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Synthesis and Crosslinking Activity of 4-*N*-(4,5′,8-Trimethylpsoralen-4′-ylmethyl)-2′-deoxycytidine-containing Oligodeoxyribonucleotides

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Photocrosslinking oligonucleotides have been developed to investigate and control gene functions without damaging living systems. Here, 4-*N*-(4,5′,8-trimethylpsoralen-4′-ylmethyl)-2′-deoxycytidine-containing oligodeoxyribonucleotide (**ODN(P)**) was newly synthesized. Photocrosslinking studies revealed that **ODN(P)** sequence-selectively crosslinked to the target with the uridine in the U–C mismatch base-pair.

Ras proteins[1] are guanine nucleotide binding proteins that transduce growth signals from the cell surface to the interior of the cell. In the past, three mutant RAS genes: K-RAS, H-RAS, and N-RAS, have been identified in humans.[2] The K-RAS gene has a G to U transversion mutation in codon 12 and is found in adenocarcinomas of the pancreas, colon, and lung.[3] K-ras protein is an oncogenic protein showing a persistent GTPase activity and activates several effectors, such as PI3 kinase, Raf-1, and RalGEFs.[4] The signaling pathways including these proteins are related to cell survival and proliferation.

Previously, we[5] and others[6] have reported sequence-selective crosslinking studies using oligonucleotides containing 4,5′,8-trimethylpsoralen derivatives. Among them, oligonucleotides containing a 4,5′,8-trimethylpsoralen derivative at the 2′-*O* hydroxy group of adenosine (2′-Ps-eom[5]) recognize one base difference in the target sequences under clinically relevant conditions. By using 2′-Ps-eom, we successfully achieved the inhibition of K-*ras*-immortalized cell proliferation (K12V) but not of Vco cells that contain the wild-type K-*ras* gene.[5] Considering potential benefits of 4,5′,8-trimethylpsoralen–nucleotide conjugates, it is important to develop oligonucleotides having 4,5′,8-trimethylpsoralen at a suitable position for the crosslinking reaction.

In 1996, Pedersen et al.[7] reported the fluorescent properties of 4-*N*-pyrenylmethyl-2′-deoxycytidine introduced in the one nucleotide bulge structure. These results suggested that a pyrene moiety was efficiently intercalated in the duplexes. In this study we newly synthesized 4-*N*-(4,5′,8-trimethylpsoralen-4′-ylmethyl)-2′-deoxycytidine (**P**) and examined the crosslinking properties of **ODN** containing **P** in a single nucleotide bulge structure with oligoRNA.[8] (Figure 1)

The synthetic route of a 4-*N*-(4,5′,8-trimethylpsoralen-4′-ylmethyl)-2′-deoxycytidine phosphoramidite unit is shown as Scheme 1. Activation of C4 of 3′,5′-bisTBDMS-2′-deoxyuridine as the tetrazolyl derivative **1** was achieved by following a previous method.[9] Conversion of **1** to 4-*N*-(4,5′,8-trimethylpsoralen-4′-ylmethyl)-2′-deoxycytidine derivative was accomplished in good yield using (4,5′,8-trimethylpsoralen-4′-yl)methylamine.[10] After removal of the TBDMS group, the chemical structure of **P** was assigned from 1D and 2D NMR measurements (Figure S1[12]). A cross-peak between CH_2 of the psoralen-4′-ylmethyl group and an amino group at the 4-position of the pyrimidine ring was observed in H–H COSY spectra, indicating that a psoralen-4′-ylmethyl group was introduced at the 4-position. Following protection of the 5′-hydroxy group and phosphitylation of the 3′-hydroxy group, a phosphoramidite unit **4** was prepared and used for the synthesis of **ODN(P)**[11] using the standard phosphoramidite DNA synthesis procedures.

