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Dynamics of Singlet Oxygen Generation by DNA-Binding Photosensitizers

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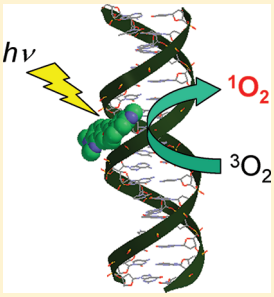
ABSTRACT: The dynamics of photosensitized singlet oxygen generation in a DNA microenvironment were examined using the DNA-binding photosensitizers berberine and palmatine. These photosensitizers generate singlet oxygen only under interaction with DNA because the singlet excited state deactivates rapidly in a nonbinding environment. A kinetic study demonstrated the reaction process whereby singlet oxygen is generated through energy transfer from the triplet excited state of DNA-binding berberine (or palmatine) to molecular oxygen. The guanine-containing sequence of DNA slightly deactivated the singlet excited state of the photosensitizers, resulting in a decrease of the singlet oxygen yield. By the steric hindrance of the DNA strand, the rate constant of the singlet oxygen generation became smaller than that of the other water-soluble photosensitizer.



INTRODUCTION

Photosensitized DNA damage is an important process in medical applications of photochemical reaction, such as photodynamic therapy (PDT). PDT is a promising treatment of cancer and some nonmalignant conditions.[1−3] Singlet oxygen ($^1O_2$) generation is the main mechanism of the photosensitized DNA damage induced by visible light.[4] The guanine residue is selectively oxidized by $^1O_2$, and the major oxidized product of guanine is 8-oxo-7,8-dihydro-2′-deoxyguanine (8-oxodGuo),[5−7] which can induce GC−TA transversion.[8] Controlling the $^1O_2$ generation based on the electron transition control may realize tailor-made PDT. $^1O_2$ generation has been controlled for a specific DNA sequence using photosensitizer/quencher/oligonucleotide systems.[9,10] The demonstrated principle of the control is to selectively place the $^1O_2$ photosensitizer close to a quenching molecule using a positioning system and then to manipulate it to change the distance between the photosensitizer and the quencher. Furthermore, the pH-regulated $^1O_2$ photosensitizer/quencher/DNA i-motif system was reported.[11] In a previous study, we reported on two photosensitizers, berberine and palmatine (Figure 1), which can easily bind to DNA through electrostatic interaction and generate $^1O_2$ only when the DNA−photosensitizer complex is formed.[7,12] Generated $^1O_2$ effectively oxidizes every guanine residue of DNA into 8-oxodGuo.[7] However, the dynamics of this $^1O_2$ generation have not been clarified. The clarification of the dynamics of this controlled $^1O_2$ generation in the DNA microenvironment should be important for a development of functional photosensitizer. In this study, the dynamics of $^1O_2$ generation in a DNA microenvironment were investigated using these DNA-binding photosensitizers and oligonucleotides as the model of the DNA microenvironment.

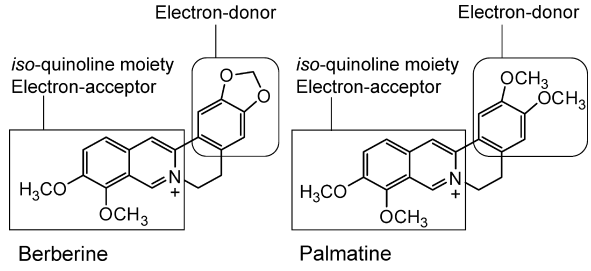


Figure 1. Structures of berberine and palmatine.

EXPERIMENTAL SECTION

Materials. Berberine chloride and sodium azide were purchased from Wako Chemicals Co. (Osaka, Japan). Palmatine chloride was from Aldrich Chemicals Co. (Milwaukee, WI). The synthesized oligonucleotides of the AT sequence (AATT: d(AAAATTTTAAAATTTT)$_2$) and the guanine-containing sequence (AGTC: d(AAGCTTTGCAAAGCTT)$_2$) were from Sigma Chemical Co. (St. Louis, MO) and used as DNA models. In this report, these oligonucleotides are described as DNA. Deuterium oxide (D_2O) was from Across Organics (Morris Plains, NJ). The spectroscopic grade water (H_2O) was from Dojin Chemicals Co. (Kumamoto, Japan) and used as received.

