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Template-directed photoreversible ligation of DNA via 7-carboxyvinyl-7-deaza-2′-deoxyadenosine

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Abstract—An efficient template-directed photoligation of oligodeoxynucleotide (ODN) using 7-deaza-2′-deoxyadenosine derivative ^{VZ}**A** is described. When ODN containing ^{VZ}**A** at the 5′ end was photoirradiated with ODNs containing a pyrimidine base at the 3′ end in the presence of template ODN, rapid and efficient ligation (cycloaddition reaction) was observed without any byproduct formation. ODNs containing ^{VZ}**A** showed an extremely high reactivity as compared with those reported in previous photoligations.

Various template-directed reactions of DNA oligonucleotides have recently been developed for the potential synthetic and biotechnological applications.[1] Modified nucleosides containing DNA backbones possessing various functional group that react after hybridization by additional reagents or by photoirradiation have been explored and extended to sequence-specific small-molecule synthesis, polymerization, and SNPs detection system.[2] In our continuing efforts to develop a novel method for template-directed DNA chemical ligation, we previously reported an efficient and reversible template-directed photoligation of ODNs using 5-substituted pyrimidine analogues.[3] By the use of ODNs containing modified pyrimidine nucleosides at the 5′ end, we have succeeded in the synthesis of complex DNA structures, such as tandem ligated DNA, branched DNA or padlocked plasmid DNA at desired sites, and the reversible photocleavage of the ligated DNA by photoirradiation at a shorter wavelength.[4] Photoirradiation at more than 360 nm does not significantly damage normal DNA and RNA, and the manipulation technique of biopolymers such as DNA by photo- or laser-irradiation has been developed with the precision of only one molecule. Therefore, our photoligation system using vinyl-substituted pyrimidine nucleosides can be a powerful tool for easy and accurate DNA handling, leading to the development of new DNA nanotechnology as represented by DNA chips and DNA computers.

Here, we report the synthesis of novel nucleosides containing a 7-deaza-2′-deoxyadenosine framework and their high reactivity in the template-directed DNA photoligation. Vinyl-substituted 7-deaza-2′-deoxyadenosine derivative **1** was synthesized due to the high molecular absorption coefficient at longer wavelength than 300 nm and their ability to form stable Watson–Crick base pair like normal adenine base (Fig. 1).

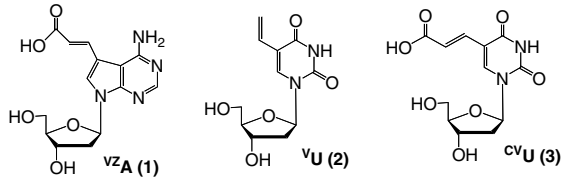

Figure 1. Structure of photoreactive nucleosides, ^{VZ}**A**, ^{V}**U** and ^{CV}**U**.

The synthesis of the phosphoramidite of 7-deaza-2′-deoxyadenosine derivative **1** is outlined in Scheme 1. 7-Iodo-7-deaza-2′-deoxyadenosine **4** was synthesized according to the protocol of Seela and Zulauf[5] **4** was coupled with methylacrylate to afford **5**.[5] Protection of the amino group of 7-deazaadenine with DMF acetal and the 5′-hydroxy group of the deoxyribose moiety

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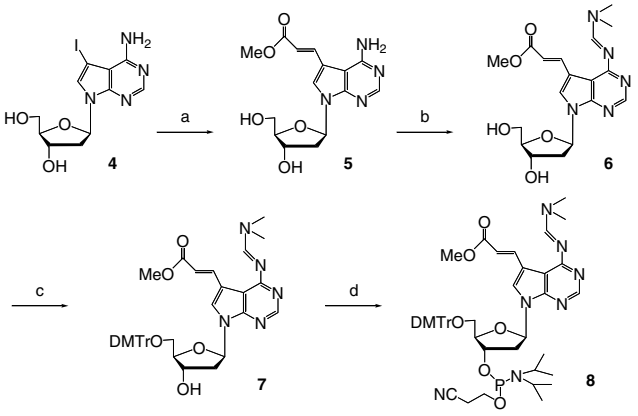

