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A Light-Controlled Reversible DNA Photoligation via Carbazole-Tethered 5-Carboxyvinyluracil

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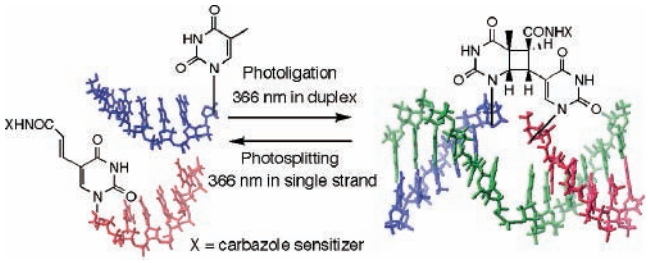
ABSTRACT



We describe a light-controlled template-directed reversible DNA photoligation via carbazole-tethered 5-carboxyvinyluracil. Carbazole-tethered 5-carboxyvinyl-2′-deoxyuridine-containing oligodeoxynucleotide (ODN) can be ligated by irradiation at 366 nm in the presence of template ODN, and the ligated ODN can be split by irradiation at 366 nm in the absence of template ODN.

Template-directed DNA ligation that proceeds under the control of specific DNA templates is an important technique for potential synthetic and biotechnological applications.[1] In recent years, template-directed DNA ligation has developed as a useful tool with applications such as nucleic acid detection,[2] sequence-specific small-molecule synthesis,[3] and the construction of nanoarchitecture.[4] In our previous work, we reported on template-directed reversible photoligation with 5-carboxyvinyl-2′deoxyuridine (^{CV}U) as a form of phototriggered DNA manipulation.[5] In this manipulation, the ligated oligodeoxynucleotide (ODN) can be split site selectively to regenerate the parent ODN by photoirradiation at 302 nm. Via these methods, we succeeded in the synthesis of complex DNA structures, such as branched DNA, end-capped DNA, or padlocked plasmid DNA, at the desired sites.[6] However, photoirradiation at 302 nm also results in fatal damage to normal DNA, due to the formation of pyrimidine dimer.[7] Thus, one problem associated with

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(1) (a) Bohler, C.; Nielsen, P. E.; Orgel, L. E. *Nature* **1995**, *376*, 578−581. (b) Herrlein, M. K.; Nelson, J. S.; Letsinger, R. L. *J. Am. Chem. Soc.* **1995**, *117*, 10151−10152. (c) Xu, Y.; Karalkar, N. B.; Kool, E. T. *Nat. Biotechnol.* **2001**, *19*, 148−152. (d) Czlapinski, J. L.; Sheppard, T. L. *J. Am. Chem. Soc.* **2001**, *123*, 8618−8619. (e) Li, Z. Y.; Zhang, Z. Y. J.; Knipe, R.; Lynn, D. G. *J. Am. Chem. Soc.* **2002**, *124*, 746−747. (f) Chen Y.; Mao, C. *J. Am. Chem. Soc.* **2004**, *126*, 13240−13241. (g) Dose, C.; Ficht, S.; Seitz, O. *Angew. Chem., Int. Ed.* **2006**, *45*, 5369−5373. (h) Kumar, R.; EI-Sagheer, A.; Tumpane, J.; Lincoln, P.; Wilhelmsson, L. M.; Brown, T. *J. Am. Chem. Soc.* **2007**, *129*, 6859−6864.

(2) Silverman, A. P.; Kool, E. T. *Chem. Rev.* **2006**, *106*, 3775−3789.

(3) Gartner, Z. J.; Kanan, M. W.; Liu, D. R. *J. Am. Chem. Soc.* **2002**, *124*, 10304−10306.

(4) Gothelf, K. V.; Brown, R. S. *Chem. Eur. J.* **2005**, *11*, 1062−1069.

(5) (a) Fujimoto, K.; Matsuda, S.; Takahashi, N.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5646−5647. (b) Yoshimura, Y.; Noguchi, Y.; Sato, H.; Fujimoto, K. *ChemBioChem* **2006**, *7*, 598−601. (c) Ogasawara, S.; Fujimoto, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 4512−4515. (d) Ogino, M.; Fujimoto, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 7223−7226. (e) Yoshimura, Y.; Noguchi, Y.; Fujimoto, K. *Org. Biomol. Chem.* **2007**, *5*, 139−142.