Absorption and Fluorescence Spectra Measurements. The absorption spectra of berberine and palmatine were measured

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with a UV−vis spectrophotometer UV-1650PC (Shimadzu, Kyoto, Japan). The samples contained 50 μM berberine or palmatine with or without DNA in a 10 mM sodium phosphate buffer (pH 7.6). The interaction between the photosensitizers, berberine and palmatine, and DNA was examined by the UV−vis absorption measurement. The apparent binding constants (K) between DNA and the photosensitizers were estimated according to the previous report,[7,12] with the following equation:

$$K = \frac{[\text{Sens}-\text{DNA}]}{[\text{Sens}][\text{DNA}]} \quad (1)$$

where [Sens] is the concentration of the nonbinding photosensitizer, berberine or palmatine, [DNA] is the base-pair concentration of DNA without a binding photosensitizer, and [Sens−DNA] is the concentration of the DNA-binding photosensitizer. This apparent constant, which represents the qualitative affinity between the photosensitizers and DNA, was determined to estimate the binding ratio of photosensitizers (x) by assuming the formation of the 1:1 complex. The value of x can be calculated using the following equation:

$$K = \frac{[\text{Sens}]_0 x}{[\text{Sens}]_0(1-x)[\text{DNA}]_0(1-x)} \quad (2)$$

where $[\text{Sens}]_0$ and $[\text{DNA}]_0$ are the initial concentrations of the photosensitizer and DNA, respectively. The observed absorbance of the sample (A_t) can be expressed using x as follows:

$$A_t = A_n(1-x) + A_b x \quad (3)$$

where A_n is the absorbance of the nonbinding photosensitizer at the initial concentration and A_b indicates the absorbance of all photosensitizers at the DNA-binding state. By using eqs 2 and 3, the values of K were calculated from the spectrum change with the DNA addition.

The fluorescence spectra of berberine and palmatine were measured with an F-4500 spectrophotometer (Hitachi, Tokyo, Japan). The fluorescence quantum yields (Φ_f) of berberine and palmatine were determined relative to riboflavin (Φ_f = 0.267 in water).[13]

Fluorescence Lifetime Measurements. Fluorescence decay was measured using a time-correlated single-photon counting method.[14] Laser excitation at 410 nm was achieved by using a diode laser (PicoQuant, LDH-P-C-410) with a power control unit (PicoQuant, PDL 800-B) in a repetition rate of 2.5 MHz. The temporal profiles of fluorescence decay were detected by using a microchannel plate photomultiplier (R3809U, Hamamatsu Photonics, Shizuoka, Japan) equipped with a TCSPC computer board module (Becker and Hickl, SPC630). The full width at half-maximum (fwhm) of the instrument response function was 51 ps. The values of χ^2 and the Durbin−Watson parameters were used to determine the quality of the fit obtained by nonlinear regression.

Measurement of the Steady-State Near-Infrared Luminescence Spectrum from $^1O_2$. $^1O_2$ generation was directly measured by near-infrared luminescence of around 1270 nm from the deactivation of $^1O_2$, which corresponds to the $^1O_2(^1\Delta_g)-^3O_2(^3\Sigma_g^-)$ transition. The sample solutions contained 50 μM berberine or palmatine with or without DNA in D_2O. A direct detection system consisting of a Nd:YAG laser (THG/355 nm, 30 Hz; Tempest-30, New Wave Research) as an excitation light source (355 nm, intensity 280 mW cm^{-2}), a quartz cuvette as an irradiation cell, a spectroscope, and a near-infrared gated multichannel detector ICCD camera (NIR-II, Hamamatsu Photonics) was built.[15] The gate time and accumulation time were 5−50 μs after the laser pulse and 128 s (total 36 J cm^{-2}), respectively.

Time Profile of $^1O_2$ Emission. The sample solutions of 2 mL contained 50 μM berberine or palmatine and 100 μM/base-pair DNA (AATT or AGTC) in a sodium phosphate buffer (pH 7.6) of H_2O or D_2O. The excitation light was the third harmonic (355 nm) of a pulsed Nd:YAG laser (5 ns, 10 Hz, Continuum Minilite-II). The beam was passed through a set of dielectric multilayer film mirrors to eliminate stray light and irradiate from the 45° direction of the surface of a 1 cm × 1 cm × 4.5 cm quartz cell. The emission from the front surface of the sample cell was collected with a set of quartz lenses, passed through a cold mirror (Sigma Koki, CLDM-50S), separated by a Bosch-Lomb Shimadzu monochromator, and then introduced into a photomultiplier (R5509-41, Hamamatsu Photonics), which was cooled to 200 K with liquid nitrogen. The signal from the photomultiplier was amplified by 75 with an amplifier (Stanford Research, SR-455) and then counted with a scaler/averager (Stanford Research, SR430). By changing the wavelength, the luminescence intensity showed a maximum at 1270 nm, confirming the detection of the phosphorescence of $^1O_2$. To analyze the time profile of $^1O_2$ emission, the signal obtained at 1270 nm was accumulated for 25 000 scans with a bin width of 320 or 40 ns.

Electrochemical Measurement. The redox potentials of berberine and palmatine were measured with a differential pulse voltammometry (Hokuto Denko, Tokyo, Japan) using a platinum working electrode, a platinum counter electrode, and a saturated calomel reference electrode (SCE) in acetonitrile.

Calculation of Intermolecular Complex between Berberines and DNA. The equilibrium geometry of an intermolecular complex between double-stranded DNA and berberine or palmatine was obtained by molecular mechanics calculation utilizing the Spartan 10′ (Wavefunction Inc., CA). The geometry of 16-mer of double-stranded DNA was constructed using Spartan 10′.

