Solution structure of the DNA complex with a quinacrine-netropsin hybrid molecule by NMR spectroscopy

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ABSTRACT
The solution structures of 1:1 complexes of a quinacrine-netropsin hybrid molecule with the self-complementary DNA duplexes, d(CGCGAATTCGCG)_2 and d(CGAATTCG)_2, have been studied by one- and two-dimensional 'H NMR spectroscopy. The NOE data indicate that the acridine ring of the hybrid intercalates into the 5'-GpA step and its netropsin moiety spans the minor groove of the central AATT region.

INTRODUCTION
The antimalarial drug quinacrine is a typical DNA intercalator and display little AT or GC base-pair preference. The spectroscopic (absorption, CD and fluorescence) behavior of quinacrine strongly depends on base pairs into which its parent acridine chromophore intercalates. Therefore, the acridine chromophore is useful as a DNA probe to obtain information on the DNA binding properties of hybrid molecules containing an intercalator and a groove binder structurally related to netropsin or distamycin.

In a previous paper, we reported the synthesis of a quinacrine-netropsin hybrid molecule (1; Fig. 1) and its interaction with DNA by spectroscopic (absorption, CD, flow dichroism and fluorescence) measurements; 1 interacts with DNA studied by both intercalation and minor-groove binding and exhibits enhanced preference for AT-rich sites.

To give an insight into the solution structure of DNA-1 complex, we have investigated the complexes of 1 with the self-complementary DNA duplexes, d(CGCGAATTCCGGC), and d(CGAATTCG)_2, by one- and two-dimensional 'H NMR spectroscopy.

EXPERIMENTAL
The synthesis of 1 has been described earlier. The DNA oligomers, d(CGCGAATTCCGGC) and d(CGAAATTCCG), were purchased from Nippon Flour Mills and used without further purification. The oligomers were dissolved in 10 mM phosphate buffer (pH 6.9) containing 0.1 M NaCl, 0.1 mM EDTA and 0.5 mM TSP-d_4 as an internal standard. The concentrations of the DNA oligomers in single strand were determined spectrophotometrically at 80°C using the calculated molar absorption coefficients at 260 nm.

All NMR experiments were performed at 500 MHz on a Bruker AVANCE 500 spectrometer. One- and two-dimensional 'H NMR spectra in 99.994% D_2O or 90%H_2O/10%D_2O were obtained at 25°C. The water resonance was suppressed by presaturation or with the WATERGATE pulse sequence.

RESULTS AND DISCUSSION
To confirm the formation of 1:1 complexes of the DNA oligomers and 1, the oligomers were titrated with 1 in D_2O. The titration altered the one-dimensional NMR spectra of the DNA oligomers and 1. We monitored the integrated intensities for the methyl signals of two thymines from the free DNA
Figure 2. The changes ($\Delta\delta = \delta_{\text{complex}} - \delta_{\text{free}}$) in the chemical shifts of the H6/H8 and H1' protons of the dodecamer upon binding of 1.

Oligomers in the NMR spectra to examine the extent of the complex formation. The intensities were gradually reduced and each degenerate signal was split into two signals (data not shown). No dyad symmetry of the DNA duplexes was held in these 1:1 complexes.

Non-exchangeable protons of d(CGCGAATTCGCG)2 in the 1:1 complex with 1 in D2O were assigned using two-dimensional NMR (COSY, TOCSY, DQF-COSY and NOESY) spectra.** Figure 2 depicts the changes in the chemical shifts of the H6/8 and H1' protons of the dodecamer upon binding of 1. The H6 resonances of T8, T7' and T8' bases and the H1' resonances within the central 5'-GAATTC region largely shift upfield, as compared to those in the free duplex. On the other hand, the chemical shifts of these protons within the terminal 5'-CGC (or 5'-GGC) region do not undergo significant changes. The intercalation of the acridine ring induces upfield-shifts of the H6/H8 aromatic protons on the neighboring base pairs (Fig. 2). The groove binding of the pyrrole ring also induces upfield-shifts of the H1' protons in the minor groove (Fig. 2). The analysis of the NOESY spectrum (mixing time = 300 msec) in H2O shows that the NH1 and NH2 protons are close to the H2 protons of A5 sugar. This suggests that the intercalation of the acridine ring into the 5'-GpA step changes the conformation of A5 sugar. This suggestion was supported by two NOE cross-peaks observed between the methoxy protons of the acridine ring and the methyl protons of T8 and T8' bases.

In the TOCSY and NOESY (mixing time = 300 msec) spectra of the 1:1 complex in D2O, two or more sets of the resonances for the aromatic protons on the acridine ring were detected. This implies that there are two or more intercalation geometries of the acridine ring.

To confirm the binding of 1 to 5'-GAATTC region, one- and two-dimensional NMR spectra of the 1:1 complex of d(CGCGAATTCGCG)2 with 1 were collected. In analogy with the dodecamer-1 complex, two NOE cross-peaks were observed between the methoxy protons of the acridine ring and the methyl protons of both T6 and T6' bases. In addition, strong NOE cross-peaks were also observed between the pyrrole H3 protons (H3-1 and H3-2) and the H2 protons of A4 (or A4') base.

The results obtained here indicate that the acridine ring of 1 intercalates into the 5'-GpA step and the netropsin moiety spans the minor groove of the central AATT region. The intercalation into the 5'-GpA step is consistent with the spectroscopic behavior of the acridine chromophore upon binding to the dodecamer.*

REFERENCES