

Effects of reaction rate of radical anion of a photosensitizer with molecular oxygen on the photosensitized DNA damage

Kiyohiko Kawai,^{*a} Yasuko Osakada,^a Mamoru Fujitsuka^a and Tetsuro Majima^{*ab}

Received (in Cambridge, UK) 6th June 2006, Accepted 4th July 2006

First published as an Advance Article on the web 4th August 2006

DOI: 10.1039/b608027h

Based on the synthesis of DNA modified with photosensitizers, direct spectroscopic measurements of the hole transfer in DNA, and quantification of the yield of the DNA oxidative damage, the reaction rate of the radical anion of the photosensitizer was demonstrated to be critically important in determining the efficiency of photosensitized DNA damage.

Photoirradiation of DNA-bound photosensitizers (Sens) triggers electron transfer from nucleobases to the Sens to produce the radical anion of the Sens (Sens^{•-}) and the radical cation of the nucleobase (hole). Prior to the charge recombination, the hole can migrate along DNA,^{1–10} and especially the rapid hole transfer between adenines (As) produces the long-lived charge-separated state.^{11–20} Before the charge recombination takes place, irreversible DNA damage can be triggered by two pathways, *i.e.*, the reaction of G^{•+} with water and the reaction of the Sens^{•-} with molecular oxygen (O₂). In the case that naphthalimide (NI) was used as a Sens, the latter pathway was proven to be critically important.²¹ Although the importance of the reaction of Sens^{•-} with O₂ was pointed out by Schuster^{22,23} and has been often discussed in the literature,^{24–26} there have been only few reports addressing the reaction rate of Sens^{•-} in the vicinity of DNA. Herein, to investigate the importance of the reaction rate of Sens^{•-} with O₂ during the photosensitized DNA damage reactions through the one-electron oxidation pathway, photosensitized DNA damage reactions were compared between two well known DNA-damaging Sens, NI and naphthaldiimide (NDI).^{27–32} NI and NDI have similar photophysical properties but differ in their redox properties,²⁷ that is the reaction rates of their radical anion with O₂ are supposed to be somewhat different. NI- and NDI-modified DNA were synthesized as reported previously (Fig. 1a),^{14,33,34} and a combination of laser flash photolysis kinetic studies and quantitative HPLC analyses of photosensitized DNA damage reactions were investigated.

To target consecutive A sequences which promote fast hole transfer between As, NI and NDI were covalently attached to AAAA (A₄) and AAAAA (A₅) sequences. Both NI and NDI can oxidize A in their singlet excited state²⁷ to promote hole transfer between the As. Therefore, the excitation of the NI- and NDI-modified DNA produces their radical anions, NI^{•-} and NDI^{•-},

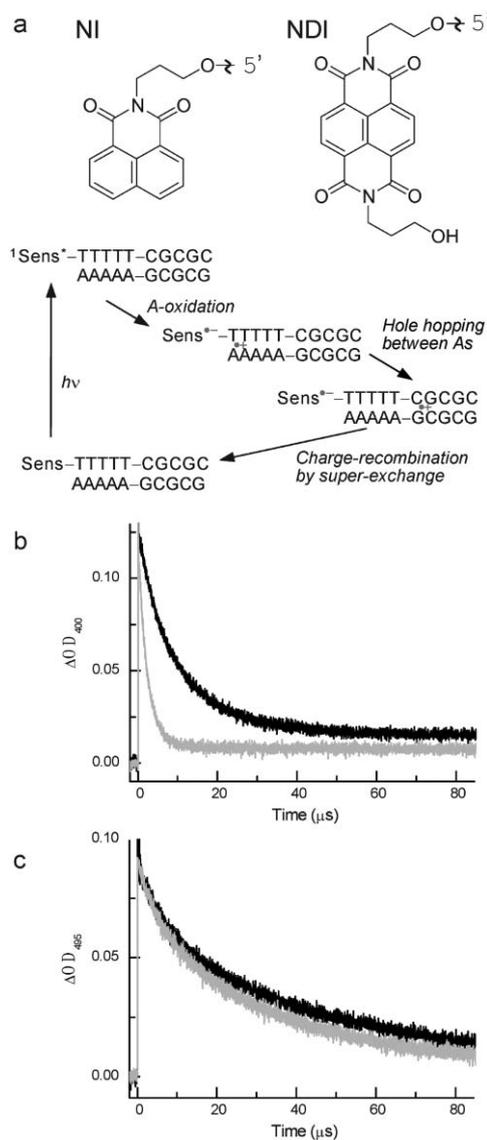


Fig. 1 (a) Structures of NI and NDI, and kinetic scheme for photo-induced one-electron oxidation of A and subsequent hole transfer between As and charge-recombination in DNA. (b) Time profiles of the transient absorption of NI^{•-} monitored at 400 nm during the 355 nm laser flash photolysis (FWHM of 4 ns, 20 mJ per pulse) of NI-A₅ under Ar (black) and under air (grey). (c) Time profiles of the transient absorption of NDI^{•-} monitored at 495 nm during the 355-nm laser flash photolysis of NDI-A₅ under Ar (black) and under air (grey). Sample solution contained 40 μM DNA in 20 mM Na phosphate buffer (pH 7.0).

