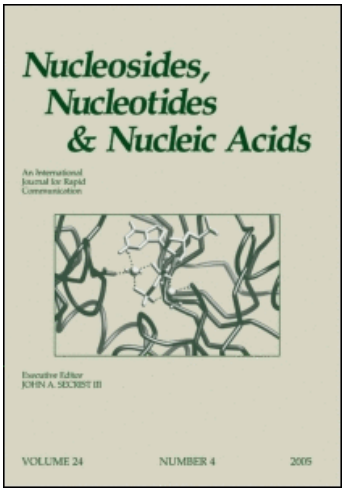

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Interactions of 5-(1-Pyrenyl)-10,15,20-tris(4-methylpyridinium)porphine with Double-Helix and Triple-Helix Nucleic Acids

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NOTE

INTERACTIONS OF 5-(1-PYRENYL)-10,15,20-TRIS(4-METHYL-PYRIDINIUM)PORPHINE WITH DOUBLE-HELIX AND TRIPLE-HELIX NUCLEIC ACIDS†

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ABSTRACT: 5-(1-Pyrenyl)-10,15,20-tris(4-methylpyridinium)porphine (H_2PTMPP) having a porphyrin ring and a pyrenyl substituent was synthesized. The compound H_2PTMPP bound to poly(dA)•poly(dT) double helix and poly(dA)•2poly(dT) triple helix in different styles. The results of H_2PTMPP binding to oligonucleotides, dA_{14}•dT_{14} and dA_{14}• $2dT_{14}$, was also shown.

In this work, we have synthesized 5-(1-pyrenyl)-10,15,20-tris(4-methyl-pyridinium)porphine (H_2PTMPP) having a porphyrin ring and a pyrenyl substituent as shown in Fig. 1, and investigated spectrophotometrically interaction of the drug with DNAs such as poly(dA)•poly(dT) and dA_{14}•dT_{14} double helices and poly(dA)•2poly(dT) and dA_{14}• $2dT_{14}$ triple helices. The absorption spectra of both drugs, 1.7×10^{-5} mol dm^{-3} H_2PTMPP and tetrakis(1-methyl-4-pyridinio)porphine (H_2TMpyP), were shown in Fig. 2. Both spectra showed one absorption peak at the Soret band (around 425 nm) due to the porphyrin ring of H_2PTMPP and H_2TMpyP. The spectrum of H_2PTMPP also showed a small absorption peak around 348 nm, while the peak was not found in the spectrum of H_2TMpyP. It suggests that the peak is due to the pyrene site of H_2PTMPP. Induced CD spectra in the Soret band (400-500 nm) of H_2TMpyP with poly(dA)•poly(dT) and poly(dA)•2poly(dT) were shown in Fig. 3. The concentration of these DNA helixes were 1.7×10^{-5} mol dm^{-3}. These two spectra are similar as an intense positive peak around 430 nm and a small negative peak around 415 nm. Induced CD spectra of H_2PTMPP with poly(dA)•poly(dT) and poly(dA)•2poly(dT)

†This paper is dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday.

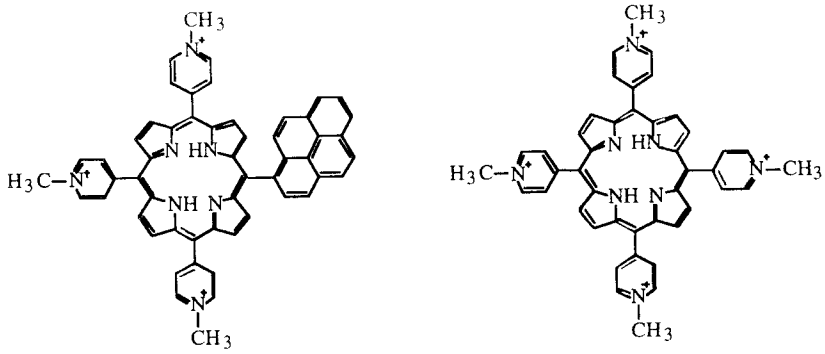

FIG. 1. Chemical structures of 5-(1-pyrenyl)-10,15,20-tris(4-methylpyridinium)-porphine (H_2PTMPP) (left) and tetrakis(1-methyl-4-pyridinio)porphine (H_2TMpyP) (right).

