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Direct Observation of Guanine Radical Cation Deprotonation in Duplex DNA Using Pulse Radiolysis

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Abstract: The dynamics of one-electron oxidation of guanine (G) base mononucleotide and that in DNA have been investigated by pulse radiolysis. The radical cation ($\mathbf{G}^{+\cdot}$) of deoxyguanosine (dG), produced by oxidation with $SO_4^{-\cdot}$, rapidly deprotonates to form the neutral G radical ($\mathbf{G(-H)}^{\cdot}$) with a rate constant of 1.8×10^7 s^{-1} at pH 7.0, as judged from transient spectroscopy. With experiments using different double-stranded oligonucleotides containing G, GG, and GGG sequences, the absorbance increases at 625 nm, characteristic of formation of the $\mathbf{G(-H)}^{\cdot}$, were found to consist of two phases. The rate constants of the faster ($\sim 1.3 \times 10^7$ s^{-1}) and slower phases ($\sim 3.0 \times 10^6$ s^{-1}) were similar for the different oligonucleotides. On the other hand, in the oligonucleotide containing G located at the 5′- and 3′-terminal positions, only the faster phase was seen. These results suggest that the lifetime of the radical cation of the G:C base pair ($\mathbf{GC}^{+\cdot}$), depending on its location in the DNA chain, is longer than that of free dG. In addition, the absorption spectral intermediates showed that hole transport to a specific G site within a 12−13mer double-stranded oligonucleotide is complete within 50 ns; that is, the rate of hole transport over 20 Å is $>10^7$ s^{-1}.

Introduction

The electron-transfer reactions of guanine (G) are central to understanding both hole transfer along DNA[1−4] and biological damage to nucleic acids.[5−8] Because G exhibits the lowest oxidation potential among the four DNA bases,[9] the electron-loss center generated in duplex DNA ultimately ends up at G sites via hole migration through the DNA duplex. Interestingly, the hole migration through the base stack has been shown to result in oxidative damage to G sites 200 Å from the site of a remotely bound oxidant.[10,11] The oxidation damage is due to further reaction of the G cation radical ($\mathbf{G}^{+\cdot}$) with H_2O to give the reaction product of 7,8-dihydro-8-oxoguanine (8-oxo-G),[12] one of the important products of "oxidative stress" in biological systems.[13] Thus, hole transport may compete with the reactions such as deprotonation or hydration of $\mathbf{G}^{+\cdot}$.[3,14]

Pulse radiolysis[15] and 193 nm laser photolysis[16] experiments show that the $\mathbf{G}^{+\cdot}$ form of deoxyguanosine (dG) has a pK_a of 3.9, so that at pH 7 the neutral oxidized radical ($\mathbf{G(-H)}^{\cdot}$) is formed by deprotonation of N1, as determined by both optical and conductance detection:[15]

$$\mathbf{G}^{+\cdot} \rightleftarrows \mathbf{G(-H)}^{\cdot} + \mathrm{H}^+ \qquad (1)$$

A kinetic isotope effect on the oxidation of G by metal complexes on the millisecond time scale indicated a proton-coupled electron transfer occurs in mononucleotides and duplex DNA.[17] However, the rate constant of the deprotonation reaction (reaction 1) has not yet been obtained directly. On the other hand, when $\mathbf{G}^{+\cdot}$ is formed in a double-stranded DNA, the effect of base pairing is to transfer the proton to N3 of cytosine (C) in the base pair. The pK_a of N3-protonated C is slightly higher (pK_a of 4.45) than that of reaction 1.[5] The theoretical study suggests that the radical cation of the G−C pair ($\mathbf{G{:}C}^{+\cdot}$) can undergo a facile proton shift along its central hydrogen bond.[18]