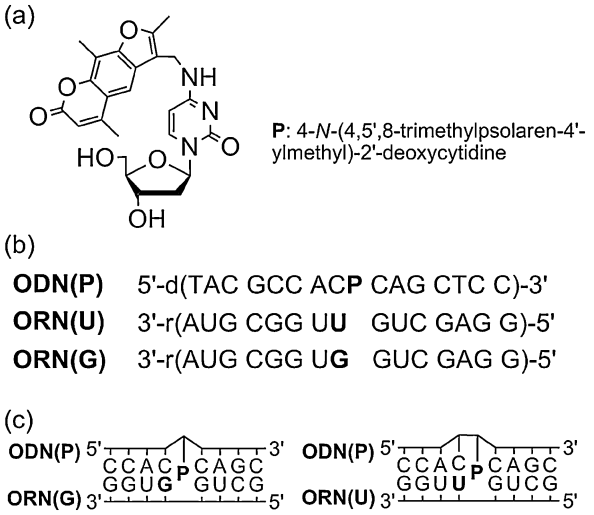
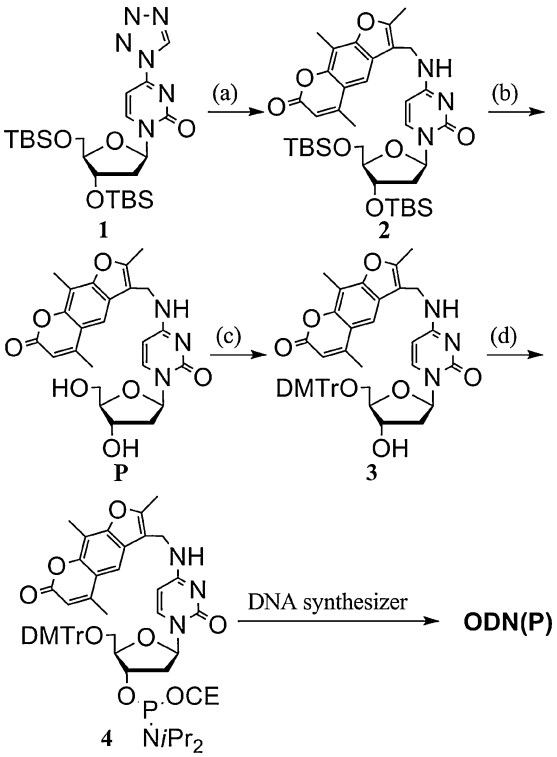


Figure 1. (a) 4-*N*-(4,5′,8-Trimethylpsolaren-4′-ylmethyl)-2′-deoxycytidine. (b) 4,5′,8-Trimethylpsoralen-containing oligodeoxynucleotides: **ODN(P)**, and target oligonucleotides: **ORN(U)** and **ORN(G)** used in this study. (c) Schematic representation of the bulge structures of the duplex formed by **ODN(P)** with **ORN(U)** or **ORN(A)**.

Photocrosslinking properties of the duplex containing a **P** in a single nucleotide bulge structure were studied (Figures 2a–2c). To estimate the reaction conditions of crosslinked duplex formation, the duplex stabilities of **ODN(P)** hybridized with **ORN(U)** or **ORN(G)**, which are 15nt oligoRNA having sequences of the K-RAS mutation, were examined. Although the duplex stability of **ODN(P)**/**ORN(U)** ($T_m = 47\,°C$) was lower than that of **ODN(P)**/**ORN(G)** ($T_m = 57\,°C$), both of the duplexes were stable under the crosslinking conditions. The photoinduced crosslinking activities of **ODN(P)** were examined in the presence of **ORN(U)** (Figure 2a) and **ORN(G)** (Figure 2b). An equimolar mixture of 5′-^{32}P-labeled **ODN(P)** and **ORN(U)** or **ORN(G)** was incubated in 100 mM phosphate buffer (0.1 M NaCl, pH 7.0) at 37 °C. After the incubation, the reaction mixtures were UV-irradiated for 0–120 min on a transilluminator (FUNAKOSHI FTI-LW, 365 nm, 1.6 mJ cm^{-2} s^{-1}), and then analyzed by denaturing PAGE. As shown in Figure 2a, a new single band in the high-molecular-



