

Long-Lived Charge-Separated State Leading to DNA Damage through Hole Transfer

Kiyohiko Kawai,* Tadao Takada, Takayoshi Nagai, Xichen Cai, Akira Sugimoto, Mamoru Fujitsuka, and Tetsuro Majima*

The Institute of Scientific and Industrial Research (SANKEN), Osaka University, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan

Received September 3, 2003; E-mail: kiyohiko@sanken.osaka-u.ac.jp; majima@sanken.osaka-u.ac.jp

The one-electron oxidation of DNA occurs during photoirradiation in the presence of photosensitizers and has been extensively studied because it leads to the formation of oxidative lesions that causes carcinogenesis and aging.^{1,2} Photosensitized DNA damage has also received attention from a therapeutic point of view, since DNA is one of the potential targets of photodynamic therapy.³ The photoirradiation of DNA-bound sensitizers produces the radical anion of the sensitizer ($S^{\bullet-}$) and radical cation of guanine ($G^{\bullet+}$) as the charge-separated state through photoinduced electron transfer.⁴ The efficiency of the photoinduced one-electron oxidation of DNA is seemingly low since the charge recombination rate is usually much faster than the process leading to the DNA strand cleavage, such as the reaction of $G^{\bullet+}$ with water.⁵⁻⁷ However, the photosensitized DNA damage does occur.⁸ Recently, we reported the kinetics of hole transfer in DNA by the adenine(A)-hopping mechanism and the weak distance dependent hole transfer with hole transfer rates faster than 10^8 s^{-1} over the distance range of 7–22 Å.^{9,10} It was suggested that a hole transfer by the A-hopping mechanism may help to separate the hole and $S^{\bullet-}$ during the photosensitized one-electron oxidation of DNA, providing a sufficiently long time for $G^{\bullet+}$ and $S^{\bullet-}$ to react with water or O_2 . Here, to clarify the effects of the hole transfer on the DNA damage during the photosensitized one-electron oxidation of DNA, the laser flash photolysis (LFP) and HPLC analysis of naphthaldiimide (NDI)-modified oligodeoxynucleotides (ODNs) were performed. The combination of a transient absorption measurement and DNA damage quantification clearly demonstrated that the yield of the DNA damage correlates well with the lifetime of the charge-separated state during the photosensitized one-electron oxidation of DNA.

NDI was selected as a photosensitizer because ¹NDI* can oxidize A to promote hole transfer by the A-hopping mechanism to eventually yield $G^{\bullet+}$.¹⁰⁻¹⁴ Several ODNs with different distances between the NDI and Gs with intervening A_n sequences (NDn) were synthesized,^{10,15} and the effect of the hole transfer on the DNA damage was investigated (Table 1). The excitation of NDI-modified ODN with the 355-nm laser (5 ns, 5 mJ pulse⁻¹) produced the NDI radical anion ($NDI^{\bullet-}$) and $ODN^{\bullet+}$ charge-separated state through the photoinduced electron transfer, and the charge separation and recombination process were examined by monitoring the formation and decay of $NDI^{\bullet-}$, as shown in Figure 1. In the case of NDn ($n = 0-2$) where Gs are near the NDI, no transient absorption was observed due to the fast charge separation and charge recombination which proceed within the laser flash duration of 5 ns. In NDn ($n = 3-5$) where the Gs are separated from NDI by more than three base pairs, the formation of the transient absorption with a maximum peak at 495 nm was observed immediately after the flash excitation, which was assigned to $NDI^{\bullet-}$.^{16,17} The yield of the formed $NDI^{\bullet-}$ was similar for NDn ($n = 3-5$). In contrast, the lifetime of the charge-separated state significantly increased with

Table 1. Decay Lifetime of $NDI^{\bullet-}$ and Consumption of G in the Photosensitized Damaging of ODNs^a

ODNs	sequence	τ (μs) ^b	-G (%) ^c
ND0	⁵ NDI-CGCGCTTTTT ³ GCGCGAAAAA	<0.005	<0.1
ND1	⁵ NDI-TCGCGCTTTTT ³ AGCGCGAAAA	<0.005	<0.1
ND2	⁵ NDI-TTCGCGCTTT ³ AAGCGCGAAA	<0.005	<0.1
ND3	⁵ NDI-TTTCGCGCTT ³ AAAGCGCGAA	0.24	0.36
ND4	⁵ NDI-TTTTCGCGCT ³ AAAAGCGCGA	0.20 (48), 7.1 (52)	2.6
ND5	⁵ NDI-TTTTTGCGCG ³ AAAAAGCGCG	6.0 (31), 60 (69)	10
NDG	⁵ NDI-TCTTTGCGCG ³ AGAAAACGCG	<0.005	1.5