RESULTS

Interaction between Berberines and DNA. The absorption spectra of photosensitizers showed the red shift through binding with DNA (Figure 2) and the estimated value of K depended on the sequence (Table 1). It has been reported that berberine preferentially binds to an AT-rich minor groove.[16] The apparent binding constant of palmatine with the AT sequence is also larger than that of the guanine-containing sequence. The binding interaction between berberines and DNA was inferred from the molecular mechanics calculation, as shown in Figure 3.

Fluorescence Enhancement of Berberines by DNA. Figure 4 shows the fluorescence spectra of berberine and palmatine with and without DNA. Although the fluorescence of the two photosensitizers was hardly observed in aqueous solution, their intensity markedly increased in the presence of DNA (Figure 4, A and C). To compare the features with the DNA-binding condition, the very weak fluorescence spectra of the two photosensitizers in the absence of DNA were enlarged to normalize the intensity and are shown in Figure 4, B and D. The fluorescence spectra of these photosensitizers became narrow through DNA binding, suggesting the thermal stabilization of photosensitizer through the fixation by the DNA

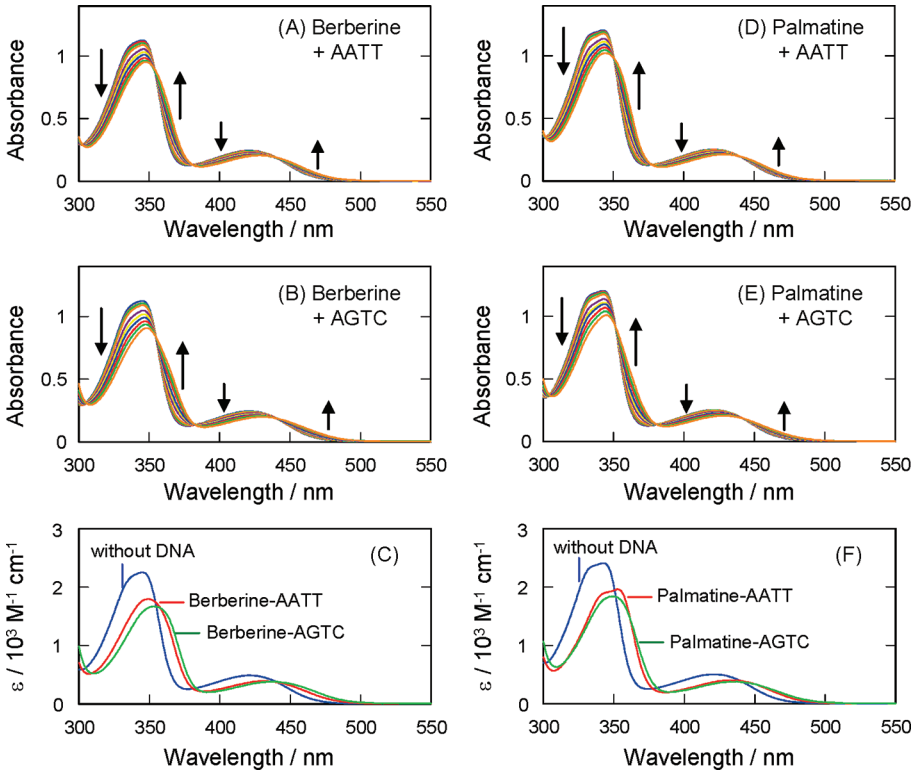

Figure 2. Absorption spectra of berberine and palmatine in the absence or presence of DNA. The sample contained 50 μM berberine (A and B) or palmatine (D and E) with DNA (0, 5, 10, 20, 50, 75, 100, 150, and 200 μM/base-pair) in a sodium phosphate buffer (pH 7.6). The absorption spectra of free berberine and palmatine in the above samples were calculated from their binding constants and concentrations of photosensitizers and DNA. The absorption spectra of the DNA–photosensitizer complexes (C and F) were estimated from the subtraction of the spectra of free photosensitizers from those of DNA-containing samples.

Table 1. Binding Constants and Photochemical Properties of Berberine and Palmatine in the Absence or Presence of DNA[a]

sensitizer	DNA	K/M^{-1}	Φ_f		τ_f/ns (relative amplitude)	
berberine			<0.001	0.05		
	AATT	13100	0.093	0.30 (0.30)	3.7 (0.42)	11.9 (0.28)
	AGTC	8200	0.043	0.12 (0.60)	1.6 (0.32)	8.0 (0.08)
palmatine			<0.001	0.04		
	AATT	9600	0.054	0.16 (0.39)	2.3 (0.45)	6.9 (0.16)
	AGTC	6600	0.031	0.14 (0.54)	1.4 (0.37)	5.9 (0.09)

[a]The concentrations of the sensitizers and DNA were 50 μM and 100 μM/base-pair, respectively.

