Synthesis and DNA-binding of acridine-netropsin hybrid molecules

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ABSTRACT
We have designed and synthesized acridine-netropsin hybrid molecules. Spectroscopic (absorption, CD, flow dichroism and fluorescence) measurements reveal that hybrid molecules interact with DNA by both intercalation and minor-groove binding and shows enhanced preference for AT-rich sites.

INTRODUCTION
Netropsin and distamycin, a family of naturally occurring oligopeptides, exhibit antiviral and anticancer activities.1 Two antibiotics bind in the minor groove of B-DNA and have high specificity for AT sites.2 Due to the fundamental biological interest and therapeutic importance, a number of analogs of netropsin and distamycin have been synthesized to understand the details of the mechanism by which these two antibiotics bind to and recognize double-stranded DNA sequences and to develop novel sequence-specific drugs.3

On the other hand, the antibiotics actinomycin and antimalarial drug quinacrine bind to DNA by intercalation. In this study, we synthesized intercalator-netropsin hybrid molecules (Fig. 1) and investigated their DNA binding properties. The parent part of quinacrine was used for an intercalating moiety. The purposes of this study are to obtain some information on the DNA binding properties of hybrid molecules for the development of new anticancer agents and to elucidate whether new absorption and CD bands are observed like the RNA-quinacrine4 and DNA-quinacrine systems.5 We report here preliminary results obtained with 1.

EXPERIMENTAL
The hybrid molecule 1 (Fig. 1) was synthesized according to the procedures described in the literatures8 and purified by both repeated recrystallizations and reversed phase column chromatography.7 Calf thymus DNA (DNA) and the DNA dodecamer d(CGCGAATTCGCG), were obtained from Sigma and Nippon Flour Mills, respectively. Absorption, CD, and fluorescence spectra were measured with a Hitachi U-3300 spectrophotometer, a Jasco J-720 spectropolarimeter, and a Hitachi MPF spectrophotofluorometer, respectively, as a function of the molar ratio of DNA base to drug (BD). Flow dichroism measurements were carried out as described elsewhere.8 All measurements were made in 5 mM phosphate buffer (pH 6.9, 25°C) with NaCl added to give desired ionic strength.

RESULTS AND DISCUSSION
Figure 1 shows the absorption and CD spectra of 1 complexed with DNA. The interaction of 1 with DNA results in hypochromism and red-shift in the absorption spectra in the visible region (380-500 nm) where the acridine moiety absorbs light (Fig. 2a). The vibrational bands of free drug become obscure with increasing BD and the CD spectrum does not resemble the corresponding absorption spectrum in shape (Fig. 2). Absorption and CD spectra in the visible region (380-500 nm) are very similar to those of quina-
Fig. 2. Absorption and CD spectra of 1 complexed with DNA in 5 mM phosphate buffer. Drug concentration was $2.14 \times 10^{-5}$ M: (-----) free, (----) $P_D = 10$, (-----) $P_D = 30$. Spectra were measured using 0.1-5 cm quartz cells and absorbances in Fig. 2a are expressed in values per 1-cm cell length.

Absorption and CD spectral behavior of the dodecamer-1 system was very similar to that of the DNA-1 system. The induced CD of bound drug around 320 nm results from the contribution of its netropsin moiety and the CD spectrum is characteristic of that of netropsin complex of (dA-dT)-containing synthetic polymers.

It is well known that the fluorescence of quina-
crine-poly(dA-dT)$_2$ complex. Absorption and CD spectral behavior of the dodecamer-1 system was very similar to that of the DNA-1 system. The induced CD of bound drug around 320 nm results from the contribution of its netropsin moiety and the CD spectrum is characteristic of that of netropsin complex of (dA-dT)-containing synthetic polymers.

The results obtained here indicate that 1 interacts with DNA by both intercalation and minor-groove binding and shows enhanced preference for AT-rich sites. Further studies using NMR spectroscopy are in progress to elucidate solution structure of the dodecamer-1 complex.

REFERENCES
7. The analytical data (C, H, N) of 1 was in agreement with calculated values and its spectroscopic data ('H NMR and MS) were consistent with the assigned structures. We are grateful to Ube Research Laboratory, Ube Industries, Ltd. for MS and elemental analyses.