Supplementary information

Sequence-dependent thymine dimerization and lifetimes of the photoexcited state of oligonucleotides

Akira Takakado[*a] and Koichi Iwata[a]

[a] Department of Chemistry, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588, Japan.

***Corresponding author**

E-mail: akira.takakado@gakushuin.ac.jp

Experimental

Samples

Oligo-deoxyribonucleotides were purchased from Macrogen Japan and FASMAC. The samples were dissolved in phosphate buffer (NaH_2PO_4 16 mM, Na_2HPO_4 34 mM, NaCl 100 mM, pH 7.5) without further purification and heated at 90°C for 5 min. by a heat block. The annealed solutions were then gradually cooled to room temperature.

Steady-state absorption spectroscopy

Steady-state absorption spectra were measured using a UV/vis spectrometer (Hitachi U3500) with a quartz black-masked microcell ((W)2 mm × (L)10 mm × (H)45 mm). To measure the changes in the absorption spectra upon UV irradiation, we obtained the absorption spectra before and after UV irradiation. Each measurement was repeated three times to confirm reproducibility. The sample solution in a 3 mm quartz cell was incubated for three hours under UV light irradiation (280 ± 5 nm, 2 mW) at room temperature. An LED (LED280W, Thorlabs) was used as the ultraviolet (UV) light source. The sample solution was stirred using a magnetic stirrer under irradiation. The concentration of oligonucleotides was 25 μM.

Transient absorption measurements

Transient absorption measurements were conducted using a femtosecond laser system. Light pulse at 800 nm from a Ti:sapphire laser (Vitesse and Legend Elite, Coherent, 80 fs, 1 kHz) were divided to generate pump and probe pulses. The wavelength of the pump pulse was converted to 280 nm via fourth-harmonic generation using an optical parametric amplifier (TOPAS-Prime, Coherent) and BBO crystals. The repetition frequency of the pump pulse was changed to 500 Hz by using an optical chopper (Model 3501, NewFocus). The probe pulse was focused on a sapphire plate to generate a white-light continuum. The intensity of the probe pulse at 532 nm was detected using a silicon photodiode (PIN-10D, OSI Optoelectronics) through a band-pass filter (VPF-25C-03-45-53200, SIGMAKOKI, 532 ± 1.5 nm). The electronic signal from the photodiode was amplified by a transimpedance amplifier (AMP102, Thorlabs, 100 kV/A) and observed with a lock-in amplifier (LI5640, NF Corporation) through electronic filters (1 kHz low-pass filter (EF110, Thorlabs) and direct-current block filter (EF500, Thorlabs)). The angle of polarization between the pump and probe lights was set to 54.7°. Measurements were performed at room temperature (24°C) using a quartz cell (optical path length of 3 mm). The sample solution was stirred using a 2 mm magnetic stirrer during measurements. A typical amount of the solution was 250 μL for each measurement. The concentration of the samples was set to 3 mM for mononucleotides and 150 μM for 20mer oligonucleotide. Transient absorption signals were analyzed using exponential functions convoluted with a Gaussian function that represents an instrumental response time of 200–300 fs.

Previous studies showed the transient absorption signals for oligonucleotides probed at several wavelengths including 250, 280, 360, and 570 nm[1,2]. These investigations indicated that the signals probed at deep-UV region included a vibrational cooling component (~2 ps). Therefore, we ruled out the vibrational cooling component by using the 532 nm detection.

