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Effect of Base Sequence and Deprotonation of Guanine Cation Radical in DNA

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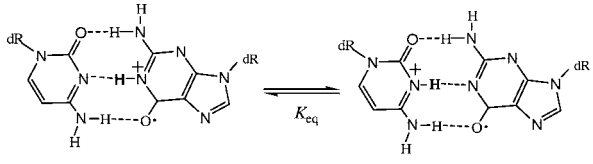
The deprotonation of guanine cation radical ($\mathbf{G}^{+\bullet}$) in oligonucleotides (ODNs) was measured spectroscopically by nanosecond pulse radiolysis. The $\mathbf{G}^{+\bullet}$ in ODN, produced by oxidation with $SO_4^{-\bullet}$, deprotonates to form the neutral G radical (**G(-H)**$^{\bullet}$). In experiments using 5-substituted cytosine-modified ODN, substitution of the cytosine C5 hydrogen by a methyl group increased the rate constant of deprotonation, whereas replacement by bromine decreased the rate constant. Kinetic solvent isotope effects on the kinetics of deoxyguanosine (dG) and ODN duplexes were examined in H_2O and D_2O. The rate constant of formation of **G(-H)**$^{\bullet}$ in dG was 1.7-fold larger in H_2O than D_2O, whereas the rate constant in the ODN duplex was 3.8-fold larger in H_2O than D_2O. These results suggest that the formation of **G(-H)**$^{\bullet}$ from $\mathbf{G}^{+\bullet}$ in the ODN corresponds to the deprotonation of the oxidized hydrogen-bridged ($\mathbf{G}^{+\bullet}$-C) base pair by a water molecule. The characteristic absorption maxima of $\mathbf{G}^{+\bullet}$ around 400 nm were shifted to a longer wavelength in the order of G < GG < GGG-containing ODNs. In contrast, the spectra of **G(-H)**$^{\bullet}$ were not affected by the sequence and were essentially similar to that of free dG. These results suggest that the positive charge in $\mathbf{G}^{+\bullet}$ in ODN is delocalized over the extended π orbitals of DNA base. The rate constant of the deprotonation was altered by the sequence of ODNs, where bases adjacent to guanine are important factors for deprotonation.

1. Introduction

In early stage of radiation-induced DNA damage, high-energy radiation ionizes nucleic acid bases, generating positive holes and electrons within DNA strand.[1,2] Identification of the DNA sites that trap holes and electrons is essential to understanding the process of DNA damage caused directly by ionizing radiation. Numerous investigations have revealed that the radical cation formed by electron loss from DNA can migrate long distance through the DNA duplex by a hopping mechanism.[3−8] Migrating guanine cation radicals ($\mathbf{G}^{+\bullet}$) participate in additional irreversible reactions with water or molecular oxygen, which may lead to oxidative damage.[9−13] A key question concerns the role played by the guanine N1 imino proton in these processes.[14] The rapid loss of the N1 proton and the subsequent reactions of the neutral G radical (**G(-H)**$^{\bullet}$) results in oxidation products.[12,13] Thus the rates of these trapping reactions primary determine overall hole migration.



C : G$^{+\bullet}$ **C(H)**$^{+}$ **: G(-H)**$^{\bullet}$ (1)

The $\mathbf{G}^{+\bullet}$ of deoxyguanosine (dG) has a pK_a of 3.9,[15,16] and it rapidly loses an N1 proton with a rate constant of 1.8×10^7 s^{-1} at pH 7.0.[17] In the guanine radical cation/cytosine base pair ($\mathbf{G}^{+\bullet}$-C), however, it is not clear whether the proton of N1 of G does or does not shift to C. Based on the pK_a values of the N1 proton of $\mathbf{G}^{+\bullet}$ (pK_a = 3.9) and N3-protonated C (pK_a = 4.3), the equilibrium for the proton transfer lies slightly to the right.[18] Theoretical studies suggest that the radical cation of the G-C pair can undergo a facile proton shift along the hydrogen bond.[19−21]

Charge transport through DNA has been described mechanistically as involving diffusive charge-hopping among low energy guanine site, since guanine is most easily oxidized among the nucleobases and the oxidation potential of guanine is further lowered for stacked bases, e.g., GG[22,23] and GGG.[24−26] Moreover, both experimental and theoretical evidence shows that the guanine base is drastically affected by the interaction fields induced by base pairing and base stacking. These effects lower oxidation potential of G[27,28] and the involvement of cytosine in charge transfer along DNA.[29] The susceptibility of G-containing sequences toward photoinduced one-electron oxidation of ODNs can be explained by these effects.[30,31] These results suggest the charge delocalization of $\mathbf{G}^{+\bullet}$ over the extended π-orbitals of DNA bases. However, the charge delocalization of DNA base has not yet established experimentally.

