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Interstrand photocrosslinking of DNA via *p*-carbamoylvinyl phenol nucleoside

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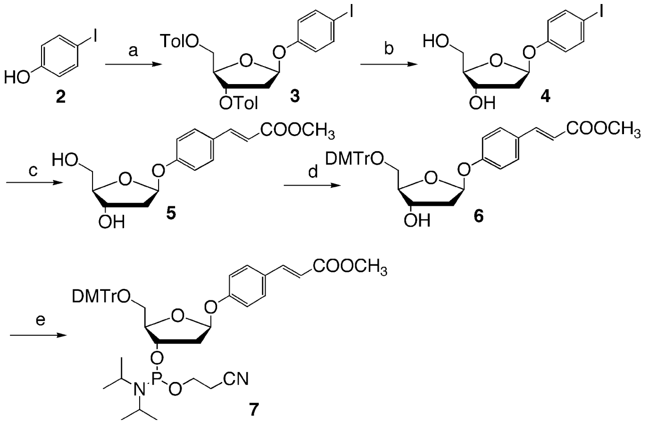
Abstract—We report a novel interstrand photocrosslinking of oligodeoxynucleotides (ODNs). In this system, a modified ODN containing *p*-carbamoylvinyl phenol nucleoside reacts by photoirradiation at 366 nm with adenine residue of a complementary template ODN to yield a crosslinked ODN in 97% yield.

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.[5]

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

The synthesis of the phosphoramidite of methyl ester of $^{p\text{-CV}}$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**. The assignments of β-stereochemistry at C1′ for **5** was based on COSY and NOESY spectra, which showed the cross-peak between H1′ and H4′. The modified ODN



Scheme 1. Reagents and conditions: (a) NaH, chlorosugar, THF, room temperature, 19 h, 62%; (b) $NaOCH_3$, THF, room temperature, 15 h, 49%; (c) methyl acrylate, $Pd(OAc)_2$, PPh_3, Et_3N, dioxane, 115 °C, 4 h, 41%; (d) 4,4′-dimethoxytrityl chloride, DMAP, pyridine, room temperature, 16 h, 47%; (e) $(iPr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, acetonitrile, room temperature, 1 h, quant.

Keywords: Photocrosslink; Nucleoside; Cycloaddition.

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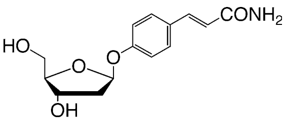
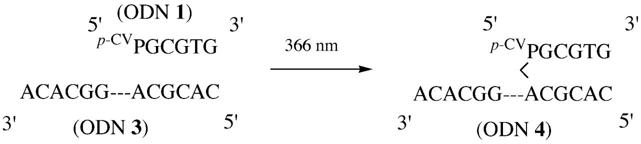
containing $^{p\text{-CV}}$P was prepared according to the standard phosphoramidite chemistry on a DNA synthesizer using phosphoramidite **7**. After HPLC purification, 5′-d($^{p\text{-CV}}$PGCGTG)-3′ (ODN **1**) was characterized by the nucleoside composition and MALDI-TOF MS [calcd 1858.3528 for $(M-H)^-$, found 1858.3695]. The modified ODN 5′-d(TGTGCC$^{p\text{-CV}}$PGCGTG)-3′ (ODN **2**) was characterized by the nucleoside composition and MALDI-TOF MS [calcd 3706.49 for $(M+H)^+$, found 3706.34] (Fig. 1).

When 5′-d($^{p\text{-CV}}$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 determined by HPLC analysis (Fig. 2).[8] The reaction mixture (total volume 60 µL) containing ODN **1** (30 µM, strand concn) in the presence of template ODN **3** (20 µM, strand concn) 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** [calcd 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–$^{p\text{-CV}}$P photoadduct that was a deaminated product of dA–$^{p\text{-CV}}$P adduct during enzymatic digestion process.[10] The dI–$^{p\text{-CV}}$P adduct was confirmed by MALDI-TOF MS [calcd 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 $^{p\text{-CV}}$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 $^{p\text{-CV}}$P and the N7–C8 double bond of adenine giving rise to the formation of aza-cyclobutane structure.



$^{p\text{-CV}}$P (**1**)

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



Scheme 2. Interstrand photocrosslink of ODN **1** in the presence of template ODN **3**.

