Electron-Transfer Oxidation Properties of DNA Bases and DNA Oligomers

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Received: September 7, 2004; In Final Form: January 6, 2005

Kinetics for the thermal and photoinduced electron-transfer oxidation of a series of DNA bases with various oxidants having the known one-electron reduction potentials (E_{red}) in an aqueous solution at 298 K were examined, and the resulting electron-transfer rate constants (k_{et}) were evaluated in light of the free energy relationship of electron transfer to determine the one-electron oxidation potentials (E_{ox}) of DNA bases and the intrinsic barrier of the electron transfer. Although the E_{ox} value of GMP at pH 7 is the lowest (1.07 V vs SCE) among the four DNA bases, the highest E_{ox} value (CMP) is only 0.19 V higher than that of GMP. The selective oxidation of GMP in the thermal electron-transfer oxidation of GMP results from a significant decrease in the pH dependent oxidation potential due to the deprotonation of $GMP^{\bullet+}$. The one-electron reduced species of the photosensitizer produced by photoinduced electron transfer are observed as the transient absorption spectra when the free energy change of electron transfer is negative. The rate constants of electron-transfer oxidation of the guanine moieties in DNA oligomers with $Fe(bpy)_3^{3+}$ and $Ru(bpy)_3^{3+}$ were also determined using DNA oligomers containing different guanine (G) sequences from 1 to 10 G. The rate constants of electron-transfer oxidation of the guanine moieties in single- and double-stranded DNA oligomers with $Fe(bpy)_3^{2+}$ and $Ru(bpy)_3^{3+}$ are dependent on the number of sequential guanine molecules as well as on pH.

Introduction

DNA is a polymer of deoxyribonucleotide units consisting of a purine or pyrimidine base bonded to a sugar, and one or more phosphate groups. Oxidation of DNA by free radicals,[1] ionizing radiation,[2,3] chemical oxidants,[4] or photoirradiation is a common source of damage.[5−9] There has been considerable interest in understanding the chemical and physical mechanisms that control the extent, location, and chemical consequences of DNA oxidation.[4−11] The oxidation of DNA bases and the oxidation mechanism have also been receiving increased attention recently in both thermal and photochemical reactions.[12−14] Among DNA bases, guanine is known to be most readily oxidized,[15−20] and guanine is the primary target of the one-electron oxidation of duplex DNA by chemical oxidation, electrochemical oxidation, or photooxidation.[4,21−24] To determine the driving force of electron-transfer reactions of DNA bases, it is necessary to know the one-electron oxidation potentials (E_{ox}) of DNA bases. There have so far been a number of reports on the E_{ox} values of DNA bases, which correspond to the one-electron reduction potentials (E_{red}) of the radical cations of DNA bases.[25−28] However, the reported E_{ox} values of DNA bases vary significantly depending on the determination methods and experimental conditions (e.g., pH). For example, the standard one-electron oxidation potential values of guanosine and adenosine were determined by pulse radiolysis as 1.58 V (vs NHE) and 2.03 V (vs NHE), respectively.[28] These values are significantly higher than the previously reported values of guanosine and adenine (1.33 and 1.73 V vs NHE, respectively).[25,28] The standard one-electron oxidation potential of guanosine was also determined by cyclic voltammetry as 1.49 V vs NHE, which is also different from the values determined by pulse radiolysis.[27] In the case of electrochemical measurements, the irreversibility of the electrode process due to the facile follow-up reactions has precluded the accurate determination of the E_{ox} values of DNA bases.[27] In any case, the facile deprotonation of guanine radical cation has precluded the direct determination of the E_{ox} value. Thus, it is highly desired to examine the electron-transfer dynamics of DNA bases in detail to determine the one-electron oxidation potentials (E_{ox}) of DNA bases under the experimental conditions that electron transfer is the rate-determining step.

On the other hand, theoretical and experimental studies have demonstrated that 5′-GG-3′ sequences in DNA are "hot spots" for oxidative damage.[29,30] The proton-coupled electron-transfer oxidation of guanine and other modified purine nucleobases has recently been reported to examine the driving force dependence of the rate constants of proton-coupled electron transfer.[31,32] However, dependence of the rates of the proton-coupled electron-transfer oxidation of single- or double-stranded DNA oligomers on various DNA base sequences has yet to be reported.

We report herein the detailed kinetic investigations on both photoinduced and thermal electron-transfer oxidation of DNA bases to determine the one-electron oxidation potentials (E_{ox}) and the intrinsic barriers of electron transfer ($\Delta G^{\ddagger}_0$), both of which would otherwise be difficult to obtain. Once the E_{ox} values of DNA bases are determined, the free energy changes (ΔG_{et}) of photoinduced electron-transfer reactions of DNA bases with various one-electron oxidants can be readily estimated. When the ΔG_{et} values are negative, the electron-transfer products would be detected as the transient absorption spectra. In contrast, no electron-transfer products would be detected when the ΔG_{et} values are positive. This is confirmed by laser flash photolysis measurements of photoinduced electron-transfer reactions of DNA bases to demonstrate the validity of the E_{ox}

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10.1021/jp0459763 CCC: $30.25
Published on Web 03/25/2005

values of DNA bases. We also report the kinetic investigations on thermal electron-transfer oxidation of single-stranded and double-stranded DNA oligomers with one-electron oxidants. By changing the number of sequential guanines, the influence of guanine sequence on the electron-transfer oxidation has been examined quantitatively. These data provide the solid energetic basis for the selective oxidation of guanine in DNA as well as a valuable insight into the oxidation mechanism.

Experimental Section

Materials. GMP (guanosine 5′-monophosphate) and AMP (adenosine 5′-monophosphate) were obtained from Nacalai Tesque, Japan. TMP (thymidine 5′-monophosphate) and CMP (cytosine 5′-monophosphate) were obtained from Sigma-Aldrich. Oligonucleotides are obtained commercially from Proligo, Japan. Duplex of the DNA oligomers were formed as a 1:1 mixture of the complementary strands by heating to 80 °C for 2 h followed by slow cooling to ambient temperature over a time of 2 h in 20 mM Tris/HCl buffer (pH 7.0). Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate [$Ru(bpy)_3Cl_2$] was obtained from Sigma-Aldrich. Tris(2,2′-bipyridyl)ruthenium(III) hexafluorophosphate [$Ru(bpy)_3(PF_6)_3$] was prepared by oxidizing $Ru(bpy)_3^{2+}$ with lead dioxide in aqueous H_2SO_4 followed by the addition of KPF_6.[33,34] Tris(2,2′-bipyridyl)iron(III) hexafluorophosphate [$Fe(bpy)_3(PF_6)_3$] was prepared by adding three equivalents of 2,2′-bipyridine to an aqueous solution of ferrous sulfate and then by oxidizing the iron(II) complex with lead dioxide in an aqueous H_2SO_4 solution, followed by the addition of KPF_6.[34] 10-Methylacridinium iodide ($AcrH^+I^-$) and 1-methylquinolinium iodide (QuH^+I^-) were prepared by the reactions of acridine and quinoline with methyl iodide in acetone,[35] the resulting iodide salts were converted to the perchlorate salts ($AcrH^+ClO_4^-$ and $QuH^+ClO_4^-$) by addition of $Mg(ClO_4)_2$ to the iodide salt, and purified by recrystallization from methanol.[36] Organic photosensitizers (9,10-dicyanoanthracene, naphthalene, triphenylene, and pyrene) were obtained commercially from Nacalai Tesque, Japan and purified by the standard method.[37] 5 mM Tris/HCl buffer (pH 7.0, 7.5 and 8.0), 5 mM potassium hydrogen phthalate [$C_6H_4(COOK)(COOH)$]/NaOH buffer (pH 5.8 and 5.0), 5 mM [$C_6H_4(COOK)(COOH)$]/HCl buffer (pH 4.0 and 3.0), and 5 mM KCl/HCl buffer (pH 2.0) were used for measurements of pH dependence of electron-transfer rate constants. The pH of the solution was determined by a pH meter (TOA; HM-20J) equipped with a glass electrode (TOA; GST-5725C). Tris(hydroxymethyl)aminomethane was obtained from Nacalai Tesque, Japan. Water was purified (18.3 MΩ cm) with a Milli-Q system (Millipore; Milli-Q Jr.). Acetonitrile was purified and dried with calcium hydride by the standard procedure, and stored under nitrogen atmosphere.[37]

Spectroscopic Measurements. Changes in the UV−vis spectra in electron-transfer reactions were monitored using a Hewlett-Packard 8453 diode array spectrophotometer. The reaction of GMP with $Fe(bpy)_3^{3+}$ was examined by measuring the change in the UV−vis spectra of $Fe(bpy)_3^{2+}$ ($\epsilon = 8400\ M^{-1}\ cm^{-1}$ at 520 nm)[38] in the presence of various concentrations of GMP (0 to 8.3×10^{-5} M). The reaction of GMP with $Ru(bpy)_3^{3+}$ was examined by measuring the change in the UV−vis spectra of $Ru(bpy)_3^{2+}$ ($\epsilon = 14600\ M^{-1}\ cm^{-1}$ at 452 nm)[38] in the presence of various concentrations of GMP (0 to 8.0×10^{-5} M).

Kinetic Measurements. Kinetic measurements were performed using a UNISOKU RSP-601 stopped-flow rapid scan spectrophotometer equipped with a MOS-type high sensitive photodiode array and a Hewlett-Packard 8453 photodiode array spectrophotometer for fast reactions with half-lives shorter than 10 s and for the slower reactions with half-lives much longer than 10 s, respectively. Rates of electron-transfer reactions from DNA bases and DNA oligomers to $Ru(bpy)_3^{3+}$ and $Fe(bpy)_3^{3+}$ in a buffer solution were followed by an increase in absorbance at $\lambda_{max} = 452$ nm due to $Ru(bpy)_3^{2+}$ and at $\lambda_{max} = 520$ nm due to $Fe(bpy)_3^{2+}$, respectively. All the kinetic measurements were carried out under pseudo-first-order conditions by using more than 10-fold excess nucleotide in a deaerated buffer solution at 298 K. Pseudo-first-order rate constants were determined by least-squares curve fit using a microcomputer.