(1) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.
(2) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253.
(3) Meggers, E.; Michel-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950.
(4) Lewis, F. D.; Wu, T.; Zhang, Y.; Letsinger, R. L.; Greenfield, S. R.; Wasielewski, M. R. *Science* **1997**, *277*, 673.
(5) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.
(6) Steenken, S. *Chem. Rev.* **1989**, *89*, 503.
(7) Angelov, D.; Spassky, A.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1997**, *119*, 11373.
(8) Cullis, P. M.; Malone, M. E.; Merson-Davies, L. A. *J. Am. Chem. Soc.* **1996**, *118*, 2775.
(9) (a) Seidel, C. A. M.; Snulz, A.; Sauer, H. M. *J. Phys. Chem.* **1996**, *100*, 5541. (b) Steenken, S.; Javanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617.
(10) (a) Núñez, M. E.; Hall, D. B.; Barton, J. K. *Chem. Biol.* **1999**, *6*, 85. (b) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.
(11) (a) Ly, D.; Sanii, L.; Schuster, G. B. *J. Am. Chem. Soc.* **1999**, *121*, 9400. (b) Gasper, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762. (c) Henderson, P. T.; Jones, D.; Hampikian, G.; Kan, Y.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8353.
(12) (a) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692. (b) Cadet, J.; Berger, M.; Buchko, G. W.; Joshi, P. C.; Raoul, S.; Ravanat, J.-L. *J. Am. Cem. Soc.* **1994**, *116*, 7403. (c) Raul, S.; Berger, M.; Buchko, G. W.; Joshi, B. M.; Weinfeld, M.; Cadet, J. *J. Chem. Soc., Perkin Trans. 2* **1996**, 371. (d) Kino, K.; Saito, I. *J. Am. Chem. Soc.* **1998**, *120*, 7373.
(13) (a) Sies, H. *Oxidative Stress*; Academic Press: Orlando, FL, 1985. (b) Helbock, H. J.; Beckman, K. B.; Shigenaga, M. K.; Walter, P. B.; Woodall, A. A.; Yeo, H. C.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 288. (c) Kasai, H.; Nishimura, S. In *Oxidative Stress*; Sies, H., Ed.; Academic Press: London, 1991; p 99. (d) Lloyd, D. R.; Phillips, D. H. *Mutat. Res.* **1999**, *424*, 23.
(14) Steenken, S. *Biol. Chem.* **1997**, *378*, 1293.
(15) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094.
(16) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1992**, *114*, 699.
(17) Weatherly, S. C.; Yang, I. V.; Thorp, H. H. *J. Am. Chem. Soc.* **2001**, *123*, 1236.
(18) (a) Colson, A.-O.; Besler, B.; Sevilla, M. D. *J. Phys. Chem.* **1992**, *96*, 9787. (b) Hutter, M.; Clark, T. *J. Am. Chem. Soc.* **1996**, *118*, 7574.

Table 1. Oligonucleotide Sequences

name	sequence (5'⟶ 3')
G_1	5'-A A A A A A G A A A A A A-3' 3'-T T T T T T C T T T T T T-5'
G_2	5'- A A A A A G G A A A A A-3' 3'- T T T T T C C T T T T T-5'
G_3	5'-A A A A A G G G A A A A A-3' 3'- T T T T T C C C T T T T T-5'
G_4	5'-A G A A A A A A A A G A-3' 3'-T C T T T T T T T T C T-5'
G_5	5'- G A A A A A A A A A A G -3' 3'- C T T T T T T T T T T C -5'
G_6	5'-A G A A A A A A A A G A-3' 3'-T C C T T T T T T C C T-5'

The proton is finally expected to be released into the solvent. In fact, in the time ranges from microsecond to millisecond, the deprotonated form for **G(−H)**· in DNA has been observed by transient absorption spectroscopy[19,20] and ESR studies.[21,22] It is well accepted that the species responsible for the fast hole transfer along DNA is the $\mathbf{G}^{+}$· prior to the deprotonation. If so, the hole transfer is interrupted by proton transfer between the base-pair radical cation.[6] There has been no previous report of the direct observation of the deprotonation process in DNA.

An efficient amount of radical cation can be formed in duplex DNA by pulse radiolysis in the presence of persulfate.[9b,15,20,23,24] The SO_4^{-}· (2.5−3.1 V)[6] is powerful enough to oxidize all four bases. Spectral identification of the resulting radical cations has been reported for the nucleic acid base species and for various nucleotides.[15,23] This paper describes application of this method with nanosecond time resolution to follow the deprotonation of $\mathbf{G}^{+}$· in duplex DNA. Differences in the dynamics of different double-stranded oligonucleotides containing G, GG, and GGG sequences are reported.

Experimental Section

Materials. The oligodeoxynucleotides **1**−**6** were synthesized and purified by HPLC at Sigma genosis biotech Co., Ltd., Japan. In Table 1, the prepared double-stranded oligonucleotides $\mathbf{G_1}$−$\mathbf{G_6}$ are shown.

The formation of double-stranded oligonucleotides was confirmed by monitoring the 260 nm absorbance. The oligonucleotide was dissolved in 10 mM potassium phosphate buffer (pH 7.0). Annealing of the two strands was accomplished by heating the samples to 90 °C for 5 min and then allowing the samples to cool slowly back to room temperature over a period of 1 h. All other reagents were of the highest purity available commercially.

Pulse Radiolysis. Samples for pulse radiolysis were prepared as follows. Aqueous solutions of the double-stranded oligonucleotides or dG contained 20 mM ammonium persulfate, 10 mM buffer, and 0.1 M *tert*-butyl alcohol (for scavenging OH radicals) and were deoxygenated in sealed cells and flushed with argon. The buffers used were 10 mM potassium acetate buffer (pH 2−6), 10 mM potassium phosphate buffer (pH 6−8), or 10 mM potassium borate (pH 8−10). The pH was adjusted with NaOH or $HClO_4$. 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.[25,26] The pulse width and energy were 8 ns and 27 MeV, respectively. The light source was a Xe flash lamp (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 by a Sony/Tektronics SCD transient digitizer. Each data point was normalized against the dose of the electron beams. The concentration of SO_4^{-}· radical generated by pulse radiolysis was determined by absorbance change at 450 nm using a millimolar extinction coefficient of 1600 M^{-1} cm^{-1}.[27] The reactions were carried out at 20 °C.