Scheme 1. Reagents and conditions: (a) methyl acrylate, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 80 °C, 8 h, 80%; (b) DMF acetal, DMF, 50 °C, 1 h, 81%; (c) 4,4′-dimethoxytrityl chloride, pyridine, room temperature, 6 h, 85%; (d) $(iPr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, acetonitrile, room temperature, 2 h, quant.

with the 4,4′-dimethoxytrityl group of **5** afforded compound **7**.[6] After conversion to cyanoethylphosphoramidite, amidite **8** was incorporated into oligonucleotide with an automated DNA synthesizer.

The synthesized ODN was deprotected with 0.4 M NaOH in 80% MeOH at room temperature for 17 h to afford ODN containing ^{VZ}**A** (**ODN 1**), which was purified by HPLC. The composition of the oligomer was determined by MALDI-TOF mass spectrometry (calcd 1900.31 for $[M - H]^-$, found 1900.07). The synthesized ODNs are summarized in Table 1.

Table 1. Oligonucleotides (ODNs) used in this study

	Sequences
ODN 1	5′-d(^{VZ}AGCGTG)-3′
ODN 2	5′-d(TGTGCT)-3′
ODN 3	5′-d(CACGCTAGCACA)-3′
ODN 4	5′-d(AGCGCT)-3′
ODN 5	5′-d(CACGCT)-3′
ODN 6	5′-d(TGTGCTAGCGTG)-3′

We initially measured the absorption spectrum of monomer **5** in methanol. The absorption maxima for **5** were observed at 270 and 322 nm. Absorbance of **5** at 366 nm was 10 times larger than that for the previously reported 5-carboxyvinyl-2′-deoxyuridine ^{CV}**U** (**3**) (ε_{366} = 820 for **5** and ε_{366} = 76 for ^{CV}**U**).

Next, we determined the feasibility of the photoligation of ^{VZ}**A**-containing ODN. When **ODN 1** and **ODN 2** were irradiated at 366 nm for 5 min in the presence of template **ODN 3**, we observed rapid appearance of the peak of **ODN A** in 93% yield as determined by HPLC with the disappearance of **ODN 1** and **ODN 2** (Figs. 2, 3).[7] MALDI-TOF mass analysis indicated that the **ODN A** isolated by HPLC purification was proven to be a ligated product of **ODN 1** and **ODN 2** (calcd 3698.55 for $[M - H]^-$, found 3698.57). From the comparison with the photoligation using ^{CV}**U**, it was apparent that ^{VZ}**A**-containing **ODN 1** was far more reactive and comparable with ODN containing conventional

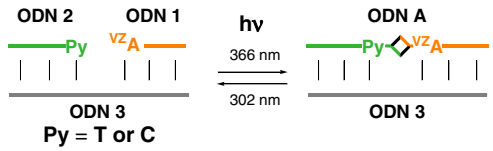

Figure 2. Schematic illustration of template-directed photoreversible ligation of DNA.

psoralen unit.[8] The selectivity of the photoligation of 5′-terminal ^{VZ}**A** to the 3′-terminal bases of **ODN 2** was also examined. A or G at the 3′ end did not undergo photoaddition with 5′-terminal ^{VZ}**A**, whereas the 3′-terminal C could react with photoexcited ^{VZ}**A** to produce ligated **ODN B** as effective as 3′-terminal T[9].

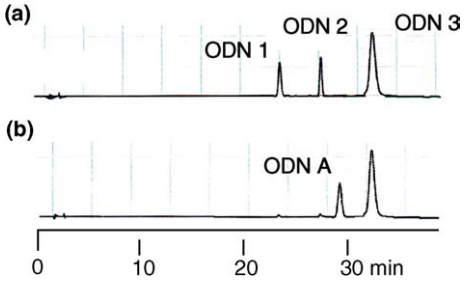

Figure 3. HPLC analysis of the irradiated **ODN 1** and **ODN 2** in the presence of template **ODN 3** with 366 nm. (a) Before photoirradiation. (b) Irradiated at 366 nm for 5 min, 93% yield.

