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Regulation of One-Electron Oxidation Rate of Guanine by Base Pairing with Cytosine Derivatives

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Abstract: Effects of base pairing on the one-electron oxidation rate of guanine derivatives, guanine, 8-bromoguanine, and 8-oxo-7,8-dihydroguanine have been studied. The one-electron oxidation rate of guanine derivatives was determined by triplet-quenching experiments, using *N*,*N*′-dibutylnaphthaldiimide (NDI) in the triplet excited state (^{3}NDI*) and fullerene (C_{60}) in the triplet excited state ($^3C_{60}$*) as oxidants. In all three guanine derivatives studied here, acceleration of the one-electron oxidation was observed upon hydrogen bonding with cytosine, which demonstrates lowering of the oxidation potential of guanine derivatives by base pairing with cytosine. When a methyl or bromo group was introduced to the C5 position of cytosine, acceleration or suppression of the one-electron oxidation relative to the guanine:cytosine base pair was observed, respectively. The results demonstrate that the one-electron oxidation rate of guanine in DNA can be regulated by introducing a substituent on base pairing cytosine.

Introduction

Site-selective oxidation of DNA is one of the goals for the design of artificial photonuclease as therapeutic and diagnostic agents.[1] The regulation of the transfer rate and direction of the hole generated in DNA by one-electron oxidation is of interest from the perspective of using DNA as a building block for electronic devices.[2−6]

The one-electron oxidation of DNA causes the oxidation of guanine with the lowest oxidation potential among four DNA bases. Various studies suggest a lower oxidation potential of guanine in duplex DNA compared to monomer guanine or guanine in single strand DNA. The lower oxidation potential for guanine in the GG and GGG sequences has been theoretically[7] and experimentally[8−10] investigated and found to result from π-stacking of guanines. As for other factors that control the one-electron oxidation rate of guanine in duplex DNA, a computational experiment has suggested that base pairing with cytosine lowers the ionization potential of guanine.[11] Recently we designed an experiment in dichloromethane to evaluate the effect of hydrogen bonding, separately from the π-stacking effect, on the one-electron oxidation of guanine.[12] It has been demonstrated that the oxidation potential of guanine is lowered by selective hydrogen bonding with cytosine. In the present work, the effect of base pairing on the one-electron oxidation rate of guanine and its derivatives, 8-bromoguanine and 8-oxo-7,8-dihydroguanine, was investigated. Furthermore, substituents were introduced to the base paired cytosine, and transmission of the electron-donating effect of the 5-methyl and electron-withdrawing effect of the 5-bromo substituent of the cytosine to guanine derivatives through hydrogen bonding of the guanine:cytosine pair was demonstrated.

Experimental Section

Materials. Nucleoside rG was obtained from Yamasa Shoyu. Nucleosides dC and T were purchased from Wako. *tert*-Butyldimethylchlorosilane (TBDMS-Cl) and 1,4,5,8-naphthalenetetracarboxylic anhydride were purchased from Tokyo Kasei. Fullerene (C_{60}) was purchased from Kanto Chemicals. Spectro-grade dichloromethane was obtained from Dojin Laboratories and used as received. Silylated nucleoside derivatives, tri-*tert*-butyldimethylsilyl guanine (**G**), tri-*tert*-butyldimethylsilyl 8-bromoguanine (**brG**), tri-*tert*-butyldimethylsilyl 8-oxo-7,8-dihydroguanine (**oxG**), di-*tert*-butyldimethylsilyl cytosine (**C**), di-*tert*-butyldimethylsilyl 5-methylcytosine (**mC**), and di-*tert*-butyldimethylsilyl 5-bromocytosine (**brC**) were synthesized according to the reported procedures.[13−15] *N*,*N*′-Dibutylnaphthaldiimide (NDI) was prepared from the reaction of 1,4,5,8-naphthalenetetracarboxylic an-