Fluorescence Quenching. Quenching experiments of the fluorescence of organic photosensitizers were carried out on a Shimadzu RF-5300 spectrofluorophotometer. The excitation wavelengths were 321, 358, 390, 311, 280, and 333 nm for 1-methylquinolinium ion (1.0×10^{-4} M), 10-methylacridinium ion (1.0×10^{-4} M), 9,10-dicyanoanthracene (5.0×10^{-5} M), naphthalene (1.0×10^{-4} M), triphenylene (1.0×10^{-4} M), and pyrene (1.0×10^{-4} M), respectively. The monitoring wavelengths were those corresponding to the maxima of the respective emission bands at 394, 453, 487, 460, 335, and 374 nm, respectively. The solutions of 5 mM Tris/HCl buffer (pH 7.0)/MeCN = 1:1 (v/v) were deoxygenated by argon purging for 10 min prior to the measurements. Relative emission intensities were measured for solution of each photosensitizer with a nucleotide quencher at various concentrations. There was no change in the shape but there was a change in the intensity of the fluorescence spectrum by the addition of a quencher. The Stern−Volmer relationship (eq 1) was obtained for the ratio of

$$I_0/I = 1 + K_{SV}[D] \tag{1}$$

the emission intensities in the absence and presence of an electron donor (I_0/I) and the concentrations of nucleotide donors used as quenchers [D]. In the case of fluorescence quenching of $AcrH^+$ by some quenchers, the Stern−Volmer plot showed a deviation from a linear correlation between I_0/I and [D] in the high concentrations of donors, which absorb light at the excitation wavelength. In such a case, the longer excitation wavelength (e.g., $\lambda = 486$ nm) was selected and the quenching constant was determined from the initial slope of the Stern−Volmer plot. Time-resolved fluorescence spectra were measured by a Photon Technology International GL-3300 with a Photon Technology International GL-302 and a nitrogen laser/pumped dye laser system equipped with a four-channel digital delay/pulse generator (Standard Research System Inc. DG535) and a motor driver (Photon Technology International MD-5020). The excitation wavelengths were 358 and 425 nm using *p*−terphenyl and POPOP (Wako Pure Chemical Ind. Ltd., Japan) as a laser dye, 311 and 284 nm for Rhodamine 650 and Rhodamine 590 (Exciton Co, Ltd.) with the output pulses were frequency doubled by GL-303 frequency doubler, and 337 nm from a nitrogen laser without laser dye. The fluorescence lifetimes τ were fitted by a single-exponential curve fit using a microcomputer. The observed quenching rate constants k_q ($=K_{SV}\tau^{-1}$) were obtained from the Stern−Volmer constants K_{SV} and the emission lifetimes τ.

Electrochemical Measurements. The cyclic voltammetry (CV) measurements were performed on a BAS 100B/W electrochemical analyzer in a deaerated aqueous solution containing 0.10 M Bu_4NClO_4 and 5 mM Na_2SO_4 as supporting electrolytes. A conventional three-electrode cell was used with a gold working electrode (surface area of 0.3 mm^2) and a platinum wire counter electrode. The gold working electrode

(BAS) was polished with BAS polishing alumina suspension and rinsed with acetone before use. The reference electrode was Ag/0.01 M $AgNO_3$ or Ag/0.01 M AgCl. The values (vs $Ag/AgNO_3$) and (vs Ag/AgCl) are converted to those versus SCE by adding 0.29 V and by subtracting 0.04 V, respectively.[39] The values vs SCE is converted to those versus NHE by subtracting 0.24 V, respectively.[39]

Photophysical Measurements. The measurements of transient absorption spectra in photoinduced electron transfer from DNA bases to pyrene were performed according to the following procedures. The degassed H_2O:MeCN = 7:3 (v/v) solution containing pyrene (5.0×10^{-4} M) and a DNA base (5.0×10^{-2} M) was excited by Nd:YAG laser (Continuum, SLII-10, 4−6 ns fwhm) at 355 nm. Time course of the transient absorption spectra were measured by using a continuous Xe-lamp (150 W) and an In GaAs-PIN photodiode (Hamamatsu 2949) as a probe light and a detector, respectively. The output from the photodiodes and a photomultiplier tube was recorded with a digitizing oscilloscope (Tektronix, TDS3032, 300 MHz). The transient spectra were recorded using fresh solutions in each laser excitation. All experiments were performed at 298 K.

Results and Discussion

Thermal Electron-Transfer Oxidation of DNA Bases. The electron-transfer oxidation properties of DNA bases are examined by thermal electron-transfer reactions with one-electron oxidants such as $Fe(bpy)_3^{3+}$ and $Ru(bpy)_3^{3+}$. The one-electron reduction potentials (E_{red}) of $Fe(bpy)_3^{3+}$ and $Ru(bpy)_3^{3+}$ in H_2O are determined by the cyclic voltammetry measurements as $E°(Fe^{3+}/Fe^{2+}) = 0.90$ V and $E°(Ru^{3+}/Ru^{2+}) = 1.18$ V (vs SCE), respectively (see Experimental Section). Among four DNA bases, only GMP can be oxidized by $Fe(bpy)_3^{3+}$. The spectral titration is shown in Figure 1, which indicates that two-electron oxidation of GMP occurs with two equivalents of $Fe(bpy)_3^{3+}$.

The rate of electron transfer from GMP to $Fe(bpy)_3^{3+}$ was determined from an increase in absorbance at 520 nm due to $Fe(bpy)_3^{2+}$. The rate obeys pseudo-first-order kinetics in the presence of large excess GMP (see Supporting Information S1). The pseudo-first-order rate constant increases linearly with increasing GMP concentration (S2). From the linear plot of the pseudo-first-order rate constant vs GMP concentration is determined the rate constant of electron transfer (k_{et}) as 3.0×10^5 M^{-1} s^{-1} at pH = 7.0. The pH dependence of k_{et} was examined, and the results are shown in Figure 2. The log k_{et} value of GMP increases with an increase in pH in the region pH > 4.0 to reach a constant value in the region, pH > 7.0. This indicates that the electron transfer from GMP to $Fe(bpy)_3^{3+}$ is endergonic and the uphill electron transfer is followed by the deprotonation of $GMP^{\bullet+}$, because the acid dissociation constant value of $GMP^{\bullet+}$ (pK_{r1}) is known to be 3.9.[40] In such a case, the overall rate constant of electron transfer increases with increasing the deprotonation rate as pH increases to reach a stage (pH > 7.0), where electron transfer becomes the rate-determining step, followed by the fast deprotonation of $GMP^{\bullet+}$, as shown in Figure 2.

When $Fe(bpy)_3^{3+}$ is replaced by a stronger one-electron oxidant, $Ru(bpy)_3^{3+}$, not only GMP but also AMP and TMP can be oxidized, but no electron-transfer oxidation of CMP takes place. The spectral titration confirms that two-electron oxidation of GMP, AMP and TMP occurs by $Ru(bpy)_3^{3+}$ (S3). The rates of electron transfer from GMP, AMP and TMP to $Ru(bpy)_3^{3+}$ in a deaerated aqueous buffer solution can be readily followed by an increase in the absorbance due to $Ru(bpy)_3^{2+}$. The rate of electron transfer also obeys pseudo-first-order kinetics with a large excess GMP. The pseudo-first-order rate constant k^1 is proportional to the GMP and AMP concentrations. The rate constants of a series of nucleotides in the electron-transfer reactions with $Fe(bpy)_3^{3+}$ at pH = 7.0 and $Ru(bpy)_3^{3+}$ at pH = 5.0 are listed in Table 1.

As the case of the electron-transfer oxidation of GMP with $Fe(bpy)_3^{3+}$, the log k_{et} value for the electron-transfer oxidation of GMP with $Ru(bpy)_3^{3+}$ increases with an increase in pH in

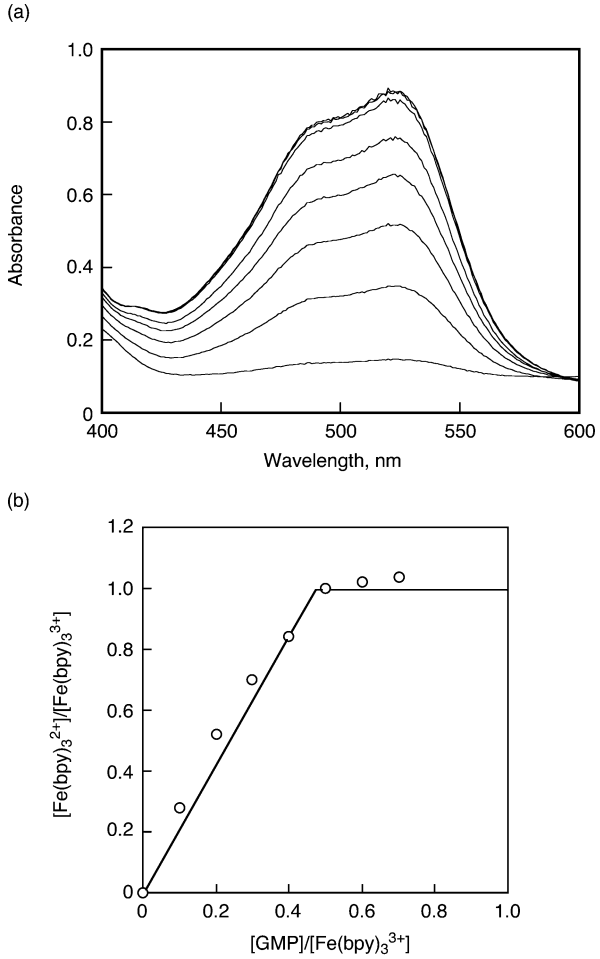

Figure 1. (a) Visible spectral changes observed upon addition of GMP (0 to 7.0×10^{-5} M) to a 5 mM Tris/HCl buffer (pH 7.0) solution of $Fe(bpy)_3^{3+}$ (1.0×10^{-4} M). (b) Plot of spectral titration for the electron-transfer oxidation of GMP (0 to 7.0×10^{-5} M) with $Fe(bpy)_3^{3+}$ (1.0×10^{-4} M) in 5 mM Tris/HCl buffer (pH 7.0) at 298 K.

