Homopyrimidine Oligonucleotides Modified by a Pyrenylmethyl Group at the Terminal Position: Enhanced Fluorescence upon Binding to Double Helical DNA

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Oligopyrimidines labeled by a pyrenylmethyl group at the terminal position exhibit high affinity for double helical DNA and enhanced fluorescence upon hybridization.

Sequence specific recognition of double helical DNA by a synthetic molecule has attracted current interest, since it may lead to new research tools useful in molecular biology, diagnosis of disease states at the level of DNA, and novel chemotherapeutic strategies *so called* antigene approach . It is known that a homopyrimidine oligonucleotide can bind to the major groove of double helical homopurine-homopyrimidine DNA through Hoogsteen base-pairings. Several reports have shown that oligopyrimidines equipped with an effector molecule such as a DNA cleaving agent,[1] a cationic moiety,[2] a reporter molecule,[3] and an intercalating aromatic fragment[4] are useful as recognition molecules with some novel functions in targeting to double helical DNA.

In this report, we describe the binding and fluorescence properties of the oligopyrimidines labeled by a pyrene fluorescence via a relatively short linker at the terminal hydroxyl function. Interesting features of the pyrene-modified oligopyrimidines are that they show relatively high affinity for double helical DNA and significant fluorescence upon binding to the major groove of DNA.

The pyrene-modified oligopyrimidines **1**-**3** which have been synthesized by the established procedure[5] are shown in Figure 1.[6] The most important feature of the oligonucleotide derivatives is the use of the methylene linker for the attachment of the pyrene into the terminal hydroxyl group of oligonucleotides.

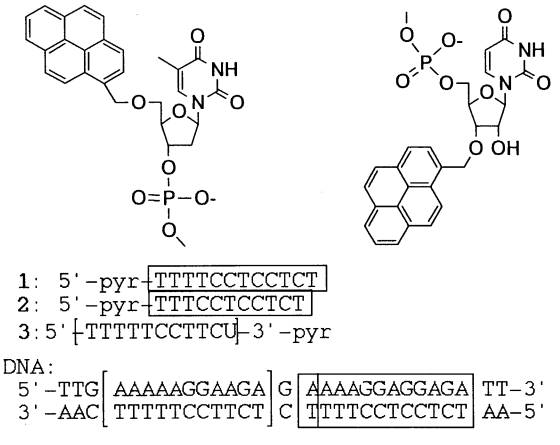

Figure 1. Structures and sequences of oligopyrimidines modified by a pyrenylmethyl group at the terminal position. The DNA sequences for binding of these oligomers are shown by box or braket. 5'-pyr-T and U-pyr-3' indicate 5'-(1-pyrenylmethyl)thymidine and 3'-(1-pyrenylmethyl)-uridine, respectively.

Table 1. Tm values and emission properties of triple helixes of pyrene-modified oligopyrimidnes **1**-**3** with DNA

oligopyrimidine	Tm (°C)	Ft/Fs
1	30.0	3.5
5'-TTTTCCTCCTCT	26.4	
2	26.8	4.0
5'-TTTCCTCCTCT	24.2	
3	30.0	2.0
5'-TTTTTCCTTCT	22.1	

UV melting experiments were carried out in a pH 5.6 buffer containing cacodylic acid (50 mmol dm^{-3}), NaCl (1 mol dm^{-3}), and spermine (1 mmol dm^{-3}) at a single strand concentration of 3.75×10^{-5} mol dm^{-3}. Fluorescence spectra were measured for the same solutions at 10°C by excitation at 328 nm. Ft/Fs indicates the relative fluorescence intensity at 400 nm for the triplex versus the single-stranded oligonucleotide. The DNA sequence used for this study is shown in Figure 1.

It is thus expected that, upon binding to double helical DNA, the pyrene attached to the oligonucleotide would be placed in the major groove rather than into the base-pairs of DNA.

