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Communication

# Photochemical Property and Photodynamic Activity of Tetrakis(2-naphthyl) Porphyrin Phosphorus(V) Complex

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**ABSTRACT:** To examine the photosensitized biomolecules damaging activity, dimethoxyP(V)tetrakis(2-naphthyl)porphyrin (NP) and dimethoxyP(V)tetraphenylporphyrin (PP) were synthesized. The naphthyl moiety of NP hardly deactivated the photoexcited P(V)porphyrin ring in ethanol. In aqueous solution, the naphthyl moiety showed the quenching effect on the photoexcited porphyrin ring, possibly through electron transfer and self-quenching by a molecular association. Binding interaction between human serum albumin (HSA), a water soluble protein, and these porphyrins could be confirmed by the absorption spectral change. The apparent association constant of NP was larger than that of PP. It is explained by that more hydrophobic NP can easily bind into the hydrophobic pockets of HSA. The photoexcited PP effectively induced damage of the tryptophan residue of HSA, through electron transfer-mediated oxidation and singlet oxygen generation. NP also induced HSA damage during photo-irradiation and the contributions of the electron transfer and singlet oxygen mechanisms were speculated. The electron transfer-mediated mechanism to the photosensitized protein damage should be advantageous for photodynamic therapy in hypoxic condition. The quantum yield of the HSA photodamage by PP was significantly larger than that of NP. The quenching effect of the naphthyl moiety is considered to suppress the photosensitized protein damage. In conclusion, the naphthalene substitution to the P(V)porphyrins can enhance the binding interaction with hydrophobic biomacromolecules such as protein, however, this substitution may reduce the photodynamic effect of P(V)porphyrin ring in aqueous media.

Photosensitized protein damage is an important process in medicinal applications of photochemical reaction, such as photodynamic therapy (PDT) of cancer. PDT is a less-invasive treatment of cancer and some non-malignant conditions.[1-3] In general, important mechanisms of biomolecules damage by PDT are the singlet oxygen ($^1O_2$) generation and photo-induced electron transfer from biomolecules such as proteins. Because oxygen concentration in cancer cells is low,[4,5] the mechanism through electron transfer is considered to be an advantageous mechanism for PDT. For this purpose, we have examined P(V)porphyrin derivatives, which can induce oxidation of protein through the electron transfer under visible-light irradiation.[6,7] Ligand-substituted derivatives of tetraphenylporphyrin P(V) complexes have been synthesized and their protein photodamaging activity were investigated. In this study, tetranaphthylporphyrin P(V) complex and its reference compound (Figure 1) were synthesized to examine the photochemical activity for PDT. A redshift of absorption band and enhancement of binding interaction with protein were expected in the case of the naphthylP(V)porphyrin.

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DimethoxyP(V)tetrakis(2-naphthyl)porphyrin (NP) and dimethoxyP(V)tetraphenylporphyrin (PP) were synthesized (Figure 1) according to the previous reports (Supporting information).[6,7] We tried to synthesize the P(V) complex of tetrakis(1-naphtyl)porphyrin (1-TNP). However, the incorporation of phosphorus atom into the free base 1-TNP could not be established, whereas this reaction could proceed relatively easily in the case of tetrakis(2-naphtyl)porphyrin (2-TNP). In general, P(V)porphyrins are sterically hindered molecules. Steric hindrance around the central atom due to an interaction between the axial ligand connecting to the central atom of porphyrin ring and the hydrogen atom of the 1-naphthyl moiety may inhibit the incorporation of phosphorus atom into 1-TNP (Figure 2). The calculated enthalpy change of this reaction of 1-TNP (191 kJ mol$^{-1}$) was larger than that of 2-TNP (110 kJ mol$^{-1}$), supporting that a steric effect inhibits the phosphorus atom incorporation into 1-TNP.

