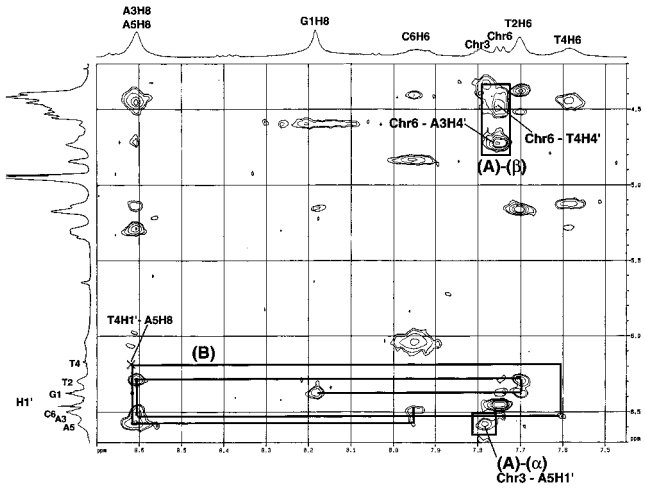

Figure 1. Expanded NOESY contour plots (mixing time, 300 ms) for the C-1027 chromophore−d(GTATAC)$_2$ complex at 40 °C in D_2O, pD 7.0, containing 100 mM NaCl. (A) The boxed intermolecular NOE cross peaks show (α) H3 of the chromophore to H1′ of A_5 and (β) H6 of the chromophore to H4′ of A_3 and T_4. (B) The line traces the distance connectivities between base and sugar H1′ protons. The cross mark points to the absence of NOEs between the H8 of A_5 and the H1′ of T_4.

Our NMR spectral data clearly indicate both intercalation and minor groove binding modes by C-1027 chromophore. In the NOESY spectra, the disruption of the sequential connectivities[16] of the base and sugar protons (H1′, H2′, and H2″) strongly supports intercalative binding at the T_4−A_5 step (Figure 1). Upon admixture of C-1027 chromophore and d(GTATAC)$_2$, we also detected broadening of the protons of the aromatic bezoxazolinate ring (methoxyl-H, 3.66 ppm; H11″, 5.64 ppm; H8″, 6.83 ppm), upfield shift of its H8″ ($\Delta\delta$, −0.35 ppm), and upfield shifting of the base-paired T_4 and T_2 imino protons[17] (supporting information). The observation is consistent with intercalation of the benzoxazolinate at the (T_4·A_3)−(A_5·T_2) step. On the other hand, strong supportable evidence for DNA minor

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(10) Previous studies with neocarzinostatin,[2] calicheamicin,[3] and esperamicin[4] suggest that the aromatized derivative binds to DNA in a manner similar to that of the enediyne chromophore.

(11) At 40 °C, 1-D NMR spectra of the complex were sharp and the chromophore binding did not disrupt the DNA symmetry, indicative of fast exchange. The T_m value of the complex was approximately 50 °C and is higher than that of the free oligomer.

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(17) Upfield shifting (1 ppm) of the T_2 and T_4 imino protons was observed in spectra acquired in H_2O (90%) / D_2O (10%) at 20 °C , which suggests slow exchange (supporting information); this supports intercalation, because groove binders typically cause downfield shifts of imino protons: (a) Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450. (b) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 7891.