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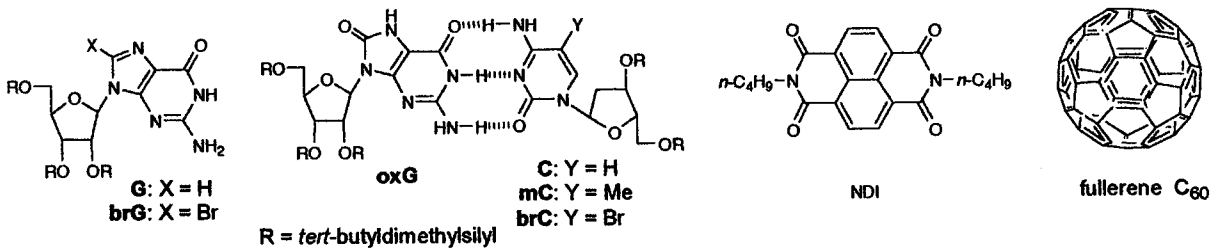


Figure 1. Structure of guanine derivatives, cytosine derivatives, and triplet sensitizers NDI and fullerene (C_{60}).

hydride with 1-aminobutane as reported[16] and recrystallized from *N*,*N*-dimethylacetamide three times.

Measurement of Association Constants. ^{1}H NMR spectra were recorded on a JEOL-JNM-EX270 (270 MHz). The ^{1}H NMR spectrum of 2 mM **G** was recorded to obtain the unbound chemical shift of N(1)H of **G** (δ_G). The concentration of **G** was kept constant at 2 mM, and the chemical shift of N(1)H was measured in the presence of 2 mM **C** (δ_{2mM}). The maximum chemical shift of N(1)H of **G** at 100% binding to the **C** (δ_{CG}) was obtained by adding additional equivalents of **C** (up to 80 mM), and the spectrum was recorded each time until the chemical shift of N(1)H no longer changed upon further addition of **C**. The data were introduced into the following equation,

$$\delta_{2mM} = \delta_G + (\delta_{CG} - \delta_G)((0.002 + 0.002 + 1/K_a) - ((0.002 + 0.002 + 1/K_a)^2 - 0.008 \times 0.002)^{1/2})/0.004$$

which provided K_a.[17] In the case of base pairing between **oxG** and cytosine derivatives, the chemical shift of N(1)H of **oxG** became almost constant at more than 2 mM of 1:1 **oxG** and cytosine derivatives. Therefore, an accurate K_a could not be obtained from NMR experiments. Thus, K_a was estimated to be larger than 2×10^4 M^{-1} for the base pair between **oxG** and cytosine derivatives.[18]

Laser Flash Photolysis. The third-harmonic oscillation (355 nm, 4 ns, 15 mJ) from a Q-switched Nd:YAG laser (Quantel Model Brilliant) was used for laser flash excitation. A xenon flash lamp (Osram, XBO-450) was focused through the sample as a probe for the transient absorption measurement. Time profiles of the transient absorption at the UV−visible region were measured with a monochromator (Nikon G250) equipped with a photomultiplier (Hamamatsu Photonics R928) and digital oscilloscope (Tektronics, TDS-380P). The monitor light for the measurement of time profiles of the transient absorption at 1000 nm was passed through an interference filter (CVI, transmittance of 40%, bandwidth of 10 nm) and its intensity was monitored with a fast InGaAs PIN photodiode equipped with an amplifier (Thorlabs, PDA255) and digital oscilloscope. For the time-resolved transient absorption spectral measurement, monitor light was focused into a quartz optical fiber (diameter = 250 μm, length = 2 m), which transported the laser induced transmittance changes to a gated-multichannel spectrometer (Unisoku TSP-601-02).

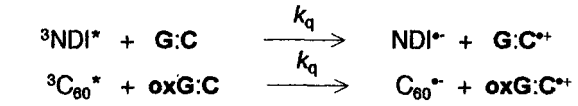
Electrochemical Measurement. Cyclic voltammetry (CV) was performed by using a CV50W potentiostat (BAS), with a single-compartment cell equipped with a Pt working electrode, a Pt-wire counter electrode, and an Ag/AgNO$_3$ reference electrode. Dichloromethane was freshly distilled over P_2O_5, and *n*-tetrabutylammonium perchlorate (0.1 M) was used as the electrolyte. The solution containing 5 mM substrates was degassed by purging with Ar for 10 min. CVs were collected at a scan rate of 100 mV/s, and referenced to ferrocene as an internal standard.[19]

