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Lifetime Regulation of the Charge-Separated State in DNA by Modulating the Oxidation Potential of Guanine in DNA through Hydrogen Bonding

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Abstract: A series of naphthalimide (NI)- and 5-bromocytosine (^{br}C)-modified oligodeoxynucleotides (ODNs) were prepared, and their lifetimes of the charge-separated states during the photosensitized one-electron oxidation of DNA were measured. Various lifetimes of the charge-separated states were observed depending on the sequence and the incorporation sites of ^{br}C, and the oxidation potential of G in the ^{br}C:G base-pair relative to that of G in the C:G base-pair and in the GGG sequence was determined by comparing the lifetimes of the charge-separated states. The change in the cytosine C5 hydrogen to bromine resulted in a 24 mV increase in the oxidation potential of G in the ^{br}C:G base-pair as compared to that of G in the C:G base-pair, the value of which is comparable to a 58 mV decrease in the oxidation potential of G in the GGG sequence. These results clearly demonstrate that hole transfer in DNA can be controlled through hydrogen bonding by introducing a substituent on the cytosine.

Introduction

Photoirradiation of DNA-bound photosensitizers (Sens) triggers electron transfer from nucleobases to the Sens to produce the radical anion of the Sens (Sens$^{\bullet-}$) and the radical cation of the nucleobase (ultimately yielding the radical cation of guanine (G$^{\bullet+}$)) as a charge-separated state. Prior to the charge recombination, the hole can migrate along DNA,[1−6] resulting in an increase in the lifetime of the charge-separated state.[7−9] Recently, we have demonstrated that the yield of the DNA damage correlates well with the lifetime of the charge-separated state during the photosensitized DNA damage.[10] That is, hole transfer helps to increase the DNA damage by providing longer times for G$^{\bullet+}$ and Sens$^{\bullet-}$ to react with water or O_2. Therefore, to further understand the mechanism of the photosensitized DNA damage, it is important to investigate factors that modulate the lifetime of the charge-separated state during the photosensitized one-electron oxidation of DNA.

The difference in the oxidation potential of G in DNA causes a shift in the equilibrium of the hole between G's, leading to the change in the lifetime of the charge-separated state. Base stacking between G's lowers the oxidation potentials of G as compared to that of a single isolated G.[11] Therefore, when the GGG sequence is located more distant from Sens than that of the initially formed G$^{\bullet+}$, a hole shift occurs from G$^{\bullet+}$ to GGG, causing the longer lifetime of the charge-separated state.[12]

As for the other factors, hydrogen bonding can play a role in regulating the oxidation potential of G in DNA. On the basis of polyacrylamide gel electrophoresis analysis, Nakatani et al. reported that *BamH*I modulates the oxidation potential of G in DNA through hydrogen bonding of the cationic guanidium group to G.[13] Previously, from the experiment in dichloromethane, we have demonstrated that the oxidation potential of G can be controlled through hydrogen bonding by introducing a substituent on the base-pairing cytosine.[14,15] The introduction of a bromo group at the C5 position of cytosine as an electron-withdrawing group (5-bromocytosine: ^{br}C) leads to suppression of the one-electron oxidation rate of G in the ^{br}C:G base-pair, according to the increase in the oxidation potential of G. Here, to investigate the hydrogen-bonding modulation of the oxidation potential of G by ^{br}C in DNA, and subsequent change in the lifetime of the charge-separated state during the photosensitized one-electron oxidation of DNA, naphthalimide (NI) was used as the Sens and laser flash photolysis of the NI- and ^{br}C-modified

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oligodeoxynucleotides (ODNs) was performed. The single bromination of C was demonstrated to cause a change in the lifetime of the charge-separated state comparable to that seen in the GGG sequence during the photosensitized one-electron oxidation of DNA. These results clearly demonstrate that the oxidation potential of G and the hole-transfer rate and direction in DNA can be controlled through hydrogen bonding by introducing a substituent on the cytosine.

Experimental Section

A-, T-, C-, G-, and ^{br}C-cyanoethyl phosphoramidites were purchased from Glen Research. Cyanoethyl phosphoramidite of *N*-(3-hydroxypropyl)-1,8-naphthalimide was synthesized as previously reported.[16]

DNA Synthesis. ODNs used in this study were synthesized with Expedite 8909 DNA synthesizer (Applied Biosystems). After the automated synthesis, the ODN was detached by treating with Na_2CO_3-saturated MeOH for 1 day. Crude ODN was purified by reverse-phase HPLC and lyophilized. The purity and concentration of all ODNs studied here were determined by complete digestion with s. v. PDE, nuclease P1, and AP to 2′-deoxyribonucleosides and *N*-(3-hydroxypropyl)-1,8-naphthalimide.

