Template-Directed Photoreversible Ligation of Deoxyoligonucleotides via 5-Vinyldeoxyuridine

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While many methods for template-directed chemical ligation of oligonucleotides via a native phosphodiester bond or non-native linkages have been demonstrated,[1] there are only a few methods for photoinduced non-enzymatic chemical ligation.[2] The merit of the photochemical ligation avoiding the need for additional reagents is obvious. Their actions are controllable within space and time by the choice of proper irradiation methods. Thus, the photoligation methods can be used as "photopadlocking" of circular DNAs,[2d] as a tool for DNA engineering and nanotechnology, and as photoregulated diagnostic and therapeutic agents. Previously reported methods for DNA photoligation utilized, (i) the thymine dimer formation by irradiation at >290 nm,[2a] (ii) photoreactions of DNA containing appended stilbenes[2b] or coumarins[2c], and (iii) the photoaddition reaction of 4-thiothymidine.[2d] However, these methods have serious problems for practical applications, such as low yields of photoligation,[2a,d] the use of short wavelength that is injurious to other components,[2a] the occurrence of undesirable photo-cross-linking reaction[2d] and the lack of photoreversibility.[2b,d] It seems therefore highly desirable to develop more efficient and clean photoligation methods that are effective at >360 nm and preferably possess high photoreversibility. We now wish to report a highly efficient and reversible template-directed photoligation of oligodeoxynucleotides (ODNs) which uses 5-vinyldeoxyuridine and which is far superior to the existing methods. We also demonstrate that several oligonucleotides are simultaneously ligated on a DNA template by 366 nm irradiation to produce a longer DNA, and the resulting ligated DNA is quantitatively reverted to the original oligonucleotides by 302 nm irradiation.

ODN **1** containing 5-vinyldeoxyuridine (^{V}U) at the 5′ end was prepared by standard automated DNA synthesis using β-cyanoethylphosphoramide of ^{V}U.[3] When ODN **1** and ODN **2** were irradiated at 366 nm[4] in the absence of template, no photoligation product was observed, but in the presence of template ODN **3**, the expected ligated 12-mer ODN **4** was produced in 80% yield as determined by densitometric assay of PAGE (Figure 1, lane 4).[5] The yield of the photoligated ODN increased up to 96% by increasing irradiation time for 12 h. HPLC analysis of the photoirradiated mixture of ODN **1** and ODN **2** in the presence of template ODN **3** indicated a clean and efficient formation of ligated ODN **4** with concomitant disappearance of ODNs **1** and **2**. ESI-TOF MS indicated that ODN **4** is a ligated product of ODN **1** with ODN **2**.[7] Enzymatic digestion of isolated ODN **4** followed by HPLC analysis indicated the formation of dC, dG, and dT in a ratio of 2:5:3 together with a new product. Spectroscopic data including ESI-TOF MS indicated that this new product was dU−^{V}U adduct **5** which was derived from deamination of initially formed dC−^{V}U adduct by the action of alkaline phosphatase.[8] In fact, photoligated ODN **4** was stable at pH 5.0−9.0 even at 90 °C, but ODN **4** was rapidly deaminated by adding alkaline phophatase to produce dU−^{V}U containing ODN. These results clearly indicated that dU−^{V}U adduct **5** was produced from deaminaion of initially formed dC−^{V}U adduct during enzymatic digestion. The structure of **5** obtained by HPLC purification was assigned as a *cis-syn* [2 + 2] adduct **5** on the basis of spectroscopic data including ^{1}H−^{1}H COSY and NOESY.[8] Molecular modeling studies of the duplex consisting of ODN **1**, ODN **2**, and template ODN **3** suggested that the vinyl group of the 5′-terminal ^{V}U of ODN **1** in its *s-trans* conformation is stacked with the 5,6-double bond of the 3′-terminal dC of ODN **2** to be able to produce the *cis-syn* [2 + 2] adduct. The selectivity of the photoaddition of 5′-terminal ^{V}U to 3′-terminal bases of ODNs to be ligated is remarkable. A and G at the 3′ end did not undergo photoaddition with 5′-terminal ^{V}U, whereas the 3′-terminal T could react with photoexcited ^{V}U to produce adducts (Scheme 1). In contrast, the 3′-terminal ^{V}U cannot undergo photoaddition with the 5′-terminal C and T as predicted by molecular modeling.

