Supporting Information

Effect of spermidine on guanine decomposition *via* photoinduced electron transfer in DNA

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Materials

HPLC-grade DNAs were purchased from Japan Bio Services Co., LTD. Crotalus adamanteus venom phosphodiesterase I (Worthington), and Alkaline Phosphatase, Calf Intestinal (Promega) were used for the enzymatic digestion of oligonucleotides. Spermidine (for Molecular Biology) was purchased from Fujifilm Wako Pure Chemical Corp. For storage, stock solutions of spermidine were refrigerated under argon atmosphere to prevent degradation. 5'-Dimethoxytrityl-5-(pyren-1-yl-ethynyl)-2'-deoxyUridine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (pyrene-dU-CE Phosphoramidite) was purchased from Glen Research. Aliquots of dsDNA samples were prepared by annealing equimolar amounts of desired DNA complements. The samples were heated at 90 °C for 3 min, then cooled slowly. Before each experiment, a concentrated aqueous buffer-salt solution of spermidine was added to a DNA sample solution. All aqueous solutions utilized ultra-pure water (Komatsu Electronics, UL-pure).

UV-vis and CD spectral measurements

Absorption spectra were obtained using a JASCO V-730BIO spectrophotometer at room temperature using 1-mm path length cell. CD measurements were carried out on a JASCO J-720W spectropolarimeter (Japan Spectroscopic Co., Ltd.) using a one-drop measurement unit (1-mm path length).

Melting temperature measurements

Melting temperatures (T_m) of all the duplexes were measured using a JASCO V-730BIO spectrophotometer with a temperature control attachment. Absorption at 260-nm (A260) of equimolar DNA complements (4.0 μM in 100 mM NaCl, 50-mM Tris-HCl buffer pH7.4) were measured every 0.5 ºC/min from 20–80 °C.

Photooxidation experiments

Aliquots (4.0-μM for DNA I-III, 16.0-μM for DNA IV, 100-mM NaCl, 50-mM Tris-HCl, pH 7.4, total volume 30 μL) were prepared for irradiation. DNA solutions were irradiated with an Xe lamp (300 W; Asahi Spectra Co. Ltd.; MAX-303) through a UV cut-off filter (LUX350). The irradiated solutions were filtered using an Amicon membrane NMWL of 3 kDa (Merck) to remove spermidine. Filtrated DNA samples were digested by incubation with both alkaline phosphatase and phosphodiesterase I at

37 ºC overnight in order to yield the free nucleosides, and digested samples were analyzed by reversed phase HPLC monitored at 254 nm. HPLC analyses were performed with a JASCO Chromatograph, Extrema using a Chemcobond 5-ODS-H column (4.6 x 150 mm) eluted with 0.05-M ammonium acetate buffer containing acetonitrile (Gradient: 3–9% over 25 min). The percentage decomposition of dG was determined using dC as an internal standard for all HPLC traces. Irradiation experiments were repeated at least three times to give average results.

Electrochemical Measurements.

Measurements of the cyclic voltammetry (CV) of pyrene-dU-CE phosphoramidite were performed at 298 K, using a BAS ALS-710D electrochemical analyzer in a deaerated DMF containing 0.1 M $TBAPF_6$ (tetra-*n*-butylammonium hexafluorophosphate) as a supporting electrolyte at 298 K. A conventional three-electrode cell was used with a grassy carbon working electrode and a platinum wire as a counter electrode. The measured potentials were recorded with respect to the $Ag/AgNO_3$ reference electrode. The E_{red} values (vs $Ag/AgNO_3$) are converted to those vs SCE by ferrocene (0.51V vs SCE in DMF)[1] as an internal reference. All electrochemical measurements were carried out under an atmospheric pressure of argon.

