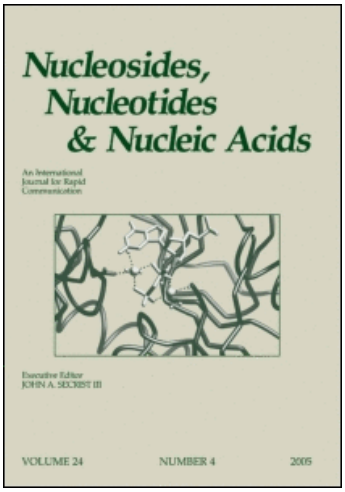

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ENHANCED FLUORESCENCE IN THE BINDING OF OLIGONUCLEOTIDES WITH A PYRENE GROUP IN THE SUGAR FRAGMENT TO COMPLEMENTARY POLYNUCLEOTIDES§

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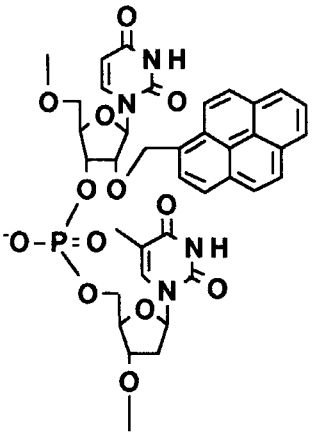
ABSTRACT: *The fluorescence intensity and lifetime of oligonucleotides with a pyrenylmethyl group at the specific sugar residue were increased upon binding to their complementary polynucleotide in aqueous solution. The present oligonucleotide-pyrene conjugates provide new fluorescent probes for detection of specific nucleic acids.*

Design and synthesis of oligonucleotide derivatives possessing a covalently attached fluorescence dye have attracted much attention because of their utility as probes in non-radioactive detection of specific nucleic acids sequences. Solution hybridizations of probes with nucleic acids being investigated would simplify the procedures for detection and lead to possible use of such probes for *in vivo* studies. For this purpose, the nucleic acids probes should be labeled specifically, so that a measurable signal is obtained only after hybridization with the complementary sequence. It has been demonstrated that a fluorescence energy transfer can be used for the measurable signal.[1,2] This approach needs two oligonucleotide probes: one is modified by the energy donor and the other is linked to the acceptor. We describe here a simpler approach to development of a new fluorescent oligonucleotide probe capable of being used for assay of nucleic acids in

§This paper is dedicated to Professor Tohru Ueda, the former editor of this journal, who passed away on September 19, 1990.



U(pyr)TTTTTTT (1)

TTU(pyr)TTTTTTT (2)

U(pyr)TTTTTTTTT (3)

FIG.1. Structure and abbreviations of oligonucleotides with pyrene group at sugar fragment.

solutions. This probe is oligonucleotides possessing a pyreneylmethyl substituent at the specific sugar residue[3] which exhibit enhanced fluorescence upon binding to their complementary polynucleotide.[4] This particular feature is in contrast to that of the oligonucleotides covalently attached to acridine[5] or pyrene[6] via linker arms at the terminal phosphate or the thymine C-5, respectively.

RESULTS AND DISCUSSION

The incorporation of a pyrenylmethyl substituent into the sugar fragment of oligonucleotides has been accomplished by the preparation of 2'-(1-pyrenylmethyl)uridine [U(pyr)] , which is then converted to the protected uridine phosphorobisdiethylamidite.[3] This reagent was used for the solid-phase synthesis of the oligonucleotide-pyrene conjugates.[3] The structure and abbreviations of the conjugates studied here are shown in figure 1.

The interactions of the oligonucleotide-pyrene conjugates with poly A in aqueous buffer solution were investigated spectrophotometorically. Figure 2 shows the absorbance change versus temperature for the mixture of oligomer **3** and poly A (1:1, mol/mol). Upon cooling the mixture, the intensity of the pyrene absorbance band (300-370 nm) was decreased and the absorption maxima were shifted to longer wavelength by 3-4 nm with isosbestic points. Similar absorbance changes were observed for the

mixtures of oligomer **1** and **2** with poly A. The UV melting curves both at 260 nm and 350 nm for the mixture of oligomer **3** with poly A are shown in figure 3. The shapes of the profiles exhibit sigmoidal curves similar to the unmodified oligothymidylate mixture with poly A. The duplexes of oligomer **1** and **2** with poly A exhibited similar melting profiles. These results indicate that the oligonucleotide-pyrene conjugates bind to the complementary polynucleotide in aqueous solution by Watson-Crick base-pairing and the interaction of the

