Relaxation Process of the Photosensitized State and Singlet Oxygen Generating Activity of Water-soluble meso-Phenanthrylporphyrin in a DNA Microenvironment

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ABSTRACT: To examine the microenvironmental effect of DNA on the photosensitized reaction, the electron-donor-antiticipating porphyrin, meso-(9-phenanthryl)-tris(N-methyl-p-pyridinio)porphyrin (Phen-TMPyP), was synthesized. Phen-TMPyP can bind to oligonucleotides with two binding modes, depending on the DNA concentration. The fluorescence lifetime measurement of Phen-TMPyP shows a shorter component than that of the reference porphyrin without the phenanthryl moiety. However, the observed value is much longer than those of previously reported similar types of electron-donor-antiticipating porphyrins, suggesting that electron-transfer quenching by the phenanthryl moiety is not sufficient. The fluorescence quantum yield of Phen-TMPyP (5 μM) decreased with an increase in DNA concentration up to 5 μM base pair (bp), possibly due to self-quenching through an aggregation along the DNA strand, increased with an increase in DNA concentration of more than 5 μM bp and reached a plateau. The fluorescence quantum yield of Phen-TMPyP with a sufficient concentration of DNA was larger than that of the reference porphyrin. The singlet oxygen (1O2) generating activity of Phen-TMPyP was confirmed by near-infrared emission spectrum measurement. The quantum yield of 1O2 generation was decreased by a relatively small concentration of DNA, possibly due to the aggregation of Phen-TMPyP, and recovered with a sufficient concentration of DNA. The recovered quantum yield was rather smaller than that without DNA, indicating the quenching of 1O2 by DNA. These results show that a DNA strand can stabilize the photosensitized state of a photosensitizer and, in a certain case, suppresses the 1O2 generation.

An important application of porphyrin photochemistry is photodynamic therapy (PDT), which is a less invasive treatment for cancer and some non-malignant conditions.1,3 Administered porphyrins induce photodynamic effect of cancer cells by the oxidation of biomacromolecules, including DNA, through the following two mechanisms during visible-light irradiation. One mechanism is the generation of singlet oxygen (1O2) through energy transfer to oxygen molecules from the photosensitized photosensitizer (Type I mechanism). Another mechanism is the electron abstraction from biomacromolecules to the photosensitized photosensitizer (Type II mechanism). The Type II mechanism is easily induced by visible-light excitation of the photosensitizer. Therefore, photosensitized 1O2 generation is an important process of PDT. Since the photosensitized reaction occurs in a microenvironment constructed by target biomacromolecules, interaction between porphyrins and biomacromolecules is important.4 Especially, DNA is one of the most important targets for PDT; we have previously reported the activity control of porphyrin photosensitizers through interaction with DNA.5,7 In the previous study, pyrene6 and anthracene5,7 were connected to the water-soluble porphyrin as the electron donor. Without DNA, the photosensitized state of porphyrin can be quenched though an intramolecular electron transfer, and the binding interaction with DNA inhibits this electron transfer-mediated quenching, leading to controlling the activity of photosensitized 1O2 generation by porphyrin photosensitizer. In this study, to design a more active photosensitizer, the microenvironmental effect of DNA on the photosensitized reaction was examined by using the novel type of electron-donor-antiticipating porphyrin, meso-(9-phenanthryl)-tris(N-methyl-p-pyridinio)porphyrin (Phen-TMPyP, Figure 1). Since more hydrophobicity was speculated with phenanthrene-connected porphyrins, strong binding interaction with hydrophobic DNA strand was expected. Furthermore, the energy level of the charge-transfer (CT) state of Phen-TMPyP is close to its singlet excited (S1) state (described in a later section). Thus, the effect of the small Gibbs energy (ΔG) of the intramolecular electron transfer was examined. The electron-donor-antiticipating cationic porphyrin, Phen-TMPyP, was synthesized in a method similar to that of previous reports5,8 and characterized with both NMR and mass spectrometer (MS). Molecular orbital (MO) calculations were performed at the Hartree-Fock 6-31G* level, utilizing Spartan 10® (Wavefunction Inc., CA, USA) to predict physophysical properties. The synthesized 16-mer oligonucleotides (AATT: d(AAAATTTTAAAA-TTTT), and AGTC: d(AAGCTTTGCAAAGCTT)) were purchased from Sigma-Aldrich Co. LLC, (St. Louis, MO USA). Photochemical properties of Phen-TMPyP with DNA were examined by spectroscopic techniques (Supporting Information).