The concentrations of DNA were determined by the absorbance at 260 nm. Optical absorption spectra were measured with a Hitachi U-3000 spectrometer connected with a temperature controller. The melting temperature (T_m) of the oligonucleotides was determined from absorbance versus temperature curves measured at 260 nm. The 10 μM duplex was measured in a buffer of 10 mM sodium phosphate (pH 7.0) from 4 to 50 °C. A typical melting curve of the oligonucleotide $\mathbf{G_4}$ is shown in the Supporting Information. We did not have any data of multiple structure in the oligonucleotides employed here, from the concentration dependence on the absorption spectra.

Results

Oxidation of Deoxyguanosine. Pulse radiolysis experiments involve the almost instantaneous generation of SO_4^{-}·, which in turn can oxidize G to the $\mathbf{G}^{+}$· without deprotonation (reaction 2).[23]

$$SO_4^{-\cdot} + G \rightarrow SO_4^{2-} + \mathbf{G}^{+\cdot} \qquad (2)$$

Because the pK_a of $\mathbf{G}^{+}$· is 3.9,[15] the deprotonation reaction in reaction 1 occurs rapidly at pH 7. If the rate-determining step in these processes is the oxidation of dG by SO_4^{-}· ($k_1 > k_2$[G]) at low concentration of dG (<1 mM), reaction 1 would not be observed. Under these conditions, the equilibrium is practically attained during the oxidation process. To discriminate the reactions individually, the dG concentration dependence of the oxidation kinetics was studied. Because the absorption spectrum of **G(−H)**· exhibits a longer wavelength from 600 to 700 nm,[16] the absorbance changes at 625 nm were measured. As shown

(19) (a) Stemp, E. D. A.; Arkin, M. R.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 2921. (b)Wagenknecht, H.-A.; Pajski, S. R.; Pascaly, M.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 4400.
(20) Shafirovich, V.; Dourandin, A.; Huang, W.; Geacintov, N. E. *J. Biol. Chem.* **2001**, *276*, 24621.
(21) Hildebrand, K.; Schulte-Frohlinde, D. *Free Radical Res. Commun.* **1990**, *11*, 195.
(22) Schiemann, O.; Turro, N. J.; Barton, J. K. *J. Phys. Chem. B* **2000**, *104*, 7214.
(23) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1993**, *115*, 2437.
(24) (a) Kawai, K.; Takada, T.; Tojo, S.; Ichinose, N.; Majima, T. *J. Am. Chem. Soc.* **2001**, *123*, 12688. (b) Kawai, K.; Miyamoto, K.; Tojo, S.; Majima, T. *J. Am. Chem. Soc.* **2003**, *125*, 912.
(25) (a) Kobayashi, K.; Hayashi, K. *J. Am. Chem. Soc.* **1990**, *112*, 6051. (b) Suzuki, S.; Kohzuma, T.; Deligeer; Yamaguchi, K.; Nakamura, N.; Shidara, S.; Kobayashi, K.; Tagawa, S. *J. Am. Chem. Soc.* **1994**, *116*, 11145. (c) Kobayashi, K.; Tagawa, S.; Mogi, T. J. *Biochemistry* **2000**, *40*, 15620. (d) Kobayashi, K.; Tagawa, S.; Daff, S.; Sagami, I.; Shimizu, T. *J. Biol. Chem.* **2001**, *276*, 39864.
(26) Seki, S.; Yoshida, Y.; Tagawa, S.; Asai, K. *Macromolecules* **1999**, *32*, 1080.
(27) McElroy, W. J. *J. Phys. Chem.* **1990**, *94*, 2435.

