Rapid Communication in Photoscience(2014), vol. 3, no. 4, pp. 81-84
http://dx.doi.org/10.5857/RCP.2014.3.4.81 **ISSN 2288-4564(Online)**

Communications

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

Kazutaka Hirakawa,*[§,†] Yusuke Ito,[§] Takashi Yamada,[§] and Shigetoshi Okazaki[‡]

[§]Department of Applied Chemistry and Biochemical Engineering, Graduate School of Engineering, Shizuoka University, Johoku 3-5-1, Naka-ku, Hamamatsu, Shizuoka 432-8561, Japan
[†]Department of Optoelectronics and Nanostructure Science, Graduate School of Science and Technology, Shizuoka University, Johoku 3-5-1, Naka-ku, Hamamatsu, Shizuoka 432-8561, Japan
[‡]Medical Photonics Research Center, Hamamatsu University School of Medicine, Handayama 1-20-1, Higashi-ku, Hamamatsu, Shizuoka 431-3192, Japan

ABSTRACT: To examine the microenvironmental effect of DNA on the photosensitized reaction, the electron-donor-connecting 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-connecting 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 of 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 ($^1O_2$) generating activity of Phen-TMPyP was confirmed by the near-infrared emission spectrum measurement. The quantum yield of $^1O_2$ 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 $^1O_2$ by DNA. These results show that a DNA strand can stabilize the photoexcited state of a photosensitizer and, in a certain case, suppresses the $^1O_2$ 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 photodamage 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 ($^1O_2$) through energy transfer to oxygen molecules from the photoexcited photosensitizer (Type II mechanism). Another mechanism is the electron abstraction from biomacromolecules to the photoexcited photosensitizer (Type I mechanism). The Type II mechanism is easily induced by visible-light excitation of the photosensitizer. Therefore, photosensitized $^1O_2$ 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, pyrene[6] and anthracene[5,7] were connected to the water-soluble porphyrin as the electron donor. Without DNA, the photoexcited 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 $^1O_2$ 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-connecting 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 (S_1) state (described in a later section). Thus, the effect of the small Gibbs energy ($-\Delta G$) of the intramolecular electron transfer was examined.

The electron-donor-connecting cationic porphyrin, Phen-TMPyP, was synthesized in a method similar to that of previous reports[6,7] 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 photophysical properties. The synthesized 16-mer oligonucleotides (AATT: d(AAAATTTTAAAA-TTTT)$_2$ and AGTC: d(AAGCTTTGCAAAGCTT)$_2$) were purchased from Sigma-Aldrich Co. LLC. (St. Lois, MO USA). Photochemical properties of Phen-TMPyP with DNA were examined by spectroscopic techniques (Supporting Information).

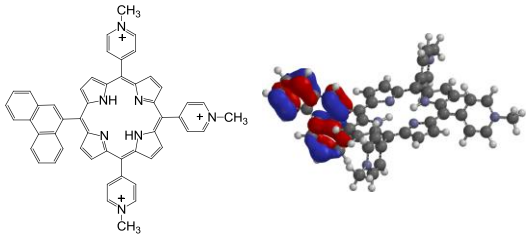

Figure 1. Structure of Phen-TMPyP. The highest occupied MO of Phen-TMPyP (right) was obtained by calculations at the Hartree-Fock 6-31G* level.

***To whom correspondence should be addressed.**
E-mail: hirakawa.kazutaka@shizuoka.ac.jp

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[5-7] suggest that the possible binding interaction is groove binding. Relevantly, minor groove binding of tetrakis(*N*-methyl-*p*-pyridinio)porphyrin (H_2TMPyP), a reference porphyrin of Phen-TMPyP, with DNA was reported.[8-10] 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 H_2TMPyP and its zinc complex (ZnTMPyP) with calf thymus DNA.[11] Under this assumption, the association constants for two binding modes, K_1 and K_2, could be expressed as the following equations[11]:

$$K_1 = \frac{[\text{Phen}-\text{TMPyP}]_0 x_1}{[\text{Phen}-\text{TMPyP}]_0(1-x_1)\dfrac{[\text{DNA}]_0}{n_1}(1-x_1)} \quad (1)$$

and

$$K_2 = \frac{[\text{Phen}-\text{TMPyP}]_0 x_2}{[\text{Phen}-\text{TMPyP}]_0(1-x_1-x_2)\dfrac{[\text{DNA}]_0}{n_2}(1-x_1-x_2)} \quad (2),$$

