

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 proteins¹ 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, we⁵ and others⁶ 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-eom⁵) 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 2DNMR measurements (Figure S1¹²). A cross-peak between CH₂ of the psoralen-

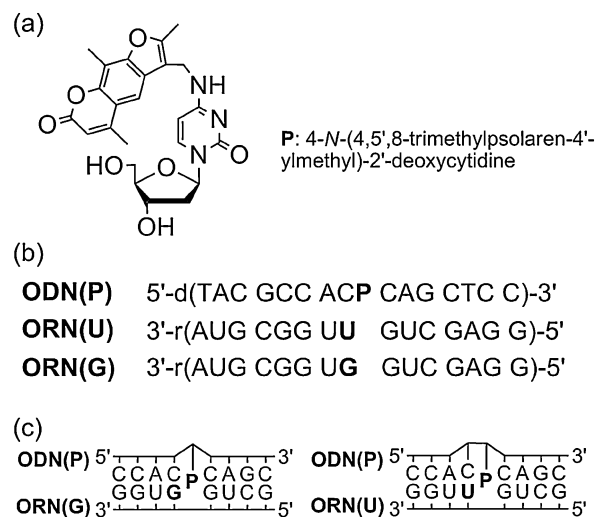
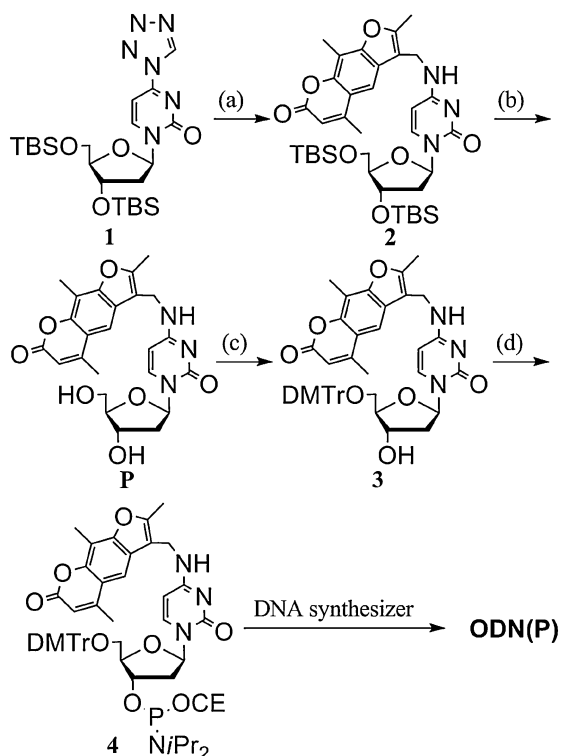


Figure 1. (a) 4-*N*-(4,5',8-Trimethylpsoralen-4'-ylmethyl)-2'-deoxycytidine. (b) 4,5',8-Trimethylpsoralen-containing oligodeoxynucleotides: **ODN(P)**, and target oligonucleotides: **ORN(U)** and **ORN(G)** used in this study. (c) Schematic representation of the bulge structures of the duplex formed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

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 phosphorylation 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.

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)** ($T_m = 47^\circ\text{C}$) was lower than that of **ODN(P)/ORN(G)** ($T_m = 57^\circ\text{C}$), 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'-³²P-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-trimethylpsoralen-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, CH₃CN, 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(G) 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 promising candidate for the photodynamic antisense drug. Biological assays of 4-*N*-(4,5',8-trimethylpsoralen-4'-ylmethyl)-2'-deoxycytidine and previously reported psoralen nucleosides⁵ are now in progress.

References and Notes

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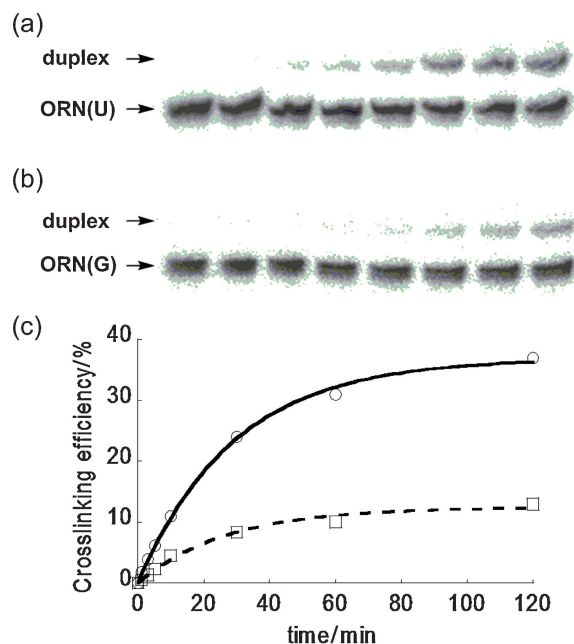


Figure 2. (a) Photocrosslinking study of ODN(P) with RNA(U). (b) Photocrosslinking study of ODN(P) with RNA(G). RNA(U) and RNA(G) were labeled at the 5'-end with ³²P and hybridized with ODN(P) in 100 mM of sodium phosphate buffer (pH 7.0) containing 150 mM of NaCl. The mixture were irradiated at 365 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>.