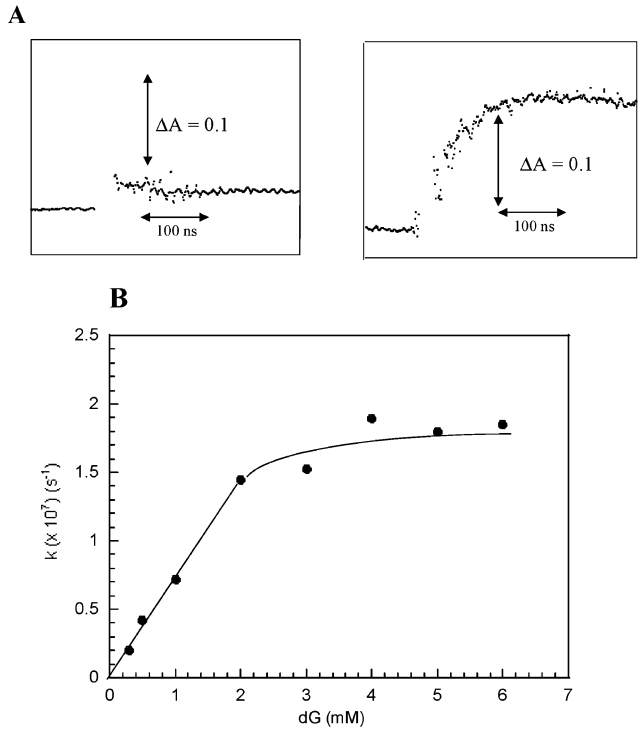

Figure 1. (A) Absorbance changes at 625 nm after pulse radiolysis of deoxyguanosine (6 mM) in the presence of ammonium persulfate (20 mM) and *tert*-butyl alcohol (0.1 M) at pH 2.1 (left) and 7.0 (right). (B) Concentration dependence of apparent rate constants from the increase of the absorbance increase at 625 nm. Samples contained deoxyguanosine, ammonium persulfate (20 mM), *tert*-butyl alcohol (0.1 M), and 10 mM potassium phosphate buffer (pH 7.0).

in Figure 1A, an absorbance increase at 625 nm at pH 7.0 was seen. In a control experiment, there was no corresponding absorbance change at pH 2.1, because deprotonation does not take place below the pK_a. Figure 1B shows the dependence of the apparent rate constants determined at pH 7.0 on the concentration of dG. The plot of rate constant versus the concentration of dG exhibits a linear relationship below 2 mM, indicating that the rate-determining step is the bimolecular reaction of dG with $SO_4^{-\cdot}$. From the slope, the second-order rate constant of the reaction with $SO_4^{-\cdot}$ is calculated to be 7.2×10^9 M^{-1} s^{-1}. The rate constant was saturated at 1.8×10^7 s^{-1} above 3 mM dG. Under these conditions, reactions 1 and 2 were kinetically resolved.

To detect the intermediates formed during the oxidation of dG, time-resolved difference spectra were measured after pulse radiolysis at a high concentration of dG (6 mM). A typical example is shown in Figure 2. The initial rapid increases in absorbance at 380 and 480 nm reflected the oxidation of dG by $SO_4^{-\cdot}$. Subsequently slower increases at 380 and 625 nm were also seen, whereas the slower absorbance change was not observed at 480 nm.

Figure 3A shows the spectra recorded at 300 ns after the pulse at pH 2.1, 3.8, and 6.5. The spectra at pH 2.1 and 6.5 can be identified as those of $\mathbf{G}^{+\cdot}$ and $\mathbf{G(-H)}^{\cdot}$, respectively, and are essentially similar to those reported before.[15] However, small differences in the range from 300 to 600 nm, except for the longer wavelengh above 600 nm, were seen in previous data,[15] whereas the absorbance around 380 and 625 nm increased with an increase of pH (Figure 3A). To confirm the pH-dependence spectra, absorbance changes after the pulse were plotted against

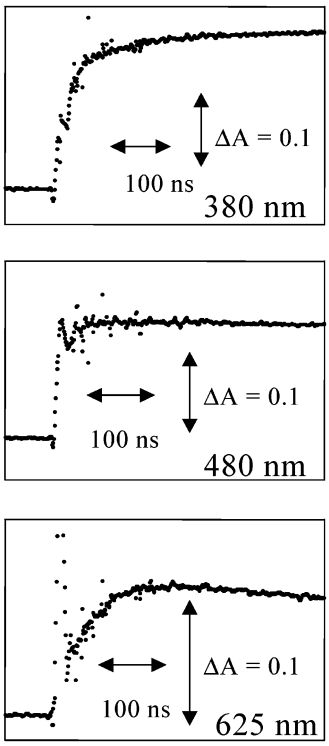

Figure 2. Absorbance changes at 380, 480, and 625 nm after pulse radiolysis of deoxyguanosine (6 mM) in the presence of ammonium persulfate (20 mM) and *tert*-butyl alcohol (0.1 M) at pH 7.0.