An efficient amount of radical cation of nucleic acid bases can be formed in duplex DNA and isolated nucleotides by pulse radiolysis in the presence of persulfate.[15,17,32−34] A previous report from our laboratory used the pulse radiolysis technique to assess the deprotonation of $\mathbf{G}^{+\bullet}$ in double-stranded ODNs.[17] From the kinetics of fast and slow components, we proposed that the slower kinetic component is associated with the deprotonation process, involving a shift of the N1 proton in $\mathbf{G}^{+\bullet}$ to its partner C, followed by the release of the proton into solution. In contrast, the faster phase has not yet been characterized. In this work, we examined one-electron oxidation duplex ODNs containing 5-methylcytosine or 5-bromocytosine in selected positions complementary to G. In addition, we examined the solvent deuterium isotope effect of these duplexes. The rates of the slower kinetic process were remarkably affected

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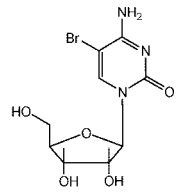
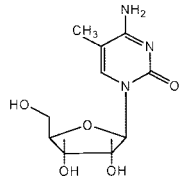
TABLE 1: Sequences of the Oligonucleotides Examined in This Work and Structures of 5-Bromocytosine (^{Br}C) and 5-Methylcytosine (^{CH_3}C)

name	Sequence (5'⟶3')
G_{1AA}	5'AAAAAAGAAAAAA3' 3'TTTTTTCTTTTTT5'
G_{2AA}	5'AAAAAGGAAAAA3' 3'TTTTTCCTTTTT5'
G_{3AA}	5'AAAAAGGGAAAAA 3' 3'TTTTTCCCTTTTT 5'
G_{22AA}	5'AAAGGAAGGAAA 3' 3'TTTCCTTCCTTT 5'
G_{22TT}	5' ATTGGTTGGTTA 3' 3'TAACCAACCAAT 5'
G_{1CC}	5'CGCGCGCGCGC 3' 3'GCGCGCGCGCG 5'
G_{1CCA}	5' ATCGCGCGGCTA 3' 3' TAGCGCGCCGAT 5'
G_{1TT}	5'TATGTTTGTAT 3' 3'ATACAAACATA 5'
G_{1TC}	5'TATGCATTAT 3' 3'ATACGTAATA 5'
^{Br}C	5'AAAAAA G AAAAAA 3' 3'TTTTTT ^{Br}CTTTTTT 5'
^{CH3}C	5'AAAAAA G AAAAAA 3' 3'TTTTTT ^{CH3}CTTTTTT 5'



^{Br}C



^{CH_3}C

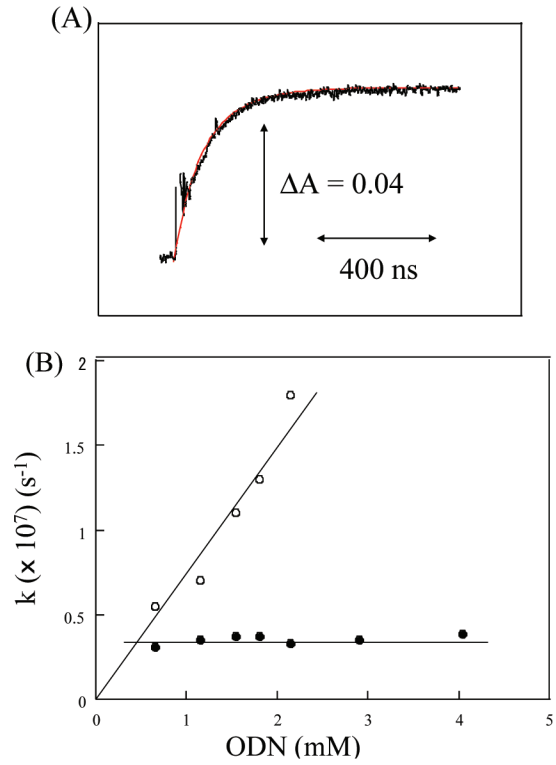


Figure 1. (A) Absorbance change at 625 nm after pulse radiolysis of ODN G_{22AA}. Samples contained ODN (1.1 mM), ammonium persulfate (20 mM), *tert*-butyl alcohol (0.1 M), NaCl (0.1 M), and phosphate buffer (20 mM, pH 7.0). (B) Concentration dependence of rate constants of the faster (○) and the slower phases (●) from the increase of the absorbance increase at 625 nm.

by these effects. We also analyzed in detail the dynamics of a systematic series of ODNs, containing G, GG, and GGG. Here, we present the transient absorption spectra and kinetics of deprotonation of these sequences of ODNs.

2. Experimental Section

Materials. ODNs were synthesized and purified by HPLC at Sigma Genosis Biotech Co., Ltd., Japan. The ODNs were dissolved in 20 mM sodium phosphate buffer (pH 7.0). The two strands were annealed by heating the samples to 90 °C for 5 min and allowing the samples to cool slowly back to room temperature over a period of 1 h. The formation of double-stranded ODNs (Table 1) was confirmed by temperature-dependent absorbance changes. The T_m values of ^{Br}C, G_{1AA}, and ^{CH3}C were identical, and thus bromination or methylation of cytosine had only slight effects on the stabilities and structures of these ODNs. All other reagents were of the highest purity available commercially.

Pulse Radiolysis. Aqueous solutions of double-stranded ODNs, containing 20 mM ammonium persulfate, 20 mM sodium phosphate buffer, 0.1 M NaCl, and 0.1 M *tert*-butyl alcohol (for scavenging OH radicals) were prepared for pulse radiolysis by deoxygenation in sealed cells and flushing with argon. The quartz cells had an optical path length of 1 cm.

Pulse radiolysis experiments were performed with a linear accelerator at the Institute of Scientific and Industrial Research, Osaka University.[17,35,36] The pulse width and pulse energy were 8 ns and 27 MeV, respectively. The light source was a Xe flash lamp, with a continuous spectrum from 300 to 1600 nm. The analyzing light was monitored with a Ritsu MC-10N monochromator and detected by PIN Si (Hamamatsu S1722) or InGaAs (Hamamatsu G3476) photodiodes. The signals were corrected using a Sony/Tektronics SCD transient digitizer. For time-resolved transient absorption spectral measurement, the monitored light was focused into a quartz optical fiber, which transported the electron pulse-induced transmittance changes to a gated spectrometer (Unisoku, TSP-601−02). The concentration of the $SO_4^{-\bullet}$ radical generated by pulse radiolysis was determined by the change in absorbance at 450 nm using an extinction coefficient of 1600 M^{-1} cm^{-1}.[37] The reactions were carried out at 25 °C.

