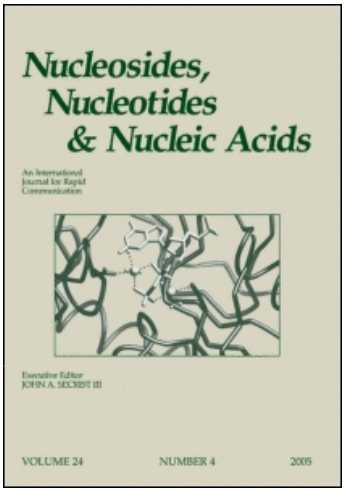

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Interaction of Pyrylium Dye with Self-Complementary DNA Oligomer as Studied by H NMR Spectroscopy

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INTERACTION OF PYRYLIUM DYE WITH SELF-COMPLEMENTARY DNA OLIGOMER AS STUDIED BY ^{1}H NMR SPECTROSCOPY‡

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ABSTRACT: Interaction of 2-methyl-4,6-bis-(4-*N,N*-dimethylaminophenyl) pyrylium salt (P2) with $[d(CGACGTCG)]_2$ was investigated by ^{1}H NMR spectroscopy. The aromatic signals of P2 and the oligomer were shifted to the upfield by forming the complex, and intermolecular NOEs were also observed between P2 and the terminal CpG base steps but not between P2 and the central CpG. These results indicate that P2 binds to the weakly stacking CpG steps in an intercalation manner.

2-Methyl-4,6-bis-(4-*N,N*-dimethylaminophenyl)pyrylium iodide (P2) is a novel fluorescent dye, which has unfused aromatic rings (SCHEME 1)[1,2]. The enhancement of fluorescence emission excited by visible light is extremely sensitive to the concentration of double-stranded DNA (dsDNA) although P2 itself does not exhibit any fluorescence in the free state. In absorption spectra, P2 shows the absorption maximum at 540 nm and the red shift of 40 nm in the presence of dsDNA[1]. Compared to ethidium bromide, one of the most popular fluorescent dyes, which shows weak fluorescence in the free state and the limited linearity of the fluorescence intensity against the dsDNA concentration, the fluorescence intensity of P2 increases concentration-dependently in a wide range of dsDNA concentration[1]. These characteristic features make it possible to utilize P2 as a fluorescence reagent, for instance, for detecting polymerase chain reaction amplification product[2].

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‡This paper is dedicated to the memory of late Professor Tsujiaki Hata.

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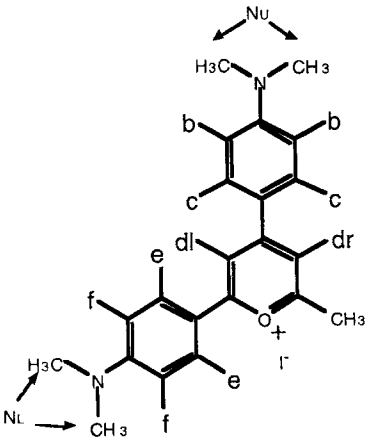

SCHEME 1. Structure of P2

Regarding the binding properties of P2 to dsDNA, although little is known about the binding mode and the sequence specificity of P2, the red shift of absorption maxima with hypochromicity and the fluorescence enhancement in the presence of dsDNA suggest that the binding mode of P2 is intercalation into dsDNA. It is of importance, therefore, to investigate the interaction between P2 and dsDNA at the atomic level.

In this study, we report a ^1H NMR study of the interaction of P2 with self-complementary DNA oligomer, $[d(CGACGTCG)]_2$. Because DNA intercalators generally bind more tightly to pyrimidine-purine sequences[3,4], this oligomer was selected as a target of P2.

MATERIALS AND METHODS

Materials

P2 was synthesized as previously described[1,2]. Dimethlyaminoacetophenone was subjected to the reaction in a mixture of acetic anhydride and conc. H_2SO_4, followed by crystallization steps. The DNA oligomer, d(CGACGTCG), was purchased from Greiner Japan (Kanagawa, Japan), and used without further purification. The purity of the DNA oligomer was checked by reversed phase liquid chromatography and ^1H NMR.