The thermal stability of the duplexes containing photoligated **ODN A** was investigated by monitoring the melting temperature (T_m). In T_m measurements of the duplex, sigmoidal curves on the change of A_{260} were obtained, and the T_m value was calculated from the first part of the curve.[10] The T_m value (32.9 °C) of **ODN A/ODN 3** was lower than that of the 12-mer duplex **ODN 6/ODN 3** containing a natural A–T base pair (54.4 °C), whereas the duplex showed a 10 °C higher thermal stability than **ODN 4/ODN 5** (T_m = 22.4 °C). These results indicate that ligated **ODN A** can form a stable duplex with the complementary **ODN 3**.

To confirm the photoreversibility of the ligated product, irradiation of the photoligated **ODN A** at 302 nm was examined. As shown in Figure 4, a rapid disappearance

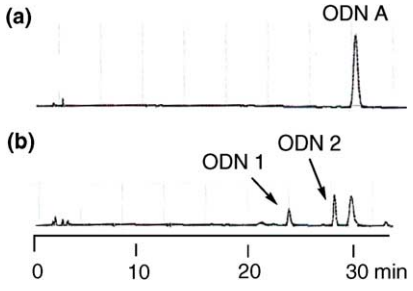

Figure 4. HPLC analysis of 302 nm irradiated **ODN 7**. (a) Before photoirradiation. (b) Irradiation at 302 nm for 30 min.

of **ODN A** was observed by 302 nm irradiation to revert to two ODNs. The analysis by MALDI-TOF mass spectrometry of newly formed ODNs obtained by HPLC purification indicated that these ODNs were **ODN 1** and **ODN 2** (**ODN 1**; calcd 1900.31 for $[M - H]^-$, found 1900.63 and **ODN 2**; calcd 1797.23 for $[M - H]^-$, found 1797.38). The reverse photoreaction produced only **ODN 1** and **ODN 2** without any byproducts. Therefore, the ODN containing ^{VZ}**A** at the 5′-terminal site has the same photoreversibility as 5-vinyl-2′-deoxyuridine ^{V}**U** (**2**) and ^{CV}**U** (**3**).

We examined molecular modeling studies of the duplexes of **ODNs 1**, **2** and **3**. As shown in Figure 5, the vinyl group of ^{VZ}**A** is stacked on C5-C6 double bond of the 3′-terminal T of **ODN 2**. The molecular weight of **ODN A** was equal to the sum of the molecular weights of **ODN 1** and **ODN 2**. As judged from the molecular modeling and the photoreversibility, it is strongly suggested that the photoligation reaction proceeded via [2 + 2] cycloaddition between the double bond of ^{VZ}**A** side chain and the C5–C6 double bond of thymine giving rise to the formation of cyclobutane structure as observed for ^{CV}**U**.[4b]

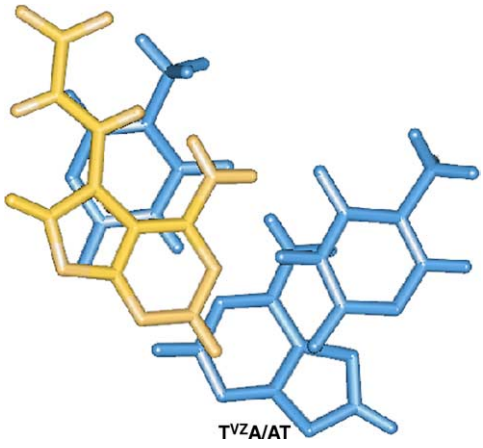

Figure 5. Molecular modeling of stacked geometry in B-form DNA. The model was optimized by AMBER* force field in water by using MacroModel version 8.1.

In conclusion, we have demonstrated that ODN containing ^{VZ}**A** can be used for the photolinking of DNA in the presence of a template DNA by 366 nm irradiation without any side reaction. The photoligated ODNs were reverted to the original ODN by 302 nm irradiation. The photoligation reaction of ODN containing ^{VZ}**A** is very rapid (irradiation time of only 5 min) and clean. Therefore, this system may be widely used for the photochemical manipulation of DNA in various aspects.