The concentrations of ODN were determined by absorbance at 260 nm. Optical absorption spectra were measured with a Hitachi U-3000 spectrometer with temperature control.

pK_a Measurements of Nucleotides. The pK_a of dC, ^{CH3}dC and ^{Br}dC were determined by measuring the changes in their UV absorption spectra in aqueous solution as a function of pH. Solutions (10^{-4}M) of the cytidine derivatives were prepared and aliquots of a dilute, standard H_2SO_4 solution were added. After each addition the pH of the solution was measured using a pH meter and the absorption spectra were recorded.

3. Results

Oxidation of ODNs. Pulse radiolysis experiments in the presence of 20 mM ammonium persulfate involve the almost instantaneous generation of $SO_4^{-\bullet}$, which, in turn, can oxidize G to $G^{\bullet+}$ in ODN. The absorbance increase observed at 625 nm fits well to a biexponential fitting curve (Figure 1A), as observed previously.[17] We also assessed the dependence of the kinetics of the absorbance changes on ODN concentration. For these experiments, the concentration of $SO_4^{-\bullet}$ was approximately 20 μM and the concentration of ODN was varied between 400 μM and 4 mM. In the faster phase, the rate constant increased

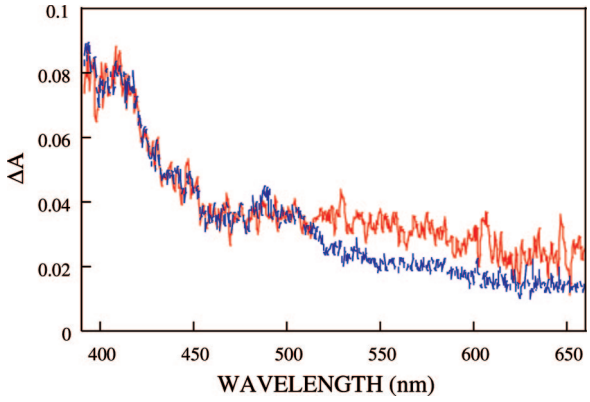


Figure 2. Kinetic difference spectra of pulse radiolysis of ODN ^{Br}**C** monitored at 100 ns (blue) and 10 μs (red) after pulse radiolysis. Samples contained 2 mM ODN ^{Br}**C**, 20 mM ammonium persulfate, 20 mM phosphate buffer (pH 7.0), 0.1 M NaCl, and 0.1 M *tert*-butyl alcohol.

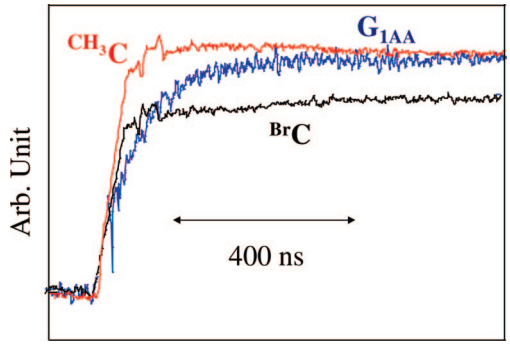


Figure 3. Kinetics of absorbance changes at 625 nm after pulse radiolysis of ODNs ^{CH3}**C**, **G**$_{1AA}$ and ^{Br}**C**. The experimental conditions were the same as described in Figure 2.

as the concentration of the ODN increased, indicating that the faster phase results from the bimolecular reaction of $SO_4^{-\bullet}$ with ODN. The second-order rate constant of the reaction was calculated to be 6×10^9 M^{-1} s^{-1}. In contrast, the rate constant of the slower phase (3.4×10^6 s^{-1}) was independent of the ODN concentration (Figure 1 (B)). The rate constants of the slower phase were determined at ODN concentrations above 2 mM, since the faster phase was completed within 20−30 ns under the condition.

Substituent Effects. The effect of the substitution by a methyl or bromine group for the cytosine C5 hydrogen on the deprotonation process of $\mathbf{G}^{+\bullet}$ in double-stranded ODNs was assessed by performing pulse radiolysis experiments using ^{CH3}C-modified or ^{Br}C-modified ODNs. Figure 2 shows transient spectra at 100 ns and 10 μs after pulse radiolysis of the ODN ^{Br}**C**. These spectra are essentially the same as those reported previously[17] and are consistent with the formation of $\mathbf{G}^{+\bullet}$ and **G(-H)**$^{\bullet}$, respectively. The decay of **G(-H)**$^{\bullet}$ thus occurred on a time scale of milliseconds (data not shown), as observed by laser pulse of ODN containing site-specific incorporation of a single 2-aminopurine.[13a]

Figure 3 compares the absorbance changes at 625 nm after pulse radiolysis of ^{CH3}**C**, **G**$_{1AA}$, and ^{Br}**C** in the presence of ammonium persulfate. We found that the absorbance increased due to the formation of **G(-H)**$^{\bullet}$. Remarkably, when a methyl group was introduced as an electron-donating group at C5 of C, the rate of the slower process was accelerated, whereas introduction of a bromo substituent on C as an-electron-accepting group reduced the rate.