The triple helix formation of the pyrene-labeled oligonucleotides with DNA was investigated by UV melting behaviors in a pH 5.6 cacodylic acid buffer (50 mmol dm^{-3}) containing NaCl (1 mol dm^{-3}) and spermine (1 mmol dm^{-3}). The triplex containing the pyrene-modified oligonucleotide **1** showed the melting temperature of 30 °C, while the unmodified triplex melted to duplex at the midpoint of transition of 26 °C. The triplexes of **2** and **3** exhibited similar melting behaviors. The tm values for the triplex to duplex transition were summarized in Table 1. All the pyrene-modified oligoprimidines showed slightly higher affinity for double helical DNA than unmodified pyrimidine oligonucleotides.

Inspection of the UV absorption due to the pyrene attached to the 5'-hydroxyl group of oligopyrimidines **1** and **2** revealed that only slight red-shift (345 nm to 346 nm) occurred upon triple helix formation. The oligonucleotide with the pyrene at the 3'-terminus **3** exhibited slight absorption change in the maximum from 347 nm to 350 nm during the triplex formation.

Upon triple helix formation with DNA, the fluorescence of the pyrene-modified oligonucleotide **1** significantly increased as shown in Figure 2. Similar fluorescence enhancement was observed for oligomer **2** and **3**. The results of fluorescence measurements were indicated in Table 1.

When pyrene and its related aromatic hydrocarbons interact with double helical DNA, there are two binding modes.[7] One involves intercalation, stacking between pyrene and nuceic acid bases, resulting in large fluorescence quenching and absorption red-shift of the pyrene (ca.10 nm , 345 nm to 354 nm), and the other is outside binding that yields little fluorescence and

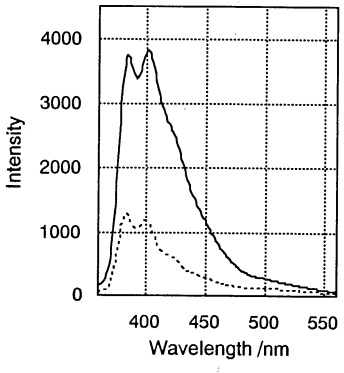

Figure 2. Fluorescence spectra for pyrene-modified oligopyrimidine **1** before (- -) and after (—) triplex formation. Measurements were carried out at 10°C at a single strand concentration of 3.75 x 10^{-5} mol dm^{-3}. Excitation wavelength was 328 nm.

absorption changes. The oligonucleotides attached to a pendant pyrene group via a long flexible linker bind to a single-stranded DNA resulting in significant fluorescence quenching and stabilization of duplexes owing to intercalation.[8] On the contrary, when the oligonucleotides possessing a pyrene group via methylene linker at the terminal position bind to a single stranded oligodeoxyribonucleotide, slight fluorescence change (ca. x 1.1-1.2 upon hybridization) occur.[5a] Based on these information, the high affinity and enhanced fluorescence in binding of the pyrene-modified pyrimidine oligonucleotides to double helical DNA are attributable to the attached pyrene placed in the major groove of DNA.[9]

We have shown some novel properties of oligopyrimidines labeled by a pyrene fluorophore via a short linker arm at the terminal position. The present pyrene-modified oligonucleotides would be useful for discrimination and detection of specific sequences in double helical DNAs.

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6 MALDI-TOFF Mass data [found(calcd)]: oligomer **1**, 3726.2 (3726.5); oligomer **2**, 3422.3(3422.3); oligomer **3**, 3454.3(3454.3).

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9 It is not precluded that the stacking interactions between the attached pyrene and the nucleic acid bases of DNA, in part, contribute to the stability and fluorescence of the triple helix. The fluorescence enhancement appears to be correlated to the relative stability of the triplex helix. The most stabilized triplex (oligomer **3**, ΔTm = 7.9 °C) exhibited less fluorescence enhancement (Ft/Fs = 2.0) when compared with oligomer **2** (ΔTm = 2.6 °C, Ft/Fs = 4.0).