Fluorescence measurement

Fluorescent spectra excited by visible light (560 nm) were recorded on a Shimadzu RF-5000. The concentration of P2 for measurement of emission spectra was 5 μM, and the temperature maintained at 5 °C.

NMR measurements

NMR samples were prepared in 10 mM phosphate buffer (pH 6.5) containing 100 mM NaCl in D_2O. Both the concentrations of d(CGACGTCG) and P2 were 4 mM. When the P2/duplex ratio was more than 1, free P2 was precipitated. All the NMR spectra were recorded on a Bruker ARX-500 spectrometer. For resonance assignments, DQF-COSY[5,6], HOHAHA (mixing time; 40 ms)[7,8] and NOESY (mixing times; 200 and 350 ms)[9,10] were run according to the time-proportional phase incrementation method[11]. For the accurate evaluation of NOE cross-peak intensities of DNA, a NOESY spectrum was acquired by applying a short mixing time of 60 ms and delay time of 5 s. Exchangeable protons were partly assigned in H_2O by jump-and-return NOESY[12]. Chemical shifts were referred to internal sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$. Unless otherwise stated, NMR measurements were carried out at 30 °C at which the DNA oligomer formed a stable duplex (T_m, ca. 65 °C). In order to investigate the chemical exchange between bound and free states, NMR spectra of the complex were measured at several temperatures between 10 and 40 °C.

RESULTS

Fluorescence intensity of P2-DNA oligomer complex

In FIG. 1, the fluorescence intensities at 615 nm excited by visible light (560 nm) are plotted versus the dsDNA concentration in base pair unit. The intensity increased in a concentration-dependent manner up to 100 μM. This result indicates that P2 can bind to $[d(CGACGTCG)]_2$ and exhibit a strong fluorescence enhancement, which is almost similar to that observed previously for Salmon testes DNA[1].

Assignment of ^1H resonances and conformation of free dsDNA oligomer

The assignment of proton resonances of the oligomers was performed by well-established procedures described in the literature[13-16]. The NOESY spectra produced at the long mixing times were used for the sequential assignments. Although stereospecific assignments of the H5'/H5" protons could not be made because of signal overlaps, assignments of the rest nonexchangeable DNA protons were conducted by analyzing the NOESY spectra by the aid of HOHAHA and DQF-COSY data. The chemical shifts in the free state of $[d(CGACGTCG)]_2$ were consistent with those reported previously[4,14]. All the chemical shifts of the dsDNA are summarized in TABLE 1. The conformation of the DNA oligomer was estimated by taking advantage of the intensity of intra- and inter-nucleotide NOEs[17,18], which were obtained from NOESY spectra acquired at mixing time of 60 ms. An overall right handed B-type structure was ascertained for the DNA duplex from the observation of H8/H6(i)-H5/CH_3(i+1) NOEs and of the NOE intensity pattern involving the H2'/2" and H8/H6 protons: H2'(i)-H8/H6(i) >> H2"(i-1)-H8/H6(i)

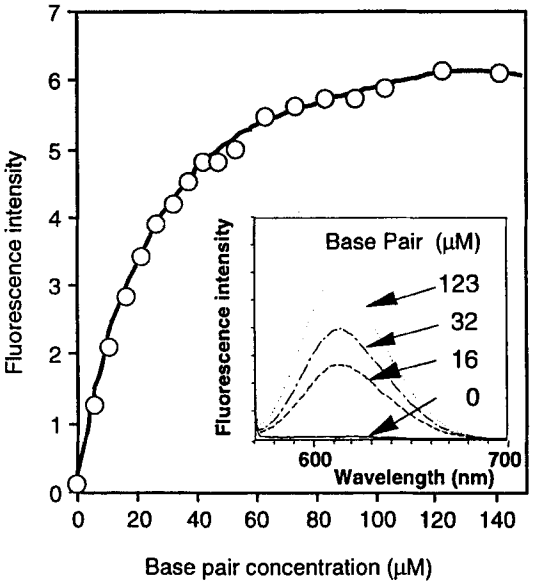

FIG. 1. Fluorescence intensity dependence of P2 on the concentration in base pair of $[d(CGACGTCG)]_2$. Fluorescence spectral change is indicated in the legend inset. The concentration of P2 was 5 μM.

