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Multiple-pyrene residues arrayed along DNA backbone exhibit significant excimer fluorescence ☆

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Abstract—Oligodeoxyribonucleotides (ODNs) carrying multi-pyrene clusters were chemically prepared by introducing a novel nucleoside–pyrene conjugate into ODNs. The multi-pyrene residues arrayed on DNA strands induced significant excimer fluorescence and the intensity was exponentially increased as the number of pyrene residues in clusters increased. The excimer fluorescence of the arrays was stable and was not quenched by duplex formation.

Strategies for arraying functional groups by using DNA backbones have been of great interest.[1,2] DNA has a wire-like structure composed of repeatedly connected structural units such as nucleobases, deoxyriboses, and phosphodiesters.[3] Thus, functional groups can be arrayed along DNA strands by attaching them to the structural units. In this letter, the synthesis of oligodeoxyribonucleotides (ODNs) carrying pyrene residues attached to the nucleobase residue is reported. The multi-pyrene clusters arrayed along DNA backbones exhibited significant excimer[4] fluorescence. A novel nucleoside–pyrene conjugate **1** (Fig. 1) was synthesized and incorporated into DNA strands. In DNA strands, stacking interactions both between nucleobases and between pyrene residues of the conjugates stabilize the arrayed structure (Fig. 1), thereby enhancing energy transfer between the pyrene residues.

The synthetic scheme for a phosphoramidite unit of the pyrene–thymidine conjugate **1** is shown in Scheme 1. According to the reported method,[5] thymidine was converted to aminoethylated derivative **3**, then **3** and a pyrene group was connected by forming an amide bond to give a conjugate **4**. According to the established procedure, **4** was converted into a phosphoramidite unit **7**, which was used for DNA synthesis on a DNA synthesizer. Oligodeoxyribonucleotides (ODNs) carrying **1** at the 5′-end, $\mathbf{I}_{(n)}$, were synthesized, deprotected and purified by the established procedures.[6]

Emission spectra of ODNs carrying the multi-pyrene arrays are shown in Figure 2. The intensity of excimer fluorescence (475 nm) of $\mathbf{I}_{(n)}$ increased as the number of pyrene residues increased (Fig. 2a). However, the intensity of the monomer fluorescence (400 nm) was not largely altered. It is noteworthy that the excimer fluorescence intensity significantly and exponentially increased as the number of pyrene residues increased. Previously, the preparation of chimera molecules of ODN residues and polyamide residues on which multi-pyrene clusters were arrayed has been reported by Tong et al.[7] However, the rate of increase of the excimer intensity with the number of pyrene residues on the polyamide backbone was less than that observed in this study. Also, an incoherence between excimer fluorescence intensity and the number of pyrene residue on polyamide, such as the excimer fluorescence intensity of the four-pyrene cluster was smaller than that of the three-pyrene cluster, was also reported. These differences may indicate that pyrene residues attached on the DNA backbones are arrayed in a more organized manner than those attached on the polyamide backbone.

Compound $\mathbf{I}_{(n)}$ formed stable duplexes with the complementary strand **II**. Sharp transition curves with T_m

Keywords: Excimer fluorescence; Pyrene; DNA; Arrayed functional group; Multi-pyrene cluster.
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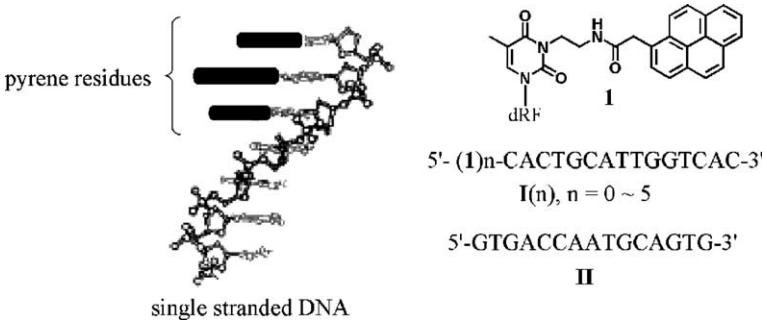
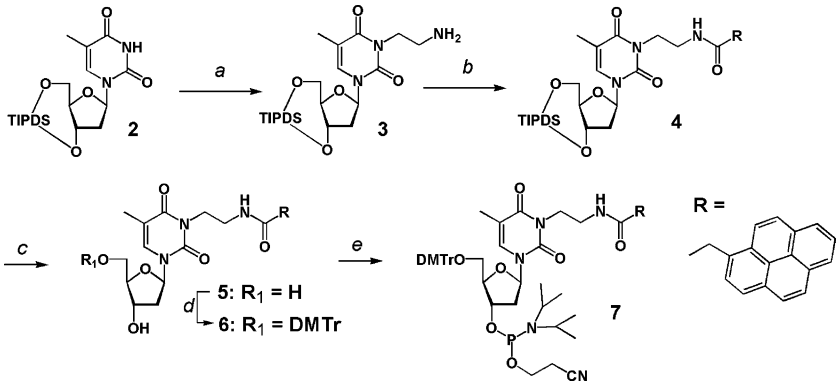


