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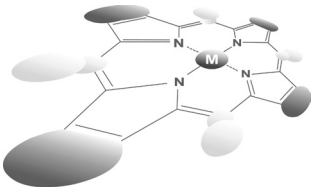

# Photosensitized damage of protein by fluorinated diethoxyphosphorus(V)porphyrin

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**ABSTRACT:** The effect of the axial ligand fluorination of the water-soluble P(V)porphyrin complex on photosensitized protein damage was examined. The activity of singlet oxygen generation by diethoxyP(V) porphyrin was slightly improved by the fluorination of the ethoxy chains. Absorption spectrum measurements demonstrated the binding interaction between the P(V)porphyrins and human serum albumin, a water-soluble protein. Photo-irradiated P(V)porphyrins damaged the amino acid residue of human serum albumin, resulting in the decrease of the fluorescence intensity from the tryptophan residue of human serum albumin. A singlet oxygen quencher, sodium azide, could not completely inhibit the damage of human serum albumin, suggesting that the electron transfer mechanism contributes to protein damage as does singlet oxygen generation. The decrease of the fluorescence lifetime of P(V)porphyrin by human serum albumin supported the electron transfer mechanism. The estimated contributions of the electron transfer mechanism are 0.57 and 0.44 for the fluorinated and non-fluorinated P(V)porphyrins, respectively. The total quantum yield of the protein photo-oxidation was slightly enhanced by this axial fluorination.

**KEYWORDS:** P(V)porphyrin, fluorination, photosensitizer, singlet oxygen, electron transfer, protein oxidation.

## INTRODUCTION

Porphyrins and their analogues are the most commonly administered photosensitizers in the photodynamic therapy (PDT), which is a promising treatment of cancer and some non-malignant conditions [1–3]. In general, administered photosensitizers damage cancer cells by the generation of singlet oxygen ($^1O_2$) (Type II mechansim), which is formed through energy transfer to molecular oxygen from the photoexcited photosensitizer. However, the phototoxic effect of $^1O_2$ on the PDT is restricted because the oxygen concentration in a cancer cell is relatively low [4]. Another important mechanism of photosensitized biomolecule damage is the oxidation reaction through electron transfer (ET) (Type I mechanism), which requires absolutely no oxygen [5]. The ET mechanism requires highly oxidative activity (a lower reduction potential) in the photoexcited state of the photosensitizer. Larger excitation energy is advantagous for the lower reduction potential of the photosensitizer in the photoexcited state. Ultra-violet photosensitizers mainly induce biomolecule photodamage through the ET mechanism, whereas a visible-light photosensitizer is not appropriate for this mechanism. Therefore, it is

◊SPP full member in good standing

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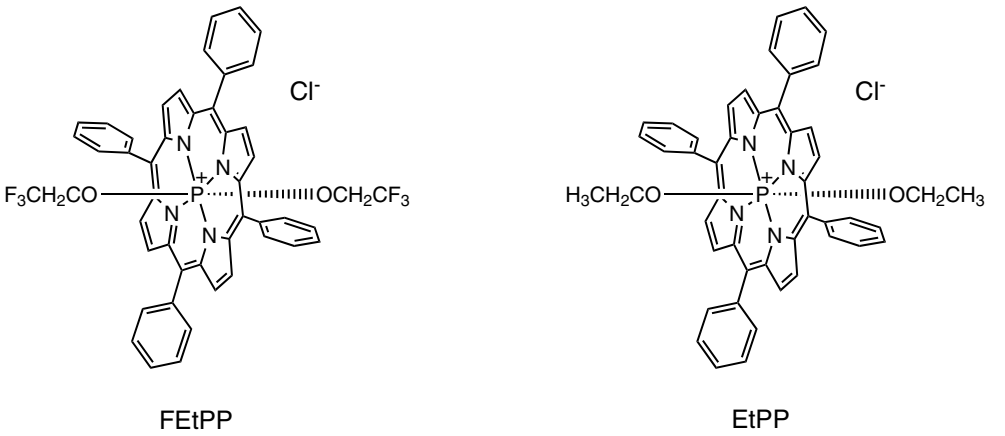