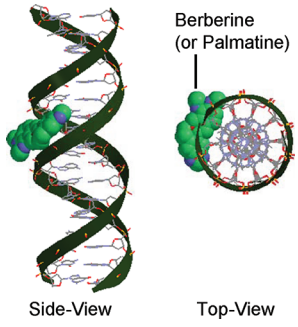

Figure 3. Speculated geometry of the DNA–berberine and –palmatine complexes.

strand. In this experiment, these photosensitizers were irradiated at an isosbestic point. Therefore, the fluorescence intensities (F) in Figure 4A,C are proportional to the binding ratio, and the value of Φ_f could then be calculated. The F can be expressed using the apparatus function (f_f), which includes the excitation light intensity, as follows:

$$F = f_f x \Phi_f \quad (4)$$

The f_f was determined by the fluorescence measurement of a reference compound, riboflavin, whose Φ_f in water has been reported[13] to be 0.267. The F values increased depending on the sequence and the concentration of DNA (Figure 5). Using eq 4 above, these plots could be analyzed, and the values of Φ_f were calculated as listed in Table 1. The Φ_f values of berberine and palmatine decreased in the case of the guanine-containing sequence.

Elongation of Fluorescence Lifetime of Berberines by DNA. Fluorescence lifetimes (τ_f) of berberines were markedly elongated through DNA binding, as shown in Figure 6. This figure represents the case of berberine-AATT. Similar results were observed in the cases of berberine-AGTC, palmatine-AATT, and palmatine-AGTC (data not shown). The fluorescence decay profiles of the DNA-binding photosensitizers were

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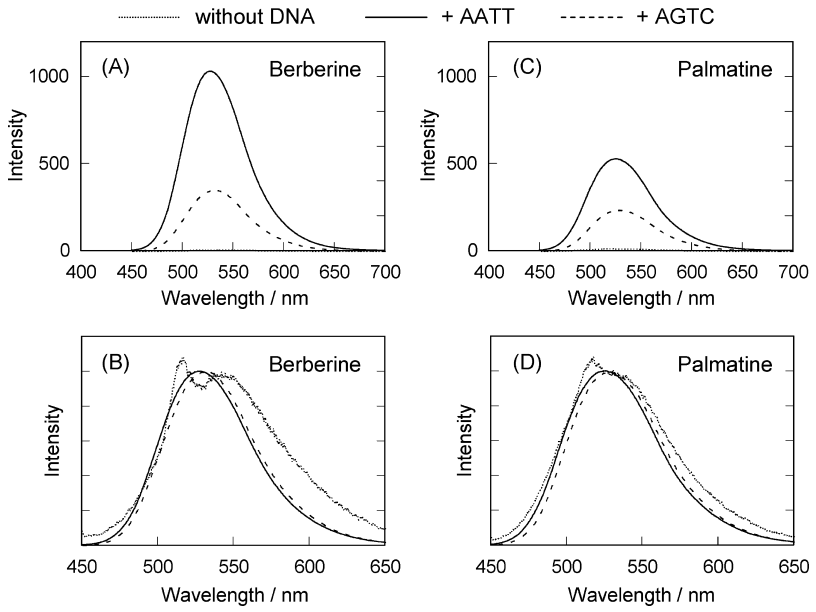

Figure 4. Fluorescence spectra of berberine and palmatine in the absence or presence of DNA. The sample contained 50 μM berberine (A) or palmatine (C) and 100 μM/base-pair DNA in a sodium phosphate buffer (pH 7.6). The fluorescence intensities were normalized at the peaks (B and D).

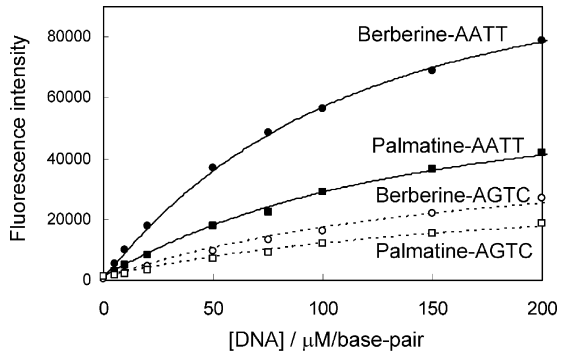

Figure 5. Fluorescence intensity of berberine and palmatine in the presence of DNA. The curves were calculated from the binding constant using least-error square method. The fluorescence intensity indicates the integral of the fluorescence spectra.