Table 1. ^{1}H NMR δ Values of the N(1)H of Guanine Derivatives and Measured Association Constants (K_a)

nucleoside analogues	δ_G[a] (ppm)	δ_{2mM}[b] (ppm)	δ_{CG}[c] (ppm)	K_a (M^{-1})
G:brC	12.12	13.22	13.37	1.9×10^4
G:C		13.51	13.79	1.2×10^4
G:mC		13.59	13.84	1.5×10^4
brG:brC	11.92	13.31	13.52	1.9×10^4
brG:C		13.79	14.01	1.9×10^4
brG:mC		13.71	14.02	1.5×10^4
oxG:brC	11.94	13.60	13.67[d]	$>2 \times 10^4$
oxG:C		14.14	14.16[d]	$>2 \times 10^4$
oxG:mC		14.20	14.18[d]	$>2 \times 10^4$

[a] Unbound chemical shift of N(1)H of guanine derivatives. [b] Chemical shift of N(1)H of 2 mM guanine derivatives measured in the presence of equivalent cytosine derivatives. [c] Chemical shift of N(1)H of base paired guanine derivatives. [d] Chemical shift of N(1)H of 10 mM **oxG** measured in the presence of equimolar cytosine derivatives.

Scheme 1



Results and Discussion

For the experiments in dichloromethane, nucleoside derivatives with a *tert*-butyldimethylsilyl group on the ribose unit were synthesized (Figure 1). To confirm the base-pair formation between guanine derivatives and cytosine derivatives under the experimental conditions, association constants (K_a) were measured (Table 1). In all nine combinations of base pairs between guanine derivatives and cytosine derivatives studied here, K_a was measured to be higher than 10^4 M^{-1}. Therefore, more than 85% of guanine derivatives are estimated to form the selective hydrogen bonding with cytosine derivatives under the present experimental conditions. The chemical shift of the N(1)H of base-paired **G** (δ_{CG}) varied with the substitution on cytosine. Relative to the **G**:**C** pair, a downfield shift in the case of the **G**:**mC** pair and an upfield shift in the case of the **G**:**brC** pair were observed, and this trend was common for all three guanine derivatives, indicating that redistribution of electron density upon hydrogen bonding leads to higher electron density of **G** in the **G**:**mC** than in the **G**:**C** base pair and lower electron density for the **G**:**brC** base pair.[20]

The one-electron oxidation rates of guanine derivatives were investigated by triplet quenching (Scheme 1). For the one-electron oxidation of **G** and **brG** proceeding slower than the

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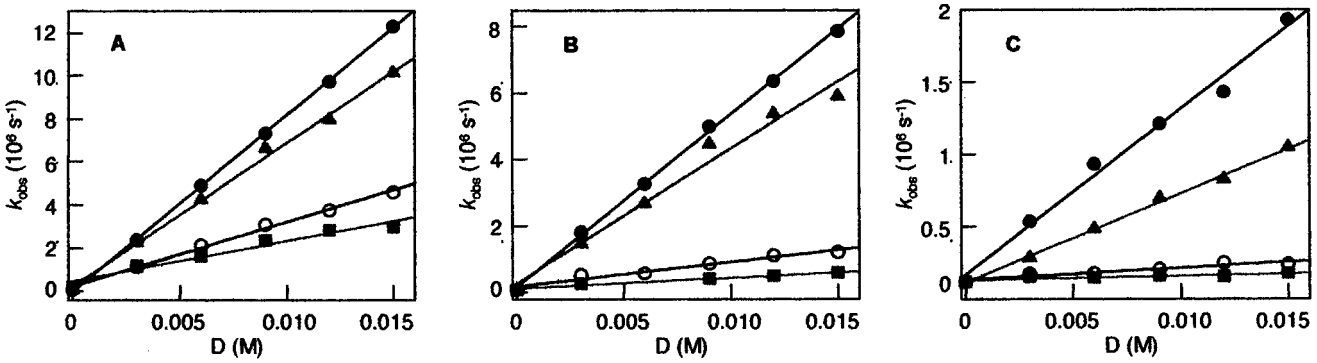

Figure 2. Bimolecular quenching plots for the reaction of triplet sensitizers with nucleoside analogues as electron donors (D): the reaction of ^{3}NDI* with **G** (A) and **brG** (B) and the reaction of $^3C_{60}$* with **oxG** (C). Guanine derivatives in the absence (square) or presence of equimolar amount of **C** (triangle), **brC** (open circle), and **mC** (closed circle).