Figure 1. Structure of Phen-TMPyP. The highest occupied MO of Phen-TMPyP (right) was obtained by calculations at the Hartree-Fock 6-31G* level.

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The UV-vis absorption spectrum of Phen-TMPyP was redshifted by the addition of DNA, indicating the binding interaction of Phen-
TMPyP with the DNA strand (Figure 2A). The absorption spectral changes in the cases of Phen-TMPyP and AATT are shown in Figure 2. The observed absorption spectral changes of Phen-TMPyP by DNA were complex. In the presence of relatively small concentrations of DNA (~5 µM base pair (bp)), the absorbance at around 450 nm was decreased, depending on the DNA concentration (Figure 2B). With higher concentrations of DNA (>5 µM bp), absorbance increased with an increase in the DNA concentration. Similar results were observed in the case of AGTC. These results could be explained by the following mechanism (Figure S1 in the Supporting Information). With relatively small concentrations of DNA, almost all Phen-TMPyP molecules aggregate around the DNA strand because the water solubility of Phen-TMPyP is small. In the presence of a sufficient concentration of DNA, Phen-TMPyP can form a stable complex with the DNA strand. The space-filling model (CPK model) and previous studies suggest that the possible binding interaction is groove binding. Relevantly, minor groove binding of tetrakis(N-methyl-p- pyridinio)porphyrin (H₂TMPyP), a reference porphyrin of Phen-TMPyP, with DNA was reported. Furthermore, intercalation of Phen-TMPyP into a DNA strand might be possible as well. Similar DNA concentration-dependent interaction has been reported in the case of H₂TMPyP and its zinc complex (ZnTMPyP) with calf thymus DNA. Under this assumption, the association constants for two binding modes, K₁ and K₂, could be expressed as the following equations:

\[
K_1 = \frac{[\text{Phen-TMPyP}]_0 \cdot x_1}{[\text{Phen-TMPyP}]_0 (1-x_1)^{[\text{DNA}]_1} n_1 (1-x_1)}
\]

and

\[
K_2 = \frac{[\text{Phen-TMPyP}]_0 \cdot x_2}{[\text{Phen-TMPyP}]_0 (1-x_1-x_2)^{[\text{DNA}]_2} n_2 (1-x_1-x_2)}
\]

where [Phen-TMPyP]₀ and [DNA]₀ are the initial concentrations of Phen-TMPyP and DNA, respectively, x₁ and x₂ are the binding ratios of the two binding modes, n₁ represents the base pairs occupied by one binding mode, and n₂ represents those occupied by another binding mode. These equations are constructed under the assumption that the groove binding (and/or intercalation) of Phen-TMPyP occurs preferentially, and another binding mode (aggregation) can be observed after the groove binding (and/or intercalation). Therefore, the equations are not symmetrical with respect to x₁ and x₂. The observed absorbance (Absₓ) can be expressed using the following equation:

\[
\text{Abs}_x = \text{Abs}_0 \cdot (1-x_1-x_2) + \text{Abs}_{b1}x_1 + \text{Abs}_{b2}x_2
\]

where Abs₀ is the absorbance of non-binding Phen-TMPyP, Abs_{b1} indicates the absorbance of groove binding (and/or intercalating) Phen-TMPyP, and Abs_{b2} is that of another binding mode. The values obtained by the least squares method are presented in Table 1. These values suggest a relatively stable interaction. The values of K₁ are comparable with those of pyrene and anthracene-connecting similar porphyrins. It could be calculated that 92% of 10 µM Phen-TMPyP bound to DNA strands in the presence of 50 µM bp DNA.