To confirm the photoreversibility of the ligation process, irradiation of the photoligated product at 302 nm was examined. As shown in Figure 1, a rapid disappearance of ligated 12-mer was observed by 302 nm irradiation to revert to 6-mers (lane 5). Further irradiation of lane 5 at 366 nm resulted in a reappearance

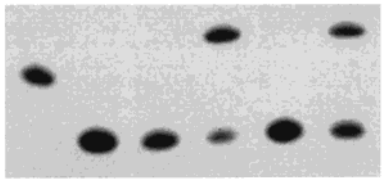
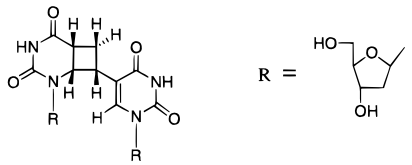


Figure 1. Autoradiogram of a denaturing polyacrylamide gel electrophoresis of photoreaction of ODN **1** (7 μM) and ^{32}P-5′-end-labeled ODN **2** (0.3 μM) in the presence of template ODN **3** (9 μM) in sodium cacodylate buffer (50 mM, pH 7.0) containing NaCl (100 mM). Photoirradiation was conducted in an Eppendorf tube with transilluminator at 366 or 302 nm at 0 °C. Lane 1, control 12-mer; lane 2, control 6-mer; lane 3, ODN **1** + ODN **2**, irradiation at 366 nm; lane 4, ODN **1** + ODN **2** + ODN **3**, irradiation at 366 nm, 3 h; lane 5, irradiation of lane 4 at 302 nm, 1 h; Lane 6, irradiation of lane 5 at 366 nm, 3 h.



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(4) ^{V}U: λ_{max} (water) 284 nm ϵ 7300 (ϵ at 366 nm, 3.3).

(5) The sensitivity of the photoligation to mismatches at the photoligation site was quite similar to those observed for enzymatic ligation with T4 DNA ligase[6] and photoligation using 4-thiothymidine.[2d] See Supporting Information.

(6) Landegren, U.; Kaiser, R.; Sanders, J.; Hood, L. *Science* **1998**, *241*, 1077.

(7) ESI-TOFF MS: calcd for ODN **4** ($C_{118}H_{150}N_{42}O_{72}P_{10}$) ($M - H^{-}$) 3615.67; found 3615.60.

(8) ESI-TOFF MS: calcd for **5** ($C_{20}H_{25}N_{40}O_{10}$)($M - H^{-}$) 481.1570; found 481.1510. See Supporting Information.

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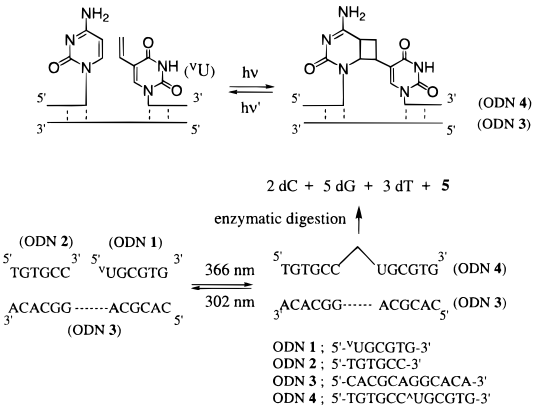
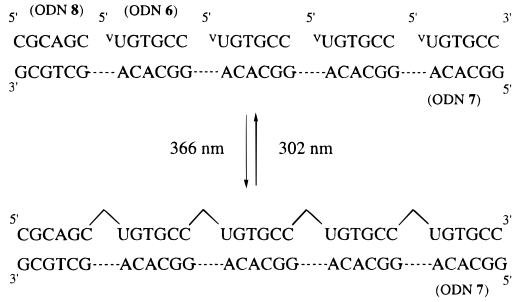
Scheme 1



