

Supporting Information for

8-Oxo-7,8-Dihydro-2'-Deoxyguanosine Produces a Long-Lived Charge-Separated State During the Photosensitized One-Electron Oxidation of DNA Resulting in Efficient and Exclusive Degradation

*Kiyohiko Kawai**, *Eri Matsutani*, and *Tetsuro Majima**

The Institute of Scientific and Industrial Research (SANKEN), Osaka University, Mihogaoka 8-1, Ibaraki,

Osaka 567-0047, Japan. Tel:(+81)6-6879-8495, Fax:(+81)6-6879-8499

Correspondence should be addressed to K. K. (kiyohiko@sanken.osaka-u.ac.jp) or T. M.

(majima@sanken.osaka-u.ac.jp.)

Material and Methods

DNA Synthesis. Cyanoethyl phosphoramidite of *N*-(3-hydroxypropyl)-1,8-naphthalimide and 8-Oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) were synthesized as previously reported.¹⁻³ All other reagents for DNA synthesis were purchased from Glen Research. DNA were synthesized on an Applied Biosystems DNA synthesizer and purified by reverse phase HPLC and lyophilized. All the NI-modified DNA studied here were characterized by MALDI-TOFF mass spectra, and their concentrations were determined by complete digestion with nuclease P1, phosphodiesterase I and AP to 2'-deoxyribonucleosides. Duplex solutions (20 mM sodium phosphate buffer (pH 7.0)) were prepared by mixing equimolar amounts of the desired DNA complements and gradually annealing with cooling from 80 °C to room temperature.

Laser flash photolysis. The nanosecond transient absorption measurements were performed using the laser flash photolysis technique.⁴⁻¹⁷ Briefly, the third-harmonic oscillation (355 nm, FWHM of 4 ns, 6 mJ/pulse) from a Q-switched Nd:YAG laser (Continuum, Surelight) was used for the excitation light which was expanded to a 1-cm diameter. The light from a xenon flash lamp (Osram, XBO-450) was focused into the sample solution for the transient absorption measurement. Time profiles of the transient absorption in the UV-visible region were measured with a monochromator (Nikon, G250) equipped with a photomultiplier (Hamamatsu Photonics, R928) and digital oscilloscope (Tektronics, TDS-580D). The quantum yield of the formation of the charge separated state (Φ_{cs}) was determined from the transient absorption of the triplet benzophenone as an actinometer during the 355 nm laser flash photolysis. The time profiles were obtained from the average of 32 laser shots. Full transient absorption spectrum and the

bimolecular rate constant between $\text{NI}^{\bullet-}$ and O_2 were measured using NI-G5 (NI-TTTTTGTCTGTTT/AAAAACAGACAAA) which shows long lifetime of charge-separated state (38 μs) (Fig. S1). The k_{O_2} was determined by comparing the time profile measured under air (Fig. S1b, cyan) with that measured under Ar (Fig. S1b, green).

Measurement of the quantum yield of DNA damage (consumption of G (Φ_{-G}) and consumption of oxG (Φ_{-oxG})). Φ_{-G} and Φ_{-oxG} was measured by using NI-TTTTTCGCGC/AAAAAGCGCG ($\Phi_{-G} = 3.3 \times 10^{-3}$) as a chemical actinometer.¹⁸ Photoirradiation was carried out in an aqueous solution containing 40 μM DNA (strand concentration) and 20 mM (pH 7.0) Na phosphate buffer. The solution mixture was photo-irradiated with a Q-switched Nd:YAG laser (355 nm, FWHM of 4 ns, 0.5 mJ / pulse, 1 Hz, Continuum, Surelight). NI-G was photo-irradiated for 15 min (0.45 J), while NI-oxG was photo-irradiated for 90 sec (0.045 J) where the consumption of 8-oxodGuo was linearly correlated with the irradiation time (Fig. S2). The reaction mixture was subjected to enzymatic digestion with nuclease P1, phosphodiesterase I, and alkaline phosphatase at 37 °C. The consumption of dGuo and 8-oxodGuo was quantified by the reverse phase HPLC on a Nacalai Tesque 5C18-PAQ HPLC column with a solvent mixture of 50 mM ammonium formate, acetonitrile 1-8%/0-15 min, and 8-100%/15-25 min at a flow rate of 1.0 ml/min and monitored at 254 nm using dA as internal standards. Φ_{-G} and Φ_{-oxG} was measured as the average of three measurements.