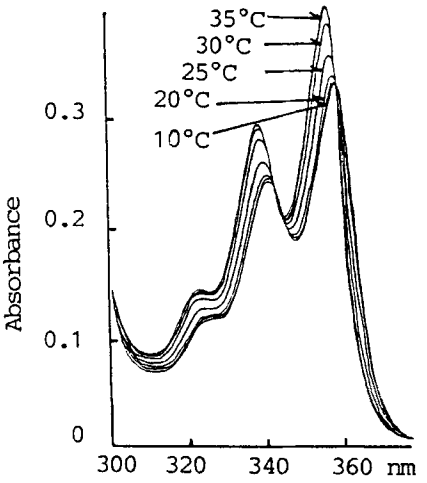


FIG.2. UV spectra of the mixture of oligonucleotide **3** with poly A (total nucleotide conc.: 6.6 x 10^{-5} M) as a function of indicated temperatures.

pyrene with the adjacent base-pairs occurs in a cooperative manner with the base-pairing. Table 1 summurizes the tm values (tms) derived from the UV melting curves at 260 nm. Inspection of the tms reveals that the introduction of the pyrene group into the 5'-end sugar of oligonucleotide slightly increases the melting temperature relative to the corressponding oligothymidylate-poly A duplex.

The conformations of duplexes of oligomer **2** and **3** with poly A were investigated by their CD spectra which are presented in figure 4. The oligonucleotide **3** which has U(pyr) unit at the 5'-end of oligonucleotide exhibits a similar CD profile to the corresponding unmodified duplex of oligothymidylate with poly A. In contrast, the oligomer **2** showed a different CD from **3**. These spectral studies indicate that the oligonucleotide with the pyrene group at the 5'-end sugar forms a duplex with poly A that has a similar conformation to the oligothymidylate-poly A duplex. It has already been shown that the oligonucleotide with an anthraquinonylmethyl group at the 2'-sugar fragment binds to the complementary segment to form a duplex with a greatly enhanced thermal stability and the conformation of the duplex is considerably distorted by the intercalation.[7] It seems reasonable that there is a difference in intercalative mode

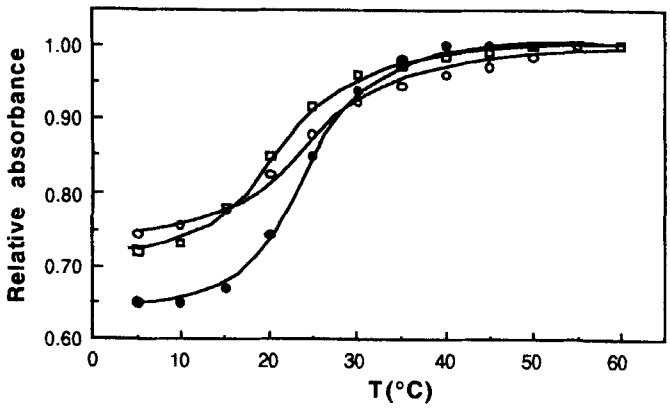


FIG.3. UV melting curves of duplexes of oligonucleotide **3** (○: 260 nm, ●: 350 nm) and decathymidylate (□: 260 nm) with poly A. Total nucleotide concentration was 6.6×10^{-5} M.

TABLE 1. Tm values(tms) for the duplexes of oligonucleotides with pyrene group at sugar fragment with poly A.

	tm(°C)
TTTTTTTT - poly A[1)]	12.5
U(pyr)TTTTTTT (**1**) - poly A[1)]	13.5
TTTTTTTTTT - poly A[2)]	23.5
TTU(pyr)TTTTTTT (**2**) - poly A[2)]	18.4
U(pyr)TTTTTTTTT (**3**) - poly A[2)]	25.1

Measurements were carried out in 0.01 M sodium phosphate and 0.1 M NaCl, adjusted to pH 7.0. 1) total nucleotide concentration : 2.0×10^{-4} M. 2) total nucleotide concentration : 6.6×10^{-5} M.