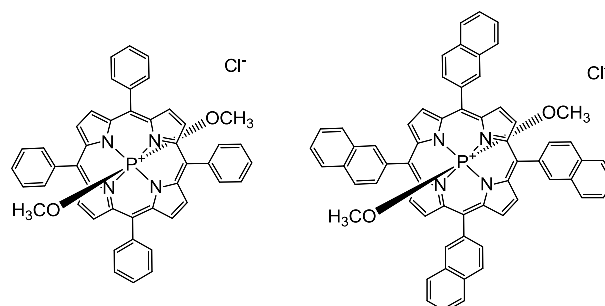


**Figure 1.** Structures of PP (left) and NP (right).

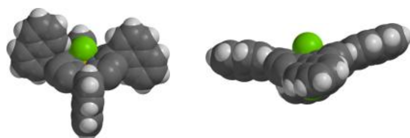

**Figure 2.** Optimized structures of P(V) complexes of 1-TNP (left) and 2-TNP (right). These structures were obtained by the semi-empirical molecular orbital calculation at the PM3 level utilizing Spartan'10 (Wavefunction Inc., CA, USA).

The photochemical properties of NP and PP were examined in ethanol. Absorption and fluorescence spectra of NP were slightly redshifted compared with those of PP (Figure 3 and Table 1). The redshift of absorption band of P(V)porphyrin photosensitizer is important for PDT. Naphthyl moiety slightly contributed to the redshift, however this effect was not large. The fluorescence quantum yield ($\Phi_f$) of NP, which was determined relative to that of bis(ethyleneglycoxy)P(V) tetraphenylporphyrin ($\Phi_f$ =0.048 in water)[8], was slightly larger than that of PP in ethanol. The time profile of fluorescence intensity of NP in ethanol could be fitted by a single exponential function, whereas that of PP could be analyzed by a double exponential function, suggesting the two different conformations of PP in ethanol solution. The fluorescence lifetime ($\tau_f$) of these porphyrins in ethanol is shown in Table 1. A quenching effect of the naphthyl moiety on the photoexcited state of the porphyrin was hardly observed in ethanol.

To examine the interaction between porphyrins and protein and their protein damaging activity, the following experiments were performed. Human serum albumin (HSA), a water soluble protein,[9] was used as target biomolecule. Sample solutions containing 5 μM porphyrins and 10 μM HSA in a 10 mM phosphate buffer (pH 7.6) containing 2.5% (vol/vol) ethanol were irradiated with a light-emitting diode (LED) (519 nm, 1 mW cm$^{-2}$). Damage of HSA was evaluated by a fluorometry of the tryptophan residue.[6,7] The excitation wavelength for this fluorometry is 298 nm.

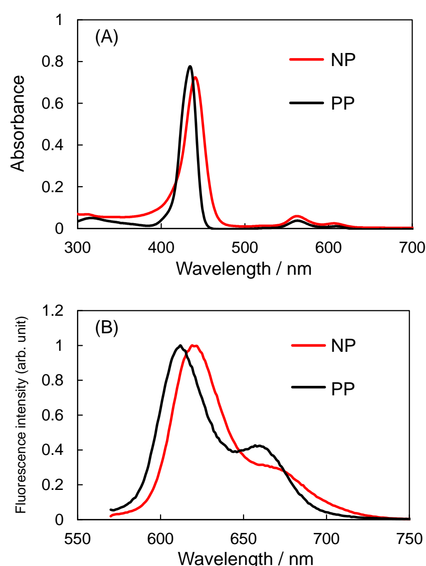


**Figure 3.** Absorption (A) and fluorescence (B) spectra of NP and PP. The sample solution contained 5 μM NP or PP in ethanol. The excitation wavelength is 400 nm. The fluorescence intensities were normalized at their peaks.

