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Consecutive Adenine Sequences Are Potential Targets in Photosensitized DNA Damage

Kiyohiko Kawai,* Yasuko Osakada, Mamoru Fujitsuka, and Tetsuro Majima*
The Institute of Scientific and Industrial Research (SANKEN)
Osaka University
Ibaraki, Osaka 567-0047
Japan

Summary

Based on direct spectroscopic measurements of hole transfer in DNA and quantification of the yield of DNA oxidative damage, consecutive adenine sequences were found to be a good launching site for photosensitizers to inject a hole in DNA, where the following rapid hole transfer between adenines causes a long-lived charge-separated state leading to DNA oxidative damage. According to the results, the essential requisites for an efficient and/or harmful photosensitizer are determined as follows: to be able to oxidize adenine to trigger hole transfer between adenines, and react rapidly with molecular oxygen following its reduction, avoiding charge recombination and making the reaction irreversible. These results will greatly help us to classify photosensitizers harmful to human health, and to design an improved photosensitizer for biochemical applications.

Introduction

Photosensitized oxidative DNA damage has been extensively studied because it leads to the formation of oxidative lesions that cause carcinogenesis and aging [1–3]. Following the absorption of light, photosensitizers (Sens) are activated to the singlet excited state, which may convert to the triplet excited state. The mechanisms of photosensitized DNA damage involve oxidation through electron transfer from DNA to the Sens in the singlet or triplet excited state (mechanism Type I), as well as oxidation mediated by singlet oxygen (mechanism Type II), which is formed through energy transfer from the Sens in the triplet excited state to molecular oxygen (O_2) [4]. Because lifetimes of Sens in the excited state or singlet oxygen are usually short in living cells, Sens should be located in the vicinity of DNA for photosensitized DNA damage to occur. And when the Sens is bound to DNA, the Type I process will be favored because, for many of the well-studied DNA-damaging Sens, the electron transfer rates from DNA to the Sens in the singlet excited state is faster compared to the rates of the intersystem crossing which leads to the formation of the Sens in the triplet excited state and subsequent singlet oxygen generation [5–10]. Hence, it is important to understand the kinetic mechanisms of the photosensitized one-electron oxidation of DNA, the Type I process [11, 12].

*Correspondence: kiyohiko@sanken.osaka-u.ac.jp (K.K.); majima@sanken.osaka-u.ac.jp (T.M.)

The photoirradiation of DNA-bound Sens triggers electron transfer from nucleobases to the excited Sens to produce the radical anion of the Sens ($Sens^{\bullet-}$) and radical cation of the nucleobase (hole) as the charge-separated state. Because guanine (G) exhibits the lowest oxidation potential among the four DNA bases, the radical cation of G ($G^{\bullet+}$) is formed, of which reaction with water and O_2 leads to oxidative DNA damage [1–3]. Apparently, it seems likely that the efficiency of the photosensitized one-electron oxidation of DNA is low because the process leading to DNA damage, such as the reaction of $G^{\bullet+}$ with water ($k_G \sim 5\ s^{-1}$ or slower; discussed below) [13–15], is much slower than the charge recombination which typically occurs with rate constants faster than $10^9\ s^{-1}$ [6–10]. A hole has been demonstrated to migrate through DNA by hopping between Gs [16–25]. The rate constants of hole transfer between Gs across A-T base pairs have been determined by Lewis et al. to be smaller than $10^8\ s^{-1}$ [6–9], which is also too slow to compete with charge recombination. Though hole transfer between consecutive Gs may proceed quickly, a hole will localize in the consecutive Gs because a hole cannot get over the A-T bridge, which results in fast charge recombination. However, photosensitized DNA damage actually occurs [2, 3].

