DNA Damage

Two-Color Two-Laser DNA Damaging**

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Photodynamic therapy (PDT) is a promising treatment for cancer based on a photosensitized oxidative reaction at the diseased tissues which results in cell death. Membranes, proteins, and DNA are considered as a potential targets.[1,2] In contrast to surgery and chemotherapy, the combination of photosensitizer (Sens) uptake in malignant tissues and selective light delivery offers the advantage of a selective method of destroying diseased tissues without damaging surrounding healthy tissues. Following the absorption of light, the photosensitizer is activated to the singlet excited state, ^{1}Sens*, which may be converted into the triplet excited state, ^{3}Sens*. The mechanisms of tumor destruction involve oxidation through electron transfer from cellular components to ^{1}Sens* or ^{3}Sens* (type I mechanism), as well as oxidation

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mediated by singlet oxygen (type II mechanism), which is formed through energy transfer from ^{3}Sens* to molecular oxygen.[3] When targeting DNA, the binding of Sens to DNA will favor the type I process, as the close association of Sens to DNA is important in the photoinduced one-electron oxidation of DNA.[4]

Excitation of DNA-bound sensitizers produces the Sens$^{\bullet-}$/DNA$^{\bullet+}$ (ultimately yielding the radical cation of guanine (G$^{\bullet+}$), the most easily oxidized base, by hole transfer) charge-separated state through photoinduced electron transfer. However, the efficiency of producing photosensitized DNA damage is low because the charge recombination rate is usually much faster than the process leading to DNA damage, such as the reaction of G$^{\bullet+}$ with water.[5–8] Thus, the absorption of a photon by Sens occasionally leads to DNA damage, but only with the aid of hole transfer, which provides time for DNA$^{\bullet+}$ and Sens$^{\bullet-}$ to react with water or O_2.[9–14] Herein, we report the first study of nanosecond-laser DNA damaging in which a combination of two-color pulses is used as a promising new strategy to reach a high DNA-damaging efficiency. The first laser pulse was applied for the production of the Sens$^{\bullet-}$ and DNA$^{\bullet+}$, and the second laser pulse for the ejection of an electron from Sens$^{\bullet-}$, making the reaction irreversible.[15]

The proposed two-color two-laser DNA damaging is represented as a two-step process in a simplified scheme (Figure 1). In this study, naphthaldiimide (NDI) was selected

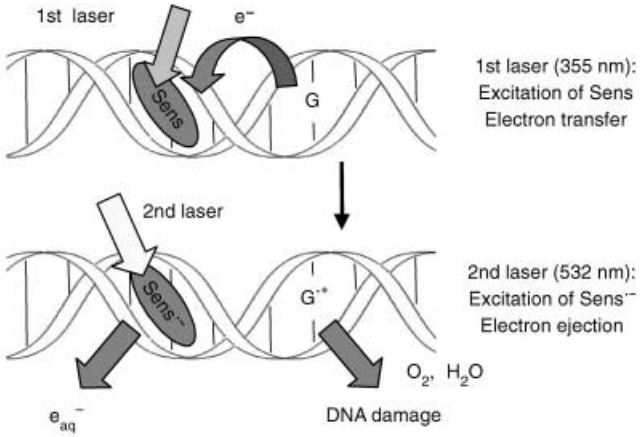

Figure 1. Schematic representation of two-color two-laser DNA damaging.

as a photosensitizer that can be excited with a first laser at a wavelength of 355 nm.[16–20] First, to assess the feasibility of electron ejection from Sens$^{\bullet-}$ bound to DNA, a pulse radiolysis–laser flash photolysis of NDI-conjugated oligodeoxynucleotide (NDI-ODN) was performed (Figure 2).[21,22] NDI$^{\bullet-}$ with a maximum absorption peak at 495 nm[16] was generated from electron attachment during the pulse radiolysis of NDI-ODN. Since Sens$^{\bullet-}$ often absorbs light at a longer wavelength than it does in its non-reduced form, laser pulses with a longer wavelength can be used for the excitation of Sens$^{\bullet-}$, and a 532-nm laser was applied as the second laser. Irradiation of NDI$^{\bullet-}$ in NDI-ODN with a 532-nm laser pulse

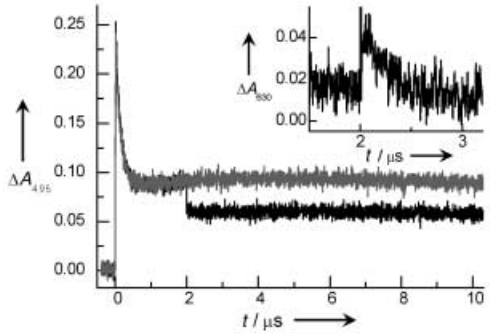

Figure 2. Electron ejection from NDI$^{\bullet-}$ promoted by a 532-nm laser pulse. NDI$^{\bullet-}$ was generated from electron attachment during the pulse radiolysis of NDI-ODN (NDI-AAAAAAGTGCGC/TTTTTTCACGCG) (gray), and photoirradiated with a 532-nm laser pulse at 2 μs after the electron pulse (black). Inset: Formation and decay of the solvated electron monitored at 630 nm.

caused a decrease in ΔOD of NDI$^{\bullet-}$ and an absorption at 630 nm assigned to a solvated electron (e_{aq}^-) immediately after the flash (inset), demonstrating the successful ejection of an electron from NDI$^{\bullet-}$ to the solvent water.