**Table 1.** Photochemical Properties of NP and PP in ethanol

| Porphyrin | $\lambda_{abs}$ / nm | $\lambda_f$ / nm | $\Phi_f$ | $\tau_f$ / ns (fraction) |
|---|---|---|---|---|
| NP | 441<br>562, 607 | 619, 674$^s$ | 0.034 | 3.6 (1.00) |
| PP | 434<br>562, 609 | 612, 654 | 0.023 | 4.7 (0.84)<br>1.1 (0.16) |

$\lambda_{abs}$: absorption maximum. $\lambda_f$: fluorescence maximum. s: shoulder.

The binding interaction between porphyrins and HSA was confirmed by the redshift of absorption spectrum of these porphyrins in the presence of HSA. The apparent association constant ($K_{ap}$) between HSA and NP or PP in a 10 mM sodium phosphate buffer (pH 7.6) plus 2.5% (vol/vol) ethanol was determined by the previously reported methods.[6,7] The $K_{ap}$ can be expressed as follows:

$$K_{ap} = \frac{[\text{Porphrin} - \text{HSA}]}{[\text{Porphyin}]\,[\text{HSA}]} \quad (1)$$

where [Porphyrin-HSA] is the concentration of the porphyrin binding with HSA, [Porphyrin] is that of the non-binding porphyrin, and [HSA] is the concentration of HSA without porphyrin. The obtained values of $K_{ap}$ were as follows: $1.8\times10^5$ $M^{-1}$ (PP) and $2.5\times10^6$ $M^{-1}$ (NP). Since NP is insoluble in water, the interaction of NP with hydrophobic pocket of HSA should be relatively strong.

Photochemical properties of NP and PP with or without HSA in a 10 mM sodium phosphate buffer (pH 7.6) containing 2.5% (vol/vol) ethanol were summarized in Table 2. The value of $\Phi_f$ for NP was smaller than that of PP and the $\tau_f$ of NP in this solution was also shorter than that of PP. These results suggest the quenching effect of the naphthyl moiety on the photoexcited porphyrin, possibly through intramolecular electron transfer from the naphthyl moiety, in aqueous buffer solution. In addition, self-quenching by an association of NP molecules may participate in the deactivation process of photoexcited NP. In the presence of HSA, the values of $\Phi_f$ and $\tau_f$ for NP increased in this solution, suggesting that the hydrophobic environment of HSA inhibits the quenching through the intramolecular electron transfer. In the case of PP, the value of $\Phi_f$ was decreased by HSA and the shorter fluorescence lifetime species were observed in the presence of HSA. These findings support that photoexcited PP can induce HSA oxidation through electron transfer mechanism.

Photosensitized $^1O_2$ generation by these porphyrins was confirmed by the measurement of near-infrared emission around 1,270 nm (Supporting information). The quantum yield of $^1O_2$ generation ($\Phi_\Delta$) by these porphyrins was determined by the comparison of the emission intensity with that of methylene blue ($\Phi_\Delta$=0.52 in water).[10] The $\Phi_\Delta$ value of PP in a 10 mM sodium phosphate buffer (pH 7.6) with 2.5% (vol/vol) ethanol was relatively large (0.93), however, $^1O_2$ generation by NP was not confirmed in this experimental condition. From the analysis of the time profile of the $^1O_2$ emission, the lifetime ($\tau_T$) of the excited triplet state ($T_1$) of PP was estimated according to the literature.[11] The result showed the elongation of the $\tau_T$ of PP through an interaction with HSA, possibly due to sterically inhibition of the quenching by oxygen molecule and the vibrational deactivation of the $T_1$ of PP.