TABLE 1. ^1H chemical shift values of $[d(CGACGTCG)]_2$ in the presence and absence of P2 (pH 6.5, 303 K).

free DNA

	H6/8	H2/5/Me	H1'	H2'	H2"	H3'	H4', 5', 5"	H1/3	H4a	H4b
C1	7.63	5.89	5.74	1.83	2.36	4.68	3.71, 3.73, 4.08			
G2	7.97		5.47	2.72	2.79	5.01	4.18, 4.23, 4.33	12.92		
A3	8.24	7.91	6.27	2.72	2.92	5.07	4.18, 4.26, 4.49			
C4	7.23	5.25	5.61	2.02	2.37	4.82	4.10, 4.18, 4.26		8.12	6.46
G5	7.84		5.97	2.61	2.78	4.94	4.14, 4.23, 4.37	12.76		
T6	7.28	1.41	6.05	2.09	2.46	4.87	4.08, 4.13, 4.23	13.77		
C7	7.50	5.71	5.76	2.03	2.38	4.83	4.13, 4.18		8.60	7.01
G8	7.92		6.15	2.63	2.39	4.68	4.10, 4.18			

P2-DNA complex

	H6/8	H2/5/Me	H1'	H2'	H2"	H3'	H4', 5', 5"	H1/3	H4a	H4b
C1	7.44	5.68	5.59	1.72	2.29	4.62	3.72, 4.02			
G2	7.82		5.37	2.66	2.71	4.96	3.94, 4.07, 4.28	12.74		
A3	8.16	7.82	6.20	2.67	2.87	5.03	4.14, 4.23, 4.45			
C4	7.16	5.18	5.59	1.99	2.33	4.78	3.94, 4.14, 4.28		8.01	6.38
G5	7.77		5.91	2.57	2.73	4.92	4.12, 4.20, 4.34	12.60		
T6	7.21	1.35	5.99	2.07	2.44	4.83	4.10, 4.19	13.61		
C7	7.41	5.60	5.68	2.01	2.33	4.80	4.06, 4.10		8.41	6.87
G8	7.82		5.98	2.57	2.29	4.64	4.06, 4.15			

> H2'(i-1)-H8/H6(i). The sugar puckering and base-orientation was found to be O1'-endo to C2'-endo and anti, respectively, on account of intranucleotide sugar-base NOE pattern [H2'(i)-H8/H6(i) >> H1'(i)-H8/H6(i) > H3'(i)-H8/H6(i)]. These NOE intensities clearly indicate that the conformation of the dsDNA belongs to the general B-type family.

Assignment of ^1H resonances and DNA conformation in the P2-dsDNA oligomer complex

The spectral behavior of DNA resonances in the P2 titration experiments demonstrated that free and bound states exchange relatively fast on the NMR time scale (FIG. 2b). The resonances of non-labile DNA protons, particularly H8/H6 protons, became broad upon addition of P2. P2 resonances in the P2-dsDNA complex also showed exchange dynamics in the fast-intermediate range, and were shifted and broadened upon binding (FIG. 2b). With increasing temperature to 40 °C, all the signals became sharp.

The assignment of the proton chemical shifts of P2 resonances in the complex was performed by analyzing NOESY spectra. The chemical shift values obtained for P2 in the presence and absence of [d(CGACGTCG)]$_2$ are summarized in TABLE 2. Upon forming the complex, *N,N*-dimethylamino group and dl protons were shifted to downfield, while the other protons were shifted to the upfield (FIG. 3a).

