Development of Photochemical DNA/RNA Manipulation Toward Its Application for Nanotechnology

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We describe a light-controlled template-directed reversible DNA photoligation via carbazole tethered 5-carboxyvinyluracil. Carbazole tethered 5-carboxyvinyl-2'-deoxyuridine (^{CV}U)-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 the template ODN.

Key words: DNA recognition, ligation reactions, carbazole, photosensitizer, photochemistry

1. Introduction

Template-directed DNA ligation proceeding under the control of specific DNA templates is an important technique for potential synthetic and nano-technological 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 nano-architecture construction [4]. While many methods for template-directed chemical ligation of DNA via a native phosphodiester bond or non-native linkages have been demonstrated, only a few exist for photoinduced non-enzymatic chemical ligation. The merit of photochemical ligation that avoids the need for additional reagents is obvious, since their actions are subsequently controllable within space and time by selecting proper irradiation methods. However, these methods also have serious problems in terms of practical applications, such as low photoligation yields, the use of short wavelengths injurious to other components, the occurrence of undesirable photocrosslinking reactions and the lack of photoreversibility. In our continuing efforts to develop a novel method for template-directed DNA photoligation, we demonstrated an efficient and reversible template-directed photoligation of ODNs using 5-substituted pyrimidine analogues [5]. Using ODNs containing modified pyrimidine nucleosides at the 5' or 3' end, we have succeeded in synthesizing complex DNA structures, such as tandem ligated DNA, branched DNA or padlocked plasmid DNA at the desired

sites, and the reversible photocleavage of the ligated DNA by photoirradiation at a shorter wavelength (Figure 1) [6]. Therefore, our photoligation system using vinyl-substituted pyrimidine nucleosides can be a powerful tool for easy and accurate DNA handling, triggering the development of new nanotechnology applications.

Fig. 1 Various DNA structures constructed by using photoligation.

In this manipulation, the ligated ODN can be split site-selectively to regenerate the parent ODN by photoirradiation at 302 nm. 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 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, capable of being 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 have 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, both with and without the ODN template.

2. Experimental

5'-*O*-(4,4'-Dimethoxytrityl)-5-(*E*)-(2-carboxy-vinyl)-2'-deoxyuridine (**4**).

To a solution of **3** (500 mg, 1.68 mmol) in dry pyridine (10 mL) was added 4,4'-dimethoxytrityl chloride (681 mg, 2.01 mmol) and *N,N*-dimethylaminopyridine (20 mg, 0.168 mmol) cooling by ice bath and the solution was stirred for 18 h at an ambient temperature. After quenching the reaction by adding methanol, the mixture was evaporated to dryness *in vacuo*. The residue was extracted with ethylacetate (20 mL x 3) and water (30 mL), and the organic layer was collected, dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography (hexane : ethylacetate = 1 : 3) to afford **4** (533 mg, 55%) as a white solid. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 11.69 (bs, 1H, NH), 8.04 (s, 1H, 6-H), 7.35 (d, 1H, *J* = 15.6 Hz, vinyl), 6.86-6.83 (m, 4H, H-ortho to OCH₃ x 4), 6.78 (d, 1H, *J* = 15.6 Hz), 6.16 (t, 1H, *J* = 7.2 Hz, 1'-H), 5.30 (bs, 1H, 3'-OH), 4.23-4.21 (m, 1H, 3'-H), 3.90-3.86 (m, 1H, 4'-H), 3.70 (d, 6H, *J* = 2.8 Hz, OCH₃), 3.24-3.13 (m, 2H, 5'-H), 2.35-2.15 (m, 2H, 2'-H); HRMS: (positive ion FAB) calcd. for C₃₃H₃₂N₂O₉ [(M+H)⁺] 600.2107, found 600.2106.

5'-*O*-(4,4'-Dimethoxytrityl)-5-(*E*)-(2-(6-(2-carbazol-9-yl-acetylamino)-hexylamino)-carbonylvinyl)-2'-deoxyuridine (**5**).