**Table 2.** Photochemical Properties of NP and PP in sodium phosphate buffer

| Porphyrin | $\Phi_f \times 10^{-3}$ | $\tau_f$ / ns (fraction) | $\Phi_\Delta$ | $\tau_T$ / μs |
|---|---|---|---|---|
| NP | 1.8 | 3.8 (0.40)<br>1.1 (0.13)<br>0.13 (0.47) | ND | ND |
| NP+HSA | 5.7 | 4.8 (0.33)<br>2.0 (0.42)<br>0.51 (0.25) | ND | ND |
| PP | 17.2 | 5.5 (0.83)<br>3.3 (0.17) | 0.93 | 2.3 |
| PP+HSA | 8.1 | 7.4 (0.29)<br>3.9 (0.61)<br>1.0 (0.10) | 0.28 | 7.6 |

Sample solution contained 5 μM NP or PP with or without 10 μM HSA in a 10 mM sodium phosphate buffer (pH 7.6) plus 2.5% (vol/vol) ethanol. The excitation wavelength is 400 nm.

The fluorescence intensity of HSA around 350 nm, assigned to tryptophan residue, was decreased by the photosensitized reaction of PP and NP, suggesting the protein photodamage. The time course of HSA damage by NP and PP during photo-irradiation was shown in Figure 4. Photosensitized damage of HSA by PP has already been reported in the slightly different experimental condition.[6] Form the previous report, protein damage photosensitized by PP could be explained by the photooxidation of tryptophan residue into oxidized products, such as *N*-formylkynulenine [12,13] through photosensitized $^1O_2$ generation, photo-induced electron transfer or both. HSA photodamage by PP was inhibited by sodium azide ($NaN_3$), which is a quencher of reactive oxygen species (ROS), $^1O_2$ [14] and superoxide anion ($O_2^{\bullet-}$) (Figure 4).[15] In general, P(V)porphyrins hardly photosensitize $O_2^{\bullet-}$ generation.[16] Therefore, the present results support the contribution of $^1O_2$. In the presence of 10 mM $NaN_3$, ROS-mediated HSA damage should be completely inhibited. Therefore, the observed HSA damage in the presence of $NaN_3$ could be explained by the electron transfer-mediated mechanism. The estimated contributions of the protein damage through electron transfer and ROS generation mechanisms by PP were 68% and 32%, respectively. The ROS generation mechanism should be explained by the $^1O_2$-mdiated process.

Apparently, HSA photodamage by NP was small. The quantum yield of HSA damage photosensitized by these porphyrins for 30 min irradiation was estimated from the decrease of the tryptophan fluorescence and the absorbed photon number by these porphyrins. The estimated value in the case of NP ($3.2\times10^{-3}$) was significantly smaller than that of PP ($3.2\times10^{-2}$). Naphthyl moiety of NP quenched the photoexcited state of NP, resulting in the suppression of protein photo-damaging activity. The contribution of the protein damaging mechanisms by NP was also calculated from the results in Figure 4. The obtained values for the electron transfer and ROS generation ($^1O_2$ generation) mechanisms were 56% and 44%, respectively. Hydrophobic NP can strongly bind to HSA, small amount of $^1O_2$ generated through the photosensitized reaction of NP in the vicinity of the tryptophan residue may effectively contribute to protein damage.

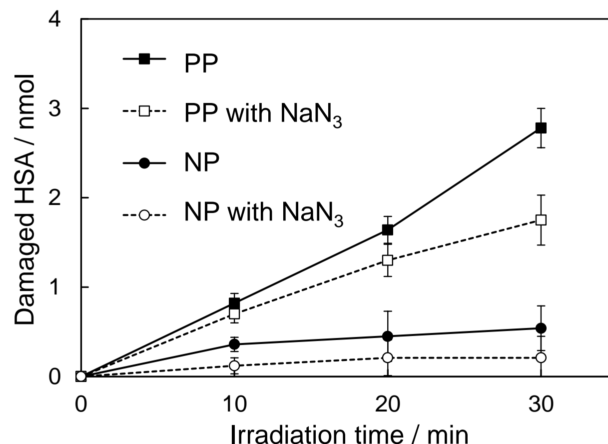


**Figure 4.** Time course of the photosensitized HSA damage by NP and PP. The sample solution (1.2 mL) contained 5 μM NP or PP and 10 μM HSA in a 10 mM sodium phosphate buffer (pH 7.6) plus 2.5% (vol/vol) ethanol. The concentration of $NaN_3$ was 10 mM. The sample solution was irradiated with an LED (519 nm, 1 mW cm$^{-2}$).