Figure 1. Structure of a pyrene–nucleoside conjugate and ODNs containing the conjugate.



Scheme 1. A schematic representation of the synthesis of the phosphoramidite building blocks **7**. Reagents and conditions: (a) (1) CF_3CONO_2, (2) Ethylenediamine. (b) RCOOH, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/HCl, 35% from **2**. (c) TBAF in THF, 76%. (d) DMTrCl in pyridine. (e) Standard procedures.

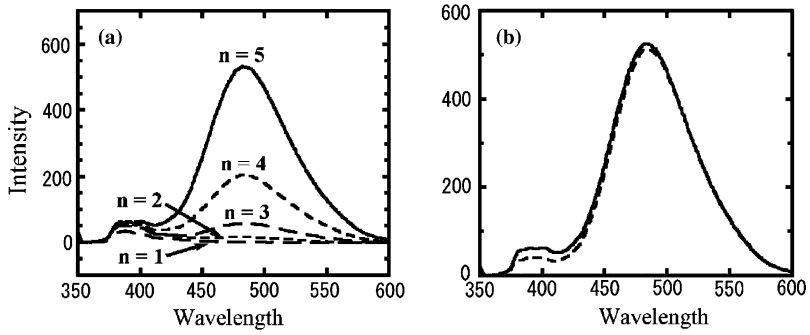


Figure 2. Emission spectra of ODNs carrying the multi-pyrene arrays. (a) $\mathbf{I}_{(n)}$. (b) -(—)- $\mathbf{I}_{(5)}$ and -(- - - -)- $\mathbf{I}_{(5)}$–**II**. Each solution contained 0.46 μM of oligomers in a buffer containing 200 mM NaCl and 10 mM Na phosphate (pH 7.0). Fluorescence spectra were measured on RF-5300PC Spectrofluorophotometers (Shimadzu, Japan) at an excitation wavelength 342 nm with excitation slit width 5 nm, emission slit width 5 nm at room temperature.

values[8] similar to the control duplex were observed in thermal denaturation profiles of $\mathbf{I}_{(5)}$–**II** (supporting information), which may indicate that the pyrene cluster attached at the 5′-end of ODN did not largely hinder the duplex structure. Fluorescence spectra of duplexes, $\mathbf{I}_{(5)}$–**II** is shown in Figure 2b. It was noteworthy that the excimer fluorescence (475 nm) of $\mathbf{I}_{(5)}$ was unaffected by duplex formation, and even the monomer fluorescence (400 nm) decreased.

The fluorescence of the pyrene monomer has been widely investigated as a potential probe of DNA and RNA secondary and tertiary structure.[9] However, in most cases, its use as a probe suffers from the efficient quenching of its fluorescence by nucleobases.[10] Pyrene excimer fluorescence, on the other hand, is less subject to quenching by donors and acceptors than is pyrene monomer fluorescence.[11] The strategy of arraying pyrene along DNA strands is useful for developing fluorescent probes since the fluorescence intensities are more stable than that of pyrene monomer in cells or in organisms. Also, the method for arraying pyrene residues along DNA backbones can be applied for arraying various functional groups such as fluorescent residues, aromatic residues, etc. Such arrays of functional groups may be useful for developing novel materials, such as

multi-fluorescent-libraries,[1,12] DNA-based nano-wires,[2] etc.

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