Laser Flash Photolysis Experiments. Nanosecond transient absorption measurements were performed using the laser flash photolysis technique for an aqueous solution containing 40 μM ODN (strand concentration) and 20 mM pH 7.0 Na phosphate buffer.[14] The third-harmonic oscillation (355 nm, fwhm of 4 ns, 20 mJ/pulse) from a Q-switched Nd:YAG laser (Continuum, Surelight) was used for the excitation light. 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). Kinetic modeling was carried out with MatLab software.

Results and Discussion

To evaluate the effect of a single substitiution of cytosine C5 hydrogen by bromine on the hole-transfer process in DNA, the laser flash photolysis of the NI- and ^{br}C-modified ODNs was performed (Figure 1). NI in the singlet excited state (^{1}NI*) can oxidize adenine (A) because of the negative free energy change for the electron transfer between ^{1}NI* and A based on the Rehm−Weller equation.[17] Therefore, by attaching the NI at the consecutive A sequences, the excitation of the NI-modified ODN produces the NI radical anion (NI$^{\bullet-}$) and A radical cation (A$^{\bullet+}$) to promote the hole transfer by the adenine(A)-hopping mechanism (Figure 2).[10,18−24] After the hole hopping between As, the hole is trapped at G with the lowest oxidation potential among the four bases nearest to NI (G_1) to form $G_1^{\bullet+}$. Because the charge recombination proceeds between NI$^{\bullet-}$ and $G_1^{\bullet+}$ by either super-exchange or A-hopping following the slow A oxidation by G$^{\bullet+}$,[22−26] the charge-separated state exhibits a lifetime sufficiently long to be observed in nanosecond LFP experiments.[10,19] Before the completion of the charge recom-

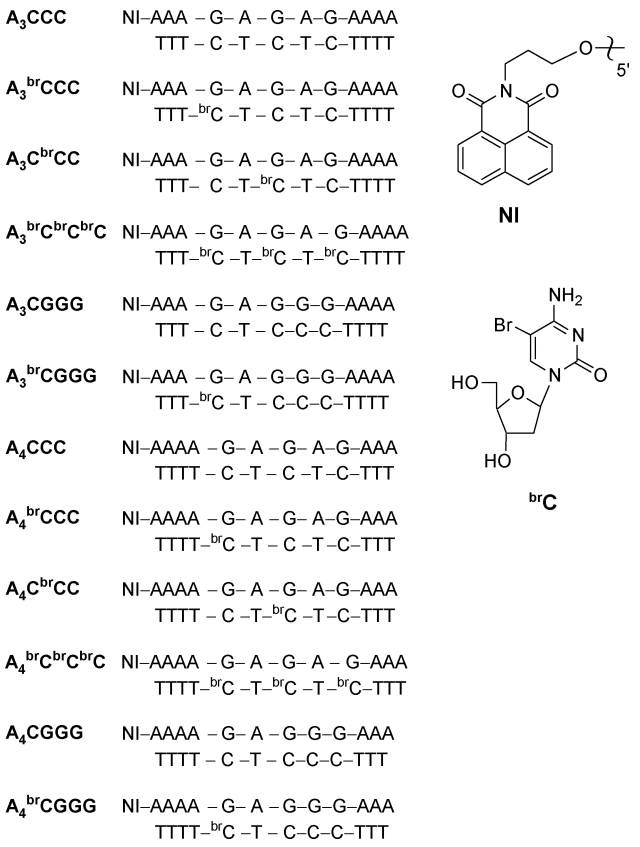

Figure 1. Structures of naphthalimide (NI) and 5-bromocytosine (^{br}C), and the sequences of NI- and ^{br}C-modified ODNs.