Reference

1 D. Dubois, G. Moninot, W. Kutner, M. T. Jones and K. M. Kadish, *J. Phys. Chem.*, 1992, **96**, 7137-7145.

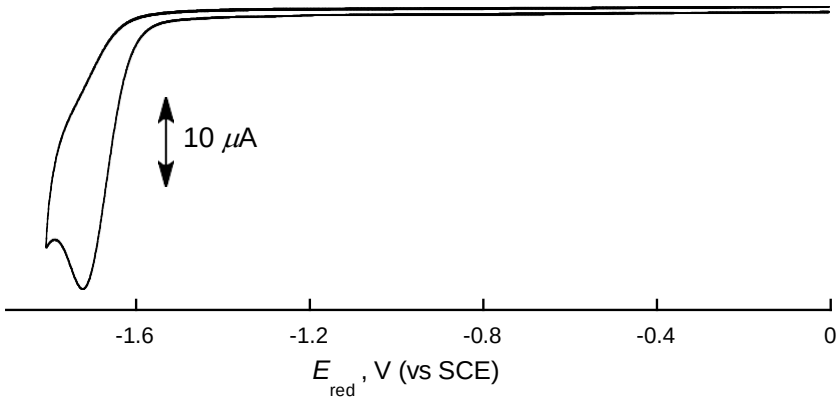

Fig. S1 Cyclic voltammogram of pyrene-dU-CE phosphoramidite (10 mM) in DMF containing 0.1 M $TBAPF_6$ as an electrolyte at 298 K under Ar. Irreversible peak is usually observed for the measurements of nucleobase measurements; See: C. A. M. Seidel, A. Schulz and M. H. M. Sauer, *J. Phys. Chem.* 1996, **100**, 5541-5553.

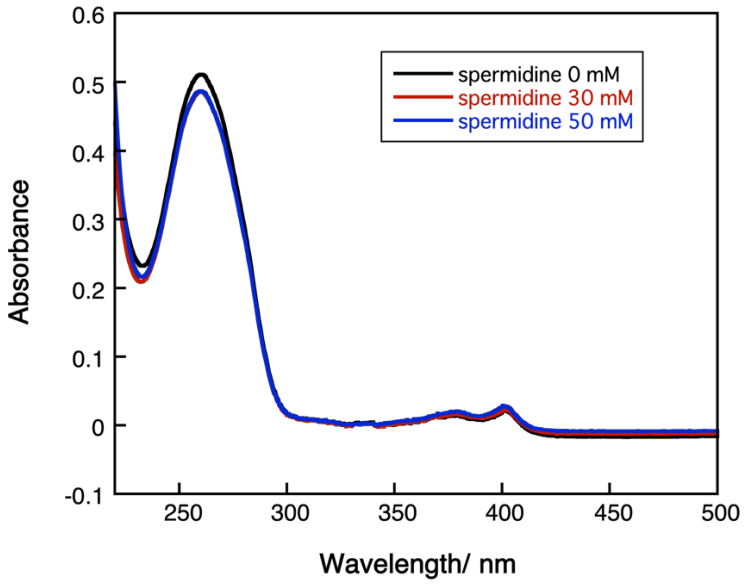

Fig. S2 UV-vis spectra of DNA I in aqueous buffer solution (black line) , 30 mM (red line), and 50 mM (blue line) spermidine-mixed solution. Experimental conditions: [DNA I] = 4.0 μM, and [NaCl] = 100 mM in pH 7.4 Tris-HCl buffer (460 mM).

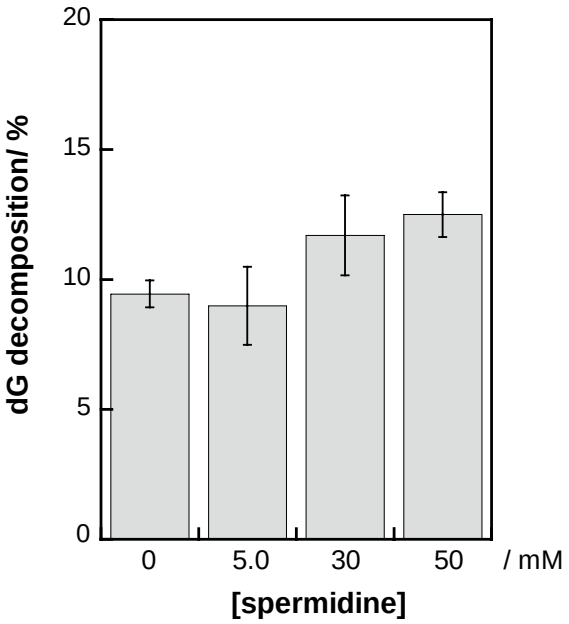

Fig. S3 dG decomposition percentages of the pyrene-modified oligonucleotides: (a) DNA I obtained from photoirradiation (λ_{ex}> 350 nm, 10 min) in the absence and presence of spermidine (5.0-50 mM). The conditions were as follows: [DNA I] = 4.0 μM in Tris-HCl buffer (460 mM), and [NaCl] = 100 mM.