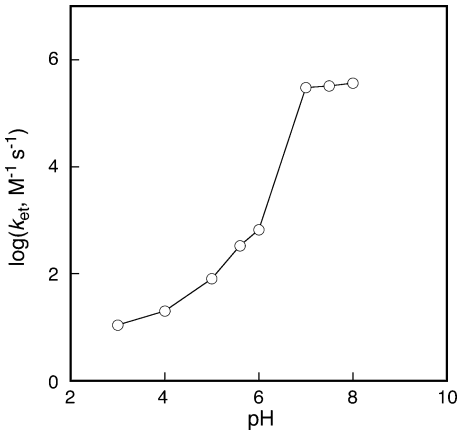

Figure 2. pH dependence of the observed second-order rate constant (k_{et}) for the electron-transfer oxidation of GMP with $Fe(bpy)_3^{3+}$ at 298 K.

TABLE 1: Rate Constants of Electron-Transfer Oxidation of DNA Bases with $Fe(bpy)_3^{3+}$ in 5 mM Tris/HCl Buffer (pH 7.0), and with $Ru(bpy)_3^{3+}$ in 5 mM $[C_6H_4(COOK)(COOH)]$/NaOH Buffer (pH 5.0) at 298 K

	k_{obs}, M^{-1} s^{-1}	
nucleotide	$Fe(bpy)_3^{3+}$	$Ru(bpy)_3^{3+}$
GMP	3.0×10^5	8.0×10^5
AMP	no reaction	2.4×10
TMP	no reaction	1.5
CMP	no reaction	no reaction

the region pH > 4.0 (S4). However, the instability of $Ru(bpy)_3^{3+}$ at pH > 5.0 has precluded the determination of k_{et} at higher pH. In contrast to the electron-transfer oxidation of GMP, there is no increase in the k_{et} value of AMP at pH > 4.0. Instead, the k_{et} value decreases with decreasing pH at pH < 4.0 (S4). Such a decrease in k_{et} at pH < 4.0 is ascribed to the protonation of AMP ($pK_{a1} = 3.7$).[41]

Photoinduced Electron-Transfer Reactions of DNA Bases. Because direct electrochemical measurements of DNA bases were complicated by the irreversible behavior upon the oxidation, we have examined the rates of photoinduced electron-transfer oxidation from which the fundamental one-electron oxidation properties can be deduced (vide infra).

The one-electron reduction potentials of singlet excited states of organic photosensitizers [E_{red}(excited)] were determined from one-electron reduction potentials at ground states [E_{red}(ground)] obtained by the cyclic voltammograms, and the singlet excitation energies obtained from eq 2, where transition energies of

$$E_{red}(\text{excited}) = E_{red}(\text{ground}) + (h\nu_{abs} + h\nu_{flu})/2 \qquad (2)$$

absorption ($h\nu_{abs}$) and fluorescence ($h\nu_{flu}$) were obtained by maxima of UV−vis and fluorescence spectra, respectively. The significant increase in the acceptor ability of electron acceptors by the electronic excitation is well-known to result in electron-transfer quenching of the singlet excited states of acceptors by the ground-state donors.[42] The emission quenching by electron transfer from an electron donor (D) to the excited-state acceptor (A*) has been formulated, as shown in eq 3, where k_{12} and k_{21}

$$D + A^* \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} (D\ A^*) \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} (D^{\bullet+}\ A^{\bullet-}) \xrightarrow{k_{30}} D + A \qquad (3)$$

are the diffusion and dissociation rate constants in the encounter complex (DA*), k_{23} and k_{32} are the rate constants of forward electron transfer from D to A* and the back electron transfer to the excited state, respectively, and k_{30} is the rate constant of back electron transfer to the ground state.[43] The overall rate constant (k_{et}) of the emission quenching by electron transfer is given by eq 4, which is reduced to eq 5 under the conditions

$$k_{et} = k_{12}k_{23}/[k_{23} + k_{21}(1 + k_{32}/k_{30})] \qquad (4)$$

$$k_{et} = k_{12}\,k_{23}/(k_{23} + k_{21}) \qquad (5)$$

that the back electron transfer to the ground state is much faster than that to the excited state, i.e., $k_{30} \gg k_{32}$.[44] From eq 5 is derived eq 6, where $\Delta G^{\ddagger}$ is the activation Gibbs energy of the

$$\Delta G^{\ddagger} = 2.3RT \log[Z(k_{et}^{-1} - k_{12}^{-1})] \qquad (6)$$

bimolecular electron-transfer process ($k_{12}k_{23}/k_{21}$), Z is the collision frequency that is taken as 1×10^{11} M^{-1} s^{-1}, the k_{12} value in H_2O is 8.0×10^9 M^{-1} s^{-1},[45] and the other notations are conventional. The dependence of $\Delta G^{\ddagger}$ on the Gibbs energy change of electron transfer (ΔG_{et}) has well been established, as given by the Gibbs energy relationship (eq 7), where $\Delta G^{\ddagger}_0$ is

$$\Delta G^{\ddagger} = (\Delta G_{et}/2) + [(\Delta G_{et}/2)^2 + (\Delta G^{\ddagger}_0)^2]^{1/2} \qquad (7)$$

the intrinsic barrier that represents the activation Gibbs energy when the driving force of electron transfer is zero, i.e., $\Delta G^{\ddagger} = \Delta G^{\ddagger}_0$ at $\Delta G_{et} = 0$.[43,46] On the other hand, the ΔG_{et} values are obtained from the one-electron oxidation potential of the donor (E_{ox}) and the one-electron reduction potential of the acceptor (E_{red}) by using eq 8, where F is the Faraday constant. From

$$\Delta G_{et} = F(E_{ox} - E_{red}) \qquad (8)$$

eqs 7 and 8 is derived a linear relation between $\Delta G^{\ddagger} + E_{red}$ and $(\Delta G^{\ddagger})^{-1}$ (eq 9).[46] The $\Delta G^{\ddagger}$ values are obtained from the quenching rate constants of photoinduced electron transfer to

$$(\Delta G^{\ddagger}/F) + E_{red} = E_{ox} + (\Delta G^{\ddagger}_0/F)^2/(\Delta G^{\ddagger}/F) \qquad (9)$$

acceptors (k_{et}) by using eq 6. We can choose appropriate acceptors whose E_{red} values are known or readily determined. Thus, the unknown values of E_{ox} and $\Delta G^{\ddagger}_0$ can be determined from the intercept and slope of the plots of $\Delta G^{\ddagger}/F + E_{red}$ vs $(\Delta G^{\ddagger})^{-1}$ by using eq 9, respectively.[46−49]

A number of rate constants (k_{et}) of photoinduced electron transfer from DNA bases to the singlet excited states of electron acceptors (QuH^+, $AcrH^+$, 9,10-dicyanoanthracene, naphthalene, triphenylene and pyrene) are determined by the emission quenching in 5 mM Tris/HCl buffer (pH 7.0) and 5 mM Tris buffer/MeCN = 1:1 (v/v) at 298 K (see Experimental Section). Typical Stern−Volmer plots (eq 1) are shown in the Supporting Information (S5, S6). The k_{et} values and the known E_{red} values of the excited states are listed in Table 2. The k_{et} values of nucleotides with the same electron acceptor increase in the order GMP > AMP > TMP > CMP.[50,51]

The pH dependence of the k_{et} value for photoinduced electron transfer from DNA bases to the singlet excited state of $AcrH^+$ was also examined by fluorescence quenching of $AcrH^+$. The pH dependence of k_{et} is shown in Figure 3, where the k_{et} values of GMP and TMP are rather constant irrespective of pH, because they are close to the diffusion-limited value. On the other hand, the k_{et} values of AMP and CMP decrease with decreasing pH in the region, pH < 4. Such a decrease in the k_{et} value may be ascribed to the protonation of AMP ($pK_{a1} = 3.7$)[41] and CMP ($pK_{a1} = 4.5$).[50]

Dependence of log k_{et} of thermal and photoinduced electron-transfer oxidation of GMP at pH 7.0 on the one-electron reduction potentials (E_{red}) of oxidants is shown in Figure 4, which exhibits typical feature of the electron-transfer process; the log k_{et} value increases with an increase in the E_{red} value to reach a plateau value corresponding to the diffusion rate constant.[52]

The unknown values of E_{ox} and $\Delta G^{\ddagger}_0$ of GMP are determined from the linear plot of $\Delta G^{\ddagger} + E_{red}$ vs $(\Delta G^{\ddagger})^{-1}$ by using eq 9. This plot is shown in Figure 5, where a linear correlation between $\Delta G^{\ddagger} + E_{red}$ and $(\Delta G^{\ddagger})^{-1}$ is obtained. The E_{ox} and $\Delta G^{\ddagger}_0$ values of GMP are obtained as 1.07 V ± 0.02 vs SCE and 23.4 ± 0.4 kJ mol^{-1} from the intercept and slope by the least-squares analysis. The E_{ox} and $\Delta G^{\ddagger}_0$ values of the other DNA bases are also determined from the intercepts and slopes of linear plots between $\Delta G^{\ddagger} + E_{red}$ and $(\Delta G^{\ddagger})^{-1}$ (S7) and the values are listed in Table 3. The E_{ox} value of GMP thus determined at pH 7.0 (1.07 V ± 0.02 vs SCE) agrees with the reported value determined by pulse radiolysis (1.29 V ± 0.03 vs NHE, which