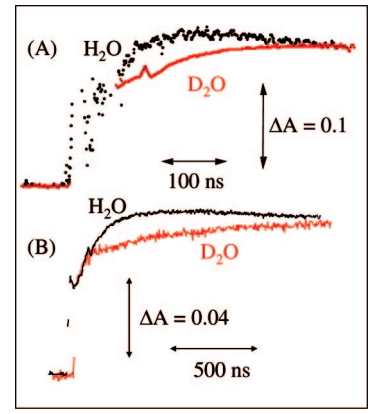


Figure 4. Absorbance changes at 625 nm after pulse radiolysis of dG (5.6 mM) (A) and ODN **G**$_{3AA}$ (2.1 mM) (B) in the presence of ammonium persulfate (20 mM), NaCl (0.1 M), and *tert*-butyl alcohol (0.1 M) in 20 mM sodium phosphate in H_2O at pH 7 (black) or in D_2O at pD 7 (red).

TABLE 2: Rate Constants of Formation of Transient Species in Double-Stranded Oligonucleotides, Determined at 625 nm

double strand	k (s^{-1})	pK_a
^{CH3}**C**	$(2.0 \pm 0.2) \times 10^7$	4.7
G$_{1AA}$	$(8.7 \pm 0.9) \times 10^6$	4.3
^{Br}**C**	$(1.2 \pm 0.1) \times 10^6$	2.8

TABLE 3: Deuterium Isotope Effect on Rate Constants of Formation of Transient Species in dG and Double-Stranded Oligonucleotides Determined at 625 nm

	k (s^{-1})		
nucleotides	H_2O	D_2O	H_2O/D_2O
dG	$(1.7 \pm 0.2) \times 10^7$	$(1.0 \pm 0.1) \times 10^7$	1.7
G$_{3AA}$	$(4.5 \pm 0.5) \times 10^6$	$(1.3 \pm 0.2) \times 10^6$	3.5
^{Br}**C**	$(1.2 \pm 0.2) \times 10^6$	$(4.0 \pm 0.4) \times 10^5$	3.0
^{CH3}**C**	$(2.0 \pm 0.2) \times 10^7$	$(6.6 \pm 0.7) \times 10^6$	3.0

As shown in Table 2, the rate constants varied in the order ^{Br}**C** < **G**$_{1AA}$ < ^{CH3}**C** depending on substitution of the base-pairing C. These results strongly suggest that electronic substituent effects on cytosine can be transmitted to the G partner through hydrogen bonding. In contrast, the faster phase was not affected by substituents on C.

Kinetic Isotope Effect. We also measured one-electron oxidation of free dG with $SO_4^{-\bullet}$ in H_2O and D_2O. The rate of deprotonation could be determined at dG concentrations of above 3 mM.[17] Figure 4A compares the absorbance changes at 625 nm after pulse radiolysis of dG in H_2O and D_2O. The absorbance change observed at 625 nm was attributable to the deprotonation of $\mathbf{G}^{+\bullet}$ to **G(-H)**$^{\bullet}$. Rate constants obtained from Figure 4A were $k_{H2O} = 1.7 \times 10^7$ s^{-1} and $k_{D2O} = 1.0 \times 10^7$ s^{-1}, making the kinetic isotope effect on the reaction rate 1.7. In contrast, the second-order rate constant of the reaction of $SO_4^{-\bullet}$ with dG in D_2O (7.0×10^9 M^{-1} s^{-1}) did not differ significantly from that of the reaction in H_2O (7.2×10^9 M^{-1} s^{-1})(data not shown).

A similar isotope effect was examined using ODN containing GGG (**G**$_{3AA}$) (Figure 4B). The time courses of the absorbance changes of **G**$_{3AA}$ in H_2O and D_2O solutions are shown in Figure 4B. The absorbance change at 625 nm consists of a fast and a slow increase. We found that the slower increase in D_2O was markedly slower than that in H_2O, though the faster phase was not significantly affected. The rate constants obtained from

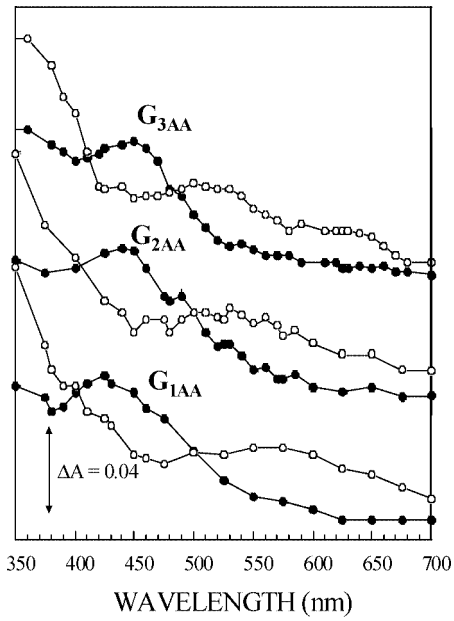


Figure 5. Kinetic difference spectra of pulse radiolysis ODN **G1AA**, **G2AA**, and **G3AA** monitored at 50 ns (●) and 500 ns (○) after pulse radiolysis. Samples contained 2 mM ODN, ammonium persulfate (20 mM), *tert*-butyl alcohol (0.1 M), NaCl (0.1 M), and 20 mM potassium phosphate buffer (pH 7.0).

Figure 4 were $k_{H2O} = 5 \times 10^6$ s^{-1} and $k_{D2O} = 1.3 \times 10^6$ s^{-1}, making the kinetic isotope effect on the reaction rate 3.8.