^a Laser flash photolysis was carried out in an aqueous solution containing 40 μM ODN (strand concentration) and 20 mM pH 7.0 Na phosphate buffer. ^b The decay lifetime of $NDI^{\bullet-}$ (pre-exponential). ^c ODNs were photoirradiated with 355 nm laser (1.6 mJ pulse⁻¹, 1500 pulses, total irradiated energy of 2.4 J) and digested with snake venom phosphodiesterase/nuclease P1/alkaline phosphatase to 2'-deoxymononucleosides. The consumption of G was quantified by HPLC using A as an internal standard.

the increasing distance between NDI and Gs, i.e., the charge recombination process is strongly distance-dependent. These results are consistent with the charge separation by the A-hopping and charge recombination by the superexchange mechanism as previously reported.^{9,18-22} The charge-separation yield is small at about 2%¹⁰ because of the fast charge recombination from the contact radical ion pair.²³ Therefore, the charge-separated state is generated by occasional escape from the charge recombination by the hole shift process. However, once a hole escapes from the Coulombic interaction, it efficiently migrates through DNA by the A-hopping mechanism.¹⁰ Insertion of a single G in the A_n sequence between NDI and Gs significantly diminished the transient absorption of $NDI^{\bullet-}$ (NDG) since the inserted G serves as a hole trap on the hole shift process, causing inhibition of consecutive A-hopping in which a hole migrates to Gs.^{24,25}

To investigate the effect of the hole transfer on the DNA damage during the photosensitized one-electron oxidation of DNA, NDn was photoirradiated and the consumption of G was quantified by HPLC (Table 1). Interestingly, the consumption of G increased with the increasing distance between NDI and Gs. In the case of NDG, where most of the generated holes recombine within the laser duration because of the inserted G in the A_n sequence, the consumption of G was small even though the remaining Gs are located far from NDI.²⁶ Thus, not the distance between the NDI and Gs, but the lifetime of the charge-separated state determines the efficiency of the DNA damage. In other words, the yield of the

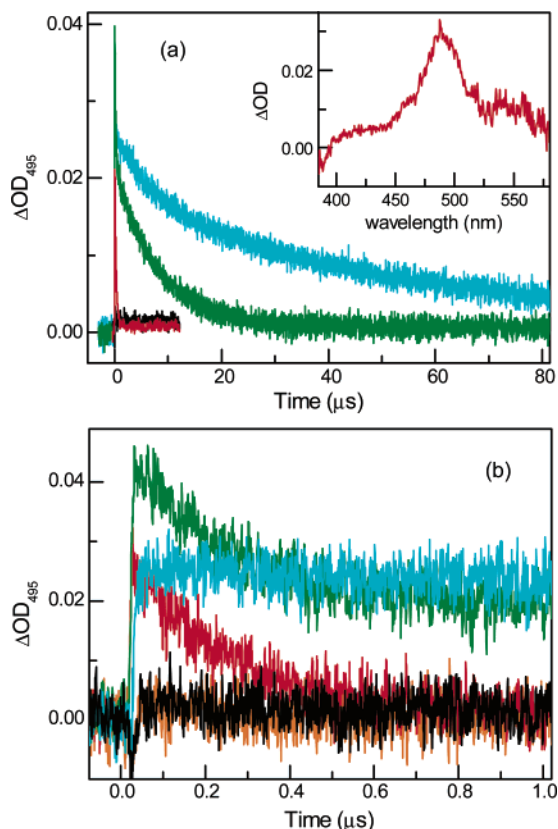


Figure 1. Time profiles of the transient absorption of NDI^{*-} monitored at 495 nm during the 355-nm laser flash photolysis of Ar-saturated aqueous solution of NDI-modified ODNs: ND2 (orange), ND3 (red), ND4 (green), ND5 (blue), and NDG (black). On the time scales of (a) 0 to 80 μs and (b) 0 to 1 μs . The inset shows the transient absorption spectrum of NDI^{*-} obtained at 100 ns after 355-nm flash excitation of ND3.

DNA damage increases with the increase in the lifetime of the charge-separated state.

In summary, the combination of the transient absorption measurement and DNA damage quantification provides the data for the effects of the hole transfer on the DNA damaged during the photosensitized one-electron oxidation of DNA. For the photosensitizers, which can only oxidize G, the close distance between the photosensitizer and G is crucial for efficient DNA damage.^{27,28} In contrast, in the case of photosensitizers, which can also oxidize A to promote the hole transfer by the A-hopping mechanism, the shorter distance between the photosensitizer and G is not necessary for efficient DNA damage. This is also true in the case of Schuster's work where anthraquinone was used as the photosensitizer which can also oxidize A.^{29,30} Our results strongly suggest that the hole transfer plays an important role in separating S^{*-} and G^{*+} during

the photosensitized one-electron oxidation of DNA, providing a sufficiently long time for G^{*+} and S^{*-} to react with water or O_2 , avoiding the charge recombination, and making the reaction irreversible.

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