Table 2. Bimolecular Rate Constants (k_q) for the Electron Transfer Quenching of ^{3}NDI* by Nucleoside Analogues

nucleoside analogues	k_q (10^8 $M^{-1}s^{-1}$)	k_q^{rel} [a]
G:mC	8.1	25
G:C	6.8	21
G:brC	3.0	9.1
G	1.9	5.8
brG:mC	5.2	16
brG:C	4.0	12
brG:brC	0.73	2.2
brG	0.33	1.0

[a] Relative to brG.

Table 3. Thermodynamic Driving Forces of the One-Electron Oxidation of Nucleosides

nucleoside	triplet sensitizer	ΔG (eV)[a]
guanine	^{3}NDI*	−0.3
8-oxo-7,8-dihydroguanine	^{3}NDI*	−0.7
8-oxo-7,8-dihydroguanine	$^3C_{60}$*	−0.2

[a] Estimated from the Rhem-Weller equation $\Delta G = [E(D^{\bullet+}/D) - E(^3A^*/A^{\bullet-})] + 0.085$, using the reported reduction potentials of ^{3}NDI* (1.81 V vs NHE)[13] and $^3C_{60}$* (1.38 V vs NHE),[22] and the oxidation potentials of guanine (1.47 V vs NHE)[25] and 8-oxo-7,8-dihydroguanine (1.09 V vs NHE).[26]

diffusion-controlled rate in dichloromethane, ^{3}NDI* was selected as an oxidant.[12,16] As has already been reported, the one-electron oxidation of **G** was accelerated upon selective base pairing with **C**. To investigate whether the electron-donating or electron-withdrawing effect of the substituent on cytosine can regulate the one-electron oxidation rate of guanine through the hydrogen bond, the quenching rate of ^{3}NDI* by **G**:**mC** and **G**:**brC** was measured and compared with that of **G**:**C**. Remarkably, when a methyl group was introduced as an electron-donating group at C5 of cytosine, the one-electron oxidation rate was accelerated (Figure 2A, Table 2). On the other hand, a bromo substituent on cytosine as an electron-accepting group led to the suppression of the one-electron oxidation rate. These results strongly suggest that the electronic substituent effects of cytosine can be transmitted to the guanine partner through hydrogen bonding.

To test the validity and the generality of this observation, the one-electron oxidation rate of **brG** in the presence of equimolar **C**, **mC**, or **brC** was measured. A slower oxidation rate was observed for **brG** compared to **G**, which arises from the higher oxidation potential of **brG** due to the electron-withdrawing effect of the bromo group. Similarly, the one-electron oxidation rate of **brG** was accelerated upon base pairing with **C** and increased in the order **brC** < **C** < **mC** according to the substituent of base-pairing partner cytosine (Figure 2B, Table 2). Interestingly, relatively larger effects on the one-electron oxidation rate were observed for **brG** upon base pairing compared to **G** (discussed below).