TABLE 2: Rate Constants for the Photoinduced Electron Transfer from Nucleotide to Organic Photosensitizer at 298 K

	k_{et}, M^{-1} s^{-1}					
nucleotide	QuH^+ [a] (2.54 V)	$AcrH^+$ [a] (2.43 V)	DCA[a] (1.95 V)	naphthalene[b] (1.48 V)	triphenylene[b] (1.43 V)	pyrene[b] (1.17 V)
GMP	8.4×10^9	1.0×10^{10}	3.9×10^9 (3.4×10^9)[c]	1.3×10^9	3.8×10^8	6.3×10^7 (5.7×10^7)[c]
AMP	6.6×10^9	6.8×10^9	2.8×10^9 (1.6×10^9)[c]	4.7×10^8	8.0×10^7	1.9×10^7 (2.2×10^7)[c]
TMP	5.1×10^9	5.0×10^9	1.6×10^9 (1.1×10^9)[c]	8.0×10^8	1.3×10^8	$<1 \times 10^7$ [d]
CMP	4.2×10^9	4.5×10^9	7.0×10^8 (5.0×10^8)[c]	3.5×10^8	7.6×10^7	$<1 \times 10^7$ [d]

[a] In Tris/HCl buffer solution (pH 7.0). [b] In Tris/HCl buffer (pH 7.0): MeCN = 1:1 (v/v) solution. [c] Determined by fluorescence decay. [d] Too slow to be determined accurately.

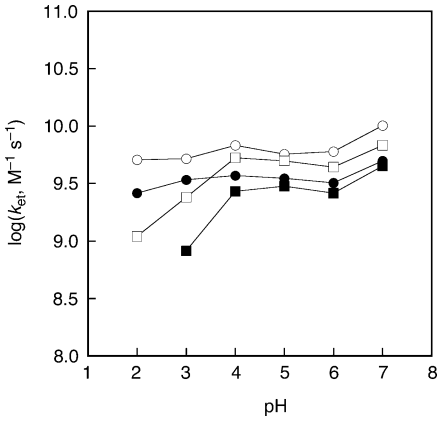

Figure 3. pH dependence of the rate constants (k_{et}) of photoinduced electron transfer from GMP (open circle), AMP (open square), TMP (closed circle) and CMP (closed square) to $AcrH^+$ in 5 mM Tris/HCl buffer (pH 7.0) at 298 K.

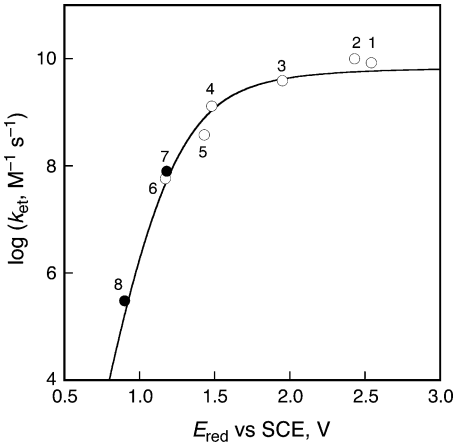

Figure 4. Plot of logarithm of the rate constants (log k_{et}) of thermal and photoinduced electron-transfer oxidation of GMP with the singlet excited state of organic sensitizers (open circles: 1, 3-$CNQuH^+$; 2, $AcrH^+$; 3, 9,10-dicyanoanthracene; 4, naphthalene; 5, triphenylene; 6, pyrene) and one-electron oxidants (closed circles: 7, $Ru(bpy)_3^{3+}$; 8, $Fe(bpy)_3^{3+}$) vs one-electron reduction potentials (E_{red}) of the singlet excited state of organic sensitizers and one-electron oxidants.

corresponds to 1.05 V ± 0.03 vs SCE).[28] The pH dependence of the E_{ox} value of GMP can be evaluated using eq 10,[28]

$$E_{pH} = E^\circ + 0.059 \times \log\left\{\frac{K_{a1}K_{a2}K_{a3} + K_{a1}K_{a2}10^{-pH} + K_{a1}10^{-2pH} + 10^{-3pH}}{K_{r1}K_{r2} + K_{r1}10^{-pH} + 10^{-2pH}}\right\} \quad (10)$$

where E° is the standard oxidation potential of GMP, $pK_{a1} = 1.9$, $pK_{a2} = 9.25$, $pK_{a3} = 12.33$ for GMP and $pK_{r1} = 3.9$, $pK_{r2} = 10.9$ for the corresponding radicals.[10a,40]

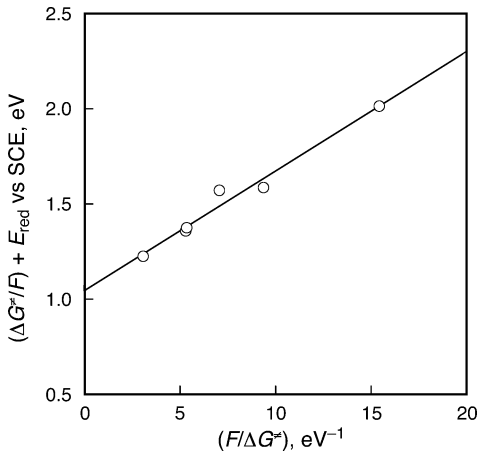

Figure 5. Plots of $(\Delta G^{\ddagger}/F) + E_{red}$ vs $(F/\Delta G^{\ddagger})$ for the thermal and photoinduced electron-transfer reaction of GMP.

TABLE 3: One-Electron Oxidation Potentials (E_{ox}) of DNA Bases and Intrinsic Barrier ($\Delta G^{\ddagger}_0$) of the Electron-Transfer Oxidation in Tris/HCl Buffer (pH 7.0)

DNA base	E_{ox} vs SCE, V	$\Delta G^{\ddagger}_0$, kJ mol
GMP	1.07 ± 0.02	23.4 ± 0.4
AMP	1.18 ± 0.02	23.8 ± 0.3
TMP	1.21 ± 0.03	25.5 ± 0.6
CMP	1.26 ± 0.02	26.3 ± 0.5

Although the E_{ox} value of GMP at pH 7.0 is the lowest among the four DNA bases (Table 3), the difference in the E_{ox} values between GMP and the other DNA bases is not so large as generally believed: the highest E_{ox} value (CMP) is only 0.19 V higher than that of GMP.[25−28,53] Thus, the selective oxidation of GMP in the thermal electron-transfer oxidation of GMP bases with $Fe(bpy)_3^{3+}$ at pH 7.0 (Figure 2) comes from a significant decrease in the pH dependent oxidation potential due to the deprotonation of $GMP^{\bullet+}$ ($pK_{r1} = 3.9$).[10a,40] The validity of E_{ox} values of DNA bases determined herein is confirmed by the laser flash photolysis experiments (vide infra).

Laser Flash Photolysis. The occurrence of photoinduced electron-transfer reactions of DNA bases is confirmed by the laser flash photolysis experiments (see Experimental Section). In the case of photoinduced electron transfer from DNA bases to the singlet excited state of $AcrH^+$ ($^1AcrH^{+}*$), the electron transfer from all types of DNA bases is highly exergonic, because the E_{red} value of $^1AcrH^{+}*$ (2.32 V vs SCE)[54] is much more positive than the E_{ox} values of DNA bases (Table 3). Laser excitation (355 nm from Nd: YAG laser) of an aqueous solution of $AcrH^+$ (5.0×10^{-5} M) and DNA bases (3.0×10^{-3} M) at pH 7.0 affords a transient absorption spectrum at 1 μs with

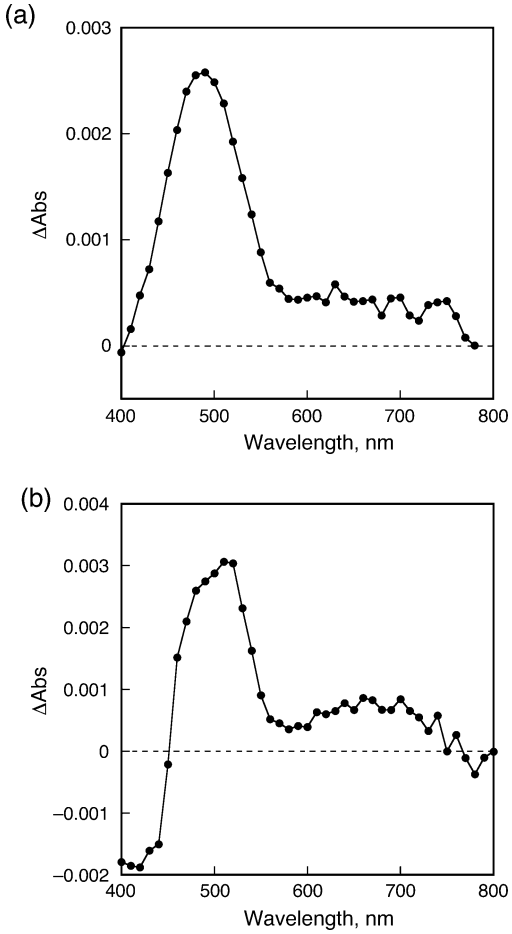

Figure 6. Transient absorption spectra observed in photoinduced electron transfer from (a) GMP (1.0×10^{-2} M) and (b) CMP (1.0×10^{-2} M) to the singlet excited state of $AcrH^+$ (5.0×10^{-5} M) in 5 mM Tris/HCl buffer (pH 7.0) at 298 K observed at 80 μs after irradiation of laser pulse at $\lambda = 355$ nm with 10 mJ/pulse.

appearance of a new absorption band at 500 nm due to $AcrH^{\bullet}$,[47b,54] as shown in Figure 6 ((a) and (b) correspond to the results of GMP and CMP, respectively). This indicates that photoinduced electron transfer from DNA bases to $^1AcrH^{+}*$ occurs to produce $AcrH^{\bullet}$ and the one-electron oxidized species of all types of DNA bases.[55] In the case of GMP (Figure 6a), the broad absorption at 500−700 nm is ascribed to the neutral oxidized radical ($GMP(-H)^{\bullet}$) produced by deprotonation of $GMP^{\bullet+}$ at pH 7.0.[40,56,57] In the case of CMP, the absorption band due to $CMP^{\bullet+}$ appears at ca. 670 nm together with the absorption at 500 nm due to $AcrH^{\bullet}$ (Figure 6b). The absorption at 500 nm due to $AcrH^{\bullet}$ decays, obeying second-order kinetics (S8). The second-order decay rate constant is determined as 7.9×10^9 M^{-1} s^{-1}, which is diffusion-limited. This indicates that $AcrH^{\bullet}$ decays via back electron transfer from $AcrH^{\bullet}$ to DNA base radical cations.