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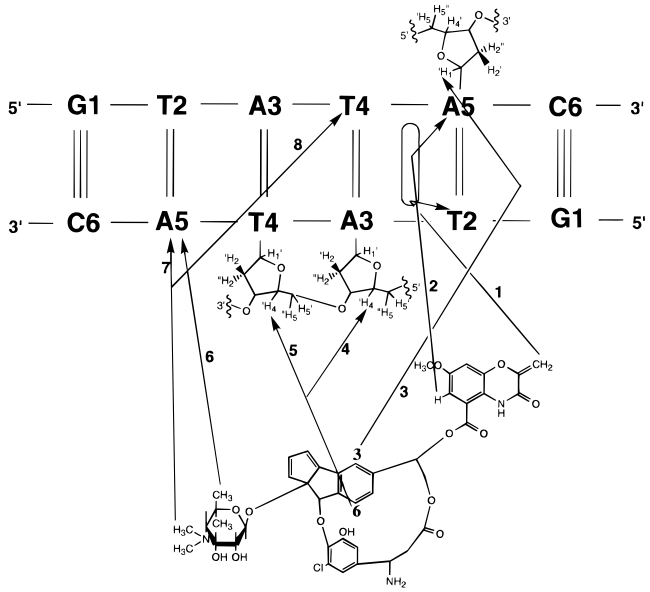

Figure 2. Schematic diagram showing typical intermolecular NOEs. Assignments: 1, ChrH11″−T_2Me; 2, ChrH8″−A_5H2″; 3, ChrH3−A_5-H1′; 4, ChrH6−A_3H4′; 5, ChrH6−T_4H4′; 6, Chr5′Me−A_5H5″; 7, Chr4′NMe−A_5H1′, H4′, and H5″; 8, Chr4′NMe−T_4H1′ and H4′.

groove binding derives from the finding of intermolecular NOEs between some protons of the chromophore (in particular, the aminosugar moiety) and DNA-H4′, -H5′, or -H5″ (Figure 2 and supporting information).

An intermolecular NOE cross peak between Chr-H3 and A_5-H1′ reveals the proximity of these protons. The Chr-H6 is also situated close to H4′ of A_3 and H4′ of T_4, as indicated by the detection of these intermolecular NOEs (Figure 1). Therefore, it is reasonably proposed that the DNA lesions by C-1027 chromophore are due to the abstraction of hydrogen atoms from C1′ of A_5 by Chr-C3 radical and from C4′ of either A_3 or T_4 by Chr-C6 radical. In fact, our previous experiment using ^{32}P-end-labeled DNA oligomer showed that the DNA damage occurs at 5′-ATA/3′-TAT with a two-nucleotide 3′-stagger of the cleaving residues.[8a] In addition, the C4′-hydrogen abstraction preferred by C-1027 chromophore has been reported in gel electrophoretic analysis.[8]

The NOESY measurements yielded 105 distinct intramolecular and 20 distinct intermolecular NOEs (Figure 2)[18] that we have been able to assign for the C-1027 chromophore−d(GTATAC)$_2$ complex (supporting information). These NOESY cross peak volumes were converted to distances by the two-spin approximation, using the cytidine H5−H6 distance of 2.45 Å for calibration. Molecular dynamics calculations were carried out by using a CHARMm force field with distance restraints.[19] The obtained model suggests an adequate structure for the C-1027 chromophore−DNA complex that is fully consistent with the observed NMR-derived distance data (Figure 3). On the basis of the present modeling calculation, the most probable stereochemistry of C8 and C9 of the chromophore is deduced to be 8*R*,9*R*.[20] The complex model also shows that the DNA