Transient absorption signals for dGMP and d(GT)$_{10}$

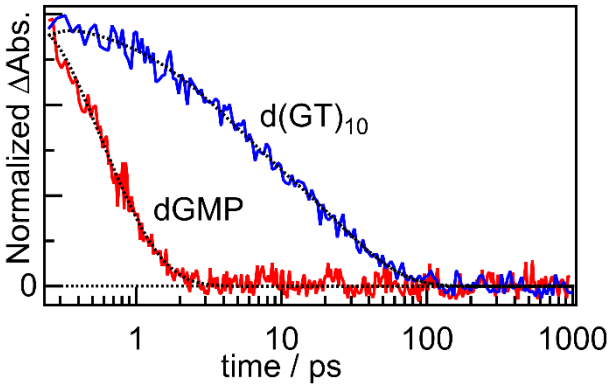

Figure S1. Transient absorption signal at 532 nm for dGMP (red) and d(GT)$_{10}$ (blue). Dotted traces indicate best-fit lines of Gaussian convoluted single-exponential (dGMP) and bi-exponential (d(GT)$_{10}$) functions.

Laser power dependence of transient absorption signal

The pump pulse energy dependence of the transient absorption signal was examined to distinguish between one-photon and two-photon processes. Figure S2(a) shows the time profiles of the transient absorption signal for dGMP pumped by high (0.62 μJ) and low (0.22 μJ) pulse energies. The long-lived state was observed for the signals excited by the higher pulse energy. The intensities of the transient signals for various pump pulse energies are plotted in Figure S2(b). Each signal represents the Δ*Absorbance* at 500 fs (red) and 3 ps (blue) after photoexcitation. At 3 ps, the trace is well reproduced by a function: $\Delta Abs. \propto (pulse\ energy)^2$, whereas the trace at 500 fs is reproduced by $\Delta Abs. \propto (pulse\ energy) + 0.6(pulse\ energy)^2$. These results suggest that the long-lived signal observed at 3 ps is derived from a two-photon reaction process because the intensity increases quadratically with respect to the pulse energy. The two-photon process is likely due to a two-photon ionization process that generates hydrated electron[3]. Hydrated electron shows a broad absorption band in the visible to near-IR wavelength region. On the other hand, the early kinetics (τ = 0.5 ± 0.1 ps; determined by the signal pumped with 0.15 μJ/pulse shown in Figure S1) was determined to be a single photon process. At 500 fs, both one-photon and two-photon processes were observed at high

pulse energies. In this study, we examined with low pump pulse energy (< 0.15 μJ/pulse) to exclude contribution of the two-photon reaction process.

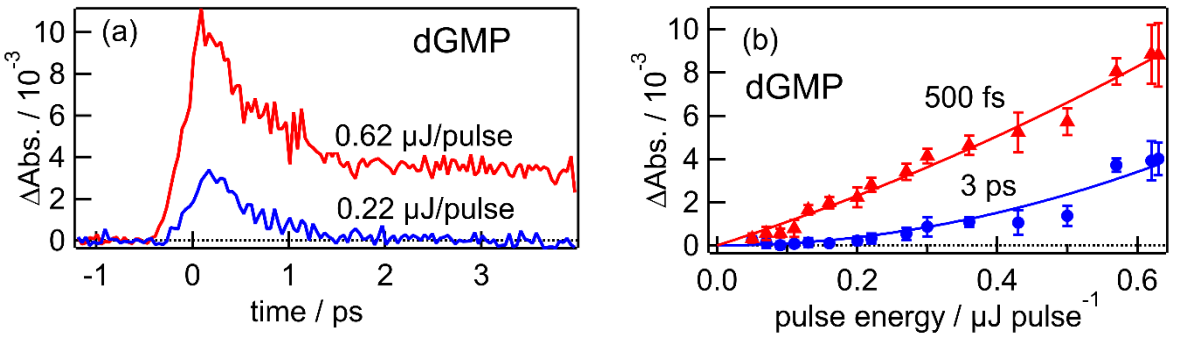

Figure S2. Pulse energy dependence of the transient absorption signal for dGMP. (a) Time-profile of the transient absorption signal excited with the pulse energy of 0.62 μJ/pulse (red) and 0.22 μJ/pulse (blue). (b) Δ*Absorbance* against the pulse energy obtained at 500 fs (red) and 3 ps (blue).

Analysis of transient absorption measurements for 20mer oligonucleotides

The transient absorption signals were analyzed using a Gaussian convoluted exponential function. The best-fit parameters are presented in Table S-1. Each measurement was repeated at least three times to confirm reproducibility.

