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Highly Selective and Sensitive Template-Directed Photoligation of DNA via 5-Carbamoylvinyl-2′-deoxycytidine

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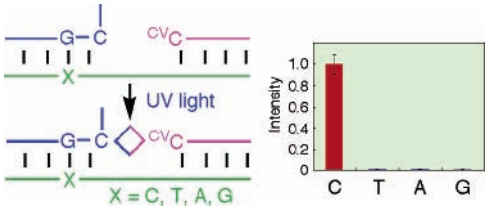
ABSTRACT



We describe a highly efficient template-directed photoligation of oligodeoxynucleotides (ODNs) through 5-carbamoylvinyl-2′-deoxycytidine (^{CV}C). When an ODN containing ^{CV}C at the 5′ end was photoirradiated with an ODN containing a pyrimidine base at the 3′ end in the presence of template DNA, efficient photoligation was observed without any byproduct formation. Single nucleotide differences can be successfully distinguished by using photoligation-based DNA chip assay.

Enzymatic ligation[1] and chemical ligation[2] have been developed in the detection of single nucleotide differences. Recently, DNA ligation on a DNA chip has drawn attention to the high-throughput method of single nucleotide polymorphisms (SNPs) detection. The rapid identification of SNPs will accelerate the identification of disease genes and diagnosis of particular genetic predispositions toward drug response.[3] Although enzymatic ligation-based SNPs assay on a DNA chip has been reported,[4] enzyme-linked assay needs to include the careful selection of the most suitable conditions, including temperature, pH, and salt concentration, because of the use of enzyme. We previously described photochemical DNA ligation-based SNPs assay and the specific detection of RNA sequences on a DNA chip.[5] Template-directed photoligation with 5-carboxyvinyl-2′-deoxyuridine (^{CV}U)[6] can be used for the detection of DNA or RNA sequences with high sensitivity and specificity. On the other hand, due to the limitation of photoligation with ^{CV}U, which is only applicable to adenine as a counter base in a duplex, we disclosed template-directed photoligation with 5-vinyl-2′-deoxycytidine (^{V}C) as shown in Figure 1A.[7]

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(1) (a) Landegren, U.; Kaiser, R.; Sanders, J.; Hood, L. *Science* **1988**, *241*, 1077−1080. (b) Luo, J.; Bergstrom, D. E.; Barany, F. *Nucleic Acids Res.* **1996**, *24*, 3071−3078.
(2) Xu, Y.; Karalkar, N. B.; Kool, E. T. *Nat. Biotechnol.* **2001**, *19*, 148−152.
(3) McCarthy, J. J.; Hilfiker, R. *Nat. Biotechnol.* **2000**, *18*, 505−508.
(4) (a) Zhong, X. B.; Reynolds, R.; Kidd, J. R.; Kidd, K. K.; Jenison, R.; Marlar, R. A.; Ward, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11559−11564. (b) Deng, J.-Y.; Zhang, X.-E.; Mang, Y.; Zhang, Z.-P.; Zhou, Y.-F.; Liu, Q.; Lu, H.-B.; Fu, Z.-J. *Biosens. Bioelectron.* **2004**, *19*, 1277−1283.
(5) (a) Yoshimura, Y.; Noguchi, Y.; Sato, H.; Fujimoto, K. *ChemBioChem* **2006**, *7*, 598−601. (b) Ogasawara, S.; Fujimoto, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 4512−4515.
(6) (a) Fujimoto, K.; Matsuda, S.; Takahashi, N.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5646−5647. (b) Fujimoto, K.; Ogawa, N.; Hayashi, M.; Matsuda, S.; Saito, I. *Tetrahedron Lett.* **2000**, *41*, 9437−9440. (c) Saito, I.; Miyauchi, Y.; Saito, Y.; Fujimoto, K. *Tetrahedron Lett.* **2005**, *46*, 97−99. (d) Ogasawara, S.; Fujimoto, K. *ChemBioChem* **2005**, *6*, 1756−1760. (e) Fujimoto, K.; Matsuda, S.; Yoshimura, Y.; Matsumura, T.; Hayashi, M.; Saito, I. *Chem. Commun.* **2006**, 3223−3225.
(7) Fujimoto, K.; Matsuda, S.; Ogawa, N.; Hayashi, M.; Saito, I. *Tetrahedron Lett.* **2000**, *41*, 6451−6454.