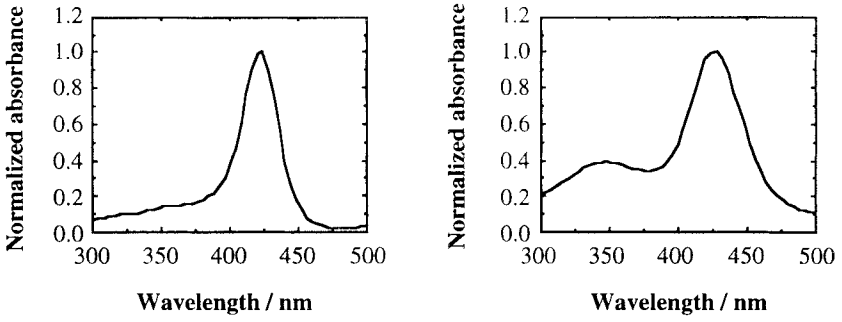

FIG. 2. Absorbance spectra of 1.7×10^{-5} mol dm^{-3} H_2TMpyP (left) and H_2PTMPP (right).

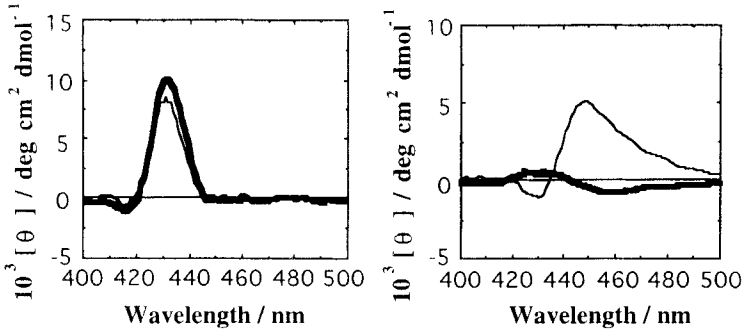

FIG. 3. CD spectra of H_2TMpyP (left) and H_2PTMPP (right) with poly(dA)•poly(dT) (——) and with poly(dA)•2poly(dT) (▬) in 0.1 mol dm^{-3} NaCl buffer. The concentration of these helixes and drugs were 1.7×10^{-5} mol dm^{-3}.

under the same condition were also shown in the same figure. The spectrum of H_2PTMPP with poly(dA)•poly(dT) showed an intense positive peak around 450 nm and a negative peak around 430 nm, which is similar to that of H_2TMpyP with the double-helix DNA. On the other hand, the spectrum of H_2PTMPP with poly(dA)•2poly(dT) showed a negative peak around 460 nm and a positive peak around 430 nm, which is very different from that of H_2TMpyP with the triple-helix DNA. Since these induced CD spectra of H_2PTMPP with the double-helix and triple-helix DNAs showed opposite shapes, it is suggested that different binding-styles of H_2PTMPP with these DNAs may be due to the presence or absence of the intercalation of the pyrene site.

In order to get information about the effect of H_2PTMPP on the stability of double-helix and triple-helix DNAs, the melting curves of dA_{14}•dT_{14} and dA_{14}•$2dT_{14}$ with/without the drug in 1.0 mol dm^{-3} NaCl buffer were measured. The obtained melting temperatures were listed in Table 1. The melting curve of 3.8×10^{-5} mol dm^{-3} dA_{14}•dT_{14} (1.9×10^{-5} mol dm^{-3} per helix) showed one denaturing point at 50.1 °C, while that of 5.7×10^{-5} mol dm^{-3} dA_{14}•$2dT_{14}$ (1.9×10^{-5} mol dm^{-3} per helix) showed two melting points at 23.9 and 51.3 °C. The low and high melting temperatures should correspond to dissociations of Hoogsteen and Watson-Crick base pairs, respectively.
The melting temperatures of the Watson-Crick base pairs were similar between the double helix (50.1 °C) and the triple helix (51.3 °C), but those of dA_{14}•dT_{14} and dA_{14}•$2dT_{14}$ were about 34 °C lower than the obtained melting temperatures (84.0 and 85.6 °C) of the polynucleotides.

The stability of these helices, dA_{14}•dT_{14} and dA_{14}•$2dT_{14}$ were greatly influenced by H_2PTMPP. The Watson-Crick base pairs were stabilized by 6.8 °C for dA_{14}•dT_{14} and 5.4 °C for dA_{14}•$2dT_{14}$ at the same concentration of described above, indicating the same increments of the melting temperatures. Surprisingly, the melting curve of dA_{14}•$2dT_{14}$ showed only one melting point as well as the double helix. It suggests that the Hoogsteen base pairs of dA_{14}•$2dT_{14}$ may be dissociated by H_2PTMPP. It was reported that ethidium affected a certain 22-mer nucleotide to destabilize Hoogsteen base pairs but stabilize Watson-Crick base pairs.[1] In this study, the addition of H_2PTMPP to dA_{14}•dT_{14} and dA_{14}•$2dT_{14}$ also showed that Hoogsteen base pairs of dA_{14}•$2dT_{14}$ were destabilized while Watson-Crick base pairs were stabilized by the drug. It is supposed that the weak Hoogsteen base pairs of dA_{14}•$2dT_{14}$ were dissociated by H_2PTMPP and the drug tends to bind to the double helix of dA_{14}•dT_{14}. Thus, it was suggested that the drug H_2PTMPP synthesized in this work has a possibility of distinguishing the double helix and the triple helix of the DNAs.