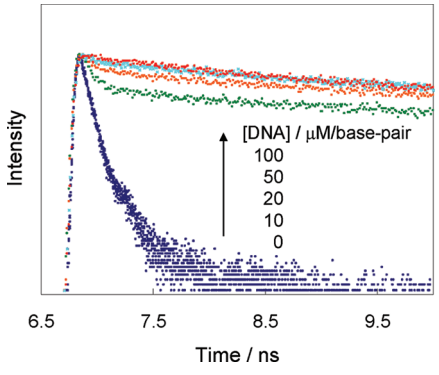

Figure 6. Time profile of the fluorescence intensity of berberine in the presence of DNA. The sample solution contained 50 μM berberine and DNA (0, 10, 20, 50, and 100 μM/base-pair) in a sodium phosphate buffer (pH 7.6).

analyzed into three components in contrast to the single component of the free photosensitizer (Table 1), reflecting the several binding environments of DNA. The amplitude of the longest fluorescence lifetime fraction of photosensitizers binding to DNA was increased with an increase in the DNA concentration and reached a plateau up to 100 μM/base-pair. The lifetimes of berberines were lower in the guanine-containing sequence than they were in the absence of guanine (Table 1), possibly due to the electron transfer from guanine, as described in the Discussion.

The transient fluorescence spectra of DNA-binding berberine and palmatine are shown in Figure 7. The spectrum of the longer lifetime species, which was assigned to the DNA-binding photosensitizer, showed a narrow feature, suggesting the stabilization of the photoexcited state of the photosensitizer through the interaction with DNA. This result coincides with that of the steady-state fluorescence spectra shown in Figure 4.

Photosensitized Singlet Oxygen Generation from DNA-Binding Berberines. To evaluate the $^1O_2$ generation activity of berberine and palmatine, the near-infrared emission spectrum was measured. The emission at around 1270 nm, assigned to the radiative deactivation of $^1O_2$ into its ground state, was clearly observed during irradiation to the DNA-binding berberines, whereas free berberines showed no emission. The emission intensities recorded for four kinds of photosensitizer–DNA systems are plotted in Figure 8 as a function of the DNA concentration. The $^1O_2$ emission increased with the concentration of DNA and depending on the sequence (Figure 8). The quantum yield of $^1O_2$ generation (Φ_Δ) from these DNA-binding photosensitizers were estimated by comparing the $^1O_2$ emission intensities of these photosensitizers and methylene blue (Φ_Δ= 0.52 in D_2O)[17] using the estimated absorbance (A) of the samples at the excitation wavelength. The $^1O_2$ emission intensity (I_Δ) is in proportion to the absorption ratio ($1 - 10^{-A}$) and the apparatus function (f_Δ), which includes the excitation light intensity. Since the absorbance of the nonbinding species affects the light absorption of the binding photosensitizer, the observed I_Δ should be decreased by the following factor:

$$\frac{A - A_0(1 - x)}{A} \tag{5}$$

where A_0 is the absorbance of the photosensitizer in the absence of DNA at the excitation wavelength and the term of "$A - A_0(1 - x)$"

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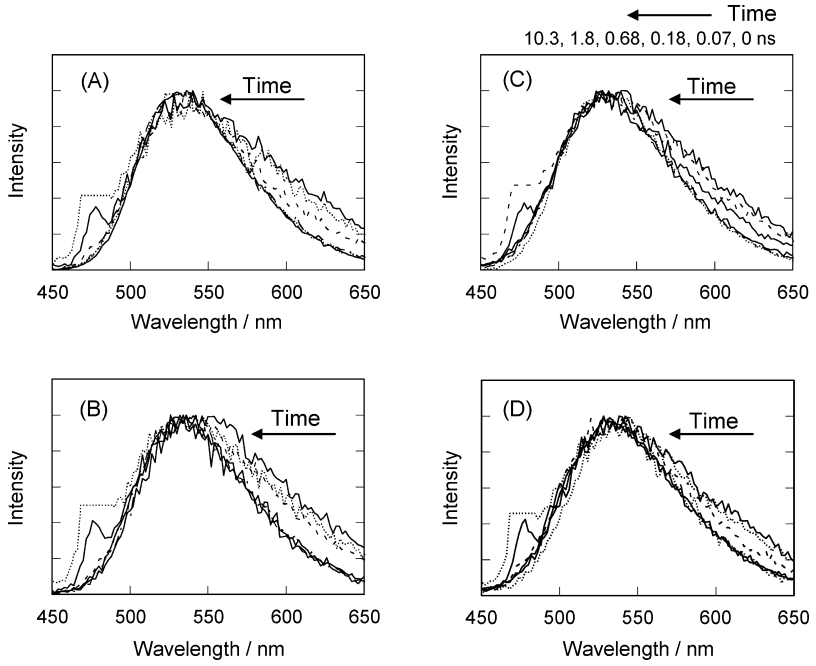

Figure 7. Transient fluorescence spectra of berberine and palmatine in the presence of DNA. The sample solution contained 50 μM berberine or palmatine and 100 μM/base-pair DNA. "Time" indicates the interval after photoexcitation (0, 0.07, 0.18, 0.68, 1.8, and 10.3 ns). (A) Berberine-AATT. (B) Berberine-AGTC. (C) Palmatine-AATT. (D) Palmatine-AGTC.