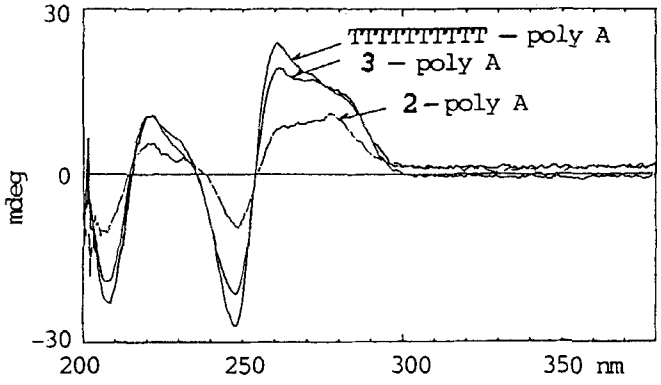


FIG.4. CD spectra (at 8°C) of duplexes of oligomers **2**, **3**, and decathymidylate with poly A. Measurements were carried out at a common total nucleotide concntration of 6.6×10^{-5} M.

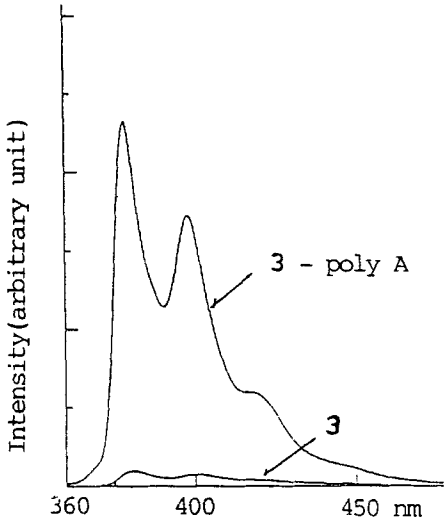


FIG.5. Emission spectra of oligonucleotide **3** (3.3×10^{-5} M in nucleotide unit) and its duplex with poly A. Measurements were carried at 2°C.

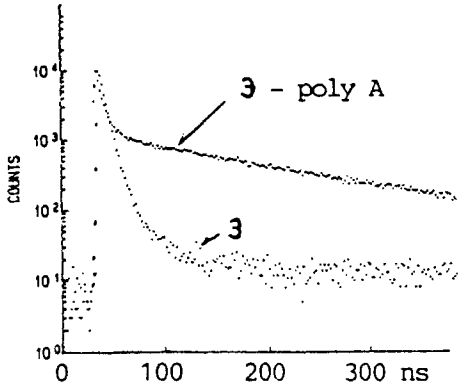


FIG.6. Fluorescence decay curves for oligonucleotide **3** and its duplex with poly A. Measurements were carried out at the same conditions as in FIG.5.

TABLE 2. Fluorescence data of pyrene derivatives, oligonucleotides with pyrene group at sugar fragment, and their duplexes with poly A.

	relative quantum yield	lifetime(ns)
pyrCH$_2$OH[1)]	485.4	
U(pyr)	41.5	
oligomer **1**	1.0	1.62(15.4%), 6.78(69.4%), 28.0(15.1%)
oligomer **2**	1.8	1.04(14.4%), 6.27(73.1%), 27.8(12.5%)
oligomer **3**	1.4	1.28(13.9%), 6.11(77.1%), 25.9(9.0%)
oligomer **1** - poly A	3.9[2)]	1.80(12.0%), 8.40(30.1%), *154*(57.9%)
oligomer **2** - poly A	1.8[2)]	1.30(36.4%), 9.11(55.2%), 62.4(8.4%)
oligomer **3** - poly A	20.4[2)]	1.28(5.6%), 7.28(15.0%), *164*(79.4%)

Measurements were carried out at 2°C in a pH 7 phosphate buffer containing 0.1 M NaCl at a concentration of 3.3 x 10^{-5} M for the pyrene derivatives and oligomers, and 6.6 x 10^{-5} M for the duplexes (total nucleotide unit). 1) 1-pyrenylmethanol.
2) relative value to the corresponding oligonucleotide-pyrene conjugate.

between the covalently attached pyrene and anthraquinone that affect the stability and global conformation of the duplexes.