Table 1. Melting temperatures (°C) of dA_{14}•dT_{14} and dA_{14}•$2dT_{14}$ in 1.0 mol dm^{-3} NaCl-phosphate buffer in the presence and absence of H_2PTMPP

	dA_{14}•dT_{14}	dA_{14}•$2dT_{14}$	
	Watson-Crick base pair	Watson-Crick base pair	Hoogsteen base pair
without H2PTMPP	50.1	51.3	23.9
with H2PTMPP	56.9	56.7	< 0

EXPERIMENTAL

Materials. 5-(1-pyrenyl)-10,15,20-tris(4-pyridyl)porphine (H_2PTPP) iodide was prepared by condensation of mixtures of pyridine-4-carboxaldehyde and 1-pyrenecarboxaldehyde with pyrrole. Crude H_2PTPP was purified by column chromatography eluted with dichloromethane/methanol 97:3. H_2PTPP (Mw: 741.86) was methylated by methyl iodide. Crude 5-(1-pyrenyl)-10,15,20-tris(4-methylpyridinium)porphine (H_2PTMPP) iodide thus obtained was dried under vacuum. Pure H_2PTMPP (Mw: 1187.68) was obtained by precipitation from methanol (14.7 % yield based on 1-pyrenecarboxaldehyde used here). The structure was established by UV-Vis, NMR, and mass spectrometry.[2] Tetrakis(1-methyl-4-pyridinio)porphine (H_2TMpyP) were purchased from Dojin Chemical Co. and used without further purification.

Polynucleotides, poly(dA) and poly(dT), were obtained from Pharmacia. Shorter deoxyribonucleotides, dA_{14} and dT_{14}, were synthesized with a phosphoramidite method on an Applied Biosystems model 391 DNA/RNA synthesizer. The cleavage and deblocking of the synthesized oligomers were done by a treatment with a concentrated ammonia at 50 °C overnight. After the detritylation, the oligonucleotides were purified by a high-performance liquid chromatography (HPLC).

All solutions were prepared in a buffer consisting of 10 mmol dm^{-3} Na_2HPO_4 and 1.0 mmol dm^{-3} Na_2EDTA. Each buffer solution was adjusted to the desired Na^+ concentration by adding NaCl and followed by adjustment of the pH to 7.0. Concentrations of the nucleic acids, H_2PTMPP, and H_2TMpyP were determined spectrophotometrically using the following extinction coefficients; ε_{259} = 6000 mol dm^{-3} cm^{-1} for poly(dA)•poly(dT),[3] ε_{260} = 8100 mol dm^{-3} cm^{-1} for poly(dT),[3] ε_{260} = 114000 mol dm^{-3} cm^{-1} for dA_{14},[4] ε_{260} = 171000 mol dm^{-3} cm^{-1} for dT_{14},[4] ε_{422} = 120000 mol dm^{-3} cm^{-1} for H_2PTMPP,[2] and ε_{424} = 226000 mol dm^{-3} cm^{-1} for

H_2TMpyP.[5] For a preparation of DNA double and triple helices, pyrimidine strands and purine strands were mixed to 1:1 or 2:1 molar ratio in an appropriate buffer.

Absorbance measurements. Absorbance measurements were done with Hitachi U-3200 and U-3210 spectrophotometers. Absorbance vs. temperature UV melting curves were measured at 260 nm on these spectrophotometers equipped with Hitachi SPR-10 thermoprogramers. The melting temperatures (T_m) were measured for the DNA double and triple helices without or with an equimolar concentration of the drugs. The heating rate was 0.2 or 0.5 °C/min. The cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid water condensation on the cuvette exterior at the low temperature range.

Circular dichroism measurements. Circular dichroism (CD) measurements were carried out on a Jasco J-600 spectropolarimeter equipped with a temperature control unit. Wavelengths were scanned from 500 to 200 nm and CD spectra were recorded at 5°C. The cuvette-holding chamber was flushed with a constant stream of dry N_2 gas.

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