The DNA resonances in the complex were assigned by the same procedure as that for DNA alone. The values are summarized in TABLE 1. Changes in the chemical shift of DNA H8/H6 proton resonances upon binding of P2 are shown in FIG. 3b. All the non-labile and labile base protons in the complex were shifted to the upfield, as compared to those in the free duplex. It was also found that the sequential NOEs were still indicative of B-form in the presence of P2 (FIG. 4).

Intermolecular NOEs between P2 and dsDNA

The NOESY spectrum (mixing time; 60 ms) of the P2-dsDNA complex showed NOE cross-peaks between some H8/H6 protons (C1, G2, C7 and G8) and *N,N*-dimethylamino protons (FIG. 5) although, on account of signal overlapping, it was not possible to determine which methyl protons (N_U or N_L) are involved in this interaction. Weak intermolecular NOEs were also observed between *N,N*-dimethylamino protons and H1' protons of C1, G2, C7 and G8. Other intermolecular NOEs were not concretely determined because of the overlapping and broadening of the peaks. Although many intermolecular NOEs were observed between P2 and dsDNA with increasing the mixing time, we did not employ them to avoid ambiguous structural information.

DISCUSSION

As described previously, the oligomer employed in the present study also showed strong enhancement of fluorescence in the presence of P2, indicative of the binding. For

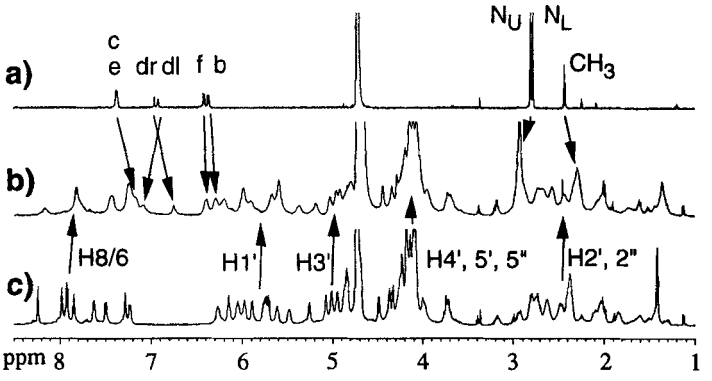

FIG. 2. ^{1}H NMR 500 MHz spectra of (a) P2 alone, (b) $[d(CGACGTCG)]_2$ in the presence of P2, and (c) $[d(CGACGTCG)]_2$ alone. The solvent was 10 mM phosphate buffer (pH 6.5) containing 100 mM NaCl, and the temperature 30 °C.

TABLE 2. Chemical shift (ppm) of P2 in the presence and absence of $[d(CGACGTCG)]_2$ (pH 6.5, 303 K).

	Nu	NL	b	c	dl	dr	e	f	CH_3
- DNA	2.78	2.81	6.36	7.36	6.93	6.96	7.38	6.42	2.42
+ DNA	2.90	2.90	6.27	7.23	7.05	6.73	7.23	6.37	2.26

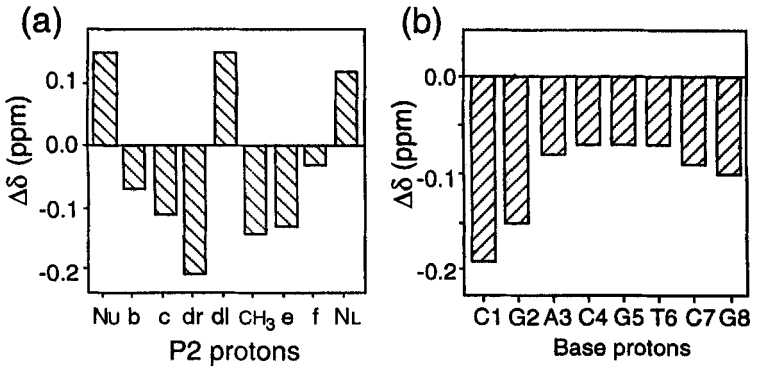

FIG. 3. Changes in the chemical shifts for (a) P2 proton resonances and for (b) H8/H6 proton resonances of $[d(CGACGTCG)]_2$ which were caused by the complex formation. Negative values indicate that the resonances were shifted to the upfield by forming the complex.