In 2001, Giese et al. revealed the role of adenine (A), the second most easily oxidized base, as the charge carriers of hole transfer in DNA [26–28]. Recently, we reported the kinetics of hole transfer between As which proceeds with a rate constant faster than $10^8\ s^{-1}$ over a distance of ~30 Å [29, 30]. These findings prompted us to suggest that this fast hole transfer between As may help to separate the $Sens^{\bullet-}$ and hole in the initial step of the photosensitized one-electron oxidation of DNA before a hole is trapped at G. To assess this hypothesis, we performed a combination of laser flash photolysis transient absorption measurements and quantitative HPLC analyses of DNA damage using naphthaldiimide (NDI)-modified oligodeoxynucleotides (ODNs). It was demonstrated that the lifetime of the charge-separated state (τ) significantly increases with the increasing number of As between NDI and Gs, and the yield of the DNA damage increases with the increasing τ during the photosensitized one-electron oxidation of DNA [31]. Thus, hole transfer was shown to produce a long-lived charge-separated state causing DNA damage by providing the time for $G^{\bullet+}$ and/or $Sens^{\bullet-}$ to react with water or O_2. Here, to further elucidate the key kinetic processes which determine the efficiency of the photosensitized DNA damage by the Type I process, ODNs modified with naphthalimide (NI) and 2′-deoxybromocytidine (^{br}C) were synthesized (Figure 1) and their τ and DNA damage yield during the photosensitized one-electron oxidation of DNA were investigated.

Results and Discussion

Photosensitized DNA Damage and τ

NI in the singlet excited state ($^1NI^*$) can oxidize A to promote hole transfer between As. Therefore, when NI

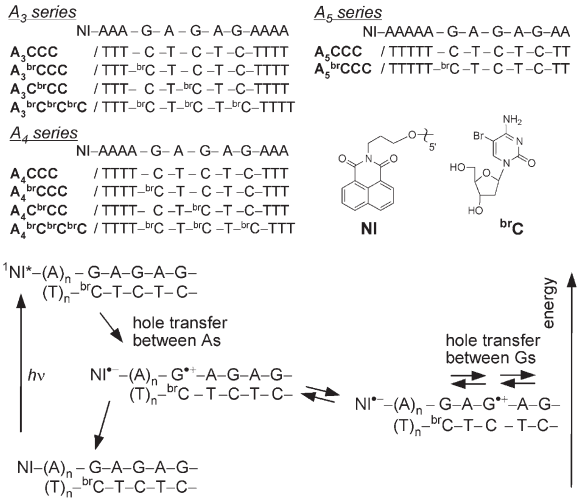

Figure 1. Sequences of NI- and ^{br}C-Modified ODNs, and a Kinetic Scheme for Photo-Induced One-Electron Oxidation of A and Subsequent Hole Transfer in DNA

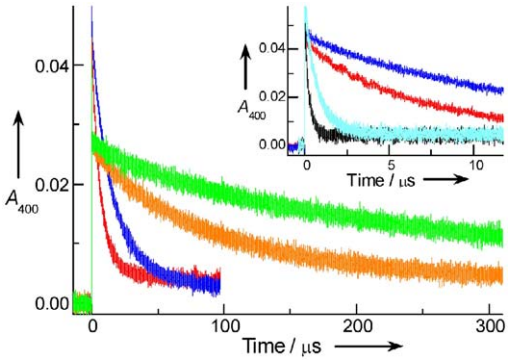

Figure 2. Lifetime of the Charge-Separated State for NI- and ^{br}C-Modified ODNs

Lifetime of the charge-separated state was monitored by the transient absorption of NI$^{\bullet-}$ at 400 nm during the 355 nm laser flash photolysis of an Ar-saturated aqueous solution of ODNs: A_3CCC (black), $A_3{}^{br}CCC$ (cyan), A_4CCC (red), $A_4{}^{br}CCC$ (blue), A_5CCC (orange), and $A_5{}^{br}CCC$ (green). Sample solution contained 40 μM ODN in 20 mM Na phosphate buffer (pH 7.0).

