Synthesis of antisense oligonucleotides containing photocleavable protecting groups on the thymine bases and their photoinduced duplex formation

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ABSTRACT
Oligonucleotides containing photocleavable protecting groups at thymine bases were synthesized to induce the duplex formation by photo-irradiation. 6-Nitroveratryloxycarbonyl (NVOC) group was used for the photocleavable protecting group at N3 position of thymidine. An oligonucleotide containing NVOC groups (NVOC-ODN2: 5'-dATG CAC CATNVOC TCTNVOC GTC TGT-3') was synthesized by phosphoramidite method. The NVOC groups were found to be removable by UV irradiation at wavelength of 365nm for 5 h. UV-melting temperature (Tm value) analysis indicated that the duplex of NVOC-ODN2 with the complementary RNA was significantly unstable compared with the unmodified DNA/RNA duplex (ΔTm = -13°C). After UV irradiation at 365nm, the Tm value of the mixture increased to the almost same as that of the unmodified duplex. These results suggest that the RNA binding ability of the NVOC-ODN2 can be induced by photocleavage of the NVOC groups.

INTRODUCTION
Regulation of gene expression by synthetic oligonucleotides has become increasingly important for elucidation of gene functions and for the development of oligonucleotide therapeutics. The methodology of temporal and spatial control of the gene expression by antisense oligonucleotides will become a new research tool for the detailed study of unknown gene functions. Recently, systems for phototriggered oligonucleotide hybridization have been reported by the use of photo-induced conformational change of azobenzene group¹ or cleavage of 1-o-nitrophenyl-1,3-propanediol linker² of the modified oligonucleotides. The control of base-pair formation of oligonucleotides using photo-cleavable protecting groups on the nucleobases are also expected to induce the duplex formation by photo-irradiation. We have chosen 6-nitroveratryloxycarbonyl (NVOC) group³ to protect the N3 position of thymidine for the induction of A-T base pairing by photo-irradiation (Figure 1). In this report, we present the synthesis of NVOC-modified oligonucleotides (NVOC-ODN) (1) and the ability of photo-induced duplex formation of NVOC-ODN with the complementary RNA.

RESULTS AND DISCUSSION
First, we prepared a thymidine phosphoramidite 5 bearing NVOC group at the N3 position (Figure 2). Reaction of 3',5'-bis-O-(t-butyldimethylsilyl)thymidine with 6-nitroveratryloxycarbonyl chloride and diisopropyl-ethylamine in CH₂Cl₂ for 24 h gave NVOC-modified derivative (2) in 56% yield. The t-butyldimethylsilyl groups were removed from 2 by treatment with TBAF in presence.
of acetic acid in THF for 17 h to obtain the 3',5'-diol (3) in 98% yield. After the 5'-dimethoxytritylation of 3 (51% yield), the 3'-phosphitylation of 4 gave the NVOC-modified thymidine phosphoramidite 5 in 76% yield.

Next, NVOC-ODNs were prepared by phosphoramidite method using 5 and other nucleoside phosphoramidites bearing easily-removable protecting groups under mild basic condition at exocyclic amino functions of purine and cytosine bases (A'Pac, C'Ac, G'PrPac, T : Pac=phenoxyacetyl, iPrPac=p-(isopropyl) phenoxyacetyl). CPG support with hydroquinone-O,O-diacetic acid linker was also used. Cleavage from CPG and deprotection of the NVOC-ODN with 0.05M of potassium carbonate in methanol for 2h gave NVOC-ODNs, NVOC-ODN1 (5'-dATG CAC CAT* TCT GTC TGT-3'; T* = thymidine having a NVOC group at N3 position) and NVOC-ODN2 (5'-dATG CAC CAT* TCT* GTC TGT-3').

The NVOC group of NVOC-ODN1 was found to be removable by UV irradiation at wavelength of 365 nm (transilluminator, 3.4 mW/cm², 11 °C) in 5 h to produce unmodified oligonucleotide (Scheme 1). The rate of photolysis for NVOC-ODN2 was the same as that of NVOC-ODN1.

To examine the effect of the NVOC groups for the destabilization of the duplex structure, we measured UV melting temperature of the mixture of NVOC-ODN and complementary RNA. The duplex of the NVOC-ODN1 with the complementary RNA was unstable compared with the unmodified ODN-RNA duplex (ΔTm = -5 °C)(Table 1). In the case of NVOC-ODN2, the duplex stability with the complementary RNA was significantly reduced (ΔTm = -13 °C)(Table 1, Figure 3). These results suggest that increased number of NVOC group on oligonucleotide facilitates the destabilization of the duplex. After UV irradiation to the mixture of the NVOC-ODN1 or NVOC-ODN2 with the complementary RNA, the Tm values were almost same as that of unmodified duplex(Table 1, Figure 3). These results indicate that the RNA binding ability of NVOC-ODN was induced by UV irradiation. Thus, the photo-induced RNA binding ability of oligonucleotides was achieved by the method for the introduction of photocleavable protecting groups at the N3 positions of thymine bases.

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**REFERENCES**