Figure 5 shows emission spectra of oligonucleotide **3** and its duplex with poly A. Fluorescence decay curves for oligomer **3** and its duplex with poly A are presented in figure 6. The fluorescence yield and lifetime of **3** were drastically increased upon binding to poly A. Table 2 summerizes the fluorescence properties of oligonucleotide-pyrene conjugates and their duplexes with poly A. The fluorescence yields of the oligonucleotides with the pyrene at the 5'-end sugar **1** and **3** were increased upon binding to poly A, whereas the degree of the fluorescence change of oligomer **2** was much smaller. Inspection of the fluorescence lifetime reveals that the enhancement of the fluorescence yield of the duplexes **1** and **3** is derived from the proportion of the long lifetime component of 154 and 164 ns, respectively No such long lifetime component for the duplex of oligomer **2** with poly A was observed.

As indicated in Table 2, the incorporation of the pyrene into the 2'-position of uridine decreased the relative fluorescence yield and further decrease in the fluorescence yield was observed in the oligonucleotide-pyrene conjugates. This fluorescence quenching of the pyrene is attributed to the stacking interaction between the pyrene and the adjacent bases.[8] The duplex formation of the oligonucleotide-pyrene conjugates with poly A resulted in an enhancement of the fluorescence yield giving a long lifetime component which is close to the reported value for a pyrene dispersed in air-saturated aqueous

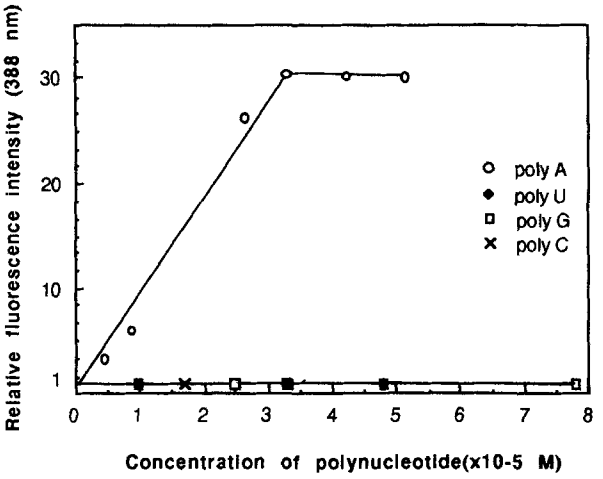


FIG.7. Specific change in fluorescence intensity of oligonucleotide **3** in the presence of different polynucleotides. Measurements were carried out in 0.01 M sodium phosphate and 1.0 M NaCl at 10 °C. Concentration of oligomer **3** was 3.3 x 10^{-5} M.

solution.[8,9] We therefore conclude that the most of the fluorescence from the duplexes of the oligomer **1** and **3** with poly A is due to the pyrene *partially intercalated into the adjacent base-pairs*, or released from the stacking interaction that is favorable in the single-stranded form. The geometrical change around the pyrene after the hybridization of these oligomers with poly A play a significant role in the fluorescence properties that we observed for the present duplexes of oligonucleotide-pyrene conjugates. The oligonucleotide **2** which has the pyrene group at the inner sugar from the both ends may lose degrees of freedom to change the local geometry around the pyrene after the hybridization.

As shown in figure 7, we confirmed that the fluorescence enhancement is observed only when the oligonucleotide-pyrene conjugate binds to the complementary polynucleotide. We anticipate that the present type of oligonucleotide-pyrene conjugates should be useful in detection of specific nucleic acids sequence in homogeneous systems. Further research to evaluate the potential and limitation of the oligonucleotide probes is in progress.

EXPERIMENTAL

The synthesis of oligonucleotides with a pyrenymethyl substituent at a specific sugar has been described elsewhere.[3] Polyribonucleotides(poly A, poly U, poly C, and poly

G) were purchased from Sigma Chemical Co. 1-Pyrenylmethanol was synthesized by reduction of 1-pyrene carboxyaldehyde with $NaBH_4$. UV spectra were recorded on a Shimadzu UV-300 spectrophotometer equipped with a thermoelectrically controlled cell holder. CD spectra were obtained on a JASCO CD J-600 spectrometer equipped with a thermoelectrically controlled cell holder. Fluorescence spectra were measured on a JASCO FP-770 spectrofluorometer. The excitation wavelength was 338 nm for the emission measurements. Fluorescence decay curves were obtained by a single photon counting method.[10] The measured decay curves were fitted to a weight sum of exponentials in a usual manner,[6] giving the lifetime of each decay component and its quantum yield(%). The buffer used for all the measurements contained 0.01 M sodium phosphate and 0.1 M NaCl, adjusted to pH 7.0, without removal of soluble oxygen.

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