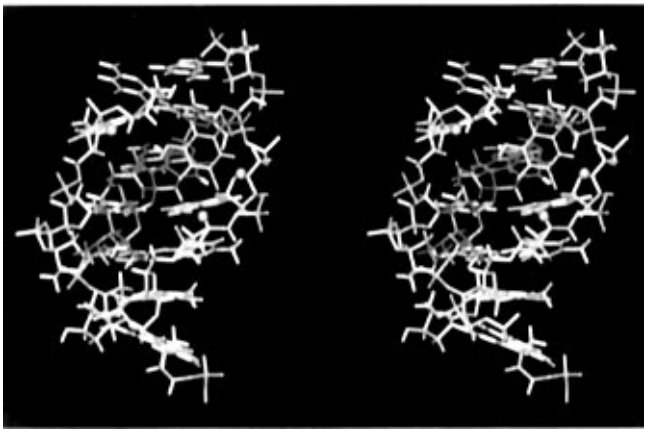

Figure 3. Stereoview of the model of the C-1027 chromophore−d(GTATAC)$_2$ complex, obtained by restrained molecular dynamics calculations. The chromophore of C-1027 (red) is positioned in the minor groove of the oligomer (blue) and intercalates through the benzoxazolinate group. The H3 and H6 sites are situated at distances of 3.67 Å from H1′ of A_5 (green ball) and of 2.68 Å from H4′ of T_4 (or 2.89 Å from H4′ of A_3) (green ball), respectively.

oligomer remains in a B-form conformation, although minor groove widths (6.04 Å) are somewhat widened by the occupation of the bulky chromophore.[21] The molecular modeling of the C-1027 chromophore−d(GTATAC)$_2$ complex allows us to suppose the functions of the bezoxazolinate and aminosugar moieties of C-1027 chromophore. The intercalation of bezoxazolinate spatially leads reactive diradical atoms (C3 and C6) of the enediyne chromophore in the vicinity of deoxyribose hydrogen atoms (H1′ and H4′) of the DNA backbone (A_5 and T_4 (or A_3)). The aminosugar assists in winding of the chromophore around the minor groove of the DNA oligomer. Further, the stereochemistry of C8 and C9, 8*R*,9*R*, induces the enediyne chromophore to fit in the DNA minor groove. The 16-membered macrocyclic part containing phenol ring did not exhibit distinct intermolecular NOEs, suggesting no clear interaction between this moiety of the chromophore and the DNA oligomer. However, some intramolecular NOEs were observed. The macrocyclic moiety may play an important role for regulation of the cycloaromatization of C-1027 chromophore.

This study shows the first structural insight into the basis of the C-1027 chromophore binding to d(GTATAC)$_2$. Further investigations employing other DNA oligomers and C-1027 chromophores are underway to refine and to develop the present interaction model.

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Supporting Information Available: Chemical structure of aromatized C-1027 chromophore; 1-D spectra of the C-1027 chromophore−d(GTATAC)$_2$ complex at 40 °C in D_2O and at 20 °C in H_2O (90%)/D_2O (10%); expanded NOESY spectra of the complex (mixing times, 150 and 300 ms) red marking the intermolecular NOEs; lists of chemical shifts and inter- and intramolecular NOEs for the complex (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(18) The strand specificity of the intermolecular NOEs in Figure 2 was determined with the assistance of model building.

(19) The initial starting structure, a DNA−C1027 chromophore docking model, was generated using Quanta. The starting structure was subjected to 500 steps of conjugate gradient minimization followed by molecular dynamics with a time step of 0.5 fs. Dynamics calculations were heated from 0 to 1000 K over 6 ps. The system was maintained at 1000 K for 16 ps, gradually cooled to 300 K over 7 ps, and maintained at 300 K for 30 ps. Coordinates were stored every 0.5 ps for the last 5 ps and then averaged. The resulting structure was then subjected to 100 steps of steepest descents minimization followed by conjugate gradient minimization until the rmsd became <0.001. The complex model reveals a low-energy structure that fully satisfies the NOE restraints. However, the retention of DNA symmetry upon the chromophore binding and small number of intermolecular NOEs suggest that the structure is not necessarily unique.

(20) Two stereoisomers of the chromophore (8*R*,9*R* and 8*S*,9*S*)[1a] were respectively located in minor groove. Only in the case of the 8*R*,9*R* isomer, the complex model satisfied the observed intermolecular NOEs.

(21) Tentative DQF-COSY spectral features are indicative of B-DNA. In the minor groove of a typical B-form DNA duplex, an average width is estimated to be 5.7 Å: Conner, B. N.; Takano, T.; Tanaka, S.; Itakura, K.; Dickerson, R. E. *Nature* **1982**, *295*, 294.