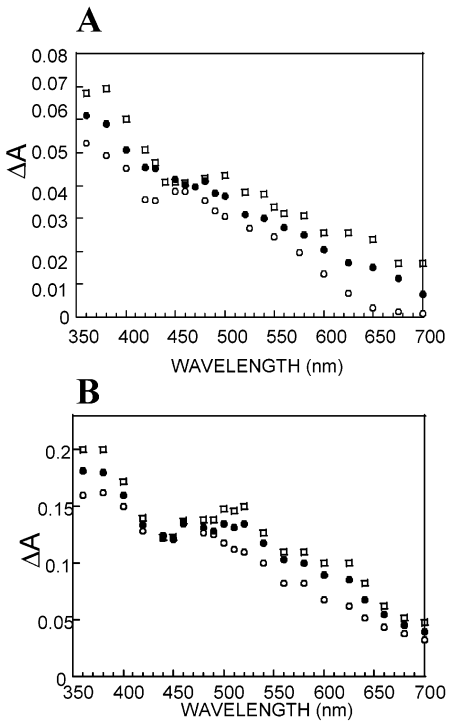

Figure 3. (A) Kinetic different spectra at 300 ns after pulse radiolysis of dG at pH 2.1 (○), pH 3.8 (●), and pH 6.5 (□). Samples contained 1 mM deoxyguanosine, and 10 mM acetate buffer (pH 2.1 and 3.8) or 10 mM phosphate buffer (pH 6.5). (B) Kinetic difference spectra of pulse radiolysis of dG monitored at 70 (○), 120 (●), 250 ns (□) after pulse radiolysis. Experimental conditions were the same as those described in the legend to Figure 1.

pH (Figure 4). These changes fit well to a pK profile with an inflection point at 3.9, whereas the absorbance changes at 480

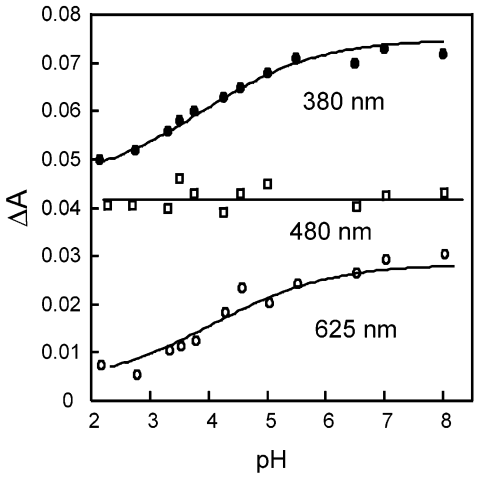

Figure 4. pH-dependent absorbance changes at 380 (●), 480 (□), and 625 nm (○) after pulse radiolysis of dG.

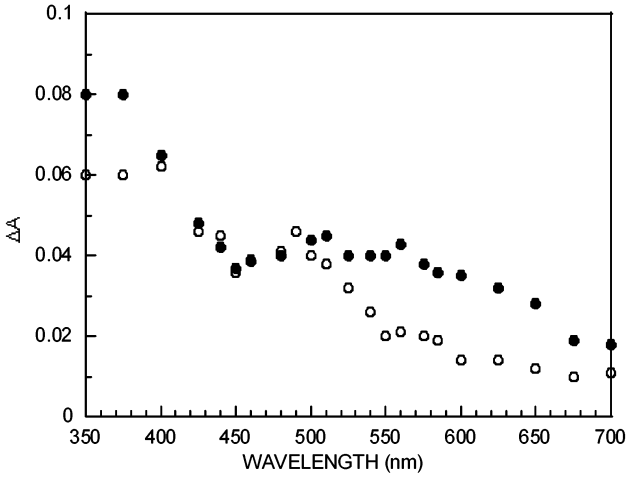

Figure 5. Kinetic difference spectra of pulse radiolysis of oligonucleotide $\mathbf{G_3}$ monitored at 70 (○) and 250 ns (●) after pulse radiolysis. Samples contained 2 nM oligonucleotide $\mathbf{G_3}$, ammonium persulfate (20 mM), *tert*-butyl alcohol (0.1 M), and 10 mM potassium phosphate buffer (pH 7.0).

nm are almost constant in this pH range. In comparison, Figure 3B shows the time-resolved spectra after pulse radiolysis of 6 mM dG at pH 7.0. The spectrum corresponding to the faster phase has an absorption maximum at 460 nm, whereas the spectra for the subsequent process have absorption increases around 380 and 625 nm. Consequently, at 70 ns after the pulse, the spectrum in Figure 3B can be identified as that of $\mathbf{G}^{+\cdot}$, on the basis of its similarity to that observed at pH 2.1. The kinetic difference spectra for the subsequent process have absorption maxima at 370, 500, and 625 nm; these are characteristic of $\mathbf{G(-H)}\cdot$. From these findings, the absorbance change seen in Figure 1 at 625 nm is attributable to the deprotonation of $\mathbf{G}^{+\cdot}$ to $\mathbf{G(-H)}\cdot$. A rate constant of 1.8×10^7 s^{-1} was obtained from Figure 1B.