References and notes

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6. Spectroscopic data for selected compounds are as follows. **6**: ^{1}H NMR (CD_3OD, 400 MHz) δ 2.36 (ddd, 1H, J = 2.8 Hz, 6.4 Hz, 13.6 Hz), 2.64 (ddd, 1H, J = 5.6, 7.6, 13.6 Hz), 3.18 (s, 3H), 3.19 (s, 3H), 3.73 (s, 3H), 3.83 (dd, 2H, J = 2.8, 12 Hz), 4.02 (ddd, 1H, J = 2.8 Hz), 4.54 (dt, 1H, J = 2.8, 5.6 Hz), 6.50 (dd, 1H, J = 6.4, 7.6 Hz), 6.79 (d, 1H, J = 16 Hz), 7.86 (s, 1H), 8.20 (s, 1H), 8.24 (d, 1H, J = 16 Hz), 8.55 (s, 1H), ^{13}C NMR (CD_3OD, 100 MHz) δ 34.6, 40.4, 40.5, 50.9, 62.5, 71.8, 85.5, 88.1, 110.0, 113.7, 115.4, 125.8, 138.8, 151.4, 152.0, 157.0, 162.0, 169.1, HRMS (FAB): calcd for $C_{15}H_{23}O_5N_5$ $[(M + H)^+]$ 390.1777, found 390.1776. **7**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.47 (ddd, 1H, J = 2.8 Hz, 6.4 Hz, 13.6 Hz), 2.58 (ddd, 1H, J = 2.8 Hz, 6.4 Hz, 13.6 Hz,), 3.16 (s, 3H), 3.26 (s, 3H), 3.73 (s, 3H), 3.73 (m, 1H), 3.74 (s, 6H), 4.16 (dt, 1H, J = 3.2, 7.6 Hz), 4.63 (dt, 1H, J = 3.2, 6.4 Hz), 6.68 (d, 1H, J = 16 Hz), 6.75 (t, 1H, J = 6.4 Hz), 6.78–6.81 (m, 4 Hz), 7.19–7.42 (m, 9H), 7.55 (s, 1H), 8.24 (d, 1H, J = 16 Hz), 8.41 (s, 1H), 8.74 (s, 1H) ^{13}C NMR ($CDCl_3$, 100 MHz) δ 35.3, 40.9, 41.0, 51.1, 55.1, 63.9, 72.5, 77.2, 83.4, 85.7, 86.5, 109.6, 113.0, 113.1, 114.0, 116.0, 123.8, 126.9, 127.8, 128.1, 129.92, 129.94, 135.5, 135.6, 138.1, 144.3, 151.9, 152.5, 156.3, 158.4, 161.5, 168.3, HRMS (FAB): calcd for $C_{39}H_{41}O_7N_5$ $[(M + H)^+]$ 692.3084, found 692.3085.
7. The reaction mixture (total volume 100 μL) containing **ODN 1** and **ODN 2** (each 20 μM, strand concn) in the presence of template **ODN 3** (24 μM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with transilluminator (366 nm) at 0 °C for 5 min and then subjected to HPLC analysis. HPLC analysis was carried out on a Chemcosorb 5-ODS-H column (4.6 × 150 mm); elution was done with 50 mM ammonium formate containing 3–10, 10–50% CH_3CN, a linear gradient (0–35 min, 35–60 min) at a flow rate of 1.0 mL/min.
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9. ALDI-TOF MS: calculated for ODN B ($C_{121}H_{151}N_{44}O_{72}P_{10}$) $[M - H]^-$ 3683.53; found 3683.95.
10. All T_m values of the duplexes (2.5 μM) were measured in 50 mM sodium cacodylate and 100 mM sodium chloride, pH = 7.0. The absorbance of the duplexes was monitored at 260 nm from 2 to 90 °C with heating rate of 1 °C/min.