Site-Specific Transition of Cytosine to Uracil via Reversible DNA Photoligation

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Supporting Information Available. Experiment details, characterization data, and PAGE chart (4 pages). See any current masthead for ordering information and Web access instructions.
**General.** $^1$H NMR spectra were measured on Varian GEMINI-200 (200 MHz) or JEOL JNM A-400 (400 MHz) spectrometers. Coupling constants ($J$ values) are reported in Hz. The chemical shifts are expressed in ppm downfield from residual chloroform ($\delta = 7.24$ in $^1$H NMR) and water ($\delta = 4.65$ in $^1$H NMR) as an internal standard. Mass spectra were recorded on a JEOL JMS DX-300. JASCO V-550 UV/VIS spectrophotometer was used for absorption spectra measurements. Irradiation was performed by VILBER LOURMAT transilluminator (TFP-35L, 366 nm, 180 W, 4000 J/cm$^2$) or FUNAKOSHI transilluminator (FTI-36M, 302 nm, 180 W). HPLC was performed on a Chemcobond 5-ODS-H column (10 × 150 mm, 4.6 × 150 mm) or a Chemcosorb 5-ODS-H column (4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 254 and 230 nm. Photodiode array HPLC was performed on a Cosmosil 5C18AR column (4.6 × 150 mm) with a Tosoh CCPE-II system equipped with a Photal MCPD-3600 system.

**Experimental procedure for site-specific transition via reversible DNA Photoligation.**

The oligonucleotides (ODNs, 400 pmol, strand concentration) were 5'-end-labeled by phosphorylation with 4 µL of $[^{32}$P]ATP and 4 µL of T4 polynucleotide kinase using standard procedures.$^{[S1]}$ 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.$^{[S2]}$ A solution (total volume 10 µL) containing $^{32}$P-5'-end-labeled ODN 1 (ca. 2 × 10$^4$ cpm, 1 µM) and ODN 2 (7 µM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM NaCl was irradiated with a transilluminator at 366 nm at a distance of 3-5 cm at 0 °C for 3 h. The reaction mixture was heated at 90 °C for 2 h and followed by irradiation at 302 nm for 1 h at 40 °C. To the reaction mixture was next added Uracil DNA glycosylase (UDG, 1 unit) and incubated at 37 °C for 1 h. The reaction mixture was treated with 1 M piperidine at 90 °C for 20 min and then evaporated under vacuum followed by coevaporation with water twice. To the reaction mixture were 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) and 10 µL of water and the samples (1-2 µL, ca. 2-4 × 10$^3$ cpm) were loaded onto 20% (19 : 1) polyacrylamide and 7 M urea denatured 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.
Synthesis and characterization of CVU-containing ODN.
CVU-containing ODN was synthesized by automated solid-phase phosphoramidaite method as reported. 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. Incorporation of CVU into oligonucleotides was confirmed by enzymatic digestion and MALDI-TOF-MS analysis. MALDI-TOF-MS: calcd. for ODN \( 2 \) (C\(_{206}\)H\(_{259}\)N\(_{75}\)O\(_{128}\)P\(_{20}\)\) [(M–H)]\(^{−}\) 6452.25; found 6452.60.


Experimental procedure for time course of the deamination reaction.
A solution (total volume 10 \( \mu \)L) containing \(^{32}\)P-5'-end-labeled ODN \( 1 \) (ca. \( 2 \times 10^4 \) cpm, 1 \( \mu \)M) and ODN \( 2 \) (7 \( \mu \)M, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM NaCl was irradiated with a transilluminator at 366 nm at a distance of 3-5 cm at 0 °C for 3 h or 4 h. The reaction mixture was heated at 90 °C for each time. The reaction samples were irradiated at 302 nm and treated with UDG and piperidine as described above. The reaction samples were loaded onto 20% (19 : 1) polyacrylamide and 7 M urea denatured 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. The gels were analyzed by autoradiography with a densitometer and BIORAD Molecular Analyst software (version 2.1). The intensities of the cleaved bands were determined by volume integration (Figure S1). The rate constant (k) for deamination of C-CVU photoadduct in the ligation site can be calculated from the equation (1).
where $x$ is the fraction of cytosine converted into uracil which is determined from the intensities of the cleaved bands and $t$ is time of incubation.

Figure S1. (a) PAGE analysis for time course of deamination. Lane 1, before photoligation; lane 2, ODN 1 + ODN 2, irradiation at 366 nm, 1 h on ice; lane 3, lane 2 + 90 °C, 0 min + 302 nm, 1 h, r.t. + UDG (37 °C, 1 h) + piperidine (90 °C, 30 min); lane 4, 5, 6, 7, 8, 9, 10, 11, under the same condition of lane 3 except for 90 °C, 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min, 120 min respectively; lane 12, treatment of ODN 4 with UDG and piperidine treatment under the same condition of lane 3. (b) Time course for the conversion of C<sup>S</sup>U adduct and the formation of U<sup>S</sup>U adduct in ligated site based on the intensities of the cleaved bands were determined by volume integration.