Judging from the E_{ox} values of DNA bases in Table 3, photoinduced electron transfer from GMP to the singlet excited state of pyrene is slightly exergonic ($\Delta G_{et} = -0.10$ eV), whereas the photoinduced electron-transfer reactions from other DNA bases are endergonic. Thus, the products of the photoinduced electron transfer would only be observed in the case of GMP. This is confirmed by the laser-flash photolysis experiments (vide infra). Laser excitation (355 nm from Nd: YAG laser) of pyrene (5.0×10^{-4} M) affords a transient absorption spectrum at 3 μs with appearance of a new absorption band 420 nm due to the triplet excited state of pyrene (^{3}pyrene*) with bleaching of pyrene at 340 nm (S9).[58] Using GMP as a quencher, the photoinduced electron transfer occurred efficiently and the absorption band at 420 nm due to ^{3}pyrene* disappeared and new absorption band at 490 nm due to $pyrene^{\bullet-}$ appeared together with the broad absorption at 500−700 nm due to the neutral oxidized radical ($GMP(-H)^{\bullet}$) produced by deprotonation of $GMP^{\bullet+}$ at pH 7.0, as shown Figure 7a.[59−62] In the case of the other nucleotides, photoinduced electron transfer does not occur and only the absorption band at 420 nm due to ^{3}pyrene* was detected, as shown in Figure 7b−d. These results indicate that only GMP has a lower E_{ox} value than the E_{red} value of the singlet excited state of pyrene ($E_{red} = 1.17$ V) and the other DNA bases have higher E_{ox} values, demonstrating the validity of the E_{ox} values of DNA bases in Table 3. No transient absorption spectra are observed in the photoinduced electron-transfer oxidation of nucleotides (1.0×10^{-2} M) by the singlet excited state of 9,10-dicyanoanthracene (DCA) at pH 7.0, because back electron transfer from radical cation of nucleotides to $DCA^{\bullet-}$ is too fast to be observed by the nanosecond laser flash measurements.[63]

Electron-Transfer Reactivity of DNA Oligomers Containing Guanine vs Guanine Monomer. The electron-transfer oxidation properties of DNA oligomers containing guanines in sequence 1, 2, 3, 4, and 10 were examined using one-electron oxidants such as $Fe(bpy)_3^{3+}$, the one-electron oxidation potential of which is 0.90 V (vs SCE). The complete sequences of the DNA oligomers are listed in Table 4. In the electron-transfer oxidation of DNA oligomers, the amount of $Fe(bpy)_3^{2+}$ produced in the electron transfer was determined from an increase in absorbance at $\lambda = 520$ nm, which increased with increasing the amount of guanine in DNA oligomer (S10). The stoichiometry of electron-transfer oxidation of DNA oligomers with $Fe(bpy)_3^{3+}$ indicates that only guanine moiety can be oxidized and that two equivalents of $Fe(bpy)_3^{3+}$ are consumed to oxidize one guanine moiety (Figure 8a). Judging from the E_{ox} values of DNA bases (1.07−1.26 V vs SCE) and the E_{red} value of $Fe(bpy)_3^{3+}$ (0.90 V vs SCE), the electron transfer from all DNA bases to $Fe(bpy)_3^{3+}$ is endergonic ($\Delta G_{et} > 0$). The facile deprotonation of the guanine radical cation makes it possible that only the guanine moiety is oxidized with $Fe(bpy)_3^{3+}$. Thus, the selective oxidation of the guanine moiety is ascribed to the facile deprotonation of the guanine radical cation rather than to the lowest E_{ox} value of guanine.

The rates of electron-transfer oxidation of DNA oligomers with $Fe(bpy)_3^{3+}$ were determined from an increase in absorbance at 520 nm due to $Fe(bpy)_3^{2+}$. The rate obeys pseudo-first-order kinetics in the presence of large excess guanines in DNA oligomers as shown in Figure 8b. The pseudo-first-order rate constant k^1 is proportional to the guanine concentrations (S11). The second-order rate constant of electron transfer (k_{obs}) is obtained from the slope of plot of k^1 vs the guanine concentration. The k_{obs} values are the rates per guanine residue. The rate constants (k_{obs}) of a DNA oligomer of AGT sequence in the electron-transfer oxidation with $Fe(bpy)_3^{3+}$ at pH 7.0 is determined as 5.6×10^3 M^{-1} s^{-1}.

When $Fe(bpy)_3^{3+}$ is replaced by a stronger oxidant, $Ru(bpy)_3^{3+}$ ($E_{red} = 1.18$ V vs SCE), not only guanine but also adenine can be oxidized with the reduction of $Ru(bpy)_3^{3+}$ to $Ru(bpy)_3^{2+}$, which has absorbance at $\lambda = 450$ nm (vide supra). The rate of electron transfer from DNA oligomers to $Ru(bpy)_3^{3+}$ was determined from an increase in absorbance at 450 nm under acidic conditions (pH 2.0) because of the instability of $Ru(bpy)_3^{3+}$ under neutral conditions. The second-order rate constant (k_{obs}) of the electron-transfer oxidation of AGT with $Ru(bpy)_3^{3+}$

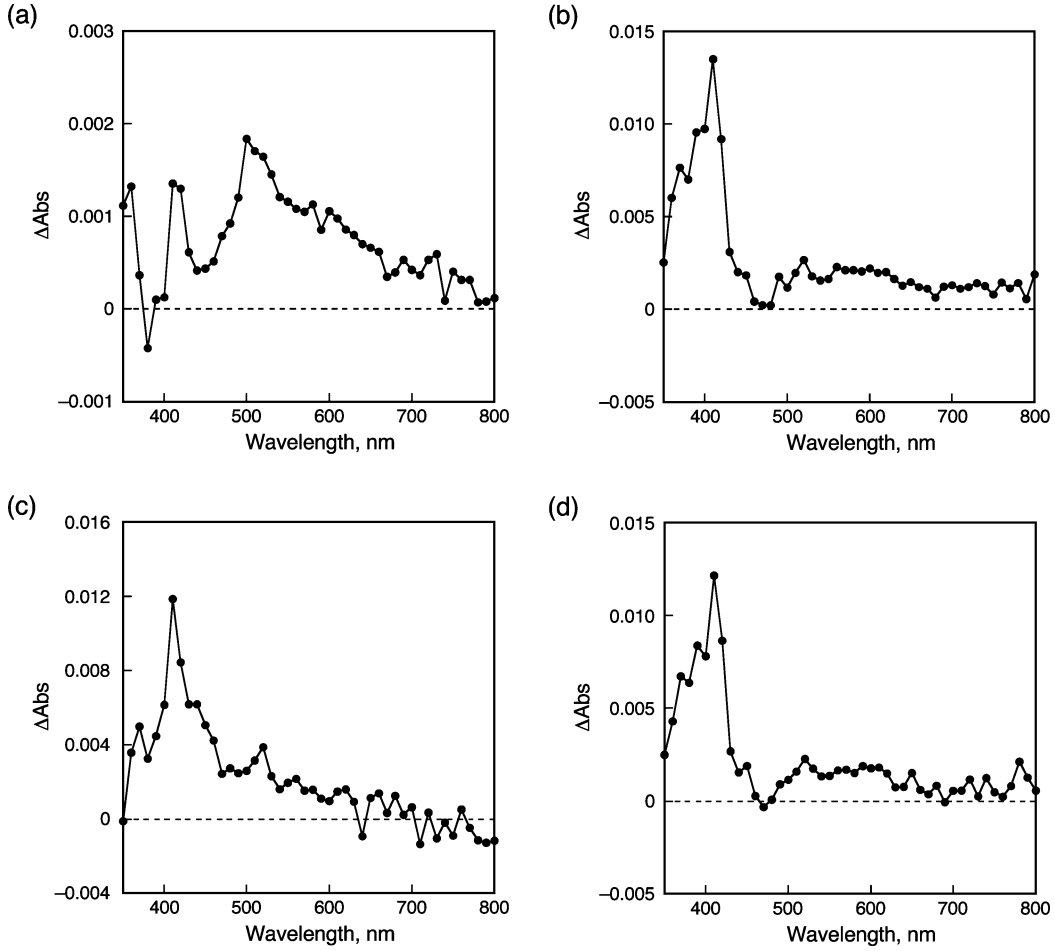

Figure 7. Transient absorption spectra observed upon laser excitation of deaerated MeCN:H_2O (3:7 v/v) solution of (a) GMP (5.0×10^{-2} M), (b) AMP (5.0×10^{-2} M), (c) TMP (5.0×10^{-2} M) and (d) CMP (5.0×10^{-2} M) in the presence of pyrene (5.0×10^{-4} M) at 298 K at 3.0 μs after irradiation of laser pulse at $\lambda = 355$ nm with 1.0 mJ/pulse.