Table S-1. Time constants and amplitudes obtained by curve fitting for $d(XY)_{10}$ sequences

	A_1 (%)	τ_1 (ps)	A_2 (%)	τ_2 (ps)	A_3 (%)	τ_3 (ps)
$d(GG)_{10}$	32 ± 13	5.0 ± 2.8	39 ± 12	32 ± 15	29 ± 2	320 ± 100
$d(GA)_{10}$	35 ± 3	1.8 ± 0.4	28 ± 2	42 ± 5	37 ± 2	340 ± 60
$d(GT)_{10}$	38 ± 1	2.6 ± 0.6	62 ± 1	25 ± 3	--	--
$d(GC)_{10}$	100	4.7 ± 0.4	--	--	--	--
$d(AA)_{10}$	50 ± 14	2.4 ± 0.8	50 ± 14	120 ± 60	--	--
$d(AT)_{10}$	49 ± 2	4.2 ± 1.9	51 ± 2	61 ± 25	--	--
$d(CA)_{10}$	43 ± 2	1.1 ± 0.2	57 ± 2	60 ± 13	--	--
$d(TT)_{10}$	82 ± 6	1.3 ± 0.3	18 ± 6	230 ± 120	--	--
$d(CT)_{10}$	75 ± 12	2.6 ± 0.8	25 ± 12	470 ± 50	--	--
$d(CC)_{10}$	55 ± 15	0.9 ± 0.3	28 ± 10	10 ± 5	17 ± 11	280 ± 80

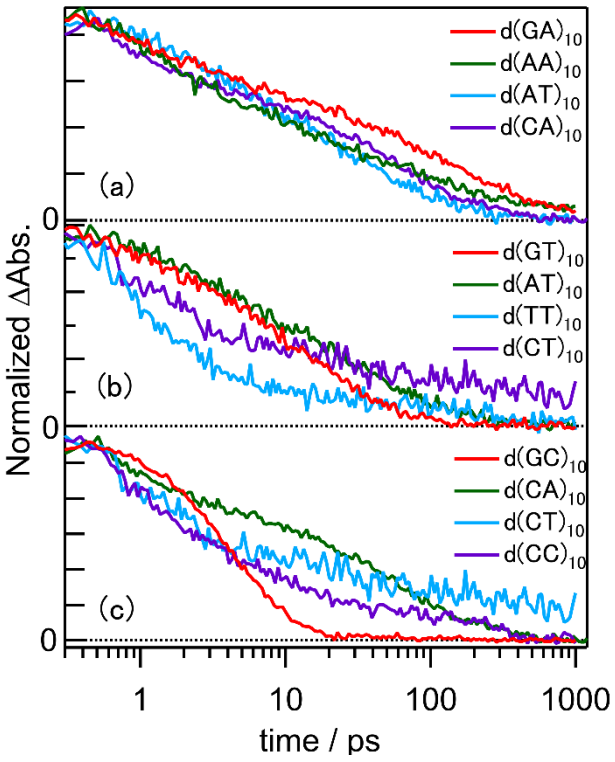

Figure S3. Transient absorption signals of $d(XY)_{10}$ sequences. Figure S3(a) shows signals of A containing sequences. Similarly, Fig S3(b) and (c) show signals T and C containing sequences, respectively. For comparison, several same traces are depicted in two figures, such as $d(GA)_{10}$ in Figure 3 and Figure S3(a).

Reference

1 C. E. Crespo-Hernández, B. Cohen and B. Kohler, *Nature*, 2005, **436**, 1141–1144.

2 C. E. Crespo-Hernández, K. de La Harpe and B. Kohler, *J. Am. Chem. Soc.*, 2008, **130**, 10844–10845.

3 J.-M. L. Pecourt, J. Peon and B. Kohler, *J. Am. Chem. Soc.*, 2001, **123**, 10370–10378.