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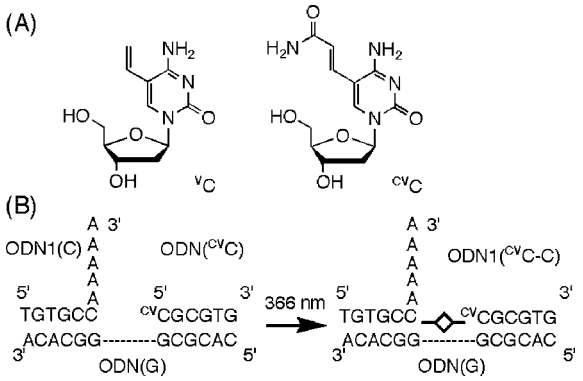

Figure 1. (A) Structure of photoresponsive nucleosides ^{V}C and ^{CV}C. (B) Schematic illustration of photoligation of ODNs with ^{CV}C.

However, in the photoligation with ^{V}C, a long photoirradiation time at 366 nm was required to complete photoligation and the 3′-terminal C could not react with 5′-terminal ^{V}C. To overcome the limitation of photoligation with ^{V}C, we now report on a highly efficient template-directed photoligation of ODNs through ^{CV}C. The availability of photoligation with ^{CV}C also allowed us to detect single nucleotide differences.

The nucleoside phosphoramidite of ^{CV}U was prepared according to a method reported in the literature.[6] The triazole derivative of ^{CV}U was prepared from the nucleoside phosphoramidite of ^{CV}U by using $POCl_3$/1,2,4-triazole in quantitative yield (see Supporting Information).[8] This monomer was incorporated into ODN using standard automated DNA synthesis protocols.[6,7] After deprotection and purification of the oligomer, ODN containing ^{CV}C, ODN(^{CV}C) (5′-d(^{CV}CGCGTG)-3′), was characterized by the nucleoside composition and MALDI−TOF−MS (calcd 1877.37 for $[M + H]^+$; found 1877.40).

We determined the feasibility of the template-directed DNA photoligation via ODN(^{CV}C).[9] When ODN(^{CV}C) and ODN1(C) (5′-d(TGTGCCAAAAA)-3′) were irradiated at 366 nm for 20 min in the presence of template ODN(G) (5′-d(CACGCGGGCACA)-3′), capillary gel electrophoresis (CGE) showed the appearance of a peak relating to ODN1(^{CV}U-C) in 97% yield along with the disappearance of ODN(^{CV}C) and ODN1(C) peaks (Figure 1B).[10] MALDI−TOF−MS indicates that the isolated ODN1(^{CV}C-C) obtained from HPLC purification was a photoligated product of ODN(^{CV}C) and ODN1(C) (calcd 5227.57 for $[M + H]^+$; found 5227.61). Enzymatic digestion of isolated ODN1(^{CV}C-C) showed the formation of dC, dG, dT, and dA in a ratio of 2:5:3:5 together with dC-^{CV}C photoadduct, which was confirmed by MALDI−TOF−MS. The structure of dC-^{CV}C photoadduct was assigned as a *cis*−*syn* [2 + 2] adduct on the basis of spectroscopic data including ^{1}H−^{1}H COSY and NOESY, as reported previously.[6,7] When ODN2(C) (5′-d(TGTGCC)-3′) was used in photoligation, we observed the appearance of the peak of ODN2(^{CV}C-C) in 97% yield as determined by CGE.[11] On the other hand, when ODN1(T) (5′-d(TGTGCTAAAAA)-3′) or ODN2(T) (5′-d(TGTGCT)-3′) was used in photoligation, the 3′-terminal T reacted with photoexcited ^{CV}C to produce a photoligated product ODN1(^{CV}C-T) or ODN2(^{CV}C-T) as effective as 3′-ternimal C, respectively (see Supporting Information).[11] As shown in Figure 2, the