TABLE 4: DNA Base Sequence of DNA Oligomers

oligomer	sequence (5′ → 3′)
AGT	5′–AGT–AGT–AGT–AGT–3′
AG(2)T	5′–AGGT–AGGT–AGGT–3′
AG(3)T	5′–AGGGT–AGGGT–AGGGT–3′
AG(4)T	5′–AGGGGT–AGGGGT–3′
AG(10)T	5′–AGGGGGGGGGGT–3′
AT	5′–AT–AT–AT–AT–AT–AT–3′
CG	5′–CG–CG–CG–CG–CG–CG–3′
sS–G(1)	5′–AGT–AAG–AGT–ATGT–3′
dS–G(1)	5′–AGT–AAG–AGT–ATGT–3′ 3′–TCA–TTC–TCA–TACA–5′
sS–G(3)	5′–AGGGT–AAGGGT–TGGGT–3′
dS–G(3)	5′–AGGGT–AAGGGA–TGGGT–3′ 3′–TCCCA–TTCCCT–ACCCA–5′

at pH 2.0 is determined as 4.7×10^3 M^{-1} s^{-1}, which is smaller than the k_{obs} value with $Fe(bpy)_3^{3+}$ (5.6×10^3 M^{-1} s^{-1}) at pH 7.0. The k_{obs} value is expected to increase with increasing pH in the region pH > 3.9, which is the pK_{r1} value of guanine radical cation.[40] The k_{obs} value of a DNA oligomer of AGT sequence at pH 2.0 (4.7×10^3 M^{-1} s^{-1}) is larger than the k_{obs} value of a guanine monomer (GMP: 3.9×10^3 M^{-1} s^{-1}) at pH 2.0. This indicates that the guanine reactivity in the electron-transfer oxidation is also enhanced in the DNA oligomer as compared with the monomer under the acidic conditions (vide supra).

Effects of Number of Contiguous Guanines on the Electron-Transfer Reactivity. The effects of adjacent nucleotides of guanine in DNA oligomers on the electron-transfer oxidation with $Ru(bpy)_3^{3+}$ at pH 2.0 are examined and the results are shown in Figure 9. Plots of the pseudo-first-order rate constants vs concentrations of guanine moiety afford virtually the same slope between DNA oligomers of AGT sequence and CG sequence. This indicates that the electron-transfer reactivity of guanine moiety in DNA oligomers is not affected by adjacent nucleotides, adenine, thymine or cytosine.

In contrast to the results in Figure 9, the electron-transfer reactivity of guanine moiety in DNA oligomers is significantly affected by the number of adjacent guanine (vide infra). The k_{obs} values of electron-transfer oxidation of DNA oligomers with $Fe(bpy)_3^{3+}$ and $Ru(bpy)_3^{3+}$ were determined using DNA oligomers containing different guanine (G) sequences: from 1 to 10 G. In the case of the oxidation by $Ru(bpy)_3^{3+}$ at pH 2.0, the k_{obs} value increases in the order AGT < AG(2)T < AG(3)T < AG(4)T = AG(10)T, as shown in Figure 10. Under such acidic conditions, the difference in the k_{obs} value is ascribed to the difference in the electron donor ability of guanine depending

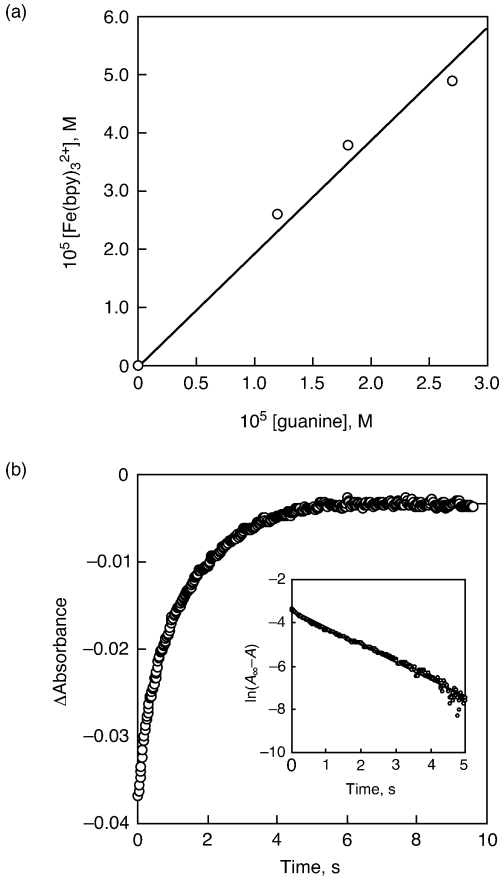

Figure 8. (a) Plot of the spectral titration for the electron-transfer oxidation of a DNA oligomer (AGT) with $Fe(bpy)_3^{3+}$ (1.0×10^{-4} M) in 5 mM Tris/HCl buffer (pH 7.0) at 298 K. (b) Time course of absorbance change at $\lambda = 520$ nm due to $Fe(bpy)_3^{2+}$ in the electron-transfer oxidation of AGT (6.0×10^{-6} M) with $Fe(bpy)_3^{3+}$ (2.0×10^{-5} M) in 5 mM Tris/HCl buffer (pH 7.0) at 298 K. Inset: First-order plot based on the absorption change at $\lambda = 520$ nm.

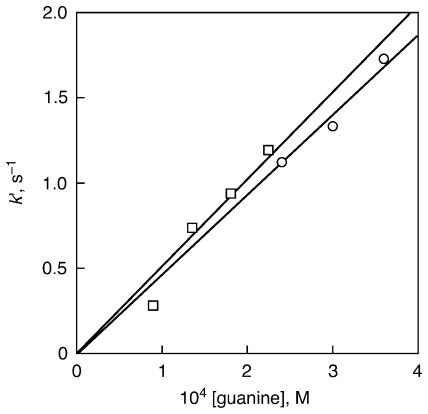

Figure 9. Plots of pseudo-first-order rate constant (k^1) vs concentration of guanine for the electron-transfer oxidation of AGT (open circle) and CG (open square) with $Ru(bpy)_3^{3+}$ in 5 mM KCl/HCl buffer (pH 2.0) at 298 K.

on the number of guanine sequence. The electron donor ability of guanine increases with increasing the number of guanine sequence to reach a constant value when the sequence number is larger than 4.

When the electron-transfer oxidation of DNA oligomers with different sequential numbers of guanine is carried out using $Fe(bpy)_3^{3+}$ as a one-electron oxidant under neutral conditions at pH 7.0, the results are quite different from those in the case of $Ru(bpy)_3^{3+}$ at pH 2.0 as shown in Figure 11. The k_{obs} value

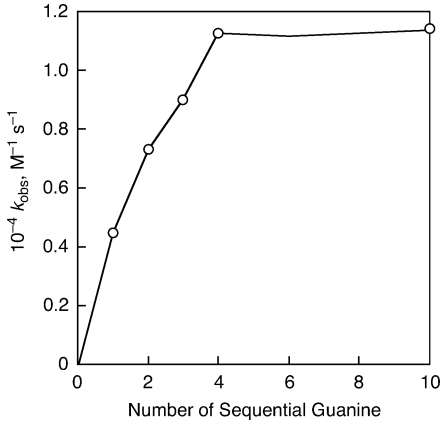

Figure 10. Sequence dependence of the observed rate constant (k_{obs}) of the electron-transfer oxidation of various DNA oligomers [AGT, AG(2)T, AG(3)T, AG(4)T and AG(10)T] with $Ru(bpy)_3^{3+}$ in 5 mM KCl/HCl buffer (pH 2.0) solution at 298 K.

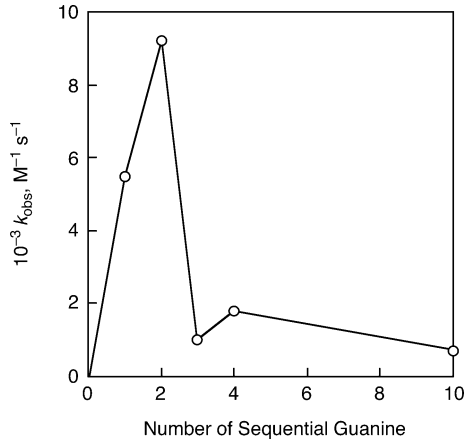

Figure 11. Sequence dependence of the observed rate constant (k_{obs}) of the electron-transfer oxidation of various DNA oligomers [AGT, AG(2)T, AG(3)T, AG(4)T and AG(10)T] with $Fe(bpy)_3^{3+}$ in 5 mM Tris/HCl buffer (pH 7.0) solution at 298 K.

increases from AGT to AG(2)T but decreases significantly with longer guanine sequences.

Electron-Transfer Reactivity of Single-Stranded DNA Oligomers vs Double-Stranded DNA Oligomers. The electron-transfer reactivity of guanine in single-stranded (sS) DNA oligomers is compared with the reactivity in the corresponding double-stranded (dS) DNA oligomer. As shown in Figure 12a, the rate constants of electron-transfer oxidation of sS-G(1) and dS-G(1) are virtually the same.[64] Thus, the electron-transfer reactivity of guanine in the single-stranded DNA oligomers is not affected by formation of the double strand. In contrast, the electron-transfer rate of dS-G(3) is 8 times faster than that of sS-G(3) as shown in Figure 12b.

