Study on the complexation between DNA and cationic porphyrin derivatives

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ABSTRACT
A water-soluble cationic porphyrin, 5,10,15,20-Tetra(N-methylpyridinium-4-yl)-21H,23H-porphyrin has been shown to intercalate selectively into the A3-G4 gap of G-quadruplexed DNA d(TTAGGG)₄.

INTRODUCTION
Characterization of the interaction between DNA and small organic compounds is of considerable importance to gain insights into the mechanism of the molecular recognition which could be highly relevant to drug design. 5,10,15,20-Tetrakis(N-methylpyridinium-4-yl)-21H,23H-porphyrin (TMPyP, structure in Fig. 1) is a water-soluble cationic porphyrin and has been under extensive study because of its unique physicochemical properties leading to interactions with nucleic acids, as well as its therapeutic application.

It has been well-documented that TMPyP interacts with G-quadruplexed DNA and the binding of TMPyP to the DNA inhibits telomerase activity. The molecular recognition between DNA and TMPyP, however, has remained to be evaluated.1-5 We present herein the results of NMR, absorption, and circular dichroism (CD) studies on the interaction of TMPyP with G-quadruplexed oligonucleotide d(TTAGGG)₄, which demonstrated that TMPyP intercalates into the A3-G4 gap of d(TTAGGG)₄.

RESULTS AND DISCUSSION
Absorption and CD spectra
We first studied the interaction between d(TTAGGG)₄ and TMPyP on the basis of the absorption and CD spectral changes of the porphyrin π-system of TMPyP. The absorption spectra of TMPyP in the presence of various concentrations of d(TTAGGG)₄ are shown in Fig. 2. Upon the addition of d(TTAGGG)₄, the Soret band exhibited a red-shift from 422 nm to 440 nm, associated with 66 % hypochromism, and an isosbestic point was observed at 434 nm. Scatchard analysis yielded the dissociation constant ($K_d = 1.62 \times 10^{-7}$ M$^{-1}$) and stoichiometry ($r = 0.79$).

Fig. 1 Molecular structure of TMPyP.

Fig. 2 Absorption spectra of TMPyP in the presence of various stoichiometric ratios of d(TTAGGG)₄ at a fixed 3 μM TMPyP. [d(TTAGGG)₄]/[TMPyP] ratios ($R$) were varied from 0.0 to 5.0.
These results indicated that d(TTAGGG)_4 and TMPyP form a stable 1:1 complex. Furthermore, a negative CD band was observed in the Soret absorption region (result not shown), suggesting a direct interaction between the π systems of G-quartets and TMPyP\(^1\).

**NMR spectra**

Some \(^1\)H NMR signals due to d(TTAGGG)_4 exhibited a progressive upfield shift and line broadening with increasing TMPyP concentration (data not shown). Especially, A3H2, A3H8, G4 imino proton signals exhibited significant line broadening, demonstrating that TMPyP binds to d(TTAGGG)_4 near A3 and G4 residues, and that the time scale of the complexation between the two is faster compared with the NMR time scale. The observed upfield shifts of the signals for d(TTAGGG)_4 in the presence of TMPyP is attributed to the ring current effect of the porphyrin of TMPyP. Furthermore, imino proton signals of d(TTAGGG)_4 in the presence of 0.2 equivalence of TMPyP were observed up to about 60 °C, while, in the absence of TMPyP, they disappeared at about 55 °C. These results indicated that the hydrogen bonds in G-quartets of d(TTAGGG)_4 were stabilized through the interaction with TMPyP.

A structure of d(TTAGGG)_4-TMPyP complex in solution could be inferred from the analysis of intermolecular NOE connectivities between them. In the NOESY spectra recorded on a mixture, [TMPyP]/[d(TTAGGG)_4] = 0.2, using a mixing time of 200 ms (Fig. 3), meta-, ortho-, and β-proton signals of TMPyP exhibited NOE connectivities with A3H2/A3H1'/G4H1', A3H2/A3H8, and A3H8 proton signals, respectively. The observed intermolecular NOE connectivities are not consistent with the intercalation of TMPyP into the adjacent planes composed of G-quartets, but with the specific binding of TMPyP to the gap between A3 and G4 of d(TTAGGG)_4.

**REFERENCES**