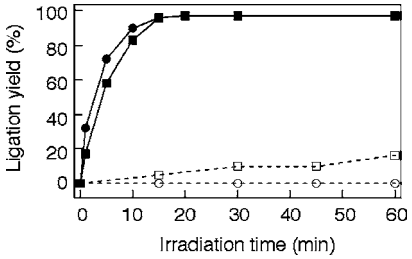

Figure 2. Comparison of photoligation rates with ODN(^{CV}C) (filled symbols) versus ODN(^{V}C) (open symbols). Circles represent ODN1(C) that had been photoligated, and squares denote ODN1-(T) that had been photoligated.

photoligation rates by using ODN(^{CV}C) were 64-fold more rapid than the corresponding ODN containing ^{V}C, ODN-(^{V}C) (5′-d(^{V}CGCGTG)-3′). Furthermore, ODN(^{CV}C) can be efficiently photoligated not only with 3′-terminal T but also with 3′-terminal C.

To demonstrate that template-directed photoligation by using ODN(^{CV}C) could be incorporated into platforms suitable for DNA chip technologies, we constructed the DNA chip by attaching amino-labeled ODN containing ^{CV}C,[12] amino-ODN(^{CV}C) (5′-d(^{CV}CGCGTG)-SSSS-NH_2-3′; here S corresponds to a hexa(ethylene glycol) linker fragment), onto the aldehyde-modified glass surface.[5]

We determined the feasibility of the template-directed photoligation through ODN(^{CV}C) on a DNA chip. A glass chip spotted with 2 μM target ODN(G) and biotin-labeled ODN1(C) (5′-biotin-TGTGCCAAAAA-3′) was irradiated at 366 nm for 1 h in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride (Figure 3A). After the chip had been washed with deionized water at 98 °C for 5 min, a phosphate-buffered saline (PBS) solution of streptavidin-Cy3 conjugate was added to the surface, and the chip

(8) (a) Xu, Y.-Z.; Zheng, Q.; Swann, P. F. *J. Org. Chem.* **1992**, *57*, 3839−3845. (b) Scherr, M.; Klebba, C.; Häner, R.; Ganser, A.; Engels, J. W. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1791−1796.

(9) ^{V}C: λ_{max} (H_2O) 289 nm, ϵ 5,400 M^{-1} cm^{-1}; 366 nm, ϵ 13 M^{-1} cm^{-1}. ^{CV}C: λ_{max} (H_2O) 301 nm, ϵ 8,770 M^{-1} cm^{-1}; 366 nm, ϵ 132 M^{-1} cm^{-1}.

(10) The reaction mixture (total volume 60 μL) containing ODN(^{CV}C) and ODN1(C) (each 20 μM, strand concentration) in the presence of template ODN(G) (24 μM, strand concentration) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with a UV-LED (366 ± 15 nm light at 700 mW/cm^2) at 0 °C for 20 min. After irradiation, the progress of the photoreaction was monitored by CGE. The yield was calculated on the basisof ODN1(C).

(11) MALDI−TOF−MS: calcd 3661.52 for ODN2(^{CV}C-C) [$(M + H)^+$], found 3661.64; calcd 5542.58 for ODN1(^{CV}C-T) [$(M + H)^+$], found 5542.49; calcd 3676.53 for ODN2(^{CV}C-T) [$(M + H)^+$], found 3676.42. Quantum yields of the formation of photoligated products were measured at 366 nm, based on the disappearance of ODN1(T) by employing valerophenone as an actinometer. The formation of ODN1(^{CV}C-T): Φ = 0.158. The formation of ODN1(^{V}C-T): Φ = 0.092.

(12) MALDI−TOF−MS: calcd 3436.67 for amino-ODN(^{CV}C) [$(M + H)^+$], found 3436.08.