Conclusions

The one-electron oxidation potentials (E_{ox}) and the intrinsic barriers of electron transfer ($\Delta G^{\ddagger}_0$) of DNA bases have been determined from the detailed kinetic investigations on both photoinduced and thermal electron-transfer oxidation of DNA bases. The E_{ox} value of GMP thus determined at pH 7.0 (1.07 V vs SCE) is the lowest among DNA bases. However, the difference in the E_{ox} values between GMP and the other DNA bases is too small to account for the selective oxidation of GMP with $Fe(bpy)_3^{3+}$. The facile deprotonation from $GMP^{\bullet+}$, followed by the electron-transfer oxidation of the deprotonated radical may be the most essential origin for the selective

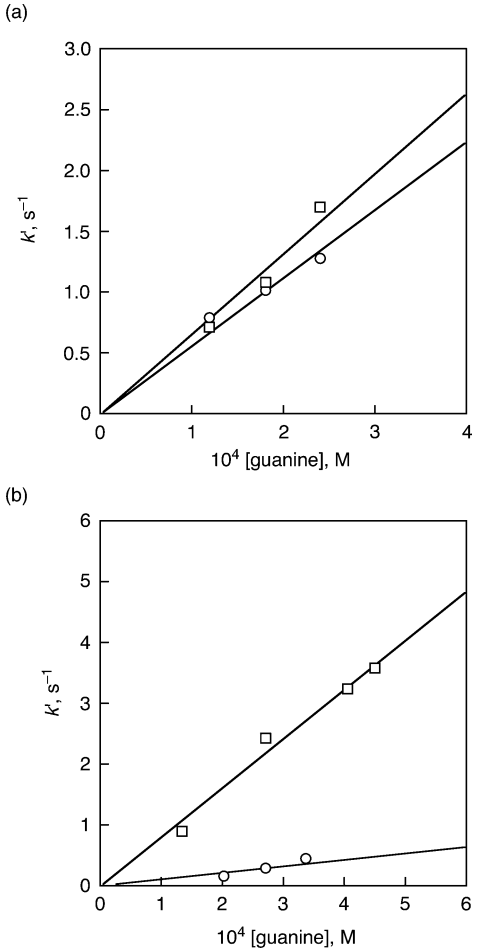

Figure 12. (a) Plot of pseudo-first-order rate constant (k^1) vs concentration of guanine for the electron transfer-oxidation of sS-G(1) (open circle) and dS-G(1) (open square) with $Fe(bpy)_3^{3+}$ in 5 mM Tris/HCl buffer (pH 7.0) at 298 K. (b) Plot of k^1 vs [guanine] for the electron-transfer oxidation of sS-G(3) (open circle) and dS-G(3) (open square) with $Fe(bpy)_3^{3+}$ in 5 mM Tris/HCl buffer (pH 7.0) at 298 K.

oxidation of GMP among DNA bases. The guanine moiety is also selectively oxidized in the electron-transfer reaction of DNA oligomers with $Fe(bpy)_3^{3+}$ via the facile deprotonation from $GMP^{\bullet+}$ following electron transfer. The rate of electron-transfer oxidation of guanine is slightly enhanced in DNA oligomers as compared with that of monomer guanine (GMP). The electron-transfer rate varies depending on the number of guanine sequences of in single- stranded and double-stranded DNA oligomers and on pH.

Acknowledgment. This work was partially supported by a Grant-in-Aid for Scientific Research (No. 16205020) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: Figures presenting spectroscopic and kinetic data (S1−S11). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Sies, H. *Oxidative Stress: Oxidants and Antioxidants*; Academic Press: London, 1991. (b) Poulsen, H. E.; Prieme, H.; Loft, S. *Eur. J. Cancer Prev.* **1998**, *7*, 9. (c) Boiteux, S.; Gellon, L.; Guibourt, N. *Free Radical Biol. Med.* **2002**, *15*, 1244.

(2) (a) Von Sonntag, C.; Schuchmann, H. P. *Int. J. Radiat. Biol.* **1986**, *49*, 1. (b) Mroczka, N.; Bernhard, W. A. *Radiat. Res.* **1993**, *135*, 155. (c) Wang, W.; Becker, D.; Sevilla, M. D. *Radiat. Res.* **1993**, *135*, 146. (d) Becker, D.; Sevilla, M. D. *Adv. Radiat. Biol.* **1993**, *17*, 121.

(3) (a) Boon, P. J.; Cullis, P. M.; Symons, M. C. R.; Wren, B. W. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1393. (b) Wolf, P.; Jones, G. D. D.; Candeias, L. P.; O'Neill, P. *Int. J. Radiat. Biol.* **1993**, *64*, 7.

(4) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109.

(5) (a) Armitage, B. *Chem. Rev.* **1998**, *98*, 1171. (b) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723.

(6) (a) Cadet, J.; Virny, P. *Bioorganic Photochemistry, Photochemistry and the Nucleic Acid*; Mirrison, H., Ed.; John Wiley and Sons: New York, 1990; Vol. 1, pp 1−272. (b) Dizdaroglu, M. *Free Radical Biol. Med.* **1991**, *10*, 225. (c) Melvin, T.; Plumb, M. A.; Botchway, S. W.; O'Neill, P.; Parker, A. W. *Photochem. Photobiol.* **1995**, *61*, 584−591.

(7) (a) Lewis, F. D. In *Electron Transfer in Chemistry*; Balzani, V., Ed.; Wiley-VCH: Weinheim, Germany, 2001; Vol. 3, pp 105−175. (b) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159. (c) Lewis, F. D.; Liu, X.; Liu, J.; Miller, S. E.; Hayes, R. T.; Wasielewski, M. R. *Nature* **2000**, *406*, 51.

(8) (a) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631. (b) Schuster, G. B. *Acc. Chem. Res.* **2000**, *33*, 253. (c) Schuster, G. B., Ed. *Long-Range Charge Transfer in DNA I, Topics in Current Chemistry*; Springer-Verlag: Berlin, 2004; Vol. 236.

(9) (a) Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746. (b) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295. (c) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.

(10) (a) Steenken, S. *Chem. Rev.* **1989**, *89*, 503. (b) Cadet, J.; Vigny, P. *Bioorganic Photochemistry: Photochemistry and the Nucleic Acids*; John Wiley and Sons: New York, 1990; Vol. 1.

(11) (a) Johnston, D. H.; Glasgow, K. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 8933. (b) Gasper, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762.

(12) Sugden, K. D.; Wetterhahn, K. E. *J. Am. Chem. Soc.* **1996**, *118*, 10811.

(13) (a) Sanii, L.; Schuster, G. B. *J. Am. Chem. Soc.* **2000**, *122*, 11545. (b) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 8747. (c) Breslin, D. T.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 2311. (d) Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845.

(14) (a) Kalsbeck, W. A.; Gingell, D. M.; Malinsky, J. E.; Thorp, H. H. *Inorg. Chem.* **1994**, *33*, 3313. (b) Croke, D. T.; Perrouault, L.; Sari, M. A.; Battionti, J. P.; Mansuy, D.; Helene, C.; Doan, T. L. *J. Photochem. Photobiol., B: Biol.* **1993**, *18*, 41. (c) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303. (d) Dunn, D. A.; Lin, V. H.; Kochevar, I. E. *Biochemistry* **1992**, *31*, 11620. (e) Nielsen, P. E.; Hiort, C.; Sonnichsen, S. H.; Buchardt, O.; Dahl, O.; Norden, B. *J. Am. Chem. Soc.* **1992**, *114*, 4967. (f) Nielsen, P. E.; Jeppesen, C.; Egholm, M.; Buchardt, O. *Biochemistry* **1988**, *27*, 6338.

(15) (a) Yoshioka, Y.; Kitagawa, Y.; Takano, Y.; Yamaguchi, K.; Nakamura, T.; Saito, I. *J. Am. Chem. Soc.* **1999**, *121*, 8712. (b) Kino, K.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 7373. (c) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406. (d) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, I.; Saito, I. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1351. (e) Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1993**, *268*, 13221.

(16) (a) Floyd, R. A.; West, M. S.; Eneff, K. L.; Schneider, J. E. *Arch. Biochem. Biophys.* **1989**, *273*, 106. (b) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283.

(17) Cullis, P. M.; Malone, M. E.; Merson-Davies, L. A. *J. Am. Chem. Soc.* **1996**, *118*, 2775.

(18) Nunez, M. E.; Hall, D. B.; Barton, J. K. *Chem. Biol.* **1999**, *6*, 85.

(19) (a) Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Helene, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2462. (b) An, Y.-Z.; Chen, C.-H. B.; Anderson, J. L.; Sigman, D. S.; Foote, C. S.; Rubin, Y. *Tetrahedron* **1996**, *52*, 5179. (c) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918. (d) Yamakoshi, Y. N.; Yagami, T.; Sueyoshi, S.; Miyata, N. *J. Org. Chem.* **1996**, *61*, 7236.

(20) Kim, N. S.; LeBreton, P. R. *J. Am. Chem. Soc.* **1996**, *118*, 3694.

(21) (a) Pyle, A. M.; Barton, J. K. *Prog. Inorg. Chem.: Bioinorg. Chem.* **1990**, *38*, 413. (b) Choi, S.; Cooley, R. B.; Hakemian, A. S.; Larrabee, Y. C.; Bunt, R. C.; Maupas, S. D.; Muller, J. G.; Burrows, C. *J. Am. Chem. Soc.* **2004**, *126*, 591.

(22) (a) Popovich, N.; Thorp, H. H. *Electrochem. Interface* **2002**, *11*, 30. (b) Gore, M. R.; Szalai, V. A.; Ropp, P. A.; Yang, I. V.; Silverman, J. S.; Thorp, H. H. *Anal. Chem.* **2003**, *75*, 6586. (c) Park, S. J.; Taton, T. A.; Mirkin, C. A. *Science* **2002**, *295*, 1503.

(23) (a) Dohno, C.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **2003**, *125*, 9586. (b) Reid, G. D.; Whittaker, D. J.; Day, M. A.; Turton, D. A.; Kayser, V.; Kelly, J. M.; Beddard, G. S. *J. Am. Chem. Soc.* **2002**, *124*, 5518. (c) Kawai, K.; Takada, T.; Tojo, S.; Majima, T. *J. Am. Chem. Soc.*

2003, *125*, 6842. (d) Kawai, K.; Takada, T.; Nagai, T.; Cai, X.; Sugimoto, A.; Fujitsuka, M.; Majima, T. *J. Am. Chem. Soc.* **2003**, *125*, 16198.