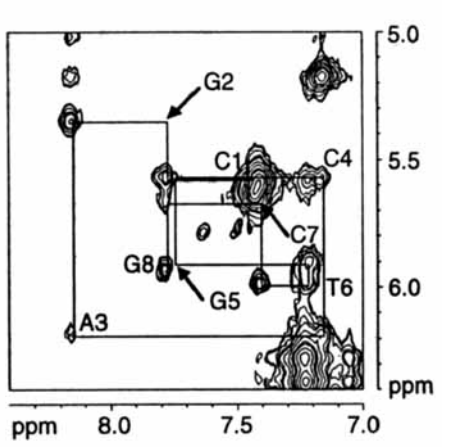

FIG. 4. Expanded region of NOESY spectrum of P2-[d(CGACGTCG)]$_2$ complex, acquired in D_2O at a mixing time of 60 ms and at 30 °C.

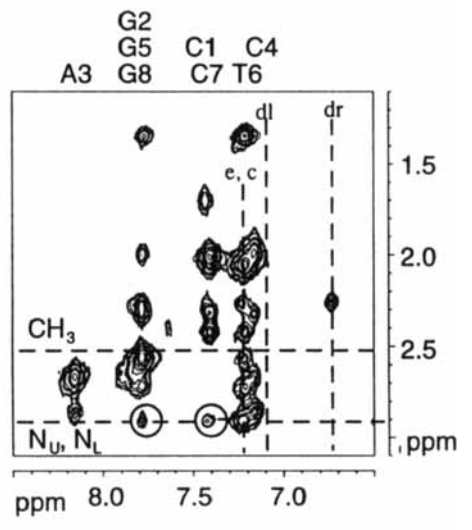

FIG. 5. Expanded NOESY spectrum (mixing time 60 ms) of P2-[d(CGACGTCG)]$_2$ complex. The intermolecular NOEs discussed in the text are enclosed in the circles.

further study of the binding mode, we used NMR technique. By NMR, two binding modes, groove binding and intercalation, have been proposed for DNA-drug complexes. The first-approximation of the distinction between these modes by NMR is the chemical shift change of the drug or DNA protons upon forming a complex. It is generally accepted that intercalators induce the upfield shifts for the imino protons of Watson-Crick pairs while drugs binding in the minor groove have the opposite effect[19,20], and that ^{1}H chemical shifts of drugs also exhibit a upfield shift upon intercalating to DNA base pairs[4,21-23]. In the present study, formation of an intercalated complex between P2 and $[d(CGACGTCG)]_2$ is strongly supported by the upfield shifts of the aromatic signals of both P2 and dsDNA which accompanied line broadening. Taking into account that the chemical shift change and intermolecular NOEs between *N,N*-dimethylamino group and the terminal CpG steps, it is suggested that P2 interacts with the loose CpG steps rather than the tightly stacked central C4pG5 step. One of the reasons for this preference of the stacking is that P2 consisting of unfused 6-members aromatic rings may prefer rather half-complete base pairs.

A fine evaluation of the structure of the complex was not achieved from intermolecular NOEs due to the poor resolution of the spectra caused by the interaction dynamics. Nevertheless, the intermolecular NOE cross-peaks between *N,N*-dimethylamino group and H8/H6 protons of DNA bases were observed in the NOESY spectra even at a short mixing time. It is deduced, therefore, that the *N,N*-dimethylamino group is located near H8/H6 protons. To make this unique local structure possible, the amino groups may interact with a phosphate group since *N,N*-dimethylamino groups could be positively charged through resonance in which the *N,N*-dimethylaminophenyl ring and pyrylium ring are partly coplanar. A strong electron-accepting character of the pyrylium ring and an electron donating one of the *N,N*-dimethylamino group suggest twisted intramolecular charge transfer (TICT)[24]. According to the previous report, the crystal structure of 2,6-diphenyl-4-*N,N*-dimethylaminophenylpyrylium salt indicated that molecules stack on each other and that the pyrylium and *N,N*-dimethylaminophenyl ring are approximately coplanar as a result of a strong through-resonance effect with the positively charged pyrylium ring[25]. Thus, we think that P2 has a strong dipole moment from the nitrogen atom of the *N,N*-dimethylamino group to the oxygen atom at the pyrylium ring, and likely has a planar structure under condition such as stacking with base pairs. Fluorescence enhancements of P2 in the presence of dsDNA may be attributed to TICT in such a unique structure of the complex: P2, a twisted form in the absence of dsDNA, possibly obtains coplanarity and a capacity for strong fluorescence when it intercalates into dsDNA.