**Table 1.** Apparent Association Constant Between Phen-TMPyP and DNA

<table>
<thead>
<tr>
<th>DNA</th>
<th>K₁ / M⁻¹</th>
<th>n₁</th>
<th>K₂ / M⁻¹</th>
<th>n₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>AATT</td>
<td>1.3×10⁷</td>
<td>2.8</td>
<td>1.0×10⁷</td>
<td>0.7</td>
</tr>
<tr>
<td>AGTC</td>
<td>1.3×10⁷</td>
<td>2.7</td>
<td>1.0×10⁷</td>
<td>0.7</td>
</tr>
</tbody>
</table>

K₁: association constant of groove binding (and/or intercalation)  
K₂: association constant of aggregation  
n₁: occupied base pairs by groove binding (and/or intercalation)  
n₂: occupied base pairs by aggregation

The MO calculation showed that the highest occupied MO (HOMO) of Phen-TMPyP was located on the phenanthryl moiety (Figure 1). This result suggests that the photoexcited state of Phen-TMPyP can be deactivated via intramolecular electron transfer from the phenanthrene moiety to the porphyrin moiety, forming a CT state. Indeed, the calculated ΔG of the electron transfer is 0.18 eV, which supports the belief that intramolecular electron transfer is possible in terms of thermodynamics. Interaction with DNA predicts a raise in CT state energy, leading to recovery of the photochemical activity. The observed fluorescence lifetime (τᵢ) of Phen-TMPyP (5.8 ns (89%) and 2.7 ns (11%)) showed a shorter component than that of the reference porphyrin without the phenanthryl moiety, H₂TMPyP (τᵢ: 5.1 ns), suggesting intramolecular electron transfer from the phenanthryl moiety to the porphyrin ring. However, the observed value is much longer than those of previously reported similar types of electron-donor-connecting porphyrins (~0.04 ns). Furthermore, Phen-TMPyP demonstrated relatively strong fluorescence in sodium phosphate buffer without DNA (fluorescence quantum yield (Φᵢ); 0.028). These results suggest that the electron transfer quenching by the phenanthryl moiety is not sufficient. Figure 3A shows the fluorescence spectral change of Phen-TMPyP by AATT. A similar change was observed in the case of AGTC. The relationship between the Φᵢ and the DNA concentration is shown in Figure 3B. The value of the Φᵢ decreased with an increase in the DNA concentration of up to 5 µM bp, possibly due to self-quenching through an aggregation. The Φᵢ increased with an increase in the DNA concentration to more than 5 µM bp and reached a plateau (0.071 for AATT and 0.065 for AGTC). The value of the τᵢ also varied similarly to that of the Φᵢ. In the presence of 50 µM bp DNA, the observed values of the τᵢ are as follows: 13.6 ns (95%) and 4.6 ns (5%) for AATT, and 12.8 ns (92%) and 4.4 ns (8%) for AGTC. These values are comparable to those of H₂TMPyP (11.5 ns for AATT, 9.1 ns (60%) and 3.2 ns (40%) for...

Figure 2. Absorption spectra of Phen-TMPyP with or without AATT (A), and the relationship between absorbance of Phen-TMPyP at 450 nm and the DNA concentration (B). The sample solution contained 5 µM Phen-TMPyP in a 10 mM sodium phosphate buffer (pH 7.6) and AATT or AGTC.
AGTC). In the DNA microenvironment, the relaxation process of the S₁ state of Phen-TMPyP should be almost the same as that of H₂TMPyP. These results indicate that the DNA microenvironment stabilizes the photoexcited state of the binding porphyrin, possibly due to suppression of the vibrational deactivation. In the case of AGTC, the Φ₁ and τ₁ of these porphyrins are smaller than those of AATT. This effect can be explained by the fact that guanine quenches the photoexcited state of porphyrins through an electron transfer because of its smallest redox potential (one electron oxidation) in the four nucleobases. 13,16

![Fluorescence spectra of Phen-TMPyP with or without AATT](image)

**Figure 3.** Fluorescence spectra of Phen-TMPyP with or without AATT (A), and the relationship between Φ₁ of Phen-TMPyP and the DNA concentration (B). The sample solution contained 5 μM Phen-TMPyP in a 10 mM sodium phosphate buffer (pH 7.6) and AATT or AGTC. The excitation wavelength is 532 nm.