FEtPP EtPP

**Fig. 1.** Structures of FEtPP (left) and EtPP (right)



important to select the appropriate molecular design to achieve ET-mediated biomolecule damage using a visible-light photosensitizer. Since high-valent porphyrin complexes demonstrate a lower reduction potential in their photoexcited state than free-base or low-valent metal complexes, these porphyrins are advantageous for the oxidative ET reaction [5–13]. Indeed, derivatives of high-valent porphyrin complexes, such as P(V) [5, 9] and Sb(V) [13] complexes, photosensitize DNA damage through two mechanisms, *i.e.* $^1O_2$ generation and the ET reaction. In this study, photosensitized protein oxidation by a porphyrin P(V) complex (Fig. 1), diethoxyP(V) tetraphenylporphyrin (EtPP) and its axial fluorinated compound (FEtPP), was examined. The specific characteristics of the porphyrin P(V) complexes are the variety of the substituted axial ligand and the relatively low redox potential of the one-electron reduction in the photoexcited state. In addition, P(V)porphyrin is cationic and water-soluble. The purpose of this study is the evaluation of a fluorination effect of the axial ligand on the photosensitized reaction. As a target protein model, human serum albumin (HSA), a water-soluble protein, was used, because its structure and property were elucidated.

## EXPERIMENTAL

### Materials

DichloroP(V)tetraphenylporphyrin chloride ($Cl_2PP$) was obtained by the phosphorus incorporation into commercially available tetraphenylporphyrin (Wako Chemicals Co., Osaka, Japan) according to the previous report [14].

EtPP was synthesized according to the previous report [6] using the following procedure. 20 mg of $Cl_2PP$ was dissolved in 2 mL of ethanol to reflux at 80 °C for 2 h. Solvent was removed under vacuum. The residue was purified by column chromatography on silica gel with an eluent of chloroform-methanol (4/1, vol/vol), resulting in a pure product with 78% yield. $^1$H NMR ($CDCl_3$, TMS): δ, ppm -2.40 ~ -2.29 (4H, m, P-OC$H_2$CH$_3$), -1.74 (6H, td, $J_{H\text{-}H}$ = 6.0 Hz, $J_{P\text{-}H}$ = 2.1 Hz, P-OCH$_2$C$H_3$), 7.78 ~ 7.81 (12H, m, *meta*- and *para*-H of phenyl group), 7.94 ~ 8.01 (8H, m, *ortho*-H of phenyl group), 9.07 (8H, d, $J_{H\text{-}H}$ = 2.7 Hz, βH). MS (FAB): *m/z* 733 (calcd. for $[M]^+$ 733). UV-vis (ethanol): $\lambda_{max}$, nm 423.5, 555.0, 594.0.

FEtPP was synthesized by the similar procedure with that of EtPP. 20 mg of $Cl_2PP$ was dissolved in 2 mL of trifluoroethanol to reflux at 80 °C for 2 h. Solvent was removed under vacuum. The residue was purified by column chromatography on silica gel with an eluent of chloroform-methanol (4/1, vol/vol), resulting in a pure product with 91% yield. $^1$H NMR ($CDCl_3$, TMS): δ, ppm -2.05 ~ -1.94 (4H, m, P-OC$H_2$CF$_3$), 7.79 (4H, t, $J_{H\text{-}H}$ = 1.8 Hz, *para*-H of phenyl group), 7.81 (8H, d, $J_{H\text{-}H}$ = 1.8 Hz, *meta*-H of phenyl group), 7.96 ~ 8.00 (8H, m, *ortho*-H of phenyl group), 9.19 (8H, d, $J_{H\text{-}H}$ = 3.0 Hz, βH). MS (FAB): *m/z* 841 (calcd. for $[M]^+$ 841). UV-vis (ethanol): $\lambda_{max}$, nm 428.5, 559.0, 600.0.