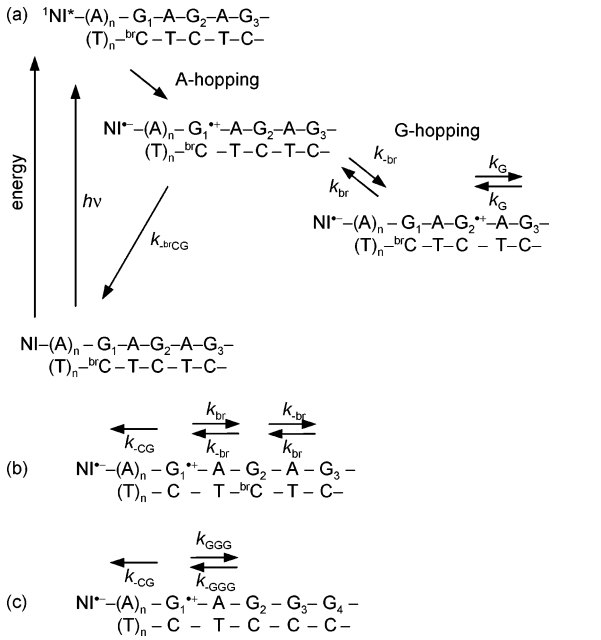

Figure 2. Kinetic scheme for photoinduced one-electron oxidation of A, hole transfer from the C:G pair to the ^{br}C:G pair (k_{br} and k_{-br}), the C:G pair to the C:G pair (k_G), single G to GGG (k_{GGG} and k_{-GGG}), and charge recombination from the C:G base-pair (k_{-CG}), and that from the ^{br}C:G base-pair (k_{-brCG}) for ODNs, (a) A$_n$^{br}CCC, (b) A$_n$C^{br}CC, and (c) A$_n$CGGG.

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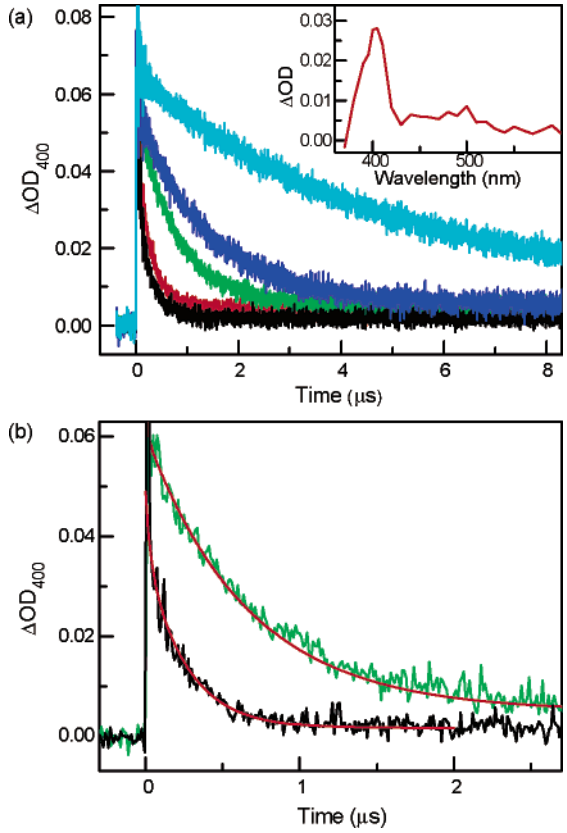

Figure 3. (a) Time profiles of the transient absorption of $NI^{\bullet-}$ monitored at 400 nm during the 355-nm laser flash photolysis of an Ar-saturated aqueous solution of NI- and ^{br}C-modified ODNs: A_3CCC (red), $A_3{}^{br}CCC$ (green), $A_3C^{br}CC$ (black), A_3CGGG (blue), and $A_3{}^{br}CGGG$ (cyan). The inset shows the transient absorption spectrum of $NI^{\bullet-}$ obtained at 400 ns after 355-nm flash excitation of A_3CCC. (b) Time profiles of the transient absorption of $NI^{\bullet-}$ for ODNs $A_3{}^{br}CCC$ (green) and $A_3C^{br}CC$ (black). The smooth red curves superimposed on the decay curves are the fit to the kinetic model depicted in Figure 2 using $k_{br} = 2.5 \times 10^7\ s^{-1}$, $k_{-br} = 6.5 \times 10^7\ s^{-1}$, and $k_G = 6 \times 10^7\ s^{-1}$.