of ligated 12-mer ODN **4**, indicating a high reversibility of the photoligation process. HPLC analysis of the photoirradiated mixture of ODN **4** at 302 nm also confirmed the clean formation of ODN **1** and ODN **2** from ODN **4** as evidenced by the retention time and the photodiodoarray assay of their HPLC peaks. HPLC analysis of enzymatic digestion mixture of photoreverted ODN **1** and ODN **2** indicated no photodamage on both ODNs by 302 nm irradiation.

To demonstrate the feasibility of this efficient and reversible photoligation, we examined a simultaneous ligation of five ODNs on template ODN **7** (Scheme 2). When ~5 equiv mol (36 μM)

Scheme 2



of 5′-^{V}UGTGCC-3′ (ODN **6**) was irradiated at 366 nm in the presence of 30-mer template ODN **7** (7 μM) together with ^{32}P-5′-end-labeled 5′-CGCAGC-3′ (ODN **8**), a clean formation of 30-mer was observed on the PAGE shown in Figure 2 (lane 5). Further irradiation of lane 5 at 302 nm resulted in a complete reversion to original 6-mers (lane 6).

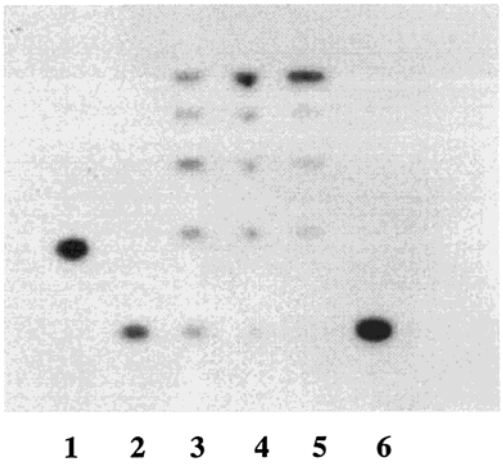


Figure 2. Autoradiogram of a denaturing polyacrylamide gel electrophoresis of the photoproducts of ^{32}P-5′-end-labeled ODN **8** (0.3 μM) and varying concentrations of ODN **6** in the presence of template ODN **7** (7 μM) after irradiation at 366 nm in sodium cacodylate buffer (50 mM, pH 7.0) containing NaCl (100 mM). Lane 1, 12-mer control; lane 2, 6-mer control; lane 3, ODN **6** (9 μM) + ODN **7** + ODN **8**, irradiation at 366 nm, 6 h; lane 4, ODN **6** (18 μM) + ODN **7** + ODN **8**, irradiation at 366 nm, 6 h; lane 5, ODN **6** (36 μM) + ODN **7** + ODN **8**, irradiation at 366 nm, 6 h; lane 6, irradiation of lane 5 at 302 nm, 0.5 h.

In summary, we have demonstrated that ^{V}U can be used to photolinking of a longer oligonucleotide from smaller oligonucleotides on a template with a high efficiency without any side reaction by 366 nm irradiation. The photoligated oligonucleotides were quantitatively reverted to the original oligonucleotides by 302 nm irradiation. Thus, the ^{V}U-mediated photoligation may find application not only to DNA−DNA ligation but also to chemical ligation with other nucleic acids and conjugates, such as DNA−RNA and DNA−PNA ligations which are inaccessible by enzyme-mediated reactions. Mechanistic aspects of the reversible photoaddition and the development of more efficient photoligation systems are currently in progress.

Supporting Information Available: Synthetic procedures for ^{V}U-containing ODN, experimental procedures for photoligation and photosplitting, spectroscopic structural determination of dU−^{V}U adduct **5**, thermal stability of photoligated ODN **4**, deamination of ODN **4** by adding alkaline phosphatase, and molecular modeling (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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