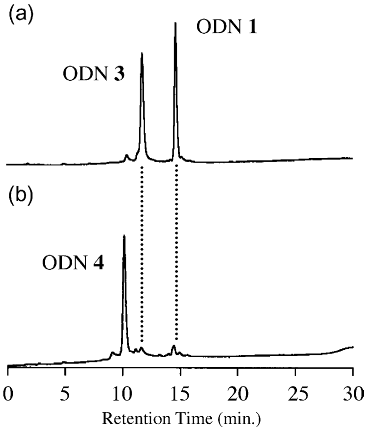


Figure 2. HPLC analysis of 366 nm irradiated ODN **1** and template ODN **3**; (a) before photoirradiation, (b) irradiation at 366 nm for 30 min, 96% yield. The progress of photoreaction was monitored by HPLC on a 5-ODS-H column (4.6 × 150 mm, elution with a solvent mixture of 50 mM ammonium formate,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

To demonstrate the feasibility of this photocrosslink, we examined two photoreactions of interstrand crosslink of the modified ODN containing $^{p\text{-CV}}$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 µL) containing ODN **1** (30 µM, strand concn) and ODN **5** (20 µM, strand concn) in the presence of template ODN **3** (20 µM, strand concn) 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 µL) containing ODN **2** (34 µM, strand concn) in the

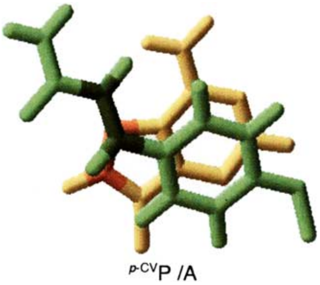
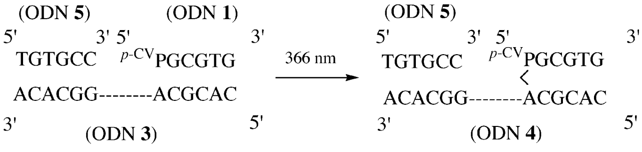
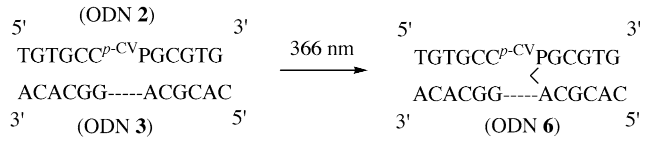


Figure 3. Molecular modeling of stacked geometry in B-form DNA. The model was optimized by AMBER* force field in water by using MacroModel version 8.1.



Scheme 3. Interstrand photocrosslink using ODN **1**.



Scheme 4. Interstrand photocrosslink using ODN **2**.

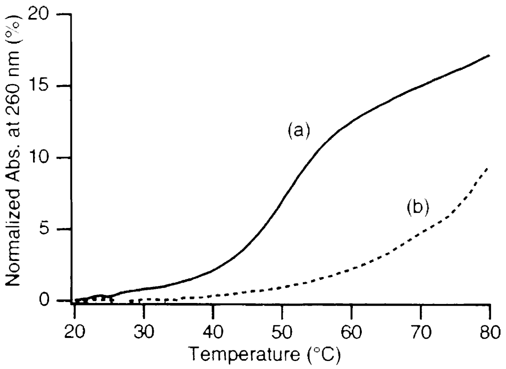


Figure 4. Melting curves of the **2** · **3** duplex (a) and ODN **6** (b). T_m values of the **2** · **3** duplex (5.0 µM) and ODN **6** (5.0 µM) were measured in 50 mM sodium cacodylate and 100 mM sodium chloride, pH 7.0.

presence of template ODN **3** (20 µM, strand concn) 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** [calcd 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 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_m of ODN, a dramatic stabilization of the duplex form.

In conclusion, we demonstrate here that a modified ODN containing $^{p\text{-CV}}$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. Compound **5**: ^{1}H NMR ($CDCl_3$, 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 $C_{15}H_{18}O_6Na$ $[(M+Na)^+]$ 317.0996, found 317.0938, UV (H_2O/CH_3OH = 1:1) λ_{max} (ε) 306 nm (14,300 M^{-1} cm^{-1}), $\varepsilon_{366} = 155$.
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(CACGCTGGCACA)-3′ or 5′-d(CACGCCGGCACA) -3′ was not successful due to furnish lower conversions of photo-induced crosslinking reactions.
10. dI–$^{p\text{-CV}}$P adduct: UV (H_2O) λ_{max} (ε) 256 nm (7540 M^{-1} cm^{-1}).
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