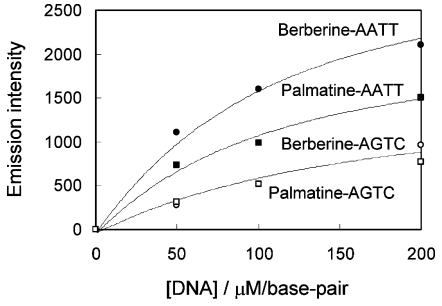

Figure 8. Intensity of the near-infrared $^1O_2$ emission of berberine and palmatine with DNA. The sample solution containing 50 μM berberine or palmatine with the indicated concentration of AATT or AGTC, respectively, in D_2O was excited by laser irradiation (355 nm). "Emission intensity" indicates the integral of the emission spectra at around 1270 nm.

indicates the absorbance part of the DNA-binding photosensitizer. Thus, I_Δ can be expressed as follows:

$$I_\Delta = f_\Delta \frac{A - A_0(1-x)}{A}(1 - 10^{-A})\Phi_\Delta \quad (6)$$

where the apparatus function, f_Δ, was determined by using the reference photosensitizer, methylene blue. The values of Φ_Δ calculated using eq 6 are listed in Table 2. Φ_Δ depended on the sequence and decreased for the guanine-containing sequence, AGTC, for both photosensitizers.

Time Profile of $^1O_2$ Emission and the Estimated Lifetime of Triplet Excited State. The time profile of $^1O_2$ emission is shown in Figure 9. The emission intensity of $^1O_2$ as a function of time, $I(t)$, can be expressed with the following equation:[18]

$$I(t) = I_0\left\{\exp\left(-\frac{t}{\tau_d}\right) - \exp\left(-\frac{t}{\tau_r}\right)\right\} \quad (7)$$

Table 2. Photosensitized $^1O_2$-Generating Activity of Berberine and Palmatine in the Absence or Presence of DNA[a]

sensitizer	DNA	Φ_Δ (D_2O)[b]	$\tau_r/\mu s$ (D_2O)[c]	$\tau_d/\mu s$ (D_2O)[c]	$\tau_d/\mu s$ (H_2O)[c]
berberine		nd	nd	nd	nd
	AATT	0.066	15	72	14
	AGTC	0.036	16	77	15
palmatine		nd	nd	nd	nd
	AATT	0.044	11	75	13
	AGTC	0.030	10	78	14

[a]The concentrations of the sensitizers and DNA were 50 μM and 200 μM/base-pair, respectively. [b]The quantum yield of $^1O_2$ generation in D_2O. [c]Parameters of the kinetic analysis of $^1O_2$ obtained from eq 7. The rise time constant of $^1O_2$ emission in H_2O was too fast to be measured.

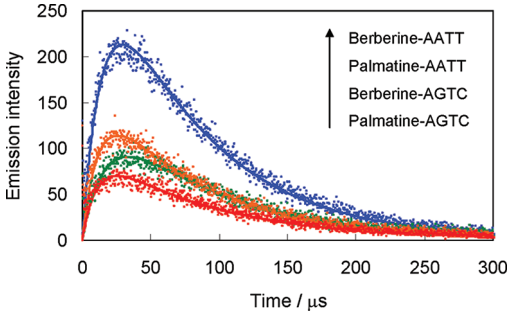

Figure 9. Time-resolved emission signal of $^1O_2$ at 1270 nm in D_2O. The sample solution containing 50 μM berberine or palmatine with 200 μM/base-pair AATT or AGTC, respectively, in D_2O was excited by laser irradiation (355 nm).

where I_0 is the pre-exponential factor, τ_d is the decay time constant of the emission, and τ_r is the rise time constant of this emission.

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When the lifetime of $^1O_2$ (τ_Δ) is longer than τ_r, τ_d corresponds to τ_Δ. In general, τ_r equals to the lifetime of the triplet excited (T_1) state of the photosensitizer (τ_T) because the T_1 state is dominantly quenched by O_2 molecules. In contrast, τ_r indicates τ_Δ, if τ_T is longer than τ_Δ. The analysis of the time-resolved $^1O_2$ emission gave the kinetic parameters, as shown in Table 2.

Table 2 lists the Φ_Δ and the values of τ_r and τ_d in D_2O in addition to the values of τ_d in H_2O measured for two photosensitizers with and without DNA (AATT and AGTC). The observed value of τ_d, around 70 μs, almost coincided with the typical lifetime of $^1O_2$ (τ_Δ) in D_2O.[19] The values of τ_r should correspond to the τ_T in D_2O. In the case of the H_2O solution (Figure 10), the emission intensity rose rapidly (τ_r < 5 μs), and the