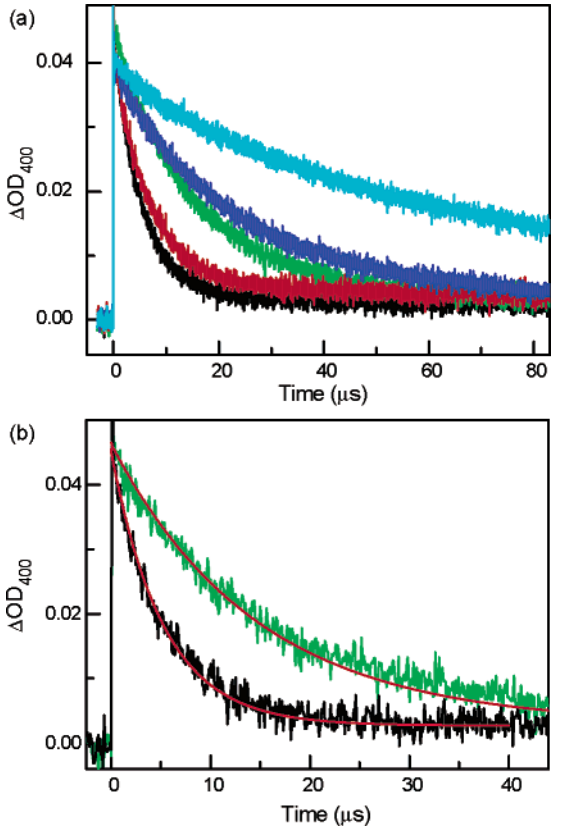

Figure 4. (a) Time profiles of the transient absorption of $NI^{\bullet-}$ monitored at 400 nm during the 355-nm laser flash photolysis of an Ar-saturated aqueous solution of NI- and ^{br}C-modified ODNs: A_4CCC (red), $A_4{}^{br}CCC$ (green), $A_4C^{br}CC$ (black), A_4CGGG (blue), and $A_4{}^{br}CGGG$ (cyan). (b) Time profiles of the transient absorption of $NI^{\bullet-}$ for ODNs $A_4{}^{br}CCC$ (green) and $A_4C^{br}CC$ (black). The smooth red curves superimposed on the decay curves are the fit to the kinetic model depicted in Figure 2 using $k_{br} = 2.5 \times 10^7\ s^{-1}$, $k_{-br} = 6.5 \times 10^7\ s^{-1}$, and $k_G = 6 \times 10^7\ s^{-1}$.

bination, the hole can migrate to more distal G's (G-hopping). The difference in the oxidation potential of G in DNA causes the shift in the hole equilibrium between G's, leading to the change in the lifetime of the charge-separated state. Therefore, the relative oxidation potential of G in the ^{br}C:G base-pair as compared to that of G in the C:G base-pair can be determined by comparing the decay curves of $NI^{\bullet-}$ for the appropriate NI- and ^{br}C-modified ODNs.

Immediately upon the excitation of the NI-modified ODN with a 355-nm laser flash, a transient absorption spectrum assigned to $NI^{\bullet-}$ with a peak maximum at 400 nm was observed (Figure 3a, inset), indicating that the charge separation (hole injection on G_1) occurred within a laser flash duration (<5 ns). The formation and decay of $NI^{\bullet-}$ for the ODN series with three intervening A−T base-pairs between NI and the G_1 (A_3 series ODNs) are shown in Figure 3, and those of the corresponding ODN series with four intervening A−T base-pairs (A_4 series ODNs) are shown in Figure 4, respectively. Because the charge recombination proceeds between $NI^{\bullet-}$ and $G_1{}^{\bullet+}$ mainly by super-exchange, the lifetimes of the charge-separated states were consistently longer for the A_4 series ODNs than those for the A_3 series ODNs. Interestingly, when a bromo group was introduced at the C_1 ($A_n{}^{br}CCC$), the lifetime of the charge-separated state increased as compared to that of A_nCCC. In contrast, bromo substitution at C_2 resulted in a decrease in the lifetime of the charge-separated state ($A_nC^{br}CC$). The lifetime of the charge-separated state is dependent on the relative concentration of the hole on G_1 and other G's. The introduction of a bromo group at C_1 ($A_n{}^{br}CCC$) caused an increase in the oxidation potential of G_1, thereby leading to a decrease in the relative hole concentration on G_1, and a subsequent increase in the lifetime of the charge-separated state. On the other hand, bromo substitution at C_2 resulted in a decrease and an increase in the relative hole concentration on G_2 and G_1, respectively, causing a decrease in the lifetime of the charge-separated state.

The lifetime of the charge-separated state increased when the A−T base-pair between G_2 and G_3 of A_nCCC was replaced with a G−C base-pair to construct a GGG hole trap (A_nCGGG). The change in the lifetime of the charge-separated state induced by the GGG hole trap was similar to that induced by a single substitution of cytosine C5 hydrogen by bromine in $A_n{}^{br}CCC$. The combination of a GGG sequence and a bromo substitution on C_1 resulted in a further increase in the lifetime of the charge-separated state ($A_n{}^{br}CGGG$). Thus, our results clearly showed that the hole equilibrium in DNA can be controlled by introducing a bromo group on cytosine C5.