is attached at the consecutive A sequences, the excitation of the NI produces the NI radical anion (NI$^{\bullet-}$) and A radical cation (A$^{\bullet+}$), followed by the fast hole hopping between As, and then a hole is trapped at G to yield G$^{\bullet+}$ as shown in Figure 1 [30–35]. The charge-separated yields (ϕ) decrease with the increasing distance between NI and G, but are weakly dependent upon the distance because the charge separation proceeds by a hopping mechanism. On the other hand, the charge recombination rates are strongly distance dependent because once G$^{\bullet+}$ is generated far from NI$^{\bullet-}$, the charge recombination proceeds by a strongly distance-dependent superexchange mechanism. Hence, τ significantly increases with the increasing number of As between NI and Gs. Before the completion of the charge recombination, the hole can migrate to the more distal Gs, where the difference in the oxidation potential of G causes the shift in the hole equilibrium between the Gs, leading to the change in τ. Recently, we demonstrated that bromine substitution of the cytosine C5 hydrogen in the C:G base pair causes a 24 mV increase in the oxidation potential of G, which resulted in a change in τ during the photosensitized one-electron oxidation of DNA (Figure 1) [33, 36]. Here, in order to investigate the τ dependence of the photosensitized DNA damage in more detail, ^{br}C was incorporated into the ODNs to construct ODNs which show various τ values. As shown in Figure 2 and Table 1, the τ systematically varied depending on the number of A bases between NI and G, and the incorporation sites of ^{br}C. To investigate the effect of τ on DNA damage during the photosensitized one-electron oxidation of DNA, these ODNs were photoirradiated and the consumption of G was quantified by HPLC (Table 1; Figure 3). When the τ was shorter than 1 μs, the yield of the DNA damage increased with the τ. Interestingly, for the ODNs with longer τ, DNA damage did not simply increase with the increasing τ but rather depended both on τ and ϕ, which was in strong contrast to the NDI-modified ODNs.

The Role of O_2

Before the charge recombination takes place, DNA damage can occur through two pathways: the reaction of G$^{\bullet+}$ with water and the reaction of Sens$^{\bullet-}$ with O_2 (Figure 4). As for the reaction of G$^{\bullet+}$ with water, pioneering work has been done by Giese and Spichty in which the k_G value was calculated to be ~6×10^4 s^{-1} based on the assumption that the hole transfer between Gs in the GTTG sequence proceeds with the rate constant of 2.5×10^6 s^{-1} [37]. After their report, this hole transfer rate was experimentally demonstrated to be slower than 1.7×10^5 s^{-1} by Lewis et al. [6], and measured as 3.6×10^4 s^{-1} by Takada et al. [32]. Hence, the k_G value should be much smaller than the value estimated by Giese and Spichty. In line with this, the lifetime of G$^{\bullet+}$ or the deprotonated G$^{\bullet+}$ was experimentally measured

Table 1. Quantum Yields of Charge Separation, Lifetime of the Charge-Separated State, and the Yields of DNA Damage during the Photosensitized One-Electron Oxidation of DNA

ODNs	ϕ ($\times 10^{-2}$)[a]	τ (μs)[b]	–G (%/min)[c]
A_3CCC	3.0	0.24	1.9
$A_3{}^{br}CCC$		0.70	3.4
$A_3C^{br}CC$		0.19	2.0
$A_3{}^{br}C^{br}C^{br}C$		0.26	2.1
A_4CCC	2.4	6.6	10.3
$A_4{}^{br}CCC$		14.2	12.6
$A_4C^{br}CC$		5.2	11.1
$A_4{}^{br}C^{br}C^{br}C$		7.0	12.9
A_5CCC	1.5	93.3	10.2
$A_5{}^{br}CCC$		182	8.8

[a] The yields of formation of the charge-separated state were determined from the absorption of NI$^{\bullet-}$ observed after the excitation using the transient absorption of the triplet benzophenone as an actinometer.
[b] τ was determined from the single exponential fit of the decay of NI$^{\bullet-}$ during the 355 nm laser flash photolysis of Ar-saturated aqueous solution of ODN described in Figure 2.
[c] The yields of photosensitized DNA damage –G (%/min) was measured as shown in Figure 3.