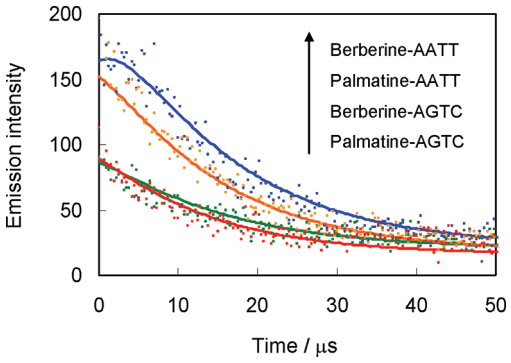

Figure 10. Time-resolved emission signal of $^1O_2$ at 1270 nm in H_2O. The sample solution containing 50 μM berberine or palmatine with 200 μM/base-pair AATT or AGTC, respectively, in H_2O was excited by laser irradiation (355 nm).

decay time, τ_d, was around 14 μs. Since the lifetime τ_Δ in H_2O is very short (2−4 μs), the τ_d in H_2O should correspond to the τ_T of berberine and palmatine. The observed values of τ_T are markedly higher than those of the singlet excited (S_1) state, indicating that $^1O_2$ was generated from the T_1 state. The τ_T values of the AATT-binding photosensitizers were similar to those of the corresponding AGTC-binding ones (Table 2).

Redox Potentials of Berberine and Palmatine. Two reversible oxidation peaks were observed for both berberine and palmatine (berberine, 1.45 and 1.70 V vs SCE; palmatine, 1.42 and 1.65 V vs SCE). The peaks at 1.70 and 1.65 V could be assigned to the one-electron oxidation of the *iso*-quinoline moieties of berberine and palmatine.[12,20,21] Because the calculation results demonstrated that the dialkoxybenzene moieties are more easily oxidized than the *iso*-quinoline moieties, the peaks at 1.45 and 1.42 V should indicate the oxidation of dialkoxybenzene moieties. These results reasonably explain that intramolecular electron transfer is possible in the cases of both berberine and palmatine.

DISCUSSION

The fluorescence lifetime of berberine and palmatine was very short, and their quantum yield was very small. In the present study, it was clarified that the redox potentials of one-electron oxidation of the electron-donating site are in fact smaller than the *iso*-quinoline moieties. Intramolecular electron transfer effectively quenches the S_1 state of the *iso*-quinoline moieties. The charge transfer (CT) state energy (E^{CT}) was estimated from their redox potentials using the following equation (Table 3):[22]

$$E^{CT} = E_{1/2}{}^{+} - E_{1/2}{}^{-} \quad (8)$$

Table 3. Energy Level of the Photoexcited and CT States of Berberine and Palmatine in the Absence or Presence of DNA

sensitizer	DNA-groove[a]	$E(S_1)$/eV[b]	E^{CT}/eV[c]	−ΔG/eV
berberine		2.34	2.07	0.27
	major		2.20	0.14
	minor		2.24	0.10
palmatine		2.34	2.09	0.25
	major		2.22	0.12
	minor		2.26	0.08

[a]"Major" and "minor" indicate the DNA microenvironments of the major groove and minor groove, respectively. [b]The S_1 energy was estimated from the fluorescence maximum (530 nm). [c]The values of E^{CT} were estimated as described in ref 25.

where $E_{1/2}{}^{+}$ and $E_{1/2}{}^{-}$ are the one-electron oxidation and reduction potentials, respectively. The estimated values were corrected using the solvent polarity.[23] The relaxation process of the S_1 state of these photosensitizers is shown in Figure 11.

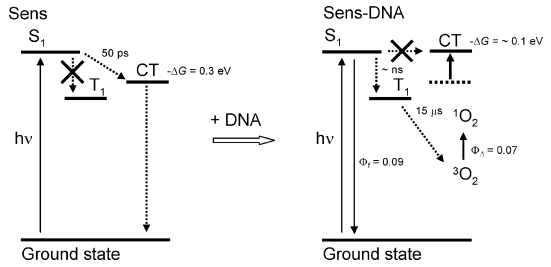

Figure 11. Relaxation process of berberine, palmatine, and their complexes with DNA. "Sens" indicates berberine or palmatine. The presented values are examples for the case of berberine (left) and berberine-AATT (right).

Berberine and palmatine bind to the DNA. The binding constant of these photosensitizers and AATT are larger than those of AGTC. These results coincide with those from a previous report, in which berberine was shown to easily bind to the AT-rich minor groove.[16] The binding interaction with DNA markedly elongates the fluorescence lifetime of these photosensitizers, resulting in the enhancement of their fluorescence quantum yields. The electrostatic interaction raises the CT state energy levels because the redox potential of palmatine was reported to be increased by 0.124 V through DNA-binding interaction.[24] Furthermore, the hydrophobic environment of the DNA strand[26,27] is unfavorable for electron transfer.[25] This negative shift of the redox potential and the hydrophobic environment of DNA should inhibit the intramolecular electron transfer (Table 3). The relatively small difference of the free energy change ($-\Delta G$) shown in Table 3 could control the relaxation process of the photoexcited photosensitizers. Relevantly, it has been reported that the electron-transfer process in the system of the pyrene−porphyrin P(V) complex could be completely controlled by a small $-\Delta G$ difference of about 0.2 eV.[28] The fluorescence lifetimes of the photosensitizers binding with the guanine-containing sequence were slightly shorter than those of the AT-only sequence. The redox potential of the one-electron oxidation of guanine is the smallest in the four nucleobases.[29] Furthermore, the redox potential of guanine becomes smaller in a DNA duplex through interaction with other nucleobases.[30] The electron transfer from the guanine residue may decrease the fluorescence lifetime