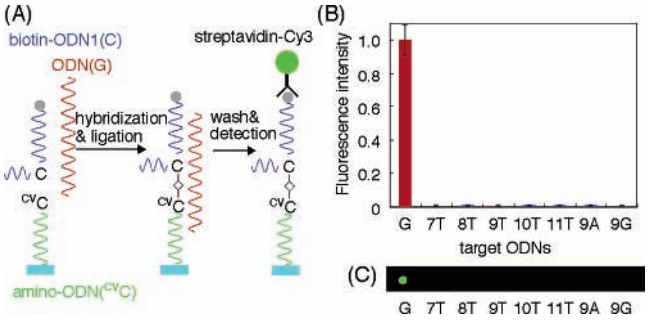

Figure 3. (A) Strategy for the detection of single nucleotide differences on a DNA chip. (B) Fluorescence intensity acquired on a microarray scanner for the product of photoligation on matched and singly mismatched target ODNs. (C) Fluorescence images.

was washed twice in PBS. Fluorescence signals were detected on a microarray scanner. As shown in Figure 3B and C, we measured the strong fluorescence signal of the photoligated product with the completely complementary case. To investigate the generality of sequence discrimination, we constructed five target ODNs with a mismatch at various positions. Results show that a single mismatch at the ninth ODN position yielded very little photoligated product, with a measured fluorescence signal that was 143-fold lower than that of the completely complementary case (Table 1). Then we constructed a set of four closely related target ODNs with a single variable base (A, T, G, or C) in the ninth position. Most mismatches give relative fluorescence signals of 1% or less than the correctly matched case.

In conclusion, we demonstrated template-directed photoligation through ^{CV}C. When an ODN containing ^{CV}C at the 5′ end was photoirradiated with an ODN containing a pyrimidine base at the 3′ end in the presence of template DNA, efficient photoligation was observed without any byproduct formation. Furthermore, single nucleotide differences at various positions can be successfully distinguished by using template-directed photoligation-based DNA chip assay. Therefore, we established the design and synthesis of four photoresponsive nucleotide analogues (C, U, A, and G), which can be photoligated with a pyrimidine base at the 3′ end.[13] We previously established the artificial site-specific

Table 1. Normalized Fluorescence Intensity for the Photoligated Product of Amino-ODN(^{CV}C), Correctly Base Paired to Eight ODN Targets That Differed in a Single Nucleotide Position.

	target ODN[a]	fluorescence intensity[b]
ODN(G)	5′-d(CACGCGGGCACA)-3′	1.0 ± 0.09
ODN(7T)	5′-d(CACGCGTGCACA)-3′	0.007 ± 0.004
ODN(8T)	5′-d(CACGCGGTCACA)-3′	0.008 ± 0.004
ODN(9T)	5′-d(CACGCGGGTACA)-3′	0.007 ± 0.004
ODN(10T)	5′-d(CACGCGGGCTCA)-3′	0.010 ± 0.005
ODN(11T)	5′-d(CACGCGGGCATA)-3′	0.009 ± 0.002
ODN(9A)	5′-d(CACGCGGGAACA)-3′	0.010 ± 0.004
ODN(9G)	5′-d(CACGCGGGGACA)-3′	0.005 ± 0.004

[a] Underlined characters indicate a mismatched base. [b] Each experiment was repeated at least three times.

transition from cytosine to uracil mediated by reversible DNA photoligation through ^{CV}U.[6] We might use three other nucleotide analogues for the site-specific transition. Template-directed photoligation through these photoresponsive nucleotide analogues can potentially be developed into a high-throughput DNA-analysis system for SNPs detection and genetic manipulation, such as the construction of DNA nanoarchitectures[6] and the site-specific transition.

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Supporting Information Available: Experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) When ODN containing 7-carboxyvinyl-7-deaza-2′-deoxyguanosine at the 5′ end was photoirradiated with ODNs containing a pyrimidine base at the 3′ end in the presence of template ODN, efficient photoligation was observed without any byproduct formation.