

Electronic Supplementary Information

Reversible Photopadlocking on Double-Stranded DNA

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Experimental Section

Materials and methods. Acetonitrile (HPLC grade) were dried over calcium hydride. The phosphoramidite of ^{CV}U analogue (Carboxy-dT), PEG building block (Spacer 18), and other reagents for the DNA synthesis were purchased from Glen Research. Calf intestine alkaline phosphatase (AP) (1000 units/mL), snake venom phosphodiesterase (s.v. PDE) (1 mg/0.5 mL), and Nuclease P1 (1 mg) were purchased from Boehringer Mannheim. Plasmid pUC 18 (10 µg, 0.25 µg/µL) was purchased from GIBCO BRL. EcoRI (10000 unit, 12 unit/µL) and HindIII (10000 unit, 15 unit/µL) were purchased from Takara Shuzo, Co., Ltd. Unmodified ODNs were purchased from Greiner Japan, Co., Ltd. T4 polynucleotide kinase was purchased from NIPPON GENE (10 units/µL) and γ-[³²P]-ATP (10 mCi/mL) was purchased from Amersham. Mass spectra of oligonucleotides were obtained on Perceptive Biosystems Mariner™ ESI-TOF MASS SPECTROMETER. JASCO V-550 UV/VIS spectra were used for absorption spectra measurements. Circular dichroism (CD) were measured on a JASCO J-720 spectropolarimeter. Photoirradiation at 366 nm was carried out by using VILBER LOURMAT TFP-35L transilluminator or COSMO BIO CSF-20AF transilluminator. Photoirradiation at 302 nm was carried out by using FUNAKOSHI FTI-36M transilluminator. The photoreactions were carried out with quartz capillary cell (7 mm diameter). The gels were analyzed by autoradiography with a BIORAD Model GS-700 imaging densitometer and BIORAD Molecular Analyst software (version 2.1).

Synthesis of oligonucleotides. Oligonucleotides were prepared by the β-cyanoethylphosphoramidite method on controlled pore glass supports (1 µmol) by using an Applied Biosystems Model 392 DNA/RNA synthesizer. The 0.1 M acetonitrile solution of Carboxy-dT and Spacer 18 phosphoramidite was used in automated synthesis, and their coupling time was extended to 999 seconds. After automated synthesis, the oligomers were cleaved from the support and deprotected by 0.4 M methanolic sodium hydroxide (methanol : water = 4 : 1) for 17 h at room temperature and purified by reverse phase HPLC. The purity and concentration of all oligodeoxynucleotides were determined by complete digestion with s.v. PDE, AP, and Nuclease P1 to 2'-deoxymononucleosides at 37 °C for 1 h. ^{CV}U-containing ODN **1** was identified by ESI-TOF mass. **1** (C₂₉₃H₄₇₆N₄₅O₂₀₇P₂₇) (M-H⁻) calcd 8772.1; found 8775.3.

Preparation of ³²P-5'-end-labeled ODN. The oligonucleotides (ODNs, 400 pmol, strand concentration) were 5'-end-labeled by phosphorylation with 4 µL of [γ-³²P]ATP and 4 µL of T4 polynucleotide kinase using standard procedures.¹ The 5'-end-labeled ODNs were recovered by ethanol precipitation and further purified by 15% denaturing gel electrophoresis and isolated by the crush and

soak method.²

Reversible photocircularization of ODN 1 on double-stranded DNA as monitored by PAGE. A solution (total volume 10 μL) containing ^{32}P -5'-end-labeled ODN 1 (*ca.* 2×10^4 cpm, 2 μM), ODN 2 (10 μM , strand conc.), and ODN 3 (10 μM , strand conc.) in 50 mM sodium acetate buffer (pH 5.0), 100 mM NaCl, and 20 mM MgCl_2 was irradiated with a transilluminator (366 nm or 302 nm) at a distance of 3-5 cm at 0 $^\circ\text{C}$ for 1 h. To the reaction mixture was added 10 μL of loading buffer (a solution of 80% v/v formamide 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue) to quench the reaction and the samples (1-2 μL , *ca.* $2\text{-}4 \times 10^3$ cpm) were loaded onto 20% (19:1) polyacrylamide and 7 M urea denaturing gel and electrophoresed at 700 V for 30 min. The gel was dried and exposed to X-ray film with intensifying sheets at -80 $^\circ\text{C}$. Mismatch experiments were also carried out as described above.

Measurement of melting temperatures. Thermal denaturation profiles were obtained with a Jasco V-550 spectrophotometer. The absorbance of the sample (1 μM strand concentration, 50 mM sodium acetate buffer, pH 5.0, 100 mM NaCl and 20 mM MgCl_2) was monitored at 260 nm from 2 $^\circ\text{C}$ to 82 $^\circ\text{C}$ at a heating rate of 1 $^\circ\text{C}$ per min before and after photoirradiation at 366 nm. Melting temperatures were determined as the maximum in a plot $\Delta\text{Abs}_{260}/\Delta\text{temp}$ vs temperature.

Measurement of CD spectra. CD spectra were measured at 4 $^\circ\text{C}$ on a JASCO J-720 spectropolarimeter with solutions (2 μM strand concentration, 50 mM sodium acetate buffer, pH 5.0, 100 mM NaCl and 20 mM MgCl_2) before and after photoirradiation at 366 nm. CD data were transformed into molar ellipticity in the units of degree cm^2/dm of monomer subunits.