HSA was from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). Ethanol and sodium azide were from Wako Chemicals Co.. Deuterium oxide ($D_2O$) was from Acrross Organics (New Jersey, USA). Sodium phosphate buffer (pH 7.6) was from Nacalai Tesque Inc. (Kyoto, Japan).

### Spectroscopic measurements

The absorption spectrum of P(V)porphyrins and HSA was measured with a UV-vis spectrophotometer UV-1650PC (Shimadzu, Kyoto, Japan). The fluorescence spectra of P(V)porphyrins and HSA were measured with an F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

### Detection of damage to HSA photosensitized by P(V) porphyrins

As a target biomacromolecule, HSA, a water-soluble protein, was used. The interaction between P(V) porphyrins and HSA was examined by a UV-vis absorption

measurement. The sample solution containing 8 μM P(V) porphyrins and 10 μM HSA in a sodium phosphate buffer (pH 7.6) was irradiated with a light-emitting diode (LED) ($\lambda_{max}$ = 519 nm, 1 mW.cm$^{-2}$, CCS Inc., Kyoto, Japan). The intensity of the LED light source was measured with a light meter (LM-331, AS ONE, Osaka, Japan). Protein damage by P(V)porphyrins was evaluated by measurement of the fluorescence intensity from the amino acid residues as previously reported [15]. The excitation and detection wavelengths were 298 and 350 nm, respectively.

### Detection of singlet oxygen

The $^1O_2$ generation was directly measured by near-infrared luminescence at around 1270 nm from $^1O_2$, which corresponds to the $^1O_2(^1\Delta_g)$–$^3O_2(^3\Sigma_g^-)$ transition. The procedure is the same as that described in an earlier report [16]. The quantum yield of $^1O_2$ generation ($\Phi_\Delta$) was estimated from the comparison of the emission intensity with that of the reference photosensitizer, methylene blue ($\Phi_\Delta$ = 0.52 in $H_2O$) [17].

The kinetics of $^1O_2$ generation and its decay were examined by the time-resolved near-infrared emission measurement. The sample solutions of 2 mL contained 8 μM P(V)porphyrins with or without HSA in a sodium phosphate buffer (pH 7.6). The excitation light was the second harmonic (532 nm) of a pulsed Nd:YAG laser (5 ns, 10 Hz, Minilite-II, Continuum, CA, USA). 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 (CLDM-50S, Sigma Koki, Tokyo, Japan), separated by a Bosch-Lomb Shimadzu monochromator, and then introduced into a photomultiplier (R5509–41, Hamamatsu Photonics, Hamamatsu, Japan), which was cooled to 200 K with liquid nitrogen. The signal from the photomultiplier was amplified by 75 with an amplifier (SR-455, Stanford Research, CA, USA) and then counted with a scaler/averager (SR430, Stanford Research). 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 20,000 scans with a bin width of 40 ns.

### Electrochemical measurements

The redox potentials of P(V)porphrins 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.

### Fluorescence lifetime measurements

Fluorescence decay was measured using a time-correlated single-photon counting method [18]. Laser excitation at 410 nm was achieved by using a diode laser (LDH-P-C-410, PicoQuant, Berlin, Germany) with a power control unit (PDL 800-B, PicoQuant) in a repetition rate of 2.5 MHz. The temporal profiles of fluorescence decay were detected by using a micro-channel plate photomultiplier (R3809U, Hamamatsu Photonics) equipped with a TCSPC computer board module (SPC630, Becker and Hickl Gmbh, Berlin, Germany). The full-width at half-maximum (FWHM) of the instrument response function was 51 ps. The values of $\chi^2$ and the Durbin-Watson parameters were used to determine the quality of the fit obtained by nonlinear regression.

