Synthesis and properties of oligonucleotides containing 2'‐pyrenylalkyluridine

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ABSTRACT
The synthesis, binding and fluorescence properties of oligonucleotides containing the uridine modified at the 2'-position by a pyrene group using different length of linker arm have been described. It is demonstrated that the oligonucleotides possessing a C₃-amide group at the 2'-position display an enhanced signal of the pyrene monomer fluorescence upon binding to DNA segments.

INTRODUCTION
We have been interested in a pyrene chromophore as a fluorescent label of oligonucleotides and developed the method for its introduction via one carbon tether into the 2'-hydroxyl functions of oligonucleotides. The pyrene-modified oligonucleotides in diluted solution exhibited fluorescence typical of pyrene monomer emission. When these oligomers bound to DNA, little change in the monomer emission intensity was observed. In contrast, it has been shown that the oligonucleotides modified with one pyrenyl residue at the appropriate sequence exhibit remarkably enhanced fluorescence upon forming duplexes with RNA. Spectroscopic analysis revealed that the observed strong fluorescence is derived from the pyrene free from the interactions with nucleic acid bases in the RNA duplexes. These findings should open the way for a design of a sensitive probe for solution-based analysis of RNA.

In this paper, we describe the synthesis, binding and fluorescence properties of oligonucleotides containing the uridine modified at the 2'-position by a pyrene group using different length of linker arms. It is demonstrated that the oligonucleotides possessing a C₃-amide group display an enhanced signal of the pyrene monomer fluorescence upon binding to DNA segments.

RESULTS AND DISCUSSION
The modification of ribonucleoside by a pyrenylalkyl group at 2'-position was carried out by the reaction of 2'-amino-2'-deoxyuridine with acid derivatives of the pyrene [pyrene-(CH₂)ₙ-COOH, n = 1 - 4]. The modified ribonucleosides were then converted to 5'-dimethoxytrityl protected nucleoside-3'-phosphoramidites which could be
used for incorporation of the pyrene-modified nucleosides into the sequences of oligonucleotides.

The UV melting behaviors indicate that all the pyrene-oligonucleotides can bind to both their complementary DNA and RNA in aqueous solution. When compared with the unmodified oligonucleotides, the pyrene modified oligonucleotides showed slightly lowered affinity for DNA. The binding of the modified oligonucleotides to RNA resulted in significantly destabilized duplexes.

The CD spectrum for the duplex of the pyrene-modified oligonucleotide containing U(pyrC₃) is shown in Figure 1. The CD profile resembles to that of the unmodified DNA duplex, indicating that the pyrene-modified DNA duplex retains its global conformation. In contrast, the pyrene-modified RNA duplex exhibited the different CD profile from that of the corresponding unmodified DNA-RNA duplex.

The pyrene-modified oligonucleotides in aqueous diluted solution exhibited fluorescence typical of pyrene monomer emission whose efficiencies are significantly lower than that of the pyrene free in solution. The hybrid formation of the oligomers containing U(pyrC₃) with DNA resulted in considerably enhanced fluorescence, as indicated in Figure 2. The little fluorescence changes were observed in binding of the pyrene-modified oligomers possessing the U(pyrC₁), U(pyrC₂), and U(pyrC₄) to DNA.

The present findings would thus lead to a novel concept for a design of a sensitive probe that may be useful in solution-based analysis of DNA.

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REFERENCES