Preparation of modified plasmid DNA.² Modified plasmid DNA 10 and 11 was constructed by insertion of ODN 2 and ODN 3 after treatment with EcoRI at the EcoRI site (+236) of the pUC 18 plasmid. ODN 2 and ODN 3 (each 100 μM , 5 μL) were treated with EcoRI (2.5 units, 5 μL) in Buffer-H (20 μL) and MilliQ (165 μL) at 37 $^\circ\text{C}$ for 15 h. After ethanol precipitation, digested ODNs (each 100 μM , 0.5 μL) and pUC18 (2.0 μL) were treated with T4 DNA ligase (0.75 units, 1.5 μL) in Ligation Buffer (0.5 mL) at 16 $^\circ\text{C}$ overnight. After color selection, modified plasmids were purified by using QIAGEN Plasmid mini.

Reversible photopadlocking on plasmid DNA. A solution (total volume 10 μL) containing ^{32}P -5'-end-labeled ODN 1 (*ca.* 2×10^4 cpm, 2 μM) and plasmid DNA (0.125 $\mu\text{g}/\mu\text{L}$) in 50 mM sodium

acetate buffer (pH 5.0), 100 mM NaCl, and 70 mM MgCl₂ was irradiated with a transilluminator (366 nm or 302 nm) at a distance of 3-5 cm at 0 °C for 3 h. To the reaction mixture was added 10 μL of loading buffer (a solution of 80% v/v formamide 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue) to quench the reaction and the samples (1-2 μL, *ca.* 2-4 x 10³ cpm) were loaded onto 15% (19:1) polyacrylamide and 7 M urea denaturing gel and electrophoresed at 700 V for 30 min. The gel was dried and exposed to X-ray film with intensifying sheets at -80 °C.

Treatment of padlocked DNA with restriction enzyme. After photoirradiation as described above, EcoRI (12 unit/μL) with a 10 x buffer or HindIII (15 unit/μL) with a 10 x buffer was added to the reaction mixture. The solutions were incubated at 37 °C for 1 h. To the reaction mixture was added 10 μL of loading buffer and analyzed as well.

Measurement of melting temperatures and CD spectra. As shown in Figure S1, thermal denaturation measurement of the reaction mixture exhibited a biphasic melting profile characteristic of triple helix. From these results ODN **1** containing ^{CV}U at its 5'-end has been shown to bind DNA double helix to form triple helix and to be linked at its own 3'-terminal to give a circular ODN **4** reversibly by photoirradiation. Based on CD studies, the structure of the complex containing ODN **4** after photoirradiation was typical B-type DNA, characterized by a positive band at 280 nm followed by a negative band 245 nm and a positive band at 225 nm. No significant structural change was observed as compared with before photoirradiation (Figure S2).³ The melting temperature (*T_m*) of the first transition increased from 45.2 °C to 49.2 °C after reaction. Thus, the circularization of ODN **1** afforded a stable triple helix because of its longer sequences hybridized with ODN **2** by joining two ODN **1** segments.

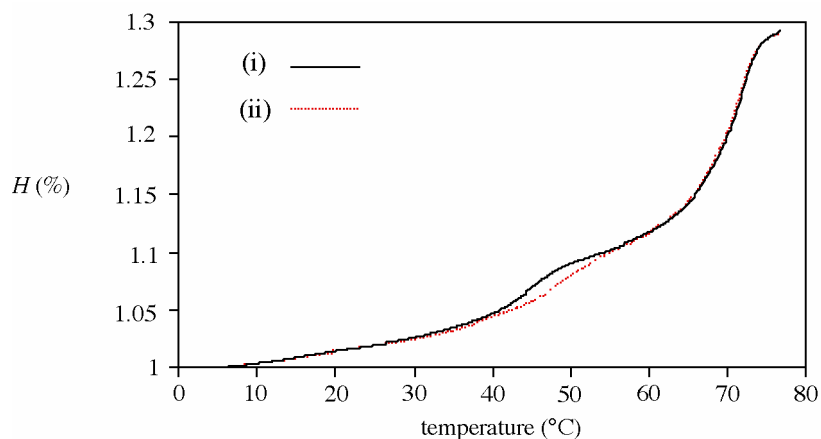


Figure S1. The UV melting curves (H = relative hyperchromicity, λ = 260 nm) of (i) before reaction, T_m = 45.2 °C, 71.3 °C (ii) after reaction, T_m = 49.2 °C, 71.3 °C in 1 μ M each strand, 100 mM NaCl, 20 mM MgCl₂, 50 mM sodium acetate (pH 5.0).

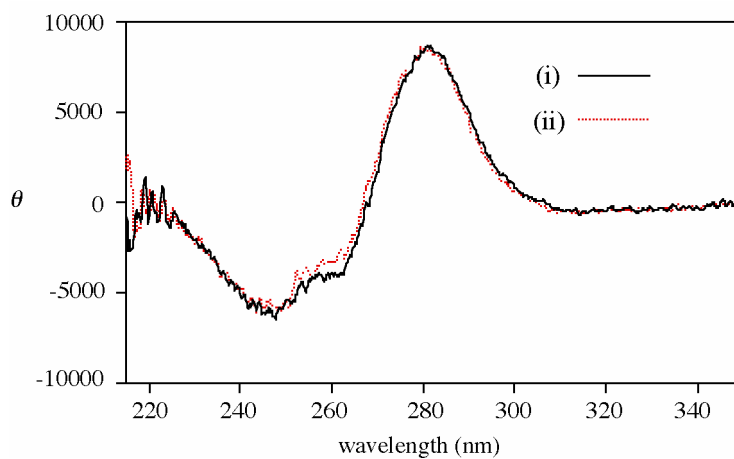


Figure S2. CD spectra (i) before reaction (ii) after reaction in 2 μ M each strand, 100 mM NaCl, 20 mM MgCl₂, 50 mM sodium acetate (pH 5.0).

References

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