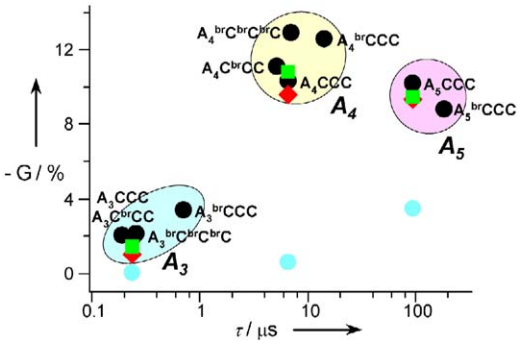

Figure 3. Correlation between the Lifetimes of the Charge-Separated State and the Yields of Photosensitized DNA Damage

The lifetime of the charge-separated state (τ) was determined as described in Table 1. For the DNA damage quantification (–G %/min), an aqueous solution containing 50 μM ODN (strand concentration) and 20 mM Na phosphate buffer (pH 7.0) was photoirradiated at 365 nm with a transilluminator under aerated conditions ([O_2] = 290 μM), and digested with nuclease P1/alkaline phosphatase to 2′-deoxyribonucleosides. The consumption of G was quantified by HPLC (●). Theoretical plots according to Equation 1 for DNA damage using the [O_2] value of 290 μM (green square), 200 μM (red diamond), and 5 μM (cyan circle) are also shown.

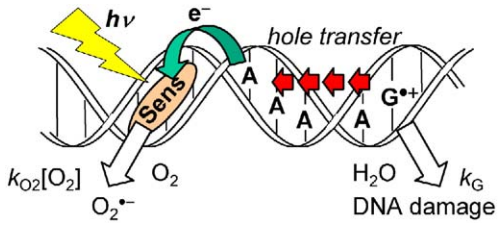

Figure 4. Schematic Representation of Photosensitized DNA Damage

by Hildenbrand and Schulte-Frohlinde to be as long as ~5 s^{-1} in calf thymus DNA [15], and measured by Shafirovich and coworkers to be 0.2 s or longer in duplex DNA [13, 14]. Therefore, the reaction of $G^{\bullet+}$ with water occurs on a much slower timescale than the τ measured in this study (τ < 200 μs). In contrast to the slow reaction of $G^{\bullet+}$ with water, the decay rate of the radical anion of NI ($NI^{\bullet-}$) was accelerated in the presence of O_2, showing that the reaction between $NI^{\bullet-}$ and O_2 rapidly proceeds, which yields $O_2^{\bullet-}$ and NI. Thus, not the reaction of $G^{\bullet+}$ with water, but the reaction between $NI^{\bullet-}$ and O_2, was suggested to be the major process that produces DNA damage, and this proves to be the very reason that, when bound to DNA, NI is a much better photosensitizer than NDI in which the radical anion reacts with O_2 only very slowly. The bimolecular reaction rate constant of $NI^{\bullet-}$ and O_2 was obtained to be k_{O2} = 1.2 × 10^9 M^{-1} s^{-1}. According to this value, the reaction of $NI^{\bullet-}$ and O_2 occurs at 2.9 μs under aerated conditions ([O_2] = 290 μM) [38]. Therefore, for the ODNs having τ longer than 2.9 μs, the DNA damage yield depends on ϕ, which decreases with the number of As (Table 1). Because k_G is much slower than $k_{O2}[O_2]$, DNA damage yield can be depicted by Equation 1.

$$[\text{DNA damage}] = (\text{const.}) \times \phi \times k_{O2}[O_2] / (k_{O2}[O_2] + 1/\tau) \quad (1)$$