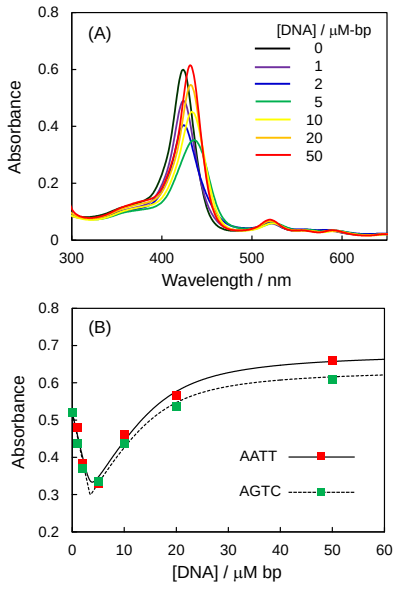

Figure 2. Absorption spectra of Phen-TMPyP with or without AATT (A), and the relationship between absorbance of Phen-TMPyP at 450 nm and the DNA concentration (B). The sample solution contained 5 μM Phen-TMPyP in a 10 mM sodium phosphate buffer (pH 7.6) and AATT or AGTC.

where $[\text{Phen-TMPyP}]_0$ and $[\text{DNA}]_0$ are the initial concentrations of Phen-TMPyP and DNA, respectively, x_1 and x_2 are the binding ratios of the two binding modes, n_1 represents the base pairs occupied by one binding mode, and n_2 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 x_1 and x_2. The observed absorbance (Abs_T) can be expressed using the following equation:

$$Abs_T = Abs_f(1-x_1-x_2) + Abs_{B1}x_1 + Abs_{B2}x_2 \quad (3),$$

where Abs_f 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 K_1 are comparable with those of pyrene-[6] and anthracene[7]-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

DNA	K_1 / M^{-1}	n_1	K_2 / M^{-1}	n_2
AATT	1.3×10^6	2.8	1.0×10^7	0.7
AGTC	1.3×10^6	2.7	1.0×10^7	0.7

K_1: association constant of groove binding (and/or intercalation)
n_1: occupied base pairs by groove binding (and/or intercalation)
K_2: association constant of aggregation
n_2: 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 $-\Delta G$ of the electron transfer is 0.18 eV,[12] 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 (τ_f) 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, H_2TMPyP (τ_f: 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).[6,7] Furthermore, Phen-TMPyP demonstrated relatively strong fluorescence in sodium phosphate buffer without DNA (fluorescence quantum yield (Φ_f): 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 Φ_f and the DNA concentration is shown in Figure 3B. The value of the Φ_f decreased with an increase in the DNA concentration of up to 5 μM bp, possibly due to self-quenching through an aggregation. The Φ_f 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 τ_f also varied similarly to that of the Φ_f. In the presence of 50 μM bp DNA, the observed values of the τ_f 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 H_2TMPyP (11.5 ns for AATT, 9.1 ns (60%) and 3.2 ns (40%) for

AGTC). In the DNA microenvironment, the relaxation process of the S_1 state of Phen-TMPyP should be almost the same as that of H_2TMPyP. 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 Φ_f and τ_f 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.[15,16]