(24) (a) Milligan, J. R.; Aguilera, J. A.; Hoang, O.; Ly, A.; Tran, N. Q.; Ward, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 1682. (b) Liu, C.-S.; Hernandez, R.; Schuster, G. B. *J. Am. Chem. Soc.* **2004**, *126*, 2877.

(25) Jovanovic, S. V.; Simic, M. G. *J. Phys. Chem.* **1986**, *90*, 974.

(26) Jovanovic, S. V.; Simic, M. G. *Biochim. Biophys. Acta* **1989**, *1008*, 39.

(27) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541.

(28) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617.

(29) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063.

(30) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.

(31) Shafirovich, V.; Dourandim, A.; Luneva, N. P.; Geacintov, N. E. *J. Phys. Chem. B* **2000**, *104*, 137.

(32) (a) Weatherly, S. C.; Yang, I. V.; Thorp, H. H. *J. Am. Chem. Soc.* **2001**, *123*, 1236. (b) Weatherly, S. C.; Yang, I. V.; Armistead, P. A.; Thorp, H. H. *J. Phys. Chem. B* **2003**, *107*, 372.

(33) DeSimone, R. E.; Drago, R. S. *J. Am. Chem. Soc.* **1970**, *92*, 2343.

(34) Fukuzumi, S.; Inada, O.; Suenobu, T. *J. Am. Chem. Soc.* **2003**, *125*, 4808.

(35) Roberts, R. M. G.; Ostovic, D.; Kreevoy, M. M. *Faraday Discuss. Chem. Soc.* **1982**, *74*, 257.

(36) Fukuzumi, S.; Koumitsu, S.; Hironaka, K.; Tanaka, T. *J. Am. Chem. Soc.* **1987**, *109*, 305

(37) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 4th ed.; Pergamon Press: Elmsford, NY, 1996.

(38) Yang, I. V.; Thorp, H. H. *Inorg. Chem.* **2000**, *39*, 4969.

(39) Mann, C. K.; Barnes, K. K. *Electrochemical Reactions in Nonaqueous Systems*; Marcel Dekker: New York, 1990.

(40) (a) Steenken, S. *Free Rad. Res. Commun.* **1992**, *16*, 349. (b) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094.

(41) Vieira, A. J. S. C.; Steenken, S. *J. Am. Chem. Soc.* **1990**, *112*, 6986.

(42) (a) Julliard, M.; Chanon, M. *Chem. Rev.* **1983**, *83*, 425. (b) Chanon, M.; Hawley, M. D.; Fox, M. A. In *Photoinduced Electron Transfer*; Chanon, M., Fox, M. A., Eds.; Elsevier: Amsterdam, 1988; Part A, p 1. (c) Kavarnos, G. J.; Turro, N. J. *Chem. Rev.* **1986**, *86*, 401.

(43) (a) Rehm, D.; Weller, A. *Ber. Bunsen-Ges. Phys. Chem.* **1969**, *73*, 834. (b) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.

(44) The photoinduced electron-transfer reactions examined in this study are exergonic or slightly endergonic, whereas the back electron transfer to the ground state is highly exergonic. In such a case the reversible exciplex formation is unlikely to occur. Moreover, the relation (eq 9) derived under the assumption that $k_{30} \gg k_{32}$ is well verified experimentally, as shown in Figure 5, where the data of the thermal electron-transfer reactions are also included.

(45) Shoute, L. C. T. *J. Phys. Chem. B.* **1997**, *101*, 5535.

(46) Fukuzumi, S.; Fujita, M.; Otera, J.; Fujita, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10271.

(47) Although the Marcus equation has usually been applied in intramolecular electron-transfer reactions with fixed distances between donor and acceptor moieties,[48,49] the application to analyze intermolecular electron-transfer reactions has been limited because of the change in the reorganization energy of intermolecular electron-transfer reactions, which varies depending on the driving force of electron transfer. See: (a) Mataga, N.; Miyasaka, H. In *Electron Transfer from Isolated Molecules to Biomolecules, Part 2*; Jortner, J., Bixon, M., Eds.; Wiley: New York, 1999; p 431. (b) Fukuzumi, S.; Ohkubo, K.; Suenobu, T.; Kato, K.; Fujitsuka, M.; Ito, O. *J. Am. Chem. Soc.* **2001**, *123*, 8459.

(48) (a) Marcus, R. A.; Eyring, H. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155. (b) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265. (c) Marcus, R. A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1111.

(49) (a) Gust, D.; Moore, T. A.; Moore, A. L. In *Electron Transfer in Chemistry*; Balzani, V., Ed.; Wiley-VCH: Weinheim, 2001; Vol. 3, pp 272−336. (b) Fukuzumi, S.; Guldi, D. M. In *Electron Transfer in Chemistry*; Balzani, V. Ed.; Wiley-VCH: Weinheim, 2001; Vol. 2, pp 270−337.

(50) Steenken, S.; Telo, J. P.; Novais, H. M.; Candeias, L. P. *J. Am. Chem. Soc.* **1992**, *114*, 4701.

(51) No fluorescence quenching via *inter*molecular electron transfer from the singlet excited state of pyrene (^{1}Py*) to CMP and TMP or CMP and TMP to ^{1}Py* occurred in our experimental conditions where the ranges of CMP and TMP concentration are 0−0.1 M. For the photoinduced electron-transfer reduction of dC and dT by 7,8,9,10-tetrahydrobenzo[*a*]pyrene, see: (1) (a) Netzel, T. L.; Zhao, M.; Nafisi, K.; Headrick, J.; Sigman, M. S.; Eaton, B. E. *J. Am. Chem. Soc.* **1995**, *117*, 9119. (b) Netzel, T. L.; Nafisi, K.; Headrick, J.; Eaton, B. E. *J. Phys. Chem.* **1995**, *99*, 17948. (c) Kerr, C. E.; Mitchell, C. D.; Headrick, J.; Eaton, B. E.; Netzel, T. L. *J. Phys. Chem. B* **2000**, *104*, 1637. (d) Kerr, C. E.; Mitchell, C. D.; Ying, Y.-M.; Eaton, B. E. Netzel, T. L. *J. Phys. Chem. B* **2000**, *104*, 2166. (e) Shafirovich, V. Y.; Levin, P. P.; Kuzmin, V. A.; Thorgeirsson, T. E.; Kliger, D. S.; Geacintov, N. E. *J. Am. Chem. Soc.* **1994**, *116*, 63.

(52) The k_{et} value of the thermal electron-transfer oxidation of GMP with $Ru(bpy)_3^{3+}$ at pH 7.0 is evaluated from the value at pH 5.0, using the pK_{r1} value of $GMP^{\bullet+}$ (3.9).[10a,40]

(53) The oxidation potential of AMP (1.18 V vs SCE = 1.42 V vs NHE) at pH 7 determined in this study also agrees well with those of AMP (1.42 V vs NHE) determined from the reduction of the corresponding neutral radical by the pulse radiolysis study.[28]

(54) Fujita, M.; Ishida, A.; Takamuku, S.; Fukuzumi, S. *J. Am. Chem. Soc.* **1996**, *118*, 8566.

(55) In the case of triphenylene, photoinduced electron transfer should also occur from all types of DNA bases to the singlet excited state of triphenylene judging from the exothermicity of the electron transfer. However, the absence of absorbance at 355 nm of tripehnylene has precluded the confirmation of the occurrence of photoinduced electron transfer from all types of DNA to the singlet excited state of triphenylene by using our laser system.

(56) The weak transient absorption at 600−800 nm in Figure 6a is due to triplet−triplet absorption of $AcrH^+$. See: Kikuchi, K.; Sato, C.; Watabe, M.; Ikeda, H.; Takahashi, Y.; Miyashi, T. *J. Am. Chem. Soc.* **1993**, *115*, 5180.

(57) The absorption spectrum of the guanine radical cation at 600−800 nm is very weak.[51b] See: O'Connor, D.; Shafirovich, V. Y.; Geacintov, N. E. *J. Phys. Chem.* **1994**, *98*, 9831.

(58) Kobayashi, K.; Tagawa, S. *J. Am. Chem. Soc.* **2003**, *125*, 10213.

(59) Hashimoto, S. *J. Phys. Chem.* **1993**, *97*, 3662.

(60) (a) Hashimoto, S. *Chem. Phys. Lett.* **1996**, *252*, 236. (b) Schomburg, H.; Weller, A. *Chem. Phys. Lett.* **1973**, *22*, 1. (c) Shida, T. *Electronic Absorption Spectra of Radical Ions*; Elsevier: Amsterdam, 1988.

(61) The broad absorption at 500−700 nm in Figure 7a is due to overlapped $Py^{\bullet-}$ (λ_{max} = 490 nm)[59] with the exciplex, ^{1}Py*−GMP. Similar transient absorption has been reported in ref 30.

(62) Transient absorption spectra of a pyrene solution were measured with low-power laser irradiation (1.0 mJ/pulse). When high-power laser irradiation (>10 mJ/pulse) is employed, the pyrene radical cation and the radical anion are also produced. See: Watkins, A. R. *J. Phys. Chem.* **1976**, *80*, 713.

(63) Singlet excited state of DCA is practically quenched in the presence of nucleotides (1.0×10^{-2} M). Transient absorption spectrum of T−T absorption of DCA (λ_{max} = 430 nm) was observed in the absence of nucleotides in Tris/HCl buffer at pH 7.0.

(64) The rates of electron-transfer oxidation of double-stranded oligonucleotide d[5′-GCAGTAGCATGTGACGAGTCG] containing no GG sequence with $Ru(bpy)_3^{3+}$ are also only slightly slower than those of the single-stranded oligonucleotide; see ref 32a.