According to Equation 1, theoretical plots for the DNA damage for A_3CCC, A_4CCC, and A_5CCC were obtained, and good agreement between the experimental and theoretical plots was found where DNA damage yield was highest for A_4 sequences (Figure 3, green square). Under Ar-bubbling conditions, consumption of G was lower than 1%/min for A_4CCC and was 2%/min for A_5CCC, which is consistent with the much smaller value of k_G compared to $k_{O2}[O_2]$ in aerated conditions, and to $1/\tau$ measured in this study. Therefore, it can be concluded that using NI as the Sens, the reaction between $Sens^{\bullet-}$ and O_2 is the dominant pathway for irreversible DNA damage to take place. Escaping from the charge recombination, $G^{\bullet+}$ can react with water or $O_2^{\bullet-}$, which eventually leads to DNA damage [13]. Thus, in general, a good photosensitizer for DNA damage should have a reduction potential more negative than O_2 (–0.32 V versus NHE), and this is the case for NI (–1.0 V versus NHE), but not for NDI (–0.22 V versus NHE) [39], where much longer τ or larger number of consecutive A bases (A_5 or longer) is needed for the irreversible DNA damage reactions to take place [31]. However, it is important to keep in mind that [O_2] is lower in the living cell [40], especially in the nuclei. The theoretical plots showing the DNA damage efficiency obtained for the cell ([O_2] = 200 μM; red diamond) [40] and that for nuclei (assuming [O_2] = 5 μM; cyan circle) are also shown in Figure 3. These plots give a good approximation of the essential number of As in the target sequences in the cell (A_4) and that in the nuclei (A_5 or longer).

Sequence Specificity of Photosensitized DNA Damage

To actually show that the consecutive A sequences serve as potential targets in photosensitized DNA damage, the consumption of G during the photosensitized DNA oxidation for ODN-A_7, having seven consecutive As, was compared with that for ODN-A_3 and ODN-A_2, with three and two consecutive As, respectively, and ODN-A_1, without consecutive As (Figure 5A). In particular, ODN-A_2 contains GG sequences, in which the ionization potential of G is lowered by a stacking interaction between Gs and which serve as a good target in photosensitized DNA damage as reported by Saito and Sugiyama [41, 42]. Interestingly, when NIN was used as a photosensitizer, the consumption of G was the highest for ODN-A_7, and was comparable for ODN-A_3 and ODN-A_2, and the lowest for ODN-A_1 (Figure 5B). In contrast, NIP showed no such preference for consecutive A sequences (Figure 5C). In the case of NIN having a high association constant with DNA due to the cationic side chain (K_a = 4.7 × 10^4 M^{-1} for ODN-A_1) [43], the one-electron oxidation of DNA mainly proceeds through the DNA-bound ^{1}NI* that can oxidize both G and A, whereas that by NIP with an anionic side chain possessing low affinity for DNA (K_a < 10^3 M^{-1} for ODN-A_1) [43] occurs through collisions between DNA and NI in

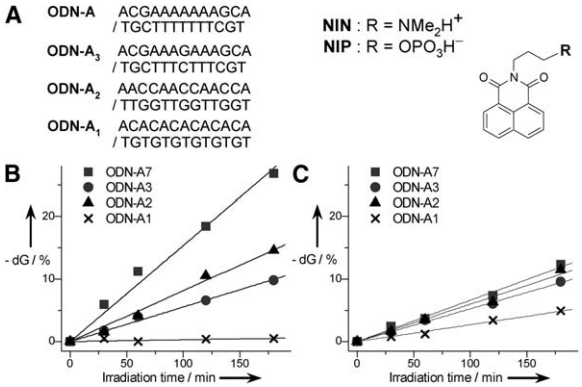

Figure 5. Sequence Specificity of Photosensitized One-Electron Oxidation of DNA

(A) Structures of NIN and NIP, and sequences of ODNs. An aqueous solution containing 50 μM ODN (strand concentration) and 20 mM Na phosphate buffer (pH 7.0) in the presence of 40 μM NIN (B) or 40 μM NIP (C) was photoirradiated at 365 nm with a transilluminator, and DNA damage was quantified as described in Figure 3.

the triplet excited state which oxidizes only G [43]. Hence, only NIN induces significant damage to the DNA with the consecutive A sequence during photosensitized DNA damage. These results clearly demonstrate that A oxidation in the consecutive A sequences is crucial for photosensitized DNA damage.

