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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. ©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.⁵

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

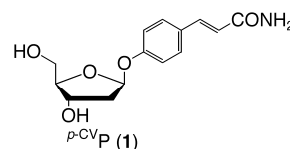
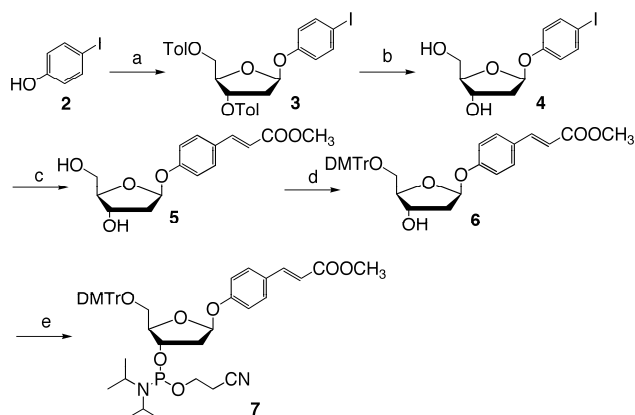


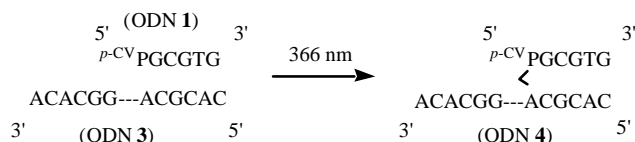
Figure 1. Structure of *p*-carbamoylvinyl phenol nucleoside (*p*-CVP).

The synthesis of the phosphoramidite of methyl ester of *p*-CVP 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 containing *p*-CVP was prepared according to the standard phosphoramidite chemistry on a DNA synthesizer using phosphoramidite **7**. After HPLC purification, 5'-d(*p*-CVPGCGTG)-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*-CVPGCGTG)-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*-CVPGCGTG)-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) (iPr₂N)₂PO(CH₂)₂CN, 1H-tetrazole, acetonitrile, room temperature, 1 h, *quant*.



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

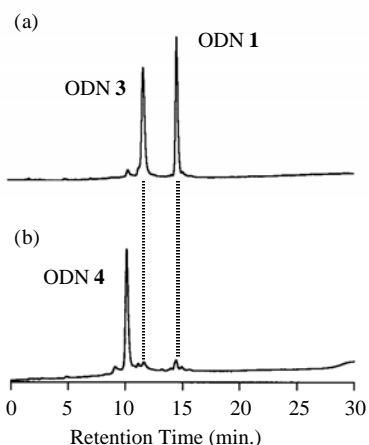


Figure 2. HPLC analysis of 366 nm irradiated ODN 1 and template ODN 3; (a) before photoirradiation, (b) irradiation at 366 nm for 30 min, 96% yield. The progress of photoreaction was monitored by HPLC on a 5-ODS-H column (4.6 × 150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0,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

determined by HPLC analysis (Figure 2).⁸ The reaction mixture (total volume 60 μl) containing ODN 1 (30 μM, strand conc.) in the presence of template ODN 3 (20 μM, 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 [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-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 [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-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.

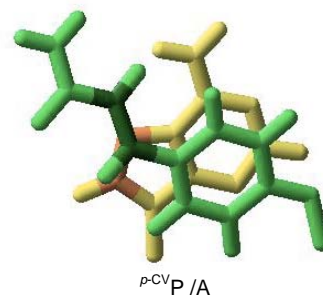
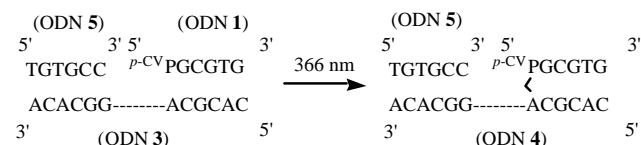
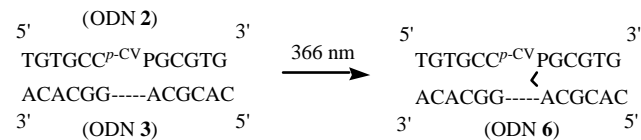


Figure 3. Molecular modeling of stacked geometry in B-form DNA. The model was optimized by AMBER* force field in water by using MacroModel version 8.1.



Scheme 3. Interstrand photocrosslink using ODN 1.



Scheme 4. Interstrand photocrosslink using ODN 2.

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 μl) containing ODN 1 (30 μM, strand conc.) and ODN 5 (20 μM, strand conc.) in the presence of template ODN

3 (20 μM , strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 $^{\circ}\text{C}$ 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 conc.) in the presence of template ODN **3** (20 μM , strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 $^{\circ}\text{C}$ 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 $(\text{M}+\text{H})^+$, found 7331.57].

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

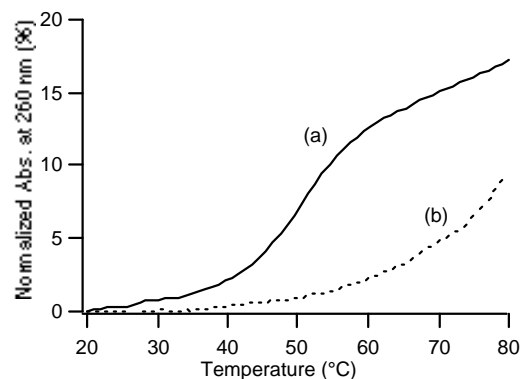


Figure 4. Melting curves of the **2•3** duplex (a) and ODN **6** (b). T_m values of the **2•3** duplex (5.0 μM) and ODN **6** (5.0 μM) were measured in 50 mM sodium cacodylate and 100 mM sodium chloride, pH 7.0.

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 $^{\circ}\text{C}$, whereas ODN **6** melted at over 80 $^{\circ}\text{C}$. 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.

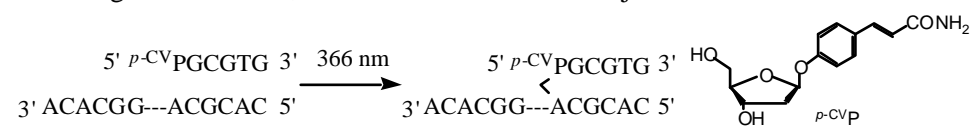
In conclusion, we demonstrate here that a modified ODN containing $p\text{-CV}P$ can be crosslinked by irradiating at 366 nm with adjacent adenine residue in a [2+2] manner. This feature of the photoreactivity may provide the intriguing methodology that is applicable to antisense and antigene.

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- Spectroscopic data for selected compounds are as follows. **5**: ^1H 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 $\text{C}_{15}\text{H}_{18}\text{O}_6\text{Na}$ $[(\text{M}+\text{Na})^+]$ 317.0996, found 317.0938, UV ($\text{H}_2\text{O}:\text{CH}_3\text{OH} = 1:1$) λ_{max} (ϵ) 306 nm ($14,300 \text{ M}^{-1}\text{cm}^{-1}$), $\epsilon_{366} = 155$.
- The yield was calculated on the basis of ODN **3**.
- 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.
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The development of a novel interstrand photocrosslink via *p*-carbamoylvinyl phenol nucleoside (*p*-CVP) is reported.