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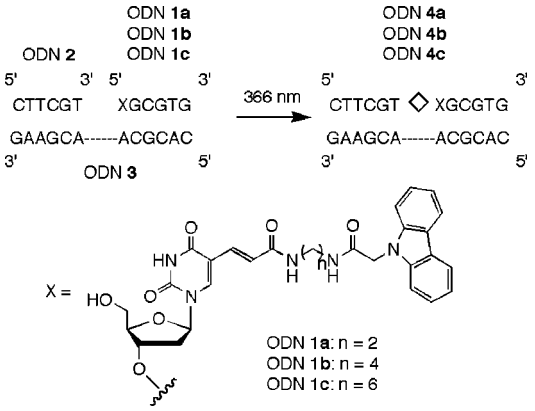
template-directed reversible DNA photoligation is the fact that the efficiency of repeated DNA photoligation declines gradually due to DNA photodamage. The development of a photoligation system, which can be repeatedly ligated and split, would represent a useful technique for DNA nanotechnology such as DNA nanoarchitecture,[8] DNA computing,[9] and DNA-based memory.[10] *N*-Methylcarbazole was known to be singlet excited-state electron donors, and featured the (6−4) pyrimidine−pyrimidone lesion repaired by photosensitized reductive electron-transfer reactions.[11] Herein, we have overcome this problem by using carbazole sensitizer-tethered ^{CV}U-containing ODN. We demonstrated that control of photoligation and photosplitting at 366 nm is possible, whether with or without the ODN template.

We initially determined the ability of the photosensitized splitting with several photosensitizers, such as benzophenone, riboflavin, *N*,*N*,*N*′,*N*′-tetramethylbenzidine, and *N*-methylcarbazole. When the photoligated ODN was irradiated at 366 nm in the presence of *N*-methylcarbazole, the photoligated ODN was efficiently photosplit (see Supporting Information). To examine the effect of varying the methylene linker arm lengths between ^{CV}U and carbazole on the efficiency of DNA photoligation, we designed three 6-mer ODNs with carbazole photosensitizer-tethered ^{CV}U (5′-d(XGCGTG)-3′; ODN **1a**, ODN **1b**, ODN **1c**) as shown in Scheme 1. The phosphoramidite of carbazole-tethered ^{CV}U was synthesized from 5-iodo-2′-deoxyuridine. These modified ODNs were synthesized according to standard phosphoramidite chemistry on a DNA synthesizer, using phosphoramidite of carbazole-tethered ^{CV}U. These modified ODNs were characterized by a nucleoside composition and MALDI-TOF-MS (see Supporting Information). When ODN **1a**, ODN **1b**, or ODN **1c** was used in the DNA photoligation, we observed the appearance of the expected ligated 12-mer ODN **4a**, ODN **4b**, and ODN **4c** in 19, 57, and 65% yields, respectively (Figure 1). These results indicate that the chain length can have a considerable effect: $-(CH_2)_6- > -(CH_2)_4- > -(CH_2)_2-$.

Scheme 1. Template-directed Photoligation of ODNs with ODN **1a**, ODN **1b**, and ODN **1c**



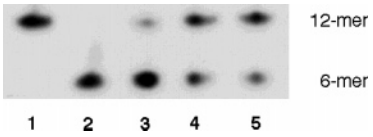


Figure 1. Autoradiogram of a denaturing 15% polyacrylamide gel electrophoresis of photoreaction of ^{32}P-5′-end-labeled ODN **2** (7 μM) and ODN **1a**, ODN **1b**, and ODN **1c** (each 7 μM) and template ODN **3** (9 μM) in a solution of NaCl (1 M) and sodium acetate buffer (50 mM, pH 5.0). (Lane 1) Authentic ^{32}P-labeled photoligated product. (Lane 2) Authentic ^{32}P-labeled ODN **2**. (Lane 3) Irradiation at 366 nm to ODN **1a** for 2 h, 0 °C, 19% yield. (Lane 4) Irradiation at 366 nm to ODN **1b** for 2 h, 0 °C, 57% yield. (Lane 5) Irradiation at 366 nm to ODN **1c** for 2 h, 0 °C, 65% yield.

Figure 2 shows a schematic chart for the light-controlled reversible DNA photoligation via carbazole-tethered modified ODNs. When ODN **1c** and ^{32}P-5′-end-labeled 6-mer (ODN **2**) were irradiated at 366 nm for 6 h in the presence of template 12-mer (ODN **3**), the expected ligated 12-mer ODN **4c** was produced at a 89% yield as determined by the

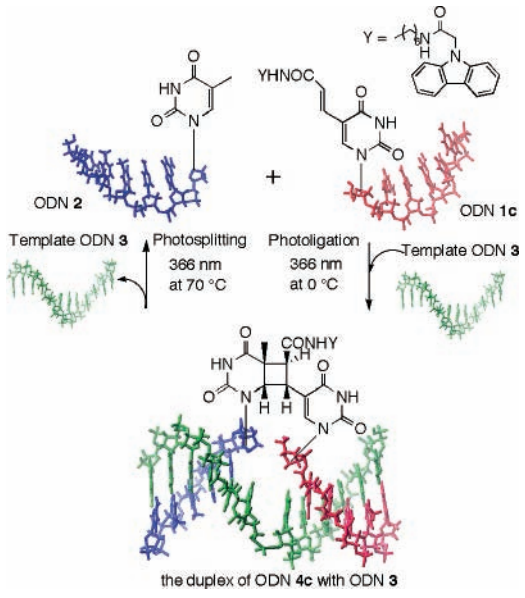


Figure 2. Schematic representation of the light-controlled reversible DNA photoligation.