Oxidation of Oligonucleotides. A similar experiment was performed using a systematic series of double-stranded oligonucleotides. Figure 5 shows the kinetic difference spectra at 70 and 250 ns after pulse radiolysis of the oligonucleotide $\mathbf{G_3}$. These spectra are essentially the same as those of dG at pH 7.0. As in the case of dG, the spectrum formed initially, which has an absorption maximum at 480 nm, is consistent with the formation of $\mathbf{G}^{+\cdot}$. The kinetic difference spectrum for the subsequent process has absorption maxima at 380, 500, 625 nm; these are characteristic of $\mathbf{G(-H)}\cdot$. From these findings, it can be concluded that, in double-stranded oligonucleotide, $\mathbf{G}^{+\cdot}$ was formed initially, followed by the deprotonation of $\mathbf{G}^{+\cdot}$ to $\mathbf{G(-H)}\cdot$. In a control experiment, a similar experiment was performed in a systematic series of single-stranded oligonucleotides containing G and A. The spectra of the single-stranded forms exhibited a composite spectrum containing G and A and were dependent on their sequences (data not shown).[29] In addition, the effect of NaCl (10−200 mM) on the spectra by the oxidation of the double-stranded oligonucleotides was not seen in the present data. Under our experimental conditions, therefore, the reactions of single-stranded forms can be excluded.

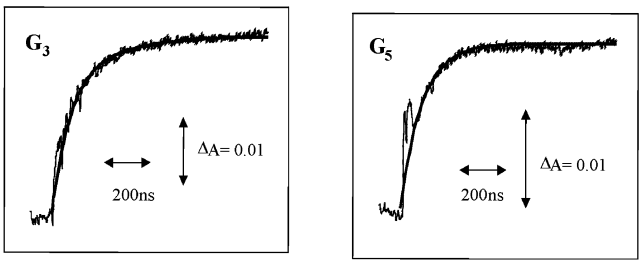

Figure 6. Kinetics of absorbance change at 625 nm after pulse radiolysis of oligonucleotides $\mathbf{G_3}$ and $\mathbf{G_5}$. Samples contained oligonucleotide (2.1 mM), ammonium persulfate (20 mM), *tert*-butylalcohol (0.1 M), and phosphate buffer (10 mM, pH 7.0).

Among the systematic series of oligonucleotides, it might be expected that the yield of $\mathbf{G}^{+\cdot}$ depends on the type and location of G in the oligonucleotides. For example, the $\mathbf{G_3}$ and $\mathbf{G_2}$ sequences contain 2 and 3 times the concentration of G than does $\mathbf{G_1}$. It is likely that G in the $\mathbf{G_5}$ can be efficiently oxidized by $SO_4^{-\cdot}$, because G is located at the 5′ and 3′-terminal positions. However, the spectra and the yield of $\mathbf{G}^{+\cdot}$ generated were not affected by the sequence of the oligonucleotides employed here (data not shown). In oligonucleotide $\mathbf{G_6}$, on the other hand, when a C·A mismatch was introduced into the $\mathbf{G_4}$ nucleotides, the yield of $\mathbf{G}^{+\cdot}$ was significantly diminished. Furthermore, the spectrum obtained following the pulse was not characteristic of the one-electron oxidized G moiety, but the spectra exhibited a mixture of base radicals (data not shown), as was observed in single-stranded oligonucleotides.

The absorbance increase observed at 625 nm with the $\mathbf{G_3}$ oligonucleotide fits well to a biexponential fitting curve (Figure 6). Similar kinetic profiles were obtained for $\mathbf{G_1}$, $\mathbf{G_2}$, and $\mathbf{G_4}$. Rate constants of $\sim 1.3 \times 10^7$ and $\sim 3.0 \times 10^6$ s^{-1} were calculated and were independent of oligonucleotide concentration (1.5−2.5 mM) within experimental error. It is noted that the rate constants were not significantly affected by the sequences, as is shown in Table 2. However, in $\mathbf{G_5}$, in which G is located at the terminal position, the observed absorbance trace fits well to a single-exponential curve (Figure 6), and the rate constant is similar to those obtained in the faster phase with the other oligonucleotides.

(28) The transient absorption spectrum of after pulse radiolysis of the oligonucleotide 5′-AAAAAGAAAAA-3′ exhibited characteristics of the one-electron oxidized A moiety with an absorption maximum at 600 nm, whereas in that of 5′-AAAAAGGGAAAAA-3′ the one-electron loss centers became localized mainly on G.

(29) The second-order rate constants of $SO_4^{-\cdot}$ with dG, dC, dA, and dT were 7.2×10^9, 7.0×10^9, 8.3×10^9, and 8.7×10^9 M^{-1} s^{-1}, respectively.