^aThe Institute of Scientific and Industrial Research (SANKEN), Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan. E-mail: kiyohiko@sanken.osaka-u.ac.jp; Fax: +81-6-6879-8499; Tel: +81-6-6879-8496

^bThe Institute of Scientific and Industrial Research (SANKEN), Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan. E-mail: majima@sanken.osaka-u.ac.jp; Fax: +81-6-6879-8499; Tel: +81-6-6879-8495

Table 1 Decay lifetime of NI^{•-} or NDI^{•-} (τ) and quantum yields of charge-separation (Φ_{CS}) and DNA damage (Φ_G)

DNA	Sequence	τ (μ s)	Φ_{CS} (10^{-2})	Φ_G (10^{-4})
NI-A4	NI-TTTTCGCGCT/AAAAGCGCGA	1.9 ^a	3.7	13
NDI-A4	NDI-TTTTCGCGCT/AAAAGCGCGA	0.20 (48), 7.1 (52) ^b	1.7	1.4
NI-A5	NI-TTTTCGCGC/AAAAGCGCG	8.2 ^a	3.1	33
NDI-A5	NDI-TTTTCGCGC/AAAAGCGCG	6.1 (31), 60 (69) ^b	1.1	6.6

^a Determined from the single exponential fit of the decay of NI^{•-} during the 355 nm laser flash photolysis of Ar-saturated aqueous solution of DNA as described in Fig. 1. ^b The decay lifetime of NDI^{•-} (pre-exponential) previously reported (ref. 15). ^c NI- and NDI-modified DNA were photoirradiated with the 355 nm laser and the quantum yield of DNA damage (consumption of G) was estimated using the absorbed photon number (P) by the sample and the molar absorption coefficient (ϵ) of these Sens at 355 nm (NI: ϵ_{355} 8.0×10^3 , NDI: 1.0×10^4 M⁻¹ cm⁻¹). P was calculated from the following equation: $P = (1 - 10^{-\epsilon ml}) ES\lambda hc$ (1), where m , l , E , S , λ , h and c are concentration of photosensitizer (2.5 μ M), length of cell (0.5 cm), irradiated energy of photon (5.25 J cm⁻²), irradiated area of sample (0.4 cm²), irradiated wavelength (355 nm), and light velocity. The photoirradiated reaction mixture was lyophilized and subjected to enzymatic digestion with P1 nuclease and alkaline phosphatase. The consumption of G was quantified as the average of three to six measurements by reverse phase HPLC using A as an internal standard.

together with an A radical cation (A^{•+}). Followed by the fast hole hopping between As, a hole is trapped at G to form G^{•+}. After G^{•+} is generated far from Sens^{•-}, the charge recombination then proceeds by a super-exchange mechanism which strongly depends on the distance or the number of intervening A–T base-pairs between Sens^{•-} and G^{•+} (Fig. 1b).^{14–19} The charge separation and the charge recombination processes were examined by monitoring the formation and decay of NI^{•-} and NDI^{•-} with a peak at 400 nm and 495 nm, respectively. As shown in Fig. 1b and 1c, rapid hole transfer between As produced a long-lived charge-separated state, and the lifetime of the charge-separated state was longer for DNA having the A₅ sequence than that having the A₄ sequence (Table 1).^{14–19}

The quantum yield of photosensitized DNA damage (Φ_G) was higher for DNA with the A₅ sequence than that for DNA with the A₄ sequence. This is because the lifetime of the charge-separated state becomes longer and provides a longer time for the irreversible reactions to take place as the number of A–T base-pairs between Sens and G increases.^{15,21} Of special interests, when Φ_G of NI- and NDI-modified DNA with the same sequence was compared, Φ_G was much higher for NI-modified DNA than that for NDI-modified DNA (Table 1). During the photosensitized one-electron oxidation of DNA, the reaction becomes irreversible when either the reaction of G^{•+} with water or the reaction of the Sens^{•-} with O₂ occurs faster than the charge recombination. It was previously proven that when NI was used as a Sens, the latter process is the dominant pathway causing the irreversible DNA damage,²¹ where the reaction of G^{•+} with water or O₂^{•-} eventually leads to DNA damage.³⁵ To measure the bimolecular reaction rate of NI^{•-} and NDI^{•-} with O₂, transient absorptions were measured under air (Fig. 1, grey) and compared with those measured under Ar (Fig. 1, black). The decay rate of NI^{•-} was accelerated by the presence of O₂, showing that NI^{•-} reacts with O₂ rapidly with a rate constant of $k_{O_2} = 1.2 \times 10^9$ M⁻¹ s⁻¹, which is close to the diffusion controlled rate in H₂O. In contrast, the presence of O₂ only slightly affected the decay of NDI^{•-}; that is, NDI^{•-} reacts with oxygen only slowly ($k_{O_2} = 4.2 \times 10^7$ M⁻¹ s⁻¹). The difference in the reaction rates was explained by the difference in the redox potentials between NI (-1.0 V vs NHE) and NDI (-0.22 V vs NHE),²⁷ where the former is more negative and the latter is more positive than the reduction potential of O₂ (-0.32 V vs NHE). Therefore, the low Φ_G observed for NDI-modified DNA can be explained by the slow reaction rate between NDI^{•-} and O₂. Hence, when bound to DNA, NI is a better Sens to produce DNA

damage especially when the numbers of consecutive As are small; that is, when the lifetime of the charge-separated state is not long enough for the reaction of G^{•+} with water to occur.

In conclusion, laser flash photolysis and DNA damage quantification by HPLC of the NI- and NDI-modified DNA were performed, and it was clearly demonstrated that the reaction rate of O₂ and Sens^{•-} is one of the critically important factors determining the efficiency of photosensitized DNA damage. These results may provide us with new strategies for the future design of the Sens for therapeutic and biochemical applications.

This work has been partly supported by a Grant-in-Aid for Scientific Research (Project 17105005, Priority Area (417), 21st Century COE Research, and others) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japanese Government.

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