Synthesis and Fluorescent Property of 2'-*O*-Methyl RNA Containing Amide-linked RNA Modified with Pyrene at the 2'-Position

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Oligonucleotides containing 2'-pyrene modified uridine (Upy) exhibit the increase of pyrene monomer emission when hybridize with the complementary RNA[1,2]. The increase of fluorescence intensity is caused by the conformational change of the pyrene residue to the minor groove of the A-form duplex. On the other hand, amide-linked RNA consists of 3'-5' methyleneamide linkages in place of 3'-5' phosphodiester linkages. The sugar conformation is restricted to C3'-*endo* form. To investigate the properties of the amide-linked RNA modified with pyrene at the 2'-position as a fluorescent probe, a DNA containing amide-linked dimer with pyrene (dCATGU$_{py}$·adCTAC) was synthesized and it showed the increase of fluorescence intensity to 7-fold at 375 nm when hybridized with RNA[3]. To improve the RNA sensitivity, we have synthesized a 9-mer of modified 2'-*O*-methyl RNA containing amide-linked uridine dimer with pyrene at the 2'-position (**1**) (Figure 1 (b)). Then the fluorescence intensity of **1** in the single strand has been compared to that of pyrene.

The solid-phase synthesis of **1** was performed by the combination of 2'-O-methyl RNA synthesis and amide-linked RNA synthesis in 38% overall coupling yield. After deprotection and HPLC purification, **1** was obtained in 53% recovery yield. The fluorescence intensity of **1** in phosphate buffer at 379 nm was 26% and 3.6% when compared to the corresponding fluorescence intensity of pyrene in MeOH or in phosphate buffer: EtOH = 4: 1 (v/v), respectively. These results suggest that the pyrene residue of **1** in the single strand is stacked with the adjacent nucleobase and the fluorescence is quenched.

In conclusion, the fluorescent property of **1** in the single strand satisfies one of the requirements for the fluorescent probe of RNA in homogeneous solution.

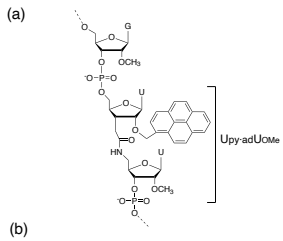

Figure 1. (a) Structure of pyrene-modified amide-linked uridine dimer region in 2'-*O*-methyl RNA, (b) Sequence of 2'-*O*-methyl RNA containing amide-linked RNA modified with pyrene (**1**).

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