To test the efficiency of two-color two-laser irradiation for DNA damaging, the consumption of G upon irradiation of ODN-G and ODN-GG bound to added *N*,*N*′-bis-[3-(*N*-dimethyl)propyl]-1,4,5,8-naphthaldiimide dichloridite (NDI-HCl) and NDI-ODN was compared with that of single-laser experiments. Interestingly, a higher consumption of G was observed with the combination of the first and second laser irradiations than with irradiation by the 355-nm pulses alone (Table 1).[23] If only 532-nm pulses were used, no DNA damage would occur because this would require a non-resonant two-photon excitation, as neither nonreduced NDI nor DNA absorbs above 450 nm.[24,25] Hence, the second laser alone cannot damage DNA, but does provide enough additional energy to cross the ionization threshold of NDI$^{\bullet-}$, which results in the transfer of the electron to the solvent and

Table 1: Consumption of G in the photosensitized damaging of ODNs.[a]

ODN		−G [%][a]		
		355 nm	532 nm	355+532 nm
ODN-G[b]	$(TG)_6T$ /$(AC)_6A$	0.6	< 0.3	2.6
ODN-GG[b]	$(TTGG)_3T$ /$(AACC)_3A$	2.5	< 0.2	16.7
NDI-ODN[c]	NDI-$T_3CGCGCT_2$ /$A_3GCGCGA_2$	0.8	0	14.2

[a] Laser flash photolysis was carried out in an aqueous solution containing ODN (strand conc. = 40 μM) and Na phosphate buffer (20 mM, pH 7.0). In the case of ODN-G and ODN-GG, NDI-HCl (40 μM) was added. For the two-wavelength irradiation, the 532-nm laser pulse (20 mJ/pulse) was synchronized with the 355 nm laser pulse (1.6 mJ/pulse) with a 10-ns delay. Photoirradiated ODNs were digested with snake venom phosphodiesterase/nuclease P1/alkaline phosphatase to 2′-deoxyribonucleosides, and the consumption of G was quantified by HPLC with A as an internal standard. [b] Photoirradiated for 20 min (355 nm: 19 J; 532 nm: 240 J). [c] Photoirradiated for 5 min (355 nm: 4.8 J; 532 nm: 60 J).

makes the reaction irreversible.[15] A higher consumption of G was observed for ODN-GG than for ODN-G; the ionization potential of G in the former is lowered by a stacking interaction between G units,[26] demonstrating that the consumption of G is based on the photoinduced electron transfer by the first laser. The acceleration of DNA damaging by the second laser was the highest for ODN covalently bonded with NDI, in which case the sequence was designed to generate a hole selectively on adenine and to have a lifetime of charge-separated state in the order of 100 ns.[14,27] Figure 3 shows the

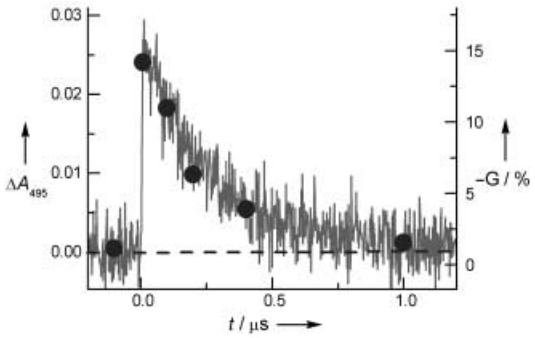

Figure 3. Formation and decay of $NDI^{\bullet-}$ and the effect of the delay time between two laser pulses on the consumption of G during the laser flash photolysis of NDI-ODN (NDI-TTTCGCGCTT/AAAGCGC-GAA). The transient absorption of $NDI^{\bullet-}$ was monitored at 495 nm following 355-nm excitation (left axis). The consumption of G is plotted as a function of the delay of the 532-nm pulse with respect to the 355 nm pulse (●: right axis). The dashed line shows the consumption of G in the absence of the 532-nm pulse.

time profile of $NDI^{\bullet-}$ in the one-color laser photolysis of NDI-ODN. Upon the first laser excitation, hole transfer by consecutive fast adenine hopping leads to a charge-separated state within the period of laser excitation (5 ns), and the charge recombination proceeds by a single-step superexchange from $G^{\bullet+}$ about 14 Å away from $NDI^{\bullet-}$ with a lifetime of 240 ns.[6,14,27] Also shown in Figure 3 is the consumption of G as a function of the delay time of the second laser pulse in the time-delayed two-color photolysis. The dependence of the consumption of G on the delay time agrees well with the decay of the transient absorption of $NDI^{\bullet-}$ obtained in the one-color laser photolysis. Thus, the acceleration caused by the second laser is clearly based on the excitation of $NDI^{\bullet-}$. The experiments were performed under low-conversion conditions, and the consumption of G was correlated linearly with the irradiation time and the power of the second laser in the present experimental arrangement.[28]

In the study described herein, we demonstrated that a combination of nanosecond laser pulses at two different colors increases DNA damage. This strategy has the advantage that the intensity of the 355-nm pulses in the first step can be kept low, and a high DNA-damaging efficiency can be attained by applying the second laser pulse at a longer wavelength with a greater depth of tissue penetration owing to reduced scattering and minimal absorption from non-pharmacological chromophores in the tissue. Two-color irradiation also offers spatial control of the reaction at the focal point of the lasers.[29–33] The photosensitizer NDI used in this preliminary experiment is not adequate for practical as since the charge-separation yield is small (about 2%) owing to the fast charge recombination from the contact radical ion pair.[27,34] Therefore, a second laser exerts its effect only on $NDI^{\bullet-}$ generated by occasional escape from the charge recombination by hole transfer. Thus, for efficient oxidative DNA damage to occur, it is necessary to increase the charge-separation yield by decreasing the rate constant for charge recombination. This can be achieved by the use of triplet sensitizers,[4] and by hole generation on adenine to promote fast hole transfer by an adenine-hopping mechanism that helps to separate the hole and $Sens^{\bullet-}$.[14,21]

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