Next, the hydrogen-bonding effect on the one-electron oxidation of **oxG** was investigated. Since 8-oxo-7,8-dihydroguanine is a major oxidation product of guanine, its biological effects[21] and redox properties[13,22−24] have been widely studied. When ^{3}NDI* was used as an oxidant for **oxG** and **oxG**:**C**, only a slight change in k_q was observed. Since the oxidation potential of 8-oxo-7,8-dihydroguanine is lower by ca. 0.4 V than that of guanine,[25,26] thermodynamic driving forces of the electron transfer (ΔG) are too negative and the one-electron oxidation rate of **oxG** reached the diffusion-controlled rate (Table 3).[27] Therefore, for the one-electron oxidation of **oxG** to proceed more slowly than the diffusion-controlled rate in dichloromethane, $^3C_{60}$* was selected as an oxidant.[22] Upon 355-nm pulsed excitation of a dichloromethane solution of C_{60}, a transient absorption spectrum with a maximum peak of 740 nm was observed at 100 ns after the laser flash (Figure 3A). The spectrum formed is characteristic of the T_1-T_n absorption of C_{60} and assigned to $^3C_{60}$*. Figure 3B shows time profiles of the transient absorption at 750 and 1000 nm during laser flash photolysis of the C_{60} solution in the presence of **oxG** and **C**. Concomitant with the decay of the transient absorption peak of $^3C_{60}$* at 750 nm, formation of transient absorption in the 900−1100 nm range was identified as the one-electron reduced form of C_{60} ($C_{60}^{\bullet-}$).[22] These results demonstrate the electron-transfer quenching of $^3C_{60}$* by **oxG**.[12] Thus, the electron-transfer quenching of $^3C_{60}$* by **oxG** in the presence of **C**, **brC**, and **mC** was examined. Interestingly, the highest effect of hydrogen bonding on k_q was observed for **oxG**, compared to those of **G** and **brG**, and as for the largest change, the one-electron

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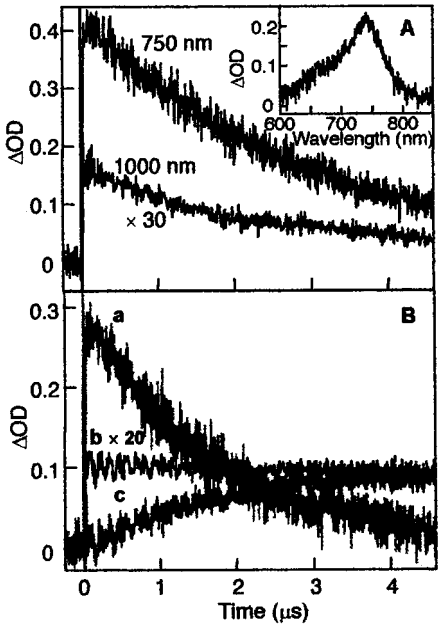

Figure 3. (A) The triplet decays observed at 750 and 1000 nm after the pulsed excitation of 50 μM C_{60} in argon-saturated dichloromethane. The inset shows the transient absorption spectrum observed at 100 ns. (B) (**a**) Decay of the transient absorption of $^3C_{60}$* observed at 750 nm. (**b**) Time profile of the transient absorption observed at 1000 nm involving decay of $^3C_{60}$* and formation of $C_{60}^{\bullet-}$ in the presence of 9 mM **oxG** and **C**. (**c**) Formation of $C_{60}^{\bullet-}$ calculated from time profiles (**a**) and (**b**). Time profile (**a**) was normalized by multiplication by 0.018 like ΔOD at $t = 0$, the same as that for (**b**). This was subtracted from time profile (**b**) to extract the rise of the $C_{60}^{\bullet-}$ component (**c** = (**b** − **a** × 0.018) × 20).

Table 4. Bimolecular Rate Constant (k_q) for the Electron-Transfer Quenching of $^3C_{60}$* by Nucleoside Analogues and Electrochemically Derived Half-Peak Oxidation Potential (E_{ox})

nucleoside analogues	k_q (10^6 $M^{-1}s^{-1}$)	k_q^{rel}	E_{ox} (mV)[b]	ΔE_{ox} (mV)
oxG:mC	114	37	540	−22
oxG:C	62	20	549	−13
oxG:brC	8.3	2.7	583	21
oxG	3.1	1	562	0

[a] Relative to oxG. [b] Referenced to ferrocene as an internal standard.[19]

oxidation rate of **oxG** was accelerated 40 times upon base pairing with **mC** (Table 4).

For all three guanine derivatives studied here, acceleration of the one-electron oxidation was observed upon base pairing with **C**. However, the extent of the change of k_q increased in the order **G** < **brG** < **oxG**. This order was explained by the difference in ΔG between these three guanine derivatives. Generally, as ΔG is more negative, k_q became less sensitive to the change of ΔG according to the Rehm−Weller equation (Figure 4). Since ΔG is the least negative for the one-electron oxidation of **oxG** by $^3C_{60}$* (Table 3), k_q is the most sensitive to the change of the oxidation potential of guanine derivatives upon hydrogen bonding. Thus, the largest acceleration was observed for **oxG** upon base pairing with **C**.