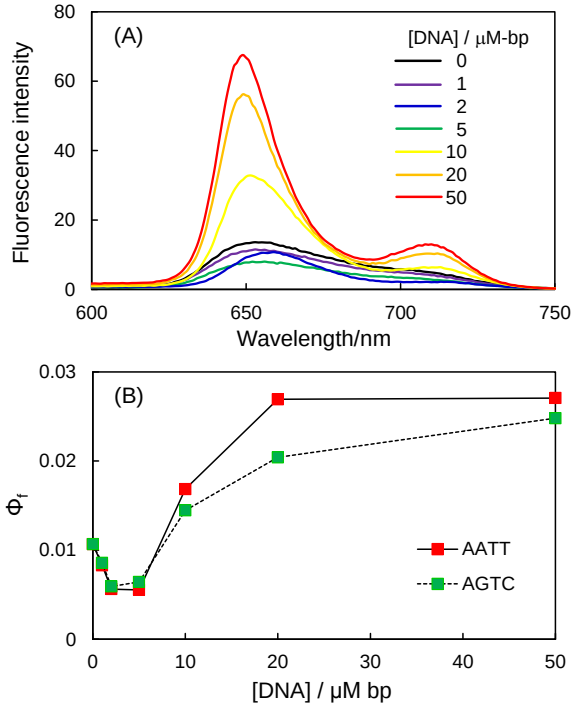

Figure 3. Fluorescence spectra of Phen-TMPyP with or without AATT (A), and the relationship between Φ_f of Phen-TMPyP and the DNA concentration (B). The sample solution contained 5 μM Phen-TMPyP in a 10 mM sodium phosphate buffer (pH 7.6) and AATT or AGTC. The excitation wavelength is 532 nm.

To evaluate the $^1O_2$ 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 $^1O_2$, was clearly observed during the photoirradiation of Phen-TMPyP without DNA (Figure 4A). The intensity of the $^1O_2$ emission depended on the AATT concentration (a similar result was observed in the case of AGTC). The quantum yield of $^1O_2$ generation (Φ_Δ) was estimated by the comparing the $^1O_2$ emission intensity of Phen-TMPyP and that of methylene blue (0.52 in water).[17] The apparent value of Φ_Δ 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 Φ_Δ and the DNA concentration. In the presence of 5 μM bp DNA, the $^1O_2$ generating activity was decreased, possibly due to aggregation (Φ_Δ=0.04 for both cases of AATT and AGTC). On the other hand, the Φ_Δ values were recovered by the addition of sufficient concentrations of DNA (50 μM bp) (Φ_Δ=0.16 and 0.14 for AATT and AGTC, respectively). However, these values were rather smaller than that without DNA. Because $^1O_2$ generation occurs in the microenvironment of DNA, the generated $^1O_2$ should interact with the DNA strand. AT-only sequences quench $^1O_2$ through a mainly physical mechanism with the rate coefficient of 4.1×10^5 $M^{-1}s^{-1}$,[18] whereas guanine can quench $^1O_2$ through a chemical process (guanine oxidation) with a larger rate coefficient (1.7×10^7 $M^{-1}s^{-1}$).[19] Therefore, the actual values of Φ_Δ may be higher than the estimated values. The observed values of Φ_Δ suggest that the chemical process contributes slightly to $^1O_2$ 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 $^1O_2$ generation and its activity. These results suggest that interaction with DNA limits the activity control of the photosensitized $^1O_2$ 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 S_1 state of Phen-TMPyP is not effectively quenched through intramolecular electron transfer from the phenanthryl moiety. The S_1 state of Phen-TMPyP is stabilized in the DNA groove binding or intercalating state. On the other hand, the activity of $^1O_2$ generation by the photosensitizer is inhibited by the DNA itself through physical and chemical deactivation processes.