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of berberine and palmatine. In addition, the fluorescence decays of the DNA-binding photosensitizers were fitted by a multi-exponential function (three components), indicating that the difference of the binding microenvironment of DNA also affects the excited-state lifetime of berberine and palmatine.

The elongation of the photoexcited-state lifetime leads to the enhancement of $^1O_2$ generation. The photosensitized $^1O_2$ generation by berberine and palmatine was observed only when the DNA−photosensitizer complex was formed. The analysis of the time profile of $^1O_2$ emission demonstrates the τ_T of photosensitizers binding to DNA. The values of τ_T with AATT for berberine and palmatine in D_2O were 15 and 10 μs, respectively, and those with AGTC showed similar values.

The present system of berberines and DNA is advantageous to investigate the dynamics of $^1O_2$ generation by DNA-binding photosensitizers. In general, not only the DNA-binding photosensitizer but also the free photosensitizer generates $^1O_2$ in solution. In the case of berberine and palmatine, only the $^1O_2$ generated from the DNA-binding photosensitizer can be observed. The estimated $^1O_2$ generation rate constants, which are equal to the energy transfer rate constants,[31] are 2.6, 2.4, 3.4, and 3.8 in the unit of 10^8 M^{-1} s^{-1} for berberine-AATT, berberine-AGTC, palmatine-AATT, and palmatine-AGTC, respectively. In general, the $^1O_2$ generation rate in aqueous solution is almost the diffusion control limit (7.4×10^9 M^{-1} s^{-1} in this experimental condition).[32] Consequently, these values indicate that the DNA strand inhibits the interaction between the binding photosensitizer and molecular oxygen. This inhibitory effect may play an important role in the prevention of photosensitized DNA damage.

The apparent values of Φ_Δ by the photosensitizer binding with the guanine-containing sequence were smaller than those of the AT-only sequence. Since τ_Δ and τ_T were less affected by guanine, the decrease of Φ_Δ could be explained by the following mechanisms. The quenching of the S_1 state of photosensitizers by guanine should decrease the intersystem crossing yield and Φ_Δ. In addition, the quenching of $^1O_2$ by the guanine residue may contribute to the apparent decrease of the value of Φ_Δ. Guanine is the only base that significantly reacts with $^1O_2$.[33] The reported rate constant of the chemical quenching of $^1O_2$ by guanine is relatively large (1.7×10^7 M^{-1} s^{-1}),[34] whereas the total quenching rate constant and that of the chemical reaction by adenine are 4.1×10^5 and 8×10^3 M^{-1} s^{-1}, respectively.[35] Therefore, the chemical quenching of $^1O_2$ by guanines may be the predominant process of the $^1O_2$ deactivation by DNA. In the case of berberine and palmatine, $^1O_2$ is necessarily generated in the vicinity of DNA. Although some parts of generated $^1O_2$ are deactivated by guanines, major parts of $^1O_2$ diffuse into the solution, as can be observed by the spectroscopic method.

CONCLUSION

The activity of photosensitized $^1O_2$ generation can be controlled through an interaction with a DNA strand depending on the sequence. The lifetime of the S_1 state of DNA-binding photosensitizers plays an important role in the yields of intersystem crossing and $^1O_2$ generation. The steric hindrance by a DNA strand suppresses the interaction between the photosensitizer and molecular oxygen to generate $^1O_2$. In the case of berberine and palmatine, the guanine-containing sequence acts as a suppressive factor for $^1O_2$ generation by quenching of their S_1 states possibly via electron transfer.

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Notes

The authors declare no competing financial interest.

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(22) The actual E^{CT} is expressed as follows: $E^{CT} = (E_{1/2}^{+} - E_{1/2}^{-}) - Z_1Z_2/4\pi\varepsilon_0\varepsilon r$, where Z_1 is the charge of the electron donor, Z_2 is the charge of the electron acceptor, ε_0 is the vacuum permeability, ε is the static dielectric constant, and r is the radius of the photosensitizer. Because the positive charge on the photosesitizers is neutralized by the electron transfer, Z_2 becomes zero, and E^{CT} can be calculated by a simple equation (eq 8).
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(32) The diffusion-controlled reaction rate coefficient was calculated using the following equation: $8000RT/3\eta$, where R is the gas constant, T is the absolute temperature, and η is the viscosity of water (8.91×10^{-4} kg m^{-1} s^{-1}).
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