To a solution of **4** (300 mg, 0.50 mmol) in acetonitrile (20 mL) was added *N*-hydroxysuccinimide (75.0 mg, 0.60 mmol) and 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide hydrochloride salt (114 mg, 0.60 mmol) cooling by ice bath, the solution was stirred for 18 h at an ambient temperature, then *N*-(6-amino-hexyl)-2-carbazol-9-yl-acetamide (161 mg, 0.50 mmol) was added and stirred for 6 h. The precipitate was removed by filtration, and evaporated. The residue was extracted with chloroform (20 mL x 3) and water (30 mL) and washed with brine (20 mL x 2). The organic layer was collected, dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The crude product was dissolved into ethylacetate and the solution was added dropwise to excess hexane for trituration. The white precipitate **5**

(348 mg, 77%) was dried *in vacuo*. ¹H-NMR (400 MHz, DMSO-*d*₆) δ 11.62 (bs, 1H, NH), 8.13 (d, 2H, *J* = 7.6 Hz), 7.94 (s, 1H, 6-H), 7.49 (d, 2H, *J* = 8.0 Hz), 7.41-7.35 (m, 4H), 7.29-7.16 (m, 9H), 7.11 (d, 1H, *J* = 15.6 Hz, vinyl-H), 7.05 (d, 1H, *J* = 15.6 Hz, vinyl-H), 6.87-6.84 (m, 4H), 6.16 (t, 1H, *J* = 6.8 Hz, 1'-H), 5.30 (d, 1H, *J* = 4.4 Hz, 3'-OH), 4.99 (s, 2H), 4.23-4.19 (m, 1H, 3'-H), 3.89-3.84 (m, 1H, 4'-H), 3.70 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 3.18-3.12 (m, 2H), 3.12-3.05 (m, 2H), 2.34-2.13 (m, 2H, 2'-H), 1.43-1.37 (m, 4H), 1.28-1.23 (m, 4H); HRMS: (positive ion FAB) calcd. for C₅₃H₅₆N₅O₉ [(M+H)⁺] 906.4078, found 906.4069.

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*O*-[2-cyanoethoxy-(*N,N*-diisopropylamino)-phosphino]-5-(*E*)-(2-(6-(2-carbazol-9-yl-acetylamino)-hexylamino)-carbonylvinyl)-2'-deoxyuridine (**6**).

To a solution of **5** (300 mg, 0.33 mmol) in acetonitrile (3 mL) was added *N,N,N'*,*N'*-tetraisopropylaminophosphorodiamidite (200 μL, 0.66 mmol), then 1*H*-tetrazole (35.0 mg, 0.50 mmol) dissolved in acetonitrile (1 mL). After being stirred for 1 h at an ambient temperature under nitrogen, the reaction mixture was concentrated, residue extracted with ethylacetate (20 mL x 3) and saturated NaHCO₃ aq. (20 mL), and the organic layer collected, dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The crude product **6** (365 mg, quant.) was dissolved in dry acetonitrile, coevaporated three times, and then used for the automated DNA synthesizer without further purification.

Photoligation of DNA as monitored by PAGE.

A solution (total volume 10 μL) containing target ODN and modified ODN (7 μM), ³²P-5'-end-labeled target ODN (7 μM), and template ODN (9 μM) in 50 mM sodium acetate buffer (pH 5.0) and 1 M NaCl was irradiate with transilluminator (366 nm) at a distance of 3-5 cm at 0 °C for 6 h.

3. Results and discussion

Scheme 1. Reagents and conditions: (a) Methyl

acrylate, $\text{Pd}(\text{OAc})_2$, PPh_3 , NEt_3 , dioxane, reflux, 1 h, 70%; (b) NaOH , HCl , H_2O , room temperature, 3 h, 81%; (c) DMTrCl , DMAP , pyridine, room temperature, 18 h, 55%; (d) NHS , EDCl , XNH_2 , CH_3CN , room temperature, 18 h, 77%; (e) $[(i\text{Pr})_2\text{N}]_2\text{POCH}_2\text{CH}_2\text{CN}$, tetrazole, CH_3CN , room temperature, 1 h, *quant*; (f) DNA synthesizer.