REFERENCES

1. Yamamoto, N.; Okamoto, T.; Kawaguchi, M. *Nucleic Acid Symp. Series,* **1993** ***29,*** 83-84.
2. Yamamoto, N.; Okamoto, T. *Nucleic Acids Res.,* **1995** ***23,*** 1445-1446.
3. Krugh, T. R.; Reinhardt, C. G. *J. Mol. Biol.,* **1975** ***97,*** 133-162.
4. Trotta, E.; D'Ambrosio, E.; Ravagnan, G.; Paci, M. *Nucleic Acids Res.,* **1995** ***23,*** 1333-1340.
5. Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.,* **1983** ***117,*** 479-485.
6. Piantini, U.; Sørensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.,* **1982** ***104,*** 6800-6801.
7. Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.,* **1983** ***153,*** 521-528.
8. Davis, D. G.; Bax, A. *J . Am . Chem . Soc .,* **1985** ***107,*** 2820-2821.
9. Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.,* **1981** ***43,*** 259-281.
10. Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.,* **1979** ***71,*** 4546-4553.
11. Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.,* **1983** ***113,*** 967-974.
12. Williamson, J. R.; Boxer, S. G. *Biochemistry,* **1989** ***28,*** 2819-2831.
13. Clore, G. M.; Gronenborn, A. M. *EMBO J.,* **1983** ***2,*** 2109-2115.
14. Hare, D. R.; Wemmer, D. E.; Chou, S.-H.; Drobny, G. *J. Mol. Biol.,* **1983** ***171,*** 319-336.
15. Scheek, R. M.; Russo, N.; Boelens, R.; Kaptein, R.; van Boom, J. H. *J. Am. Chem. Soc.,* **1983** ***105,*** 2914-2916.
16. Reid, D. G.; Salisbury, S. A.; Bellard, S.; Shakked, Z.; Williams, D. H. *Biochemistry,* **1983** ***22,*** 2019-2025.
17. Gronenborn, A. M.; Clore, G. M. *Prog. Nucl. Magn. Resn. Spectorsc.,* **1985** ***17,*** 1-33.
18. Clore, G. M.; Gronenborn, A. M. *FEBS Lett.,* **1985** ***179,*** 187-198.
19. Leupin, W.; Feigon, J.; Denny, W. A.; Kearns, D. R. *Biophys. Chem.,* **1985** ***22,*** 299-305.
20. Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.,* **1984** ***27,*** 450-465.
21. Delbarre, A.; Delepierre, M.; Garbay, C.; Igolen, J.; Le Pecq, J.-B.; Roques, B. P. *Proc. Natl. Acad. Sci. USA,* **1987** ***84,*** 2155-2159.
22. Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Wydra, R. L.; Jones, R. L.; Boykin, D. W.; Strekowski, L. *Anti-cancer Drug Design,* **1990** ***5,*** 31-42.
23. Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Jones, R. L.; Fox, K.; Wydra, R. L.; Strekowski, L. *Biochemistry,* **1990** ***29,*** 8452-8461.
24. Haucke, G.; Czerney, P.; Cebulla, F. *Ber. Bunsenges. Phys. Chem.,* **1992** ***96,*** 880-886.
25. Turowska-Tyyk, I.; Krygowski, T. M.; Milart, P. *J. Mol. Struc.,* **1991** ***263,*** 235-245.