Similarly, we assessed the isotope effect of using ^{CH3}C-modified and ^{Br}C-modified ODNs. The rate constants of **^{CH3}C** and **^{Br}C** ODNs in D_2O were 7.5×10^6 s^{-1} and 4×10^5 s^{-1}, respectively. The kinetic isotope effect on the reaction were about 3.0−3.6, similar to **G1AA**.

Dynamics of G$^{+\bullet}$ in ODN. It was of particular interest to study ODN sequences showing dependence on spectroscopy changes of the intermediates and the rate constants of the deprotonation of **G$^{+\bullet}$**. We compared the transient spectra, corresponding to the faster and the slower phases (Figure 5). Spectra corresponding to the faster phase, which have absorption maxima around 450 nm, are consistent with the formation of **G$^{+\bullet}$**. The spectra for the slower phase have absorption around 380 and 550 nm; characteristic to **G(-H)$^\bullet$**.

The characteristic absorption maxima of **G$^{+\bullet}$** intermediate around 450 nm were found to differ. These spectra shifted to longer wavelengths in the order of G < GG < GGG. For further data analysis, a similar experiment was performed with other sequences of ODNs containing G. However, we did not observed significant differences among the spectra of the ODNs **G1CC**, **G1TT**, **G1TC**, and **G1AA** (data not shown), unlike findings for the ODNs **G1AA**, **G2AA**, and **G3AA**. The spectra of ODNs **G22AA** and **G22TT** containing two GGs are the same as **G2AA** (data not shown). Therefore, it is concluded that that the difference in the spectra in Figure 5 is attributable to the stacked bases of two or three consecutive guanine bases. In contrast, the spectral changes of **G(-H)$^\bullet$** were not altered among these sequences and were essentially the same as that of free dG.[17]

Figure 6 shows that the increased absorbance at 625 nm due to the deprotonation of **G$^{+\bullet}$** was affected by the sequences of ODNs. The rate constants of the deprotonation increased from 3.0×10^6 s^{-1} for **G22TT** to 2.1×10^7 s^{-1} for **G1CC** (Table 4).

4. Discussion

We previously reported that one-electron oxidation of double-stranded ODNs produced **G$^{+\bullet}$**, followed by deprotonation to yield **G(-H)$^\bullet$**.[17] The kinetics of the formation of **G(-H)$^\bullet$** had two components, a fast and a slow phase. The results presented here clearly show that the faster and the slower phases can be attributed to the bimolecular reaction of $SO_4^{-\bullet}$ with ODN and the deprotonation of **G$^{+\bullet}$** to **G(-H)$^\bullet$**, respectively. This was confirmed by ODN concentration dependence of reaction kinetics, (Figure 1), and by our finding that the slower kinetic process was affected by introducing a substituent on C and by the solvent deuterium isotope effect. In contrast, the faster kinetic phase was not altered by these effects.

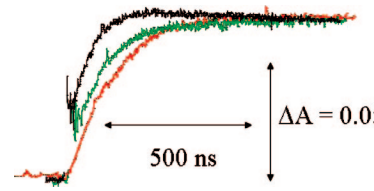
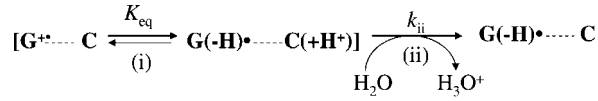


Figure 6. Kinetics of absorbance changes at 625 nm after pulse radiolysis of ODN **G3AA** (red), **G1AA** (green), and **G1CC** (black). The experimental conditions were same as described in Figure 2.

TABLE 4: Rate Constants of Deprotonation in Various Oligonucleotides

double strand	k (s^{-1})
G1CC	$(2.0 \pm 0.2) \times 10^7$
G1CCA	$(1.4 \pm 0.2) \times 10^7$
G1TC	$(1.0 \pm 0.1) \times 10^7$
G1TT	$(1.1 \pm 0.1) \times 10^7$
G1AA	$(8.7 \pm 0.9) \times 10^6$
G2AA	$(5.6 \pm 0.6) \times 10^6$
G3AA	$(4.5 \pm 0.5) \times 10^6$
G22AA	$(3.4 \pm 0.3) \times 10^6$
G22TT	$(3.0 \pm 0.3) \times 10^6$

SCHEME 1



Following the oxidation of ODN on the 10 ns time scale, absorbance changes due to deprotonation occurred within $10^6 \sim 10^7$ s^{-1}. The resulting product of the deprotonated form for **G(-H)$^\bullet$** in ODN has been observed by transient absorption spectroscopy[13a,38] and EPR studies[14b,39] in the milliseconds time range. However, the deprotonation of **G$^{+\bullet}$** in double-stranded DNA is composed of several steps, including proton transfer from G to C in the base-pair radical cation (**G$^{+\bullet}$**-C) (step i) and release of the proton from C(+H$^+$) into the solution (step ii) (Scheme 1): where K_{eq} ($=k_i/k_{-i}$) is the equilibrium constant and k_{ii} is the rate constant of the release of proton. Since proton transfer from the N1 proton of G to N3 of C could, in principle, occur very rapidly on the order of 10^{14} s^{-1},[18,40,41] the rate limiting step in these processes is the deprotonation of **C(+H$^+$)** by a water molecule ($k_{ii} \ll k_{-i}$). The rate constant of the deprotonation can be expressed as $k = k_{ii} K_{eq}$. This suggests that the absorbance changes at 625 nm observed would correspond to the proton loss from the oxidized proton-shifted resonance structure (**G$^{+\bullet}$:C ↔ G(-H)$^\bullet$:C(+H$^+$)**) by a water molecule; this is supported by the fact that the process was affected by the accessibility of water molecule.[17] The results presented here show that the step (ii) is greatly affected by the substitutent of C and the kinetic solvent isotope. A bromo substituent on C, as an electron accepting group, suppressed the process ca. 20-fold compared with a methyl substituent on C, as an electron donating group. The proton transfer from **G$^{+\bullet}$** ($pK_a = 3.9$) to ^{Br}C is thermodynamically unfavorable, since the pK_a of ^{Br}dC was 2.8 as

