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Author(s)	Yoshimura, Yoshinaga; Ito, Yoshiaki; Fujimoto, Kenzo
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Interstrand Photocrosslinking of DNA via *p*-Carbamoylvinyl Phenol Nucleoside

Yoshinaga Yoshimura^{b,*}, Yoshiaki Ito^a and Kenzo Fujimoto^{a,b,*}

^aThe School of Materials Science, Japan Advanced Institute of Science and Technology, Ishikawa 923-1292, Japan, ^bPRESTO, Japan Science and Technology Agency, Ishikawa 923-1292, Japan

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Abstract—We report a novel interstrand photocrosslinking of oligodeoxynucleotides (ODNs). In this system, a modified ODN containing *p*-carbamoylvinyl phenol nucleoside reacts by photoirradiation at 366 nm with adenine residue of a complementary template ODN to yield a crosslinked ODN in 97% yield. ©2004 Elsevier Science Ltd. All rights reserved.

The linkage of DNA binding agents to ODNs has become a popular strategy to enhance the efficiency of antisense and triplex inhibition of cellular processes.¹ Psoralens have been used for many years as interstrand photocrosslinker of nucleic acids.² Psoralens form a reversible intercalative complex with DNA and can undergo subsequent photoactivation with near-UV light and then crosslink through a [2+2] cycloaddition with thymine residues. However, interstrand photocrosslink occurs between thymine residues at the duplex site 5'-TA-3' preferentially.³ To overcome such a sequence dependence, the study of photocrosslinker has been developed. The photochemical [2+2] cycloaddition of cinnamic acid and its methyl esters with alkenes is one of the most extensively investigated and synthetically useful of photochemical reactions.⁴ These photoreactions of cinnamic acid are expected to use as photocrosslinker for the study of interstrand photocrosslink of nucleic acids. Template directed reversible DNA photoligation via 5-vinyldeoxyuridine has already reported as a tool for DNA engineering and nanotechnology."

Here we report the development of a novel interstrand photocrosslink via *p*-carbamoylvinyl phenol nucleoside (^{*p*-CV}P) in duplex DNA.⁶ We also demonstrate that the modified ODN containing ^{*p*-CV}P was photocrosslinked with adjacent adenine residue by UV irradiation.



Figure 1. Structure of *p*-carbamoylvinyl phenol nucleoside (^{*p*-CV}P).

The synthesis of the phosphoramidite of methyl ester of $p^{-CV}P$ is outlined in Scheme 1. Compound 3 was synthesized from 4-iodophenol and Hoffer's α chlorosugar. Deprotection of 3 with sodium methoxide afforded 4. Compound 4 was coupled with methylacrylate to afford $5.^7$ Compound 5 was dimethoxytritylated, and converted into the nucleoside phosphoramidite 7. assignments The of βstereochemistry at C1' for 5 was based on COSY and NOESY spectra, which showed the cross-peak between H1' and H4'. The modified ODN containing $p^{-CV}P$ was prepared according to the standard phosphoramidite chemistry on a DNA synthesizer using phosphoramidite 7. After HPLC purification, $5'-d(^{p-CV}PGCGTG)-3'$ (ODN 1) was characterized by the nucleoside composition and MALDI-TOF MS [cald 1858.3528 for (M-H), found 1858.3695]. The modified ODN 5'd(TGTGCC^{*p*-CV}PGCGTG)-3' (ODN 2) was characterized by the nucleoside composition and MALDI-TOF MS [cald 3706.49 for $(M+H)^+$, found 3706.34].

When 5'-d($^{p-CV}$ PGCGTG)-3' (ODN 1) was irradiated at 366 nm for 30 min in the presence of template ODN 3 (Scheme 2), ODN 4 was produced in 96% yield as



Scheme 1. *Reagents and conditions*: a) NaH, chlorosugar, THF, room temperature, 19 h, 62%; b) NaOCH₃, THF, room temperature, 15 h, 49%; c) methyl acrylate, Pd(OAc)₂, PPh₃, Et₃N, dioxane, 115 °C, 4 h, 41%; d) 4,4'-dimethoxytrityl chloride, DMAP, pyridine, room temperature, 16 h, 47%; e) (*i*Pr₂N)₂PO(CH₂)₂CN, 1*H*-tetrazole, acetonitrile, room temperature, 1 h, *quant*.





Figure 2. HPLC analysis of 366 nm irradiated ODN **1** and template ODN **3**; (a) before photoirradiation, (b) irradiation at 366 nm for 30 min, 96% yield. The progress of photoreaction was monitored by HPLC on a 5-ODS-H column (4.6×150 mm, elution with a solvent mixture of 50 mM animonium formate, pH 7.0, linear gradient over 30 min from 3% to 20% acetonitrile at a flow rate 1.0 mL/min).