## RESULTS AND DISCUSSION

### Interaction between P(V)porphyrins and HSA

In the presence of HSA, the hyperchromic effect and red-shift were observed in the UV-vis absorption spectra of FEtPP (Fig. 2), indicating the static interaction between FEtPP and the protein. The analysis of the absorption spectrum suggests the 4:1 complex formation between both P(V)porphyrins and HSA (inset of Fig. 2). Similar results were observed in the case of EtPP. Job's plot of the absorption change showed the intersection points at *ca*. 0.2 in the both cases of FEtPP and EtPP, supporting the 0.8:0.2 (= 4:1) complex formation (Fig. 3). The apparent association constant ($K_{ap}$) between P(V)poprhyrins and HSA was evaluated under an assumption of the following equation:

$$K_{ap} = \frac{[\text{P(V)porphyrin-HSA}]}{[\text{P(V)porphyrin}][\text{HSA}]_b} \quad (1)$$

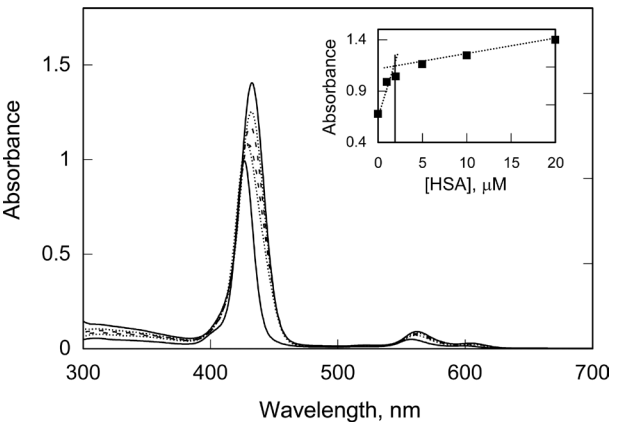


**Fig. 2.** Absorption spectra of FEtPP in the presence of HSA. The sample solution contained 8 μM FEtPP and HSA (1, 2, 5, 10, or 20 μM) in a 10 mM sodium phosphate buffer (pH 7.6). The inset indicates the relationship between the absorbance of P(V)porphyrin at 440 nm and the concentration of HSA. The intersection point of two asymptotes indicates almost 2 μM of HSA, suggesting the 4:1 complex formation between P(V) porphyrin and HSA

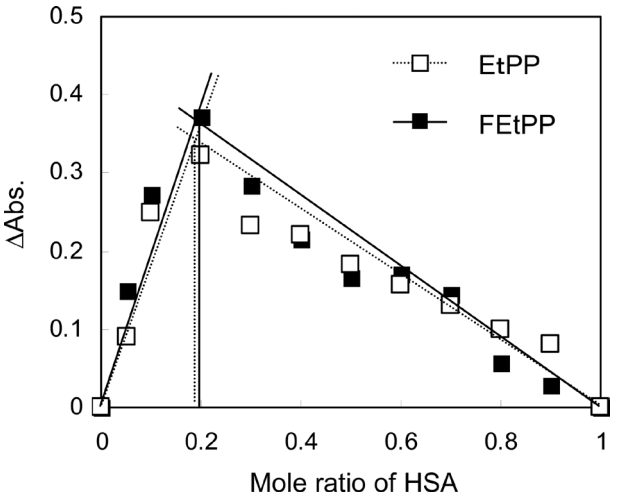


**Fig. 3.** Job's plots of the Soret band peak of P(V)porphyrins with HSA. The sample solution contained 0 ~ 10 μM FEtPP or EtPP and 0 ~ 10 μM HSA in a 10 mM sodium phosphate buffer (pH 7.6). The total concentration of P(V)porphyrin and HSA was 10 μM

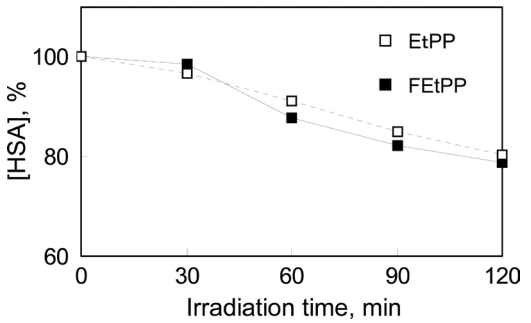


**Fig. 4.** Time course of HSA damage photosensitized by FEtPP and EtPP. The sample solution contained 8 μM P(V)porphyrins and 10 μM HSA in a 10 mM sodium phosphate buffer (pH 7.6). The vertical axis "[HSA]" indicates the relative concentration of non-damaged HSA