determined by titration in solution (data not shown). The equilibrium of ^{Br}**C** in eq 1 is far to the left, and the equilibrium constant (K_{eq}) of the proton transfer in $\mathbf{G}^{+\bullet}$-^{Br}C is estimated to be 0.08, suggesting that a large portion of the oxidized G in ^{Br}**C** remains protonated after oxidation. Thus, the difference in the rate constants can be explained primarily by the pK_a values of C (Table 2).

It should be noted that a kinetic isotope effect ($k_H/k_D = 3.8$) of the deprotonation in ODN $\mathbf{G_{3AA}}$ radiolysis was about twice that of the kinetic isotope effect on deprotonation of free dG ($k_H/k_D = 1.7$). The isotope effect on free dG reflects the release of the N1 proton of dG to the surrounding water after rapid oxidation. A similar kinetic isotope effect was obtained on the oxidation of G in DNA ($k_H/k_D = 1.3-2.1$).[42−44] In both cases, these reactions can be considered in terms of proton-coupled electron transfer reactions, coupling of the deprotonation with the guanine electron transfer. In contrast, the kinetic isotope effect of 3.8 measured here thus means that the formation of **G(-H)**$^{\bullet}$ in ODN may be associated with the loss of tightly bound protons in DNA. Thus this process is consistent with step ii in Scheme 1.

Recently, two alternative schemes to explain the deprotonation behavior of $\mathbf{G}^{+\bullet}$ in DNA have been proposed, using either pulse radiolysis or a theoretical model. In one scheme, $\mathbf{G}^{+\bullet}$ first decays to the iminic form of **G(-H)**$^{\bullet}$, which then undergoes water assisted tautomerization.[45] In the other scheme, there is an intramolecular rearrangement of the initially formed C radical resulting in a G radical.[46] Under our experimental conditions, however, we identified **G(-H)**$^{\bullet}$ as the deprotonation species of $\mathbf{G}^{+\bullet}$.

Importantly, we have shown the spectra of $\mathbf{G}^{+\bullet}$ in ODNs differ from that of the isolated nucleotide and are affected by the ODN sequence (Figure 5). These results demonstrate that the transiently formed $\mathbf{G}^{+\bullet}$ is stabilized by base pairing with C and the stacking interaction of neighborhood nucleobases. These findings also provide direct spectroscopic evidence of the delocalization of the positive charge along the extended π orbitals of DNA bases.[29−31,47] The spectral shift among $\mathbf{G_{1AA}}$, $\mathbf{G_{2AA}}$, and $\mathbf{G_{3AA}}$ can be understood as the stacking interaction of two or three consecutive guanine bases, though the spectral assignment of $\mathbf{G}^{+\bullet}$ at 400 nm is not known. We did not observe the spectral shift of $\mathbf{G}^{+\bullet}$ in GA, GC, and GT, findings consistent with the theoretical calculation of the energy level of four stacked nucleobases.[22] In contrast, the spectra of **G(-H)**$^{\bullet}$ in ODNs are essentially identical to that of dG and are not affected by the ODN sequence. This strongly suggests that radical orbital of **G(-H)**$^{\bullet}$ is essentially localized on a preferential specific guanine base site. The selectivity of ODN sequence containing GGG toward one electron oxidation can be explained by the mechanism.[31]

Interestingly, the rate constants of deprotonation were affected by ODN sequences. Unexpectedly the difference among G, GG, and GGG were not distinct, but rather depended on the neighboring bases. We found that the rate constant increased in the order CGC > TGT > AGA > AGG > GGG, an order that may correlate with the calculated ionization potential of the nucleobases.[31,48] The finding, that the rate decreases as the oxidation potential decreases, may reflect on the stability of the radical cation. Another important factor affecting deprotonation was the accessibility of water molecule, allowing release and acceptance from the exterior aqueous environment. A water molecule can easily access the G:C moiety located in the terminal position of ODN, and the deprotonation occur very rapidly, as previously suggested.[17] Some support for this possibility is taken from the difference in the deprotonation of the rates between $\mathbf{G_{1CC}}$ and $\mathbf{G_{1CCA}}$ (Table 4). In $\mathbf{G_{1CC}}$, rapid hole transfer between interstranded guanines occurs within 10^8 s^{-1},[49] followed by the formation of $\mathbf{G}^{+\bullet}$ in the terminal position of the ODN. This may result in the fastest rate of the deprotonation among the ODNs examined here. In contrast, $(GC)_n$ sequences are separated by two A/T base pairs from the terminal position of ODN $\mathbf{G_{1CCA}}$. Similarly, it was shown that solvent-exposed G residues has the greater reactivity on the oxidation with $CO_3^{-\bullet}$ radicals.[13b] In a such case, the subsequent reaction of $\mathbf{G}^{+\bullet}$ in the solvent-exposed residues may occur easily.