The Gibbs free energy ($-\Delta G$) for the electron transfer oxidation of the tryptophan residue by the photoexcited P(V)porphyrins was roughly calculated from the following equation:

$$-\Delta G = E_{0\text{-}0} - e(E_{ox} - E_{red}) \quad (2),$$

where $E_{0\text{-}0}$ is the 0-0 transition energy of porphyrins calculated from the fluorescence maxima (2.00 eV for NP and 2.03 eV for PP), $e$ is charge of the electron, $E_{ox}$ is the oxidation potential of tryptophan (0.65 V vs SCE),[17] and $E_{red}$ is the reduction potential of the P(V)porphyrin ring (-0.50 V vs. SCE).[6,7,18] Because the charge of the P(V) porphyrin is neutralized by the electron transfer, the factor of the distance between the electron donor and acceptor is negligible.[18,19] The estimated values of $-\Delta G$ (0.85 eV for NP and 0.88 eV for PP) suggest that the oxidation of the tryptophan residue of HSA through the electron transfer by photoexcited these porphyrins is thermodynamically possible (Figure 5). The photoexcited state of PP and NP ($S_1$) should induce the oxidation of tryptophan residue of HSA, resulting in the formation of the charge transfer state (CT) including the tryptophan cation radicals. The formed radicals should undergo further reaction with water and oxygen molecule, leading to the formation of the final oxidized products such as *N*-formylkynulenine.[12,13] Alternately, the $^1O_2$ generation from their $T_1$ also contribute to the tryptophan oxidation. In the case of the $^1O_2$-mediated oxidation also produces the formation of *N*-formylkynulenine.

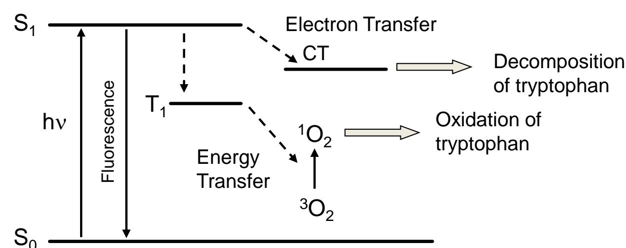


**Figure 5.** Scheme of the relaxation process of the photoexcited P(V)porphyrins and the mechanism of protein damage.

In summary, photoexcited NP and PP can induce damage of the tryptophan residue of HSA. The quantum yield of HSA photodamage by PP was significantly larger than that by NP in a sodium phosphate buffer. This result can be explained by the quenching of the photoexcited P(V)porphyrin ring of NP through intramolecular electron transfer from the naphthyl moiety and self-quenching by a molecular association, though the quenching effect of the naphthyl moiety was hardly observed in ethanol solution. This study suggests that the naphthyl moiety of P(V)porphyrin reduce the photosensitized biomolecule damage in aqueous media. However, protein damage through the electron transfer mechanism was confirmed in the case of NP. The electron transfer-mediated mechanism to the photosensitized biomolecule damage should be advantageous for PDT in hypoxic condition, such as cancer cell. In addition, the recognition of protein by P(V)porphyrin photosensitizer and the binding constant could be improved by the naphthalene substitution to the P(V)porphyrins.

**KEYWORDS**: Porphyrin P(V) complex, Naphthylporphyrin, Photodynamic therapy, Singlet oxygen, Electron transfer, Protein damage

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**SUPPORTING INFORMATION**

Experimental procedures; synthesis of porphyrins; measurements of absorption and fluorescence spectra and fluorescence lifetime, measurement of near-infrared emission spectra of singlet oxygen, calculations.

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