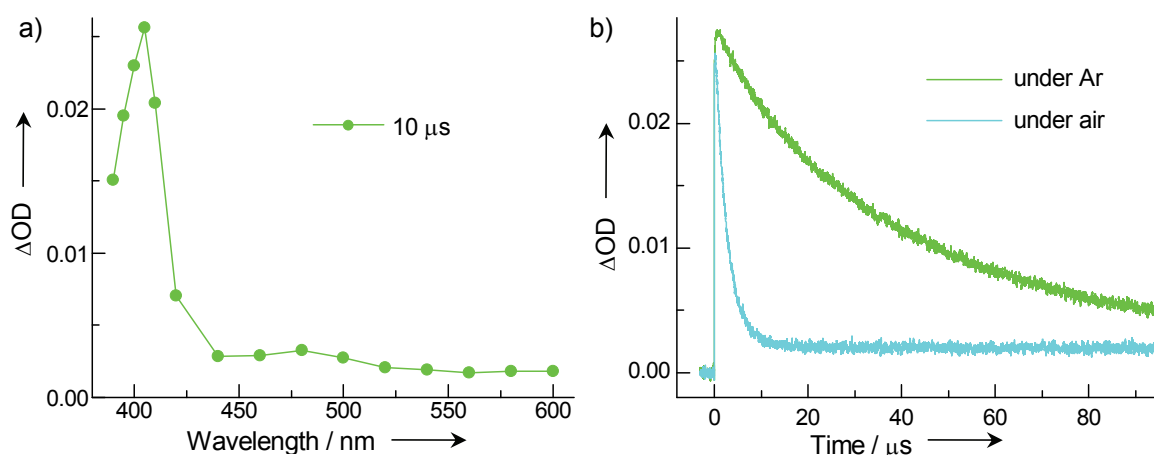


Figure S1. (a) The transient absorption spectrum of NI-G5 obtained at 10 μs after the 355-nm laser flash excitation under Ar. (b) Time profiles of the transient absorption of $\text{NI}^{\bullet-}$ monitored at 400 nm during the laser flash photolysis of NI-G5 under Ar (green) and under air (cyan). The sample aqueous solution contained 40 μM DNA and 20 mM Na phosphate buffer (pH 7.0).

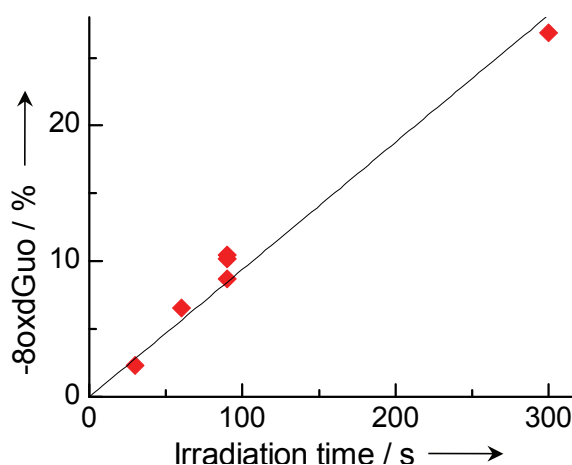


Figure S2. The time dependent consumption of 8-oxodGuo during the photosensitized one-electron oxidation of NI-oxG. An aqueous solution containing 40 μM NI-oxG (strand conc.) and 20 mM pH 7.0 Na phosphate buffer was photo-irradiated with a Q-switched Nd:YAG laser (355 nm, FWHM of 4 ns, 0.5 mJ / pulse, 1 Hz, Continuum, Surelight). The reaction mixture was directly subjected to enzymatic digestion with nuclease P1, phosphodiesterase I, and alkaline phosphatase at 37 $^{\circ}\text{C}$. The consumption of 8-oxodGuo was quantified by the reverse phase HPLC.

References

1. J. E. Rogers and L. A. Kelly, *J. Am. Chem. Soc.*, 1999, **121**, 3854-3861.
2. K. Kawai, T. Takada, S. Tojo, N. Ichinose and T. Majima, *J. Am. Chem. Soc.*, 2001, **123**, 12688-12689.
3. K. Kawai, T. Kimura, K. Kawabata, S. Tojo and T. Majima, *J. Phys. Chem. B*, 2003, **107**, 12838-12841.
4. K. Kawai, H. Kodera, Y. Osakada and T. Majima, *Nature Chem.*, 2009, **1**, 156-159.
5. Y. Osakada, K. Kawai, M. Fujitsuka and T. Majima, *Nucleic Acids Res.*, 2008, **36**, 5562-5570.
6. Y. Osakada, K. Kawai, M. Fujitsuka and T. Majima, *Chem. Commun.*, 2008, 2656-2658.
7. K. Kawai, Y. Osakada, M. Fujitsuka and T. Majima, *Chem. Eur. J.*, 2008, **14**, 3721-3726.
8. Y. Osakada, K. Kawai, M. Fujitsuka and T. Majima, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 18072-18076.
9. T. Takada, K. Kawai, M. Fujitsuka and T. Majima, *Angew. Chem., Int. Ed.*, 2006, **45**, 120-122.
10. K. Kawai, Y. Osakada, M. Fujitsuka and T. Majima, *Chem. Biol.*, 2005, **12**, 1049-1054.
11. T. Takada, K. Kawai, M. Fujitsuka and T. Majima, *Chem. Eur. J.*, 2005, **11**, 3835-3842.
12. T. Takada, K. Kawai, M. Fujitsuka and T. Majima, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 14002-14006.
13. K. Kawai, Y. Osakada, T. Takada, M. Fujitsuka and T. Majima, *J. Am. Chem. Soc.*, 2004, **126**, 12843-12846.
14. K. Kawai, X. Cai, A. Sugimoto, S. Tojo, M. Fujitsuka and T. Majima, *Angew. Chem., Int. Ed.*, 2004, **43**, 2406-2409.
15. T. Takada, K. Kawai, X. Cai, A. Sugimoto, M. Fujitsuka and T. Majima, *J. Am. Chem. Soc.*, 2004, **126**, 1125-1129.
16. K. Kawai, T. Takada, S. Tojo and T. Majima, *J. Am. Chem. Soc.*, 2003, **125**, 6842-6843.
17. K. Kawai, T. Takada, T. Nagai, X. Cai, A. Sugimoto, M. Fujitsuka and T. Majima, *J. Am. Chem. Soc.*, 2003, **125**, 16198-16199.
18. K. Kawai, Y. Osakada, M. Fujitsuka and T. Majima, *Chem. Commun.*, 2006, 3918-3920.