determined by HPLC analysis (Figure 2).⁸ The reaction mixture (total volume 60 μ l) containing ODN **1** (30 μ M, strand conc.) in the presence of template ODN **3** (20 μ M, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 °C for 30 min.⁹ MALDI-TOF MS indicated that isolated ODN **4** obtained from HPLC purification was a crosslinked product of ODN **1**

and ODN **3** [cald 5485.74 for $(M+H)^+$, found 5485.98]. The isolated ODN 4 was digested with AP, svPDE, and P1 nuclease at 37 °C for 4 h. Enzymatic digestion of isolated ODN 4 showed the formation of dC, dG, dI, and dT in a ratio of 6:6:3:1 together with $dI_{-p}^{-CV}P$ photoadduct that was a deaminated product of $dA^{-p-CV}P$ adduct during enzymatic digestion process.¹⁰ The dI-^{*p*-} ^{CV}P adduct was confirmed by MALDI-TOF MS [cald 532.2043 for (M+H)⁺, found 532.1096]. We examined molecular modeling studies of the duplex 1.3. As shown in Figure 3, the vinyl group of $p^{-CV}P$ is stacked on N7-C8 double bond of adjacent adenine residue of ODN 3. It is known that photoreaction of adenine-thymine dinucleotide analog proceeds via [2+2] cycloaddition between the C5-C6 double bond of thymine and the N7-C8 double bond of adenine.¹¹ As judged from the molecular modeling, it is strongly suggested that the photocrosslink reaction proceed via [2+2] cycloaddition between the double bond of ${}^{p-CV}P$ and the N7-C8 double bond of adenine giving rise to the formation of azacyclobutane structure.



Figure 3. Molecular modeling of stacked geometry in B-form DNA. The model was optimized by AMBER* force field in water by using MacroModel version 8.1.





To demonstrate the feasibility of this photocrosslink, we examined two photoreactions of interstrand crosslink of the modified ODN containing ^{*p*-CV}P. When ODN **1** was irradiated at 366 nm for 30 min in the presence of template ODN **3** together with ODN **5**, ODN **4** was produced in 96% yield as determined by HPLC analysis (Scheme 3).⁸ The reaction mixture (total volume 60 μ l) containing ODN **1** (30 μ M, strand conc.) and ODN **5** (20 μ M, strand conc.) in the presence of template ODN

3 (20 μ M, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 °C for 30 min. When ODN **2** was irradiated at 366 nm for 30 min in the presence of template ODN **3**, ODN **6** was produced in 97% yield as determined by HPLC analysis (Scheme 4).⁸ The reaction mixture (total volume 60 μ l) containing ODN **2** (34 μ M, strand conc.) in the presence of template ODN **3** (20 μ M, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 °C for 30 min. MALDI-TOF MS indicated that isolated ODN **6** obtained from HPLC purification was a crosslinked product of ODN **2** and ODN **3** [cald 7330.92 for (M+H)⁺, found 7331.57].

To examine the influence of photocrosslink reaction on the thermal stability, the melting temperature (T_m) of the



Figure 4. Melting curves of the **2•3** duplex (a) and ODN **6** (b). $T_{\rm m}$ values of the **2•3** duplex (5.0 μ M) and ODN **6** (5.0 μ M) were measured in 50 mM sodium cacodylate and 100 mM sodium chloride, pH 7.0.

duplex 2•3 or ODN 6 was determined by UV-monitored thermal denaturation. As shown in Figure 4, the duplex 2•3 showed a melting temperature of 50.5 °C, whereas ODN 6 melted at over 80 °C. Example of this behavior has been seen for crosslinked ODNs by the introduction of thiol groups.¹² Thus, photocrosslinking increased $T_{\rm m}$ of ODN, a dramatic stabilization of the duplex form.

In conclusion, we demonstrate here that a modified ODN containing ^{p-CV}P can be crosslinked by irradiating at 366 nm with adjacent adenine residue in a [2+2] manner. This feature of the photoreactivity may provide the intriguing methodology that is applicable to antisense and antigene.

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7. Spectroscopic data for selected compounds are as follows. **5**: ¹H NMR (CDCl₃, 300 MHz) δ 2.32 (dt, 1H, *J* = 14.2 Hz, 5.7 Hz), 2.55 (ddd, 1H, *J* = 14.2 Hz, 6.9 Hz, 1.7 Hz), 3.61-3.75 (m, 2H), 3.77 (s, 3H), 4.07-4.11 (m, 1H), 4.62-4.68 (m, 1H), 5.90 (dd, 1H, *J* = 5.7 Hz, 1.7 Hz), 6.30 (d, 1H, *J* = 16.2 Hz), 7.05 (d, 2H, *J* = 8.6 Hz), 7.44 (d, 2H, *J* = 8.6 Hz), 7.62 (d, 1H, *J* = 16.2 Hz), HRMS (MALDI): calcd for C₁₅H₁₈O₆Na [(M+Na)⁺] 317.0996, found 317.0938, UV (H₂O:CH₃OH = 1:1) λ_{max} (ϵ) 306 nm (14,300 M⁻¹cm⁻¹), $\epsilon_{366} = 155$.

8. The yield was calculated on the basis of ODN 3.

9. Isolation of each interstrand crosslinked photoproducts that were produced in two photoreactions of ODN **1** in the presence of template ODN 5'-d(CACGC<u>T</u>GGCACA)-3' or 5'-d(CACGC<u>C</u>GGCACA) -3' was not successful due to furnish lower conversions of photo-induced crosslinking reactions.

10. $dI_{-p}^{-CV}P$ adduct: UV (H₂O) λ_{max} (ϵ) 256 nm (7,540 M⁻¹cm⁻¹).

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The development of a novel interstrand photocrosslink via *p*-carbamoylvinyl phenol nucleoside (^{*p*-CV}P) is reported.