To evaluate the ¹⁰O₂ generating activity of Phen-TMPyP, we measured the near-infrared emission in sodium phosphate buffer (pH 7.6). The typical near-infrared emission spectrum at around 1,270 nm, which is assigned to the emission of ¹⁰O₂, was clearly observed during the photoirradiation of Phen-TMPyP without DNA (Figure 4A). The intensity of the ¹⁰O₂ emission depended on the AATT concentration (a similar result was observed in the case of AGTC). The quantum yield of ¹⁰O₂ generation (Φₐ) was estimated by the comparing the ¹⁰O₂ emission intensity of Phen-TMPyP and that of methylene blue (0.52 in water). 17 The apparent value of Φ₂ by Phen-TMPyP without DNA was relatively large (0.38). This result also indicates that the quenching effect of the phenanthryl moiety is not sufficient. Figure 4B shows the relationship between Φ₂ and the DNA concentration. In the presence of 5 μM bp DNA, the ¹⁰O₂ generation activity was decreased, possibly due to aggregation (Φ₂=0.04 for both cases of AATT and AGTC). On the other hand, the Φ₂ values were recovered by the addition of sufficient concentrations of DNA (50 μM bp) (Φ₂=0.16 and 0.14 for AATT and AGTC, respectively). However, these values were rather smaller than that without DNA. Because ¹⁰O₂ generation occurs in the microenvironment of DNA, the generated ¹⁰O₂ should interact with the DNA strand. AT-only sequences quench ¹⁰O₂ through a mainly physical mechanism with the rate coefficient of 4.1×10⁵ M⁻¹s⁻¹. 18 whereas guanine can quench ¹⁰O₂ through a chemical process (guanine oxidation) with a larger rate coefficient (1.7×10⁴ M⁻¹s⁻¹). 19 Therefore, the actual values of Φ₂ may be higher than the estimated values. The observed values of Φ₂ suggest that the chemical process contributes slightly to ¹⁰O₂ deactivation and the physical process plays a major role in the total deactivation process. In addition, the DNA strand may prevent energy transfer from the photoexcited Phen-TMPyP to the oxygen molecule. Therefore, the DNA microenvironment acts as a suppressor of ¹⁰O₂ generation and its activity. These results suggest that interaction with DNA limits the activity control of the photosensitized ¹⁰O₂ generation.

In conclusion, Phen-TMPyP aggregates around the relatively small concentration of a DNA strand, whereas stable binding interaction becomes possible with a sufficient concentration of DNA. The S₁ state of Phen-TMPyP is not effectively quenched through intramolecular electron transfer from the phenanthryl moiety. The S₁ state of Phen-TMPyP is stabilized in the DNA groove binding or intercalating state. On the other hand, the activity of ¹⁰O₂ generation by the photosensitizer is inhibited by the DNA itself through physical and chemical deactivation processes.

![Near-infrared emission spectra of ¹⁰O₂ generated by the photosensitized reaction of Phen-TMPyP](image)

**Figure 4.** Near-infrared emission spectra of ¹⁰O₂ generated by the photosensitized reaction of Phen-TMPyP with or without AATT (A), and the relationship between Φ₂ and the DNA concentration (B). The sample solution contained 5 μM Phen-TMPyP and the indicated concentration of AATT or AGTC in a 10 mM sodium phosphate buffer (pH 7.6). The excitation wavelength is 532 nm.

**KEYWORDS:** Porphyrin, Phenanthrene, DNA, Photosensitizer, Singlet oxygen, Electron transfer

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SUPPORTING INFORMATION
Experimental procedures; synthesis of Phen-TMPyP, characterization, and the proposed image of binding interaction between Phen-TMPyP and DNA.

REFERENCES AND NOTES
12. The \( \Delta G \) was roughly estimated using the following equation: \( \Delta G = E_{0,0} - e (E^* - E) \), where \( E_{0,0} \) is the S1 energy calculated from the fluorescence maximum (650 nm), \( e \) is the charge of the electron, \( E^* \) is the oxidation potential of the phenanthrene (1.50 V vs. saturated calomel electrode; SCE)\(^{13} \) and \( E \) is the reduction potential of the porphyrin ring (-0.23 V vs SCE)\(^{14} \).