The rate constants of hole transfer across one A−T base-pair have been determined by Lewis et al. to be in the range of 10^6−$10^8\ s^{-1}$.[7,9] Hence, for the A_4 series ODNs, the charge recombination rate is much slower than the hole-transfer rate

between G's. Therefore, the decay rate of $NI^{\bullet-}$ (k_{obs}) reflects the equilibration of the hole over all Gs and can be described by eq 1.

$$k_{obs} = k_{-}[G_1^{\bullet+}]/([G_1^{\bullet+}] + [G_2^{\bullet+}] + [G_3^{\bullet+}]) \quad (1)$$

In the case of ODN A_4CCC and $A_4{}^{br}C{}^{br}C{}^{br}C$, all G's are located in the identical sequence context 5′-AGA-3′. Therefore, it can be assumed that all of the G's in these ODNs have an identical oxidation potential and that the hole is equally distributed in three G's ($[G_1^{\bullet+}] = [G_2^{\bullet+}] = [G_3^{\bullet+}]$).[27,28] Therefore, k_{-} is given by eq 2.

$$k_{-} = 3k_{obs} \quad (2)$$

The k_{-} value was slightly greater for A_4CCC ($k_{-CG} = 4.59 \times 10^5\ s^{-1}$) than for $A_4{}^{br}C{}^{br}C{}^{br}C$ ($k_{-{}^{br}CG} = 4.26 \times 10^5\ s^{-1}$). The faster charge recombination between $NI^{\bullet-}$ and $C{:}G^{\bullet+}$ is consistent with the smaller free energy change for the electron transfer in the Marcus inverted region.[29] Using the equilibrium constant K_{br} ($= k_{-br}/k_{br}$) for hole transfer between the C:G base-pair and ^{br}C:G base-pair, k_{obs} for $A_4{}^{br}CCC$ ($k_{{}^{br}CCC}$) and $A_4C{}^{br}CC$ ($k_{C{}^{br}CC}$) can be described by eqs 3 and 4, respectively.

$$k_{{}^{br}CCC} = k_{-{}^{br}CG}/(1 + 2K_{br}) \quad (3)$$

$$k_{C{}^{br}CC} = k_{-CG}/(2 + 1/K_{br}) \quad (4)$$

According to eqs 3 and 4, K_{br} was determined to be 2.5. Based on the kinetic scheme shown in Figure 2, and by using the values $k_{br} = 2.5 \times 10^7\ s^{-1}$, $k_{-br} = 6.5 \times 10^7\ s^{-1}$ (where k_{-br}/k_{br} is 2.5), and $k_G = 6 \times 10^7\ s^{-1}$, theoretical curves for the decay of $NI^{\bullet-}$ were obtained for the ODNs, $A_n{}^{br}CCC$, and $A_nC{}^{br}CC$, which are shown in Figures 3b and 4b, respectively. Good agreement between the experimental and theoretical curves was found for both the A_3 and the A_4 ODN series, demonstrating that K_{br} truly represents the hole equilibrium between the C:G and ^{br}C:G base-pair in DNA. Similarly, according to the decay curve of A_4CGGG, the equilibrium constant for hole transfer between the isolated G and GGG was determined to be $K_{GGG} = 9.6$.[30] The relative oxidation potentials for ^{br}C:G, C:G, and GGG obtained using the A_4 series ODNs in this study, and those for G and GGG reported by Lewis et al.,[12] are shown in Table 1. It was shown that the change in cytosine C5 hydrogen to bromine causes a 24 mV increase in the oxidation potential of G in the ^{br}C:G base-pair as compared to that of G in the C:G base-pair in DNA. The obtained value was consistent with the 34 mV increase in the oxidation potential of oxG in the ^{br}C:oxG base-pair as compared to the C:oxG base-pair which was determined from the experiments in dichloromethane.[14] These results clearly demonstrate that the bromo substituent on the cytosine C5 is as important as the GGG sequences in the hole-transfer process in DNA.

Table 1. Relative Populations of Holes ($[G^{\bullet+}]$), and Relative Oxidation Potentials (E_{rel}) for G in ^{br}C:G, C:G, and GGG

	$[G^{\bullet+}]^a$	E_{rel} (mV)^a
^{br}C:G	0.39	+24
C:G	1	0
GGG	9.6 (20)	−58 (−77)

[a] The numbers in parentheses are the values reported by Lewis et al. using the synthetic DNA hairpins with stilbenedicarboxamide linkers (ref 12).

Conclusions

Laser flash photolysis of the NI- and ^{br}C-modified ODNs was performed to investigate the hydrogen-bonding modulation of the oxidation potential of G by ^{br}C in DNA, and the subsequent change in the lifetime of the charge-separated state during the photosensitized one-electron oxidation of DNA. The results 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 in DNA, which resulted in a significant change in the lifetime of the charge-separated state during the photosensitized one-electron oxidation of DNA.

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