Scheme 1. (a) 1 equiv of (4,5′,8-trimethylpsolaren-4′-yl)methylamine, DMF, rt, 6 h, 80%. (b) 2 equiv of tetrabutylammonium fluoride, THF, rt, 3 h, 78%. (c) 3 equiv of 4,4′-dimethoxytrityl chloride, pyridine–DMSO (3:1, v/v), rt, 4 h, 81%. (d) 1.2 equiv of 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, 1.1 equiv of 1*H*-tetrazole, CH_3CN, rt, 4 h, 49%.

weight region was observed at the first 1 min incubation, and the intensity of that band increased in a time-dependent manner with a concomitant decrease in that of **ORN(U)**. To quantify the crosslinking reaction between **ODN(P)** and **ORN(U)** or **ORN(G)**, the crosslinking efficiency is estimated and summarized in Figure 2c. The crosslinking yield of **ODN(P)** to **ORN(U)** reached 37% after 2 h, whereas that to **ORN(U)** was less than 10%. By using an LED spot light (HLV2, 365 nm, 400 mW cm^{-2}, CCS Inc.), both crosslinking reactions reached equilibrium states within 2 min. These results suggest that **ODN(P)** favorably reacts with RNA having a mutation and is a promissing candidate for the photodynamic antisense drug. Biological assays of 4-*N*-(4,5′,8-trimethylpsoralen-4′-ylmethyl)-2′-deoxycytidine and previously reported psoralen nucleosides[5] are now in progress.

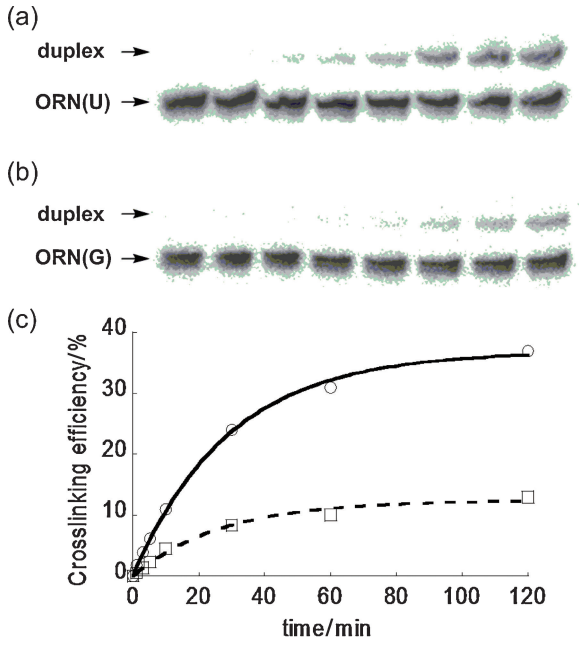


Figure 2. (a) Photocrosslinking study of **ODN(P)** with **RNA(U)**. (b) Photocrosslinking study of **ODN(P)** with **RNA(G)**. **RNA(U)** and **RNA(G)** were labeled at the 5′-end with ^{32}P and hybridized with **ODN(P)** in 100 mM of sodium phosphate buffer (pH 7.0) containing 150 mM of NaCl. The mixture were irradiated at 365 nm up to 600 s and analyzed using autoradiograms of 20% polyacrylamide/7 M urea gel. Lane 1, without UV irradiation; Lane 2, 1 min; Lane 3, 3 min; Lane 4, 5 min; Lane 5, 10 min; Lane 6, 30 min; Lane 7, 60 min; Lane 8, 120 min. (c) Time course of crosslinking efficiency of **ODN(P)**. Crosslinking efficiencies were obtained as follows: (band density of duplex)/(band density of duplex + band density of **RNA(U or G)**). Black solid line: **RNA(U)**, black broken line: **RNA(G)**.

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11 **ODN(P)** was purified by reversed-phase HPLC and analyzed by ESI-TOFMS (m/z): $[M - 5H]^{5-}$ calcd 840.2, found 840.2.

12 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