One important issue is whether hole transfer through duplex DNA is affected by the rate of the deprotonation of N1 in $\mathbf{G}^{+\bullet}$. A kinetic isotope effect for G oxidation[42−44] and for charge transfer in DNA[7] indicates a concerted proton-coupled electron transfer involving the N1 proton. Hole transfer through DNA has been shown to be inhibited when proton loss from G is facilitated.[50] The data presented here indicate that the decrease of C basicity and the solvent isotope effect significantly decrease the rate of deprotonation of N1. In such circumstance, if the deprotonation of $\mathbf{G}^{+\bullet}$ contributes to the rate-determining step for hole transfer, the decrease of C basicity and D_2O isotope effect would be inhibited. Using by strand cleavage analysis, however, the incorporation of a methyl-substituted C[51] or 5-fluoro-substituted C[52] did not measurably affect the efficiency of hopping. Moreover, isotope effects for the hole-transfer in GAAG ($k_H/k_D = 1.2$) or GAG ($k_H/k_D = 1.0$) sequences are very small.[53] Thus, hole hopping efficiency is little affected by the deprotonation process observed here. In contrast to the effect on hole transfer efficiency, irreversible chemical trapping of the radical cation was inhibited by substitution with 5-fluoro-2′-deoxycytidine,[52] a finding consistent with ours, that deprotonation is inhibited by a decrease in the basicity of C.

The spectra of oxidized G in early investigation of pulse radiolysis[15] are essentially similar to those reported here. However, pH-dependent spectral changes[15] from $\mathbf{G}^{+\bullet}$ to **G(-H)**$^{\bullet}$ are different from our previous paper[17] and the spectra in Figure 5. Especially, the spectra reported here have marked increase above 600 nm. The difference may be due to the optical detection system and high dose of electron pulse in our apparatus. In most kinetic data, photomultiplier was frequently used as a detection system, because of its sensitivity. In our system, on the other hand, the analyzing light was detected by PIN Si or InGaAs photodiode. Thus we can obtain absorbance changes in the wide wavelength ranges from 300 to 1600 nm.[36] In addition, pulse of 8 ns were used that supplied doses such that 200 μM in maximum radicals were produced. On the other hand, in early investigation of pulse radiolysis, 1−2 μM radicals were produced with 400 ns pulse.[15] Thus, the absorbance changes observed here can be obtained clearly.

Conclusions

We observed spectrphotometrically that $\mathbf{G}^{+\bullet}$ in double-stranded ODN was formed transiently and deprotonated to yield the neutral G radical (**G(-H)**$^{\bullet}$). The process of ODN observed here corresponds to the deprotonation of the oxidized hydrogen-bridged ($\mathbf{G}^{+\bullet}$-C) base pair by a water molecule. Among a systematic series of G-containing ODNs, the characteristic absorption maxima of $\mathbf{G}^{+\bullet}$ intermediates were affected by the ODN sequence, whereas the spectra of **G(-H)**$^{\bullet}$ were not affected. The rate constant for deprotonation was dependent on the ODN sequence, especially on the sequence of bases adjacent to the guanine base. These results establish a sequence specificity in the stability of holes.