The phosphoramidite of carbazole tethered $^{\text{CVU}}$ was synthesized from 5-iodo-2'-deoxyuridine (Scheme 1). Compound **3** was prepared according to a method reported in literature [12]. These modified ODNs were synthesized according to the standard phosphoramidite chemistry on a DNA synthesizer, using phosphoramidite of carbazole tethered $^{\text{CVU}}$. These modified ODNs were characterized by a nucleoside composition and MALDI-TOF-MS. Figure 2 shows a schematic chart for the light-controlled reversible DNA photoligation via carbazole tethered modified ODNs. When ODN **1**, 5'-d($^{\text{XCV}}$ UGCGTG)-3', and ^{32}P -5'-end-labeled 6-mer ODN **2**, 5'-d(CTTCGT)-3', were irradiated at 366 nm for 6 h at 0 °C in the presence of template 12-mer ODN **3**, 5'-d(CACGCAACGAAG)-3', the expected ligated 12-mer ODN **4** was produced at an 89% yield, as determined by the densitometric assay of PAGE (Figure 3, lane 3) [12]. As shown in Figure 4, The enzymatic digestion of isolated ODN **4**, obtained from HPLC purification, showed the formation of dC, dG, and dT in a ratio of 3:4:3 together with $^{\text{XCVU}}$ -dT photoadduct, which was confirmed by MALDI-TOF-MS (calcd. 846.89 for $[\text{M} - \text{H}]^-$; found 846.81) [13]. As shown in Figure 5, the structure of $^{\text{XCVU}}$ -dT photoadduct obtained from HPLC purification was assigned as a *cis-syn* [2 + 2] adduct on the basis of the spectroscopic data, including ^1H - ^1H COSY and NOESY. In the case of photoirradiation at 366 nm in the presence of ODN **3** at 70 °C, no photoligated product was observed (Figure 3, lane 5). This result clearly shows that the photoligation via ODN **1** proceeded in a template-directed manner.

To examine the role of the carbazole sensitizer in reversible DNA photoligation, photoirradiation at 366 nm of ligated ODN **4** was performed and analyzed by 15% PAGE. When isolated ODN **4** was irradiated at 366 nm for 6 h in the absence of template ODN **3**, we observed the appearance of ODN **2** in 91% yield, as determined by PAGE, along with the disappearance of ODN **4** (Figure 6, lane 6).

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

Fig. 3 Autoradiogram of a denaturing 15% polyacrylamide gel electrophoresis of a photoreaction of ^{32}P -5'-end-labeled ODN **2** (7 μM) and ODN **1** (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.

Fig. 4 (a) HPLC analysis of the enzymatic digestion of isolated ODN **4**. (b) UV spectrum of $^{\text{XCVU}}$ -T adduct.

Fig. 5 (a) Structure of ^{13}C -labeled ODN 4 photoadduct. (b) NOESY spectrum of ^{13}C -labeled ODN 4 photoadduct.

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 4 with ODN 3 was higher than that of ODN 3 and the photoligated product from ^{13}C -containing ODN (5'-d(CTTCGT $\leftrightarrow$ ^{13}C UGCGTG)-3') (44.9 °C). Furthermore, we performed fluorescence titration of ODN 4 by ODN 3 in order to study the environment of the carbazole [14]. The fluorescence intensity of the carbazole was gradually increased by 2.7-fold compared to the single-stranded ODN 4. These results suggested that the carbazole in the duplex ODN 4 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. The pathway in Scheme 2 illustrates the mechanistic proposal for the sensitized photosplitting at 366 nm and the photosplitting at 302 nm. In the sensitized photosplitting, carbazole absorbs a photon and the electron flow is from the sensitizer to the photoligated site. Thus the introduction of carbazolyl group to ^{13}C is important to improve the reactivity of photosplitting.