(6) (a) Fujimoto, K.; Yoshimura, Y.; Ikemoto, T.; Nakazawa, A.; Hayashi, M.; Saito, I. *Chem. Commun*. **2005**, 3177−3179. (b) Ogasawara, S.; Fujimoto, K. *ChemBioChem* **2005**, *6*, 1756−1760. (c) Fujimoto, K.; Matsuda, S.; Yoshimura, Y.; Ami, T.; Saito, I. *Chem. Commun*. **2007**, 2968−2970.

(7) (a) Heelis, P. F.; Hartman, R. F.; Rose, S. D. *Chem. Soc. Rev*. **1995**, *24*, 289−297. (b) Carell, T.; Burgdorf, L. T.; Kundu, L. M.; Cichon, M. *Curr. Opin. Chem. Biol*. **2001**, *5*, 491−498.

(8) (a) Shih, W. M.; Quispe, J. D.; Joyce, G. F. *Nature* **2004**, *427*, 618−621. (b) Goodman, R. P.; Schaap, I. A. T.; Tardin, C. F.; Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. *Science* **2005**, *310*, 1661−1665.

(9) (a) Su, X.; Smith, L. M. *Nucleic Acids Res*. **2004**, *32*, 3115−3123. (b) Weizmann, Y.; Elnathan, R.; Lioubashevski, O.; Willner, I. *J. Am. Chem. Soc*. **2005**, *127*, 12666−12672.

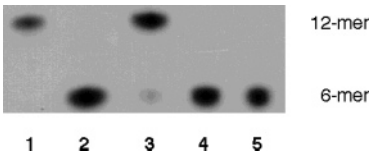


Figure 3. Autoradiogram of a denaturing 15% polyacrylamide gel electrophoresis of photoreaction of ^{32}P-5′-end-labeled ODN **2** (7 μM) and ODN **1c** (7 μM) and template ODN **3** (9 μM) in a solution of NaCl (1 M) and sodium acetate buffer (50 mM, pH 5.0). (Lane 1) Authentic ^{32}P-labeled photoligated product. (Lane 2) Before photoligation. (Lane 3) Irradiation at 366 nm for 6 h, 0 °C, 89% yield. (Lane 4) Irradiation at 366 nm for 6 h without template ODN **3**. (Lane 5) Irradiation at 366 nm for 6 h, 70 °C.

densitometric assay of PAGE (Figure 3, lane 3). The enzymatic digestion of isolated ODN **4c**, obtained from HPLC purification, showed the formation of dC, dG, and dT in a ratio of 3:4:3 together with ^{YCV}U-dT photoadduct, which was confirmed by MALDI-TOF-MS (calcd 846.89 for $[M - H]^-$; found 846.81). The structure of ^{YCV}U-dT photoadduct obtained from HPLC purification was assigned as a *cis*−*syn* [2 + 2] adduct on the basis of spectroscopic data, including ^{1}H−^{1}H COSY and NOESY (see Supporting Information). In the case of photoirradiation at 366 nm in the presence of ODN **3** at 70 °C, no photoligated product was observed (lane 5). This result clearly shows that the photoligation via ODN **1c** proceeded in a template-directed manner.

To examine the role of carbazole sensitizer in reversible DNA photoligation, photoirradiation at 366 nm of ligated ODN **4c** was performed and analyzed by 15% PAGE. When isolated ODN **4c** was irradiated at 366 nm for 6 h in the absence of template ODN **3**, we observed the appearance of ODN **2** in 90% yield, as determined by PAGE, along with the disappearance of ODN **4c** (Figure 4, lane 3). This

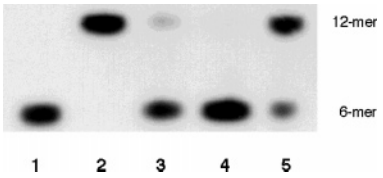


Figure 4. Autoradiogram of a denaturing 15% polyacrylamide gel electrophoresis of photoreaction of ODN **4c** (5 μM). (Lane 1) Authentic ^{32}P-5′-end-labeled ODN **2**. (Lane 2) Before photosplitting. (Lane 3) Irradiation at 366 nm for 6 h in water at ambient temperature, 90% yield. (Lane 4) Irradiation at 366 nm for 1 h in water/CH_3CN = 1:1 at ambient temperature, 99% yield. (Lane 5) Irradiation of lane 3 at 366 nm for 6 h with template ODN **3** at 0 °C, 69% yield.