Significance

Our results clearly demonstrate that consecutive A sequences serve as a good target in photosensitized DNA damage, or G adjacent to such sequences may be a potential hot spot of oxidative DNA damage. During the photosensitized one-electron oxidation of DNA, the reaction becomes irreversible when either the reaction of $G^{\bullet+}$ with water or the reaction of $Sens^{\bullet-}$ with O_2 occurs faster than the charge recombination. In this report, the latter pathway was proven to be critically important. The best DNA binding photosensitizer for DNA damage or the most harmful to human health is the photosensitizer powerful enough to oxidize A (E_{ox} = 1.42 V versus NHE) [44] but weak enough to give up an electron to oxygen (E_{rdn} = −0.32 V versus NHE) [38] from its radical anion. Biological roles of hole transfer in DNA have been extensively investigated by Barton and coworkers [45–50]. Our results clearly show that hole transfer between adenines promotes photosensitized DNA damage, which may provide one answer for the biological consequences of hole transfer in DNA.

Experimental Procedures

DNA Synthesis

A-, T-, C-, G-, and ^{br}C-cyanoethyl phosphoramidites were purchased from Glen Research (Sterling, VA). Cyanoethyl phosphoramidite of *N*-(3-hydroxypropyl)-1,8-naphthalimide was synthesized as previously reported [51]. ODNs used in this study were synthesized with an Expedite 8909 DNA synthesizer (Applied Biosystems, Foster City, CA). After the automated synthesis, the NI-modified ODN was detached and deprotected by treating with EtOH/concentrated aqueous NH_3 1/3 (v/v) for 8 hr at 55°C. Crude ODN was purified by reverse phase HPLC and lyophilized. Purity and concentration of all ODNs studied here were determined by complete digestion with snake venom phosphodiesterase, nuclease P1, and alkaline phosphatase to 2′-deoxyribonucleosides and *N*-(3-hydroxypropyl)-1,8-naphthalimide. Duplex solutions (20 mM sodium phosphate buffer [pH 7.0]) were prepared by mixing equimolar amounts of the desired ODN complements and gradually annealing with cooling from 80°C to room temperature.

Laser Flash Photolysis

Nanosecond transient absorption measurements were performed using the laser flash photolysis technique [29–31, 33–35] for an aqueous solution containing 40 μM ODN (strand concentration) and 20 mM Na phosphate buffer (pH 7.0). The third-harmonic oscillation (355 nm, FWHM of 4 ns, 20 mJ/pulse) from a Q-switched Nd:YAG laser (Surelight; Continuum, Santa Clara, CA) was used for the excitation light, which was expanded to a 1 cm diameter. The light from a xenon flash lamp (Osram, München, Germany) 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, Tokyo, Japan) equipped with a photomultiplier (Hamamatsu Photonics, Shizuoka, Japan) and digital oscilloscope (Tektronics, Beaverton, OR).

DNA Damage Quantification

Photoirradiation was carried out in an aqueous solution containing 50 μM ODN (strand concentration) and 20 mM (pH 7.0) Na phosphate buffer. In the case of the experiments in Figure 5, 40 μM NIN (B) and NIP (C) was added, respectively. The solution mixture was photoirradiated with a transilluminator (365 nm, 1.2 mW/cm^2) for 1–3 min (Figure 3) and for 30–180 min (Figure 5), where the consumption of G was linearly correlated with the irradiation time. The reaction mixture was directly subjected to enzymatic digestion with nuclease P1 and alkaline phosphatase. The consumption of G was quantified by reverse phase HPLC using T and A as internal standards. For the experiments in Figure 3, DNA damage yield was described as –G%/min.

Acknowledgments

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