To test whether the acceleration of k_q upon base pairing truly reflects the lowering of the oxidation potential of guanine derivatives, the redox chemistry of **oxG** was investigated electrochemically using cyclic voltammetry.[28,29] One-electron oxidation of **oxG** was irreversible and results were expressed as the half-peak potential with E_{ox} of 0.56 V (vs ferrocene) in dichloromethane (Figure 5). The shifts in E_{ox} observed for **oxG**

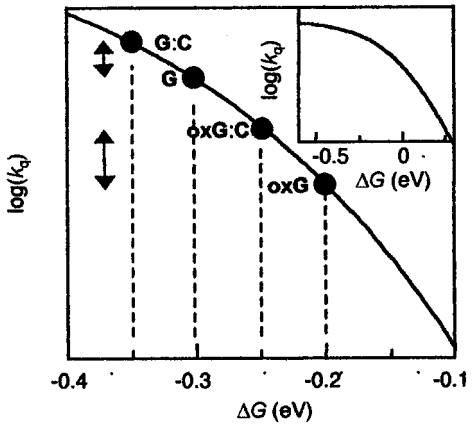

Figure 4. Schematic representation of the relationship between bimolecular quenching rate constant (k_q) vs ΔG in the range of −0.4 to −0.1 eV according to the Rehm−Weller equation. Inset: Calculated rate constants vs ΔG in the wider range (−0.6 to 0.3 eV) according to the Rehm−Weller equation.

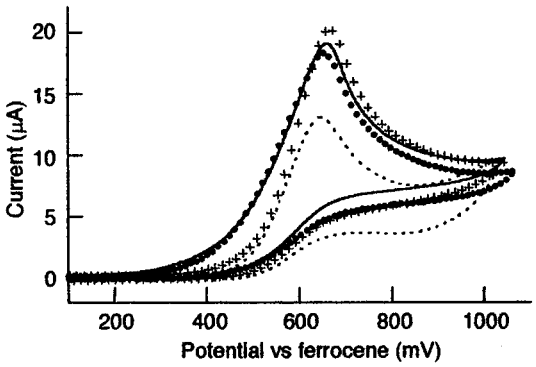

Figure 5. Cyclic voltammograms of 5 mM **oxG** (dashed line) and **oxG** in the presence of **brC** (+), **C** (solid line), and **mC** (●) in dichloromethane, *n*-tetrabutylammonium perchlorate carrier (0.1 M).

upon addition of equimolar cytosine derivatives are listed in Table 4. The addition of equimolar **C** to **oxG** resulted in a 13 mV negative shift in the oxidation potential of **oxG**. Furthermore, **mC** gave a larger negative shift compared to that of the **oxG**:**C** pair, and in contrast, the addition of **brC** resulted in a positive shift. The lowering of the oxidation potential of guanine for the GG and GGG sequence is reported as 52 and 77 mV, respectively.[10] Therefore, the results indicate that the effect of hydrogen bonding on one-electron oxidation of guanine is roughly as important as π-stacking. These results demonstrate that the observed effect of cytosine derivatives on k_q strongly reflects the oxidation potential of **oxG**.[30]

Conclusions

It has been demonstrated that the one-electron oxidation rate of guanine derivatives can be controlled by base pairing with cytosine derivatives. Although all guanines in DNA form a base pair with cytosine, the results suggest that the oxidation potential of guanine can be controlled by introducing a substituent on cytosine on the complementary strand. To elucidate the effects of the methyl or bromo group at C5 of cytosine on the one-

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(30) In the case of the **oxG**:**brC** pair, although the one-electron oxidation rate was accelerated, a positive shift of E_{ox} was observed compared to **oxG**. Therefore, some other factors, such as an increase of the cross section upon base pairing, may also contribute on the change of k_q.

electron oxidation rate of guanine, and the hole transfer rate in DNA, laser flash photolysis and pulse radiolysis experiments of 5-methylcytosine and 5-bromocytosine containing oligodeoxynucleotides are now underway.

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