Table 2. Rate Constants of Formation of Transient Species Determined at 625 nm

double strand	k_1 (s^{-1})	k_2 (s^{-1})	T_m (°C)
G_1	1.5×10^7	3.5×10^6	34.9
G_2	1.4×10^7	3.6×10^6	34.4
G_3	1.4×10^7	3.3×10^6	41.4
G_4	1.5×10^7	3.3×10^6	29.6
G_5	1.3×10		30.9

Discussion

Hole-Transfer Process. The present experiment showed that $\mathbf{G}^{+\cdot}$ was formed within 50 ns after pulse radiolysis of double-stranded DNA in the presence of persulfate. However, the reaction scheme after the pulse can be interpreted by the following sequence events. The initial step is reaction of radiolytically generated $SO_4^{-\cdot}$ with bases unselectively within double-stranded DNA.[29] Subsequently, hole transfer from the initially formed loss centers to G sites occurs. The essentially quantitative formation of $\mathbf{G}^{+\cdot}$, based on the initial yield of $SO_4^{-\cdot}$, suggests that hole transfer occurs efficiently through 13−15mer DNA. In the $\mathbf{G_6}$ oligonucleotide, the formation of $\mathbf{G}^{+\cdot}$ was diminished by introducing a C·A mismatch. This result suggests that local perturbation of the duplex structure results in incomplete hole migration.[30]

Kinetic measurements of hole transport in DNA have been carried out to determine rates using various hole donors and acceptors. Lewis et al. reported that rates of hole transport are from $\sim 2 \times 10^5$ to 5×10^7 s^{-1} between G and GG with AA, A, or T intervening.[31] Similar kinetic data (10^4-10^5 s^{-1}) were observed in several systems.[24,33] In the present experiments, on the other hand, hole transfer from radical cations occurs long before 50 ns. Thus, this means that the rate of the hole transfer is greater than 2×10^7 s^{-1} and is considerably faster than previously observed.[24,31] However, it is important to realize that the rates were obtained under different conditions. Our measured rates of $\mathbf{G}^{+\cdot}$ formation by the hole transport from the initially formed loss center across the $(A-T)_n$ sequence differ from the indirect spectroscopic measurement of hole transport by Lewis and co-workers.[31] Very recently, it has been shown that rapid radical formation of methylindole, incorporated as an artificial base, by DNA charge transport occurs at the rate $\geq 10^7$ s^{-1} and is independent of distance over 17−37 Å.[32] This observation is compatible with our results.

Deprotonation Process. We observed spectrophotometrically that free guanine radical cation, $\mathbf{G}^{+\cdot}$, was found transiently in neutral aqueous solution and deprotonated rapidly to form a neutral radical $\mathbf{G(-H)}^{\cdot}$ with a half time of 35 ns. The spectrum of $\mathbf{G}^{+\cdot}$ exhibits absorption bands at around 480 and 400 nm, whereas $\mathbf{G(-H)}^{\cdot}$ has absorption maxima at around 500 and 380 nm, together with a characteristic shoulder in the spectral range of 600−700 nm. With double-stranded oligonucleotide, similar types of transient absorption spectra were obtained after the pulse. Therefore, in the case of DNA, it is concluded that the conversion from $\mathbf{G}^{+\cdot}$ to $\mathbf{G(-H)}^{\cdot}$ takes place with concomitant release of the proton on time scales similar to that observed in free dG at neutral pH.

The deprotonation of $\mathbf{G}^{+\cdot}$ in double-stranded DNA can be interpreted by the following steps. The initial step is the formation of $\mathbf{G}^{+\cdot}\mathbf{:C}$ (step 1). Proton transfer from the base-pair radical cation then gives deprotonated G radical and the C base pair ($\mathbf{G(-H)}^{\cdot}\mathbf{:C(+H^+)}$) (step 2). Finally, the proton of the $\mathbf{C(+H^+)}$ is expected to be picked up by a water molecule (step 3). In these processes, the proton transfer from G to C in step 2 could, in principle, be very fast, because it involves the movement of proton along a preexisting hydrogen bond.[14,34] Thus, the formation of $\mathbf{G}^{+\cdot}\mathbf{:C}$ as an initial product would not be observed, and the initial spectrum obtained immediately after the pulse may be a proton-shifted resonance structure ($\mathbf{G}^{+\cdot}\mathbf{:C} \leftrightarrow \mathbf{G(-H)}^{\cdot}\mathbf{:C(+H^+)}$). From the p$K_a$ values of the N1 proton of $\mathbf{G}^{+\cdot}$ (pK_a = 3.9) and N3 protonated C (pK_a = 4.3), the equilibrium constant (K_{eq}) for the proton transfer is estimated to be 2.5. This suggests that the oxidized G remains protonated to some extent. In the present study, however, the significant difference in the initial spectra obtained between free dG and DNA was not seen in Figures 2 and 3. Deprotonation of the N1 proton of G in DNA and subsequent deprotonation of $\mathbf{C(+H^+)}$ would give deprotonated G radical and C base-pair $\mathbf{(G^{\cdot}:C)}$, which could account for the spectrum, Figure 5, 250 ns after the pulse. It is noteworthy that the depronation process in DNA monitored at 625 nm revealed the existence of two component kinetics. We could not identify definitely these spectral changes, but it is likely that the slower kinetic component is attributed to the deprotonation of $\mathbf{C(+H^+)}$ by a water molecule; this is supported by the fact that the slower process was diminished in the $\mathbf{G_5}$ oligonucleotide. A water molecule can easily access the G:C moiety located in the terminal position of $\mathbf{G_5}$, and thus the process will occur very rapidly. In the other oligonucleotides, on the other hand, the G:C moiety is well buried in π-stacked bases of DNA. The process is presumed to reflect on the T_m values of DNA. However, the rates were not correlated with T_m values of oligonucleotides (Table 2).