Fig. 6 Autoradiogram of a denaturing 15% polyacrylamide gel electrophoresis of photoreaction of ^{32}P -5'-end-labeled ODN 4 (5 μM).

polyacrylamide gel electrophoresis of photoreaction of ^{32}P -5'-end-labeled ODN 4 (5 μM). Lane 1: authentic ^{32}P -labeled ODN 2; Lane 2: before photosplitting; Lane 3: 366 nm irradiation for 30 min., 14% yield; Lane 4: 366 nm irradiation for 60 min., 31% yield; Lane 5: 366 nm irradiation for 2 h, 82% yield; Lane 6: 366 nm irradiation for 6 h, 91% yield; Lane 7: 366 nm irradiation for 30 min., in water:MeCN = 1:1, 84% yield; Lane 8: 366 nm irradiation for 60 min., in water:MeCN = 1:1, 96% yield.

Scheme 2. (a) Carbazole-sensitized photosplitting of the photoligated ODN. (b) Photosplitting of the photoligated ODN at 302 nm.

4. Conclusion

In conclusion, we have demonstrated the fact that carbazole tethered ^{13}C -containing ODN can be ligated by irradiation at 366 nm in the presence of template ODN, and that the 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.

Acknowledgement

This work was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

- [1] (a) C. Bohler, P. E. Nielsen and L. E. Orgel, *Nature*, **376**, 578–581 (1995). (b) Y. Xu, N. B. Karalkar and E. T. Kool, *Nat. Biotechnol.*, **19**, 148–152 (2001).
- [2] A. P. Silverman and E. T. Kool, *Chem. Rev.*, **106**, 3775–3789 (2006).
- [3] Z. J. Gartner, M. W. Kanan and D. R. Liu, *J. Am. Chem. Soc.*, **124**, 10304–10306 (2002).
- [4] K. V. Gothelf and R. S. Brown, *Chem. Eur. J.*,

11, 1062–1069 (2005).

[5] (a) K. Fujimoto, S. Matsuda, N. Takahashi and I. Saito, *J. Am. Chem. Soc.*, **122**, 5646–5647 (2000). (b) Y. Yoshimura, Y. Noguchi, H. Sato and K. Fujimoto, *ChemBioChem*, **7**, 598–601 (2006). (c) S. Ogasawara and K. Fujimoto, *Angew. Chem., Int. Ed.*, **45**, 4512–4515 (2006).

[6] (a) K. Fujimoto, Y. Yoshimura, T. Ikemoto, A. Nakazawa, M. Hayashi and I. Saito, *Chem. Commun.*, 3177–3179 (2005). (b) S. Ogasawara, Y. Yoshimura, M. Hayashi, I. Saito and K. Fujimoto, *Bull. Chem. Soc. Jpn.*, **80**, 2124–2130 (2007). (c) K. Fujimoto, S. Matsuda, Y. Yoshimura, T. Ami and I. Saito, *Chem. Commun.*, 2968–2970 (2007).

[7] T. Carell, L. T. Burgdorf, L. M. Kundu and M. Cichon, *Curr. Opin. Chem. Biol.*, **5**, 491–498 (2001).

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

[9] Y. Weizmann, R. Elnathan, O. Lioubashevski and I. Willner, *J. Am. Chem. Soc.*, **127**, 12666–12672 (2005).

[10] J.-S. Shin and N. A. Pierce, *Nano Lett.*, **4**, 905–909 (2004).

[11] A. Joseph, G. Prakash and D. E. Falvey, *J. Am. Chem. Soc.*, **122**, 11219–11225 (2000).

[12] M. Ashwell, A. S. Jones, A. Kumar, J. R. Sayers, R. Walker, T. Sakuma and E. De Clercq, *Tetrahedron*, **43**, 4601–4608 (1987).

[13] ^{xcv}U-dT photoadduct: UV (H₂O) λ (ϵ) 366 nm (36 M⁻¹ cm⁻¹).

[14] R. M. Hartshorn and J. K. Barton, *J. Am. Chem. Soc.*, **114**, 5919–5925 (1992).

(Received December 10, 2008; Accepted April 17, 2009)