photosplit ODN **1c** can be ligated effectively to regenerate the parent ODN **4c** by photoirradiation at 366 nm in the presence of template ODN **3** (lane 5). To demonstrate the feasibility of this efficient and reversible photoligation, we examined photoligation of ODNs **1c** and **2** in the presence of template ODN **3**. When ODNs **1c** and **2** were irradiated at 366 nm for 6 h in the presence of template ODN **3**, the clean formation of ODN **4c** was observed by the densitometric assay of PAGE (Figure 5, lane 3). Further irradiation

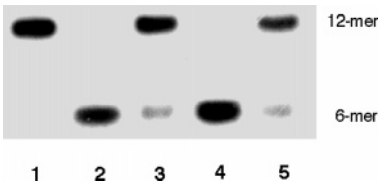


Figure 5. Autoradiogram of a denaturing 15% polyacrylamide gel electrophoresis of ^{32}P-5′-end-labeled ODN **2** (7 μM) and ODN **1c** (7 μM) and template ODN **3** (9 μM) in a solution of NaCl (1 M) and sodium acetate buffer (50 mM, pH 5.0). (Lane 1) Authentic ^{32}P-labeled photoligated product. (Lane 2) Before photoligation. (Lane 3) Irradiation at 366 nm for 6 h, 0 °C, 83% yield. (Lane 4) Irradiation of lane 3 at 366 nm for 1 h in water/CH_3CN = 1:1 at 70 °C, 97% yield. (Lane 5) Incubation of lane 3 for 1 h in water/CH_3CN = 1:1 at 70 °C.

of lane 3 at 366 nm at 70 °C resulted in a complete reversion to original ODN **2** (lane 4). Thus, these results indicate that carbazole-tethered ^{CV}U-containing ODN can be used for repeated DNA photoligation by the light-controlled photoreaction without any side reaction, such as the formation of pyrimidine dimer. To examine the environment of the carbazole in a DNA duplex, UV melting profiles were obtained. The T_m value (46.8 °C) of the duplex of ODN **4c** with ODN **3** was higher than that of ODN **3** and the photoligated product from ^{CV}U-containing ODN (5′-d(CTTCGT^{CV}UGCGTG)-3′) (44.9 °C). Further, we performed fluorescence titration of ODN **4c** by ODN **3** in order to study the environment of the carbazole (see Supporting Information).[12] The fluorescence intensity of carbazole was gradually increased by 2.7-fold compared to that of the single-stranded ODN **4c**. These results suggested that the carbazole in the duplex ODN **4c** with ODN **3** intercalated between bases and kept away from the photoligated position, meaning DNA photoligation at 366 nm can proceed effectively in the duplex without carbazole-sensitized photosplitting. An important feature of this reversible DNA photoligation is the fact that photoligation and photosplitting at 366 nm can be controlled by the difference between the single-stranded and duplex structures.

In conclusion, we have demonstrated that carbazole-tethered ^{CV}U-containing ODN can be ligated by irradiation at 366 nm in the presence of template ODN, and that the

(10) (a) Shin, J.-S.; Pierce, N. A. *Nano Lett.* **2004**, *4*, 905−909. (b) Le, J. D.; Pinto, Y.; Seeman, N. C.; Musier-Forsyth, K.; Taton, T. A.; Kiehl, R. A. *Nano Lett.* **2004**, *4*, 2343−2347.

(11) (a) Scannell, M. P.; Fenick, D. J.; Yeh, S.-R.; Falvey, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 1971−1977. (b) Joseph, A.; Prakash, G.; Falvey, D. E. *J. Am. Chem. Soc.* **2000**, *122*, 11219−11225.

(12) (a) Hartshorn R. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 5919−5925. (b) Chang, C.-C.; Wu, J.-Y.; Chien, C.-W.; Wu, W.-S.; Liu, H.; Kang, C.-C.; Yu, L.-J.; Chang, T.-C. *Anal. Chem.* **2003**, *75*, 6177−6183.

ligated ODN can be split by irradiation at 366 nm in the absence of template ODN. This light-controlled template-directed reversible DNA photoligation may provide a unique methodology for DNA engineering, DNA nanotechnology, and photochemical DNA manipulation.

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Supporting Information Available: Synthetic procedures for carbazole-tethered modified ODNs, experimental procedures for the photoligation and photosplitting, spectroscopic structural determination of ^{YCV}U-dT photoadduct, detailed experimental data of fluorescence titration. This material is available free of charge via the Internet at http://pubs.acs.org.

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