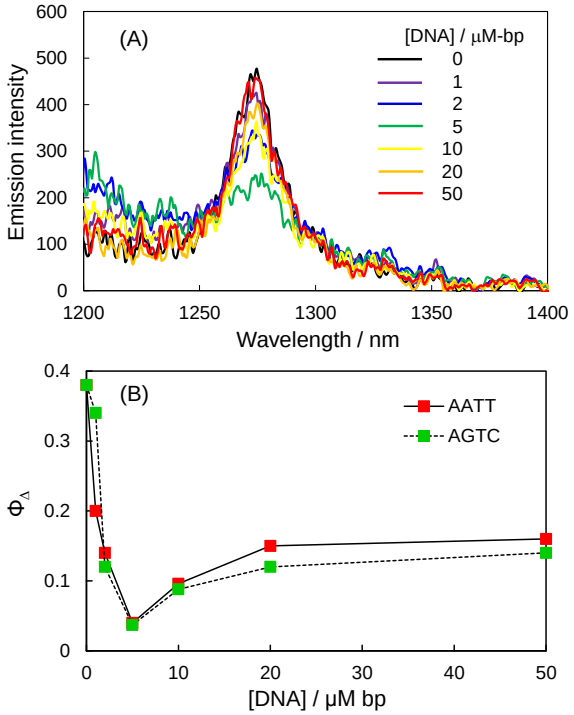

Figure 4. Near-infrared emission spectra of $^1O_2$ generated by the photosensitized reaction of Phen-TMPyP with or without AATT (A), and the relationship between Φ_Δ and the DNA concentration (B). The sample solution contained 5 μM Phen-TMPyP and the indicated concentration of AATT or AGTC in a 10 mM sodium phosphate buffer (pH 7.6). The excitation wavelength is 532 nm.

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

Received December 23, 2014; Accepted December 28, 2014

ACKNOWLEDGEMENT

We are grateful to the Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government for support of this research.

SUPPORTING INFORMATION

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

REFERENCES AND NOTES

1. Dolmans, D. E. J. G. J.; Fukumura, D.; Jain, R. K. *Nat. Rev. Cancer* **2003**, *3*, 380–387.
2. Castano, A. P.; Mroz, P.; Hamblin, M. R. *Nat. Rev. Cancer* **2006**, *6*, 535–545.
3. Wilson, B. C.; Patterson, M. S. *Phys. Med. Biol.* **2008**, *53*, R61–R109.
4. Lang, K.; Mosinger, J.; Wagneroviá, D. M. *Coordination Chem. Rev.* **2004**, *248*, 321–350.
5. Hirakawa, K.; Hirano, T.; Nishimura, Y.; Arai, T.; Nosaka, Y. *Photochem. Photobiol.* **2011**, *87*, 833–839.
6. Hirakawa, K.; Harada, M.; Okazaki, S.; Nosaka, Y. *Chem. Commun*. **2012**, *48*, 4770–4772.
7. Hirakawa, K.; Nishimura, Y.; Arai, T.; Okazaki, S. *J. Phys. Chem. B* **2013**, *117*, 13490–13496.
8. Jin, B.; Lee, H. M.; Lee, Y.-A.; Ko, J. H.; Kim, C.; Kim, S. K. *J. Am. Chem. Soc*. **2005**, *127*, 2417–2424.
9. Kim, Y. R.; Gong, L.; Park, J.; Jang, Y. J.; Kim, J.; Kim, S. K. *J. Phys. Chem*. *B* **2012**, *116*, 2330–2337.
10. Gong, L.; Bae, I.; Kim, S. K. *J. Phys. Chem. B* **2012**, *116*, 12510–12521.
11. Hirakawa, K.; Nakajima, S. *Recent Adv. DNA and Gene Seq*. **2014**, *8*, 35–43.
12. The $-\Delta G$ was roughly estimated using the following equation: $-\Delta G = E_{0\text{-}0} - e\,(E^{+} - E^{-})$, where $E_{0\text{-}0}$ is the S_1 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]
13. Yoshimi, Y.; Hayashi, S.; Nishikawa, K.; Haga, Y.; Maeda, K.; Morita, T.; Itou, T.; Okada, Y.; Ichinose, N.; Hatanaka, M. *Molecules* **2010**, *15*, 2623–2630.
14. Kalyanasundaram, K.; Neumann-Spallart, M. *J. Phys. Chem.* **1982**, *86*, 5163–5169.
15. Lewis, F. D.; Wu, Y. *J. Photochem. Photobiol. C: Photochemistry Rev.* **2001**, *2*, 1–16.
16. Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541–5553.
17. Usui, Y.; Kamogawa, K. *Photochem. Photobiol.* **1974**, *19***,** 245–247.
18. Petroselli, G.; Dántola, M. L.; Cabrerizo, F. M.; Capparelli, A. L.; Lorente, C.; Oliveros, E.; Thomas, A. H. *J. Am. Chem. Soc.* **2008**, *130*, 3001–3011.
19. Petroselli, G.; Erra-Balsells, R.; Cabrerizo, F. M.; Lorente, C.; Capparelli, A. L.; Braun, A. M.; Oliveros, E.; Thomas, A. H. *Org. Biomol. Chem.* **2007**, *5*, 2792–2799.