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References and Notes

(1) Steenken, S. *Biol. Chem.* **1997**, *378*, 1293–1297.
(2) (a) Debije, M. G.; Bernhard, W. A. *J. Phys. Chem. A* **2002**, *106*, 4608–4615. (b) Bernhard, W. A.; Close, D. M. In *Charged Particles and Photon Interactions with Matter*; Mozunder, A., Hatano, Y., Eds.; Marcel Dekker, Inc: New York and Basel, 2004; p 431. (c) Cai, Z.; Sevilla, M. D. In *Long Charge Transfer in DNA II*; Schuster, G. B., Ed.;*Top. Curr. Chem.* **2004**, *237*, 103.
(3) Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8353–8358.
(4) O'Neill, M. A.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 11471–11483.
(5) Lewis, F. D.; Letinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159–170.
(6) Giese, B.; Amaudrut, J.; Kohler, A. K.; Spomann, M.; Wessely, S. *Nature* **2001**, *412*, 318–320.
(7) Shafirovich, V.; Cadet, J.; Gasparutto, D.; Dourandin, A.; Huang, W.; Geacintov, N. E. *J. Phys. Chem. B* **2001**, *105*, 586–592.
(8) Takada, T.; Kawai, K.; Fujitsuka, M.; Majima, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14002–14006.
(9) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692–9694.
(10) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109–1152.
(11) Cadet, J.; Douki, T.; Gasparutto, D.; Ravanat, J.-L. *Mutat. Res.* **2003**, *531*, 5–23.
(12) (a) Kino, K.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 7373–7374. (b) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **2001**, *123*, 9681–9682.
(13) (a) Misiaszek, M.; Crean, C.; Joffe, A.; Geacintov, N. E.; Shafirovich, V. *J. Biol. Chem.* **2004**, *279*, 32106–32115. (b) Lee, Y. A.; Yun, B. H.; Kim, S. K.; Margolin, Y.; Dedon, P. C.; Geacintov, N. E.; Shafirovich, V. *Chem. Eur. J.* **2007**, *13*, 4571–4581. (c) Crean, C.; Uvaydov, Y.; Geacintov, N. E.; Shafirovich, V. *Nucleic Acids Res.* **2008**, *36*, 742–755.
(14) (a) Sun, L.; Bu, Y. *J. Phys. Chem. B* **2005**, *109*, 593–600. (b) Adhikary, A.; Kumar, A.; Backer, D.; Sevilla, M. D. *J. Phys. Chem. B* **2006**, *110*, 24171–24180.
(15) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094–1099.
(16) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1992**, *114*, 699–704.
(17) Kobayashi, K.; Tagawa, S. *J. Am. Chem. Soc.* **2003**, *125*, 10213–10218.
(18) Hutter, M.; Clark, T. *J. Am. Chem. Soc.* **1996**, *118*, 7574–7577.
(19) Li, X.; Cai, Z.; Sevilla, M. D. *J. Phys. Chem. B* **2001**, *105*, 10115–10123.
(20) Gervasio, F. L.; Laio, A.; Iannuzzi, M.; Parrinello, M. *Chem. Eur. J.* **2004**, *10*, 4846–4852.
(21) Weatherly, S. C.; Yang, I. V.; Armistead, P. A.; Thorp, H. H. *J. Phys. Chem. B* **2003**, *107*, 372–378.
(22) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063–7068.
(23) Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845–846.
(24) Lewis, F. D.; Liu, X.; Liu, J.; Hayes, R. T.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2000**, *122*, 12037–12038.
(25) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5893–5894.
(26) Sistare, M. F.; Codden, S.; Codden, S. J.; Heimlich, G.; Thorp, H. H. *J. Am. Chem. Soc.* **2000**, *122*, 4742–4749.
(27) Kawai, K.; Wata, Y.; Ichinose, N.; Majima, T. *Angew. Chem., Int. Ed.* **2000**, *39*, 4327–4329.
(28) Caruso, T.; Carotenuto, M.; Vasca, E.; Peluso, A. *J. Am. Chem. Soc.* **2005**, *127*, 15040–15041.
(29) Shao, F.; O'Neill, M. A.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17914–17919.
(30) Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686–12687.
(31) Yoshioka, Y.; Kitagawa, Y.; Takano, Y.; Yamaguchi, K.; Nakamura, T.; Saito, I. *J. Am. Chem. Soc.* **1999**, *121*, 8712–8719.
(32) Shafirovich, V.; Dourandin, A.; Huang, W.; Geacintov, N. E. *J. Biol. Chem.* **2001**, *276*, 24621–24626.
(33) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1993**, *115*, 2437–2440.
(34) Kawai, K.; Takada, T.; Tojo, S.; Ichinose, N.; Majima, T. *J. Am. Chem. Soc.* **2001**, *123*, 12688–12689.
(35) (a) Kobayashi, K.; Hayashi, K. *J. Am. Chem. Soc.* **1990**, *112*, 6051–6053. (b) Suzuki, S.; Kohzuma, T.; Deligeer; Yamaguchi, K.; Nakamura, N.; Shidara, S.; Kobayashi, K.; Tagawa, S. *J. Am. Chem. Soc.* **1994**, *116*, 11145–1146. (c) Kobayashi, K.; Tagawa, S.; Daff, S.; Sagami, I.; Shimizu, T. *J. Biol. Chem.* **2001**, *276*, 39864–39871. (d) Kobayashi, K.; Mustafa, G.; Tagawa, S.; Yamada, M. *Biochemistry* **2005**, *44*, 13567–13572.
(36) (a) Seki, S.; Yoshida, Y.; Tagawa, S.; Asai, K. *Macromolecules* **1999**, *32*, 1080–1086. (b) Seki, S.; Koizumi, Y.; Kawaguchi, T.; Habara, H.; Tagawa, S. *J. Am. Chem. Soc.* **2004**, *126*, 3521–3528.
(37) McElroy, W. J. *J. Phys. Chem.* **1990**, *94*, 2435–2441.
(38) (a) Stemp, E. D. A.; Arkin, M. R.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 2921–2925. (b) Wagenknecht, H-A.; Rajski, S. R.; Pascaly, M.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 4400–4407.
(39) Schiemann, O.; Turro, N. J.; Barton, J. K. *J. Phys. Chem. B* **2000**, *104*, 7214–7220.
(40) Douhal, A.; Kim, S. K.; Zewail, A. H. *Nature* **1995**, *378*, 260–263.
(41) Zewail, A. H. *J. Phys. Chem.* **1996**, *100*, 12701–12724.
(42) Shafirovich, V.; Dourandin, A.; Luneva, N. P.; Geacintov, N. E. *J. Phys. Chem. B* **2000**, *104*, 137–139.
(43) Shafirovich, V.; Dourandin, A.; Geacintov, N. E. *J. Phys. Chem. B* **2001**, *105*, 8431–8435.
(44) Weatherly, S. C.; Yang, I. V.; Thorp, H. H. *J. Am. Chem. Soc.* **2001**, *123*, 1236–1237.
(45) Chatgilialoglu, C.; Caminal, C.; Altieri, A.; Vougioukalakis, G. C.; Mulazzani, Q. G.; Gimisis, T.; Guerra, M. *J. Am. Chem. Soc.* **2006**, *128*, 13796–13805.
(46) Anderson, R. F.; Shinde, S. S.; Maroz, A. *J. Am. Chem. Soc.* **2006**, *128*, 15966–15967.
(47) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406–6407.
(48) Voityuk, A. A.; Jortner, J.; Bixon, M.; Rŏsch, N. *Chem. Phys. Lett.* **2000**, *324*, 430–434.
(49) Osakada, Y.; Kawai, K.; Fujitsuka, M.; Majima, T. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 18072–18076.
(50) Giese, B.; Wessely, S. *Chem. Commun.* **2001**, *2001*, 2108–2109.
(51) Kanvah, S.; Schuster, G. B. *J. Am. Chem. Soc.* **2004**, *126*, 7341–7344.
(52) Ghosh, A. K.; Schuster, G. B. *J. Am. Chem. Soc.* **2006**, *128*, 4172–4173.
(53) Takada, T.; Kawai, K.; Fujitsuka, M.; Majima, T. *Chem. Eur. J.* **2005**, *11*, 3835–3842.

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