The results presented here provide the following important implication for the hole transfer in DNA. It is accepted that charges are transported over a long distance through a multistep hopping reaction; the "G-hopping" involves positive charges moving between G.[2,35−38] The transfer rates decrease with increasing separation of G and compete with the deprotonation of $\mathbf{G}^{+\cdot}$ in DNA. The results presented here show that the deprotonation of $\mathbf{G}^{+\cdot}$ in DNA occurs within 1 μs, suggesting that the hole transfer along DNA in this time range occurs prior to the deprotonation. In this case, the rate of hole transfer should be larger than 10^6 s^{-1}. In contrast, the oxidized species of G responsible for the hole transfer in the time range from microsecond to millisecond is the deprotonated form, $\mathbf{G(-H)}^{\cdot}$

(30) Bhattacharya, P. K.; Barton, J. K. *J. Am. Chem. Soc.* **2001**, *123*, 8649.
(31) (a) Lewis, F. D.; Liu, X.; Liu, J.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. *Nature* **2000**, *406*, 51. (b) Lewis, F. D.; Liu, X.; Liu, J.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. *Nature* **1999**, *406*, 51. (c) Lewis, F. D.; Wu, T.; Liu, X.; Letsinger, R. L.; Greenfield, S. R.; Miller, S. E.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2000**, *122*, 2889. (d) Lewis, F. D.; Zuo, X.; Liu, J.; Hayes, R. T.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2002**, *124*, 4568.
(32) (a) Pascaly, M.; Yoo, J.; Barton, J. K. *J. Am. Chem. Soc.* **2002**, *124*, 9083. (b) Yoo, J.; Delaney, S.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **2003**, *125*, 6640.
(33) Shafirovich, V.; Cadet, J.; Gasparutto, D.; Dourandin, A.; Huang, W.; Geacintov, N. E. *J. Phys. Chem. B* **2001**, *105*, 586.
(34) (a) Douhal, A.; Kim, S. K.; Zewail, A. H. *Nature* **1995**, *378*, 260. (b) Zewail, A. H. *J. Phys. Chem.* **1996**, *100*, 12701.
(35) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631.
(36) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159.
(37) Jortner, J.; Bixon, M.; Lagenbacher, T.; Michel-Beyerle, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12759.
(38) Berlin, Y. A.; Burin, A. L.; Ratner, M. A. *J. Am. Chem. Soc.* **2001**, *123*, 260.

neutral radical. Because the neutral G radicals in DNA are stable and decay on millisecond to second time scales,[20,22,33] long-range hole transfer is possible. Thus, the long distance migration of oxidative damage in double-stranded DNA is more likely to be induced by neutral radical than by radical cation. A similar mechanism has been proposed by Shafirovich et al.[33] In the study, the hole transfer in DNA from the base analogue 2-aminopurine (2-AP) to 8-oxy-dG reveals the existence of two-component oxidation kinetics of a rapid component (<100 ns) of the radical cation ($2\text{-AP}^{+\cdot}$) and a slower component of the deprotonation product ($2\text{-AP}(-\text{H})^{\bullet}$) in microseconds.

The reactivity and the selectivity of G base are drastically affected by the interaction fields induced by base pairing and base stacking, which has been shown by the experimental observation of preferential cleavage at multiple G-containing sites and in a theoretical model.[39] However, present data show that the deprotonation process was independent of the sequence of the oligonucleotides (Table 2). This suggests that the difference in the electron population of the G base is not an important factor for the proton-transfer process.

Conclusion

We observed spectrophotometrically that free guanine radical cation, $\mathbf{G}^{+\cdot}$, was found transiently in neutral aqueous solution and deprotonated rapidly to form a neutral radical $\mathbf{G(-H)}^{\cdot}$. With double-stranded oligonucleotide, similar types of transient absorption spectra were obtained. The conversion from $\mathbf{G}^{+\cdot}$ to $\mathbf{G(-H)}^{\cdot}$ takes place with concomitant release of the proton on time scales similar to that observed in free dG.

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Supporting Information Available: Figure of absorbance changes of oligonucleotide $\mathbf{G_4}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(39) (a) Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc*. **1998**, *120*, 12686. (b) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc*. **1995**, *117*, 6406. (c) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc*. **1996**, *118*, 7063. (d)Yoshioka, Y.; Kitagawa, Y.; Takano, Y.; Yamaguchi, K.; Nakamura, T.; Saito, I. *J. Am. Chem. Soc*. **1999**, *121*, 8712. (e) Nakatani, K.; Dohno, C.; Saito, I. *J. Am. Chem. Soc*. **2000**, *122*, 5893.