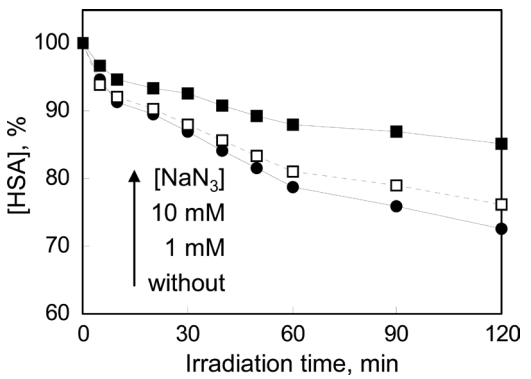


**Fig. 5.** Effect of sodium azide ($NaN_3$) on HSA photo-oxidation by FEtPP. The sample solution contained 8 μM FEtPP, 10 μM HSA and indicated concentration of $NaN_3$ in a 10 mM sodium phosphate buffer (pH 7.6). The vertical axis "[HSA]" indicates the relative concentration of non-damaged HSA

where [P(V)porphyrin] is the concentration of the non-binding photosensitizer, FEtPP or EtPP, $[HSA]_b$ is the concentration of the binding sites of HSA without a binding porphyrin (four times the actual concentration of free HSA), and [P(V)porphyrin-HSA] is the concentration of the HSA-binding photosensitizer. The estimated values of $K_{ap}$ were $4.6 \times 10^4$ $M^{-1}$ and $2.6 \times 10^4$ $M^{-1}$ for FEtPP and EtPP, respectively. The affinity between FEtPP and HSA is slightly larger than that of EtPP.

### Photosensitized damage of HSA by P(V)porphyrins

The intensity of HSA fluorescence around 350 nm, assigned to the tryptophan residue, was decreased by photo-irradiation in the presence of these P(V)porphyrins. The fluorescence decrement of HSA can be explained by the amino acid oxidation through the photosensitized reaction [15]. The observed extent of this HSA damage by the fluorinated P(V)porphyrin, FEtPP, was almost the same as that of the EtPP (Fig. 4). The quantum yields of tryptophan degradation photosensitized by P(V) porphyrins for 120 min irradiation were estimated from the decrease of the tryptophan fluorescence and the absorbed photon number by the porphyrins. The estimated yields were $2.9 \times 10^{-5}$ and $2.2 \times 10^{-5}$ for FEtPP and EtPP, respectively. The quantum yield of HSA photodamage by FEtPP was slightly larger than that of EtPP.

This HSA damage was partially inhibited by sodium azide, a physical quencher of $^1O_2$ [19] (Fig. 5). Furthermore, HSA damage was enhanced in $D_2O$ (data not shown), in which the lifetime of $^1O_2$ is markedly elongated (about 2 ~ 4 μs in $H_2O$ to 70 μs in $D_2O$) [20]. These findings suggest HSA oxidation by $^1O_2$. However, HSA damage was not completely inhibited by an excess amount of sodium azide (~10 mM). These results suggest that the ET mechanism is partly responsible for HSA photodamage, as is the $^1O_2$ mechanism. Because the almost all $^1O_2$ can be quenched by 10 mM sodium azide, the damage of HSA photosensitized by P(V) porphyrins with 10 mM sodium azide should be due to the ET mechanism. The quenching rate coefficient of $^1O_2$ by sodium azide is almost diffusion control limit ($k_{dif}$), which is calculated as follows:

$$k_{dif} = \frac{8000 R T}{3\eta} \quad (2)$$

where R is the gas constant, $T$ is the absolute temperature, and η is the viscosity of water ($8.91 \times 10^{-4}$ $kg.m^{-1}.s^{-1}$). The quenching efficiency of $^1O_2$ by sodium azide ($Ef_q$) can be calculated from the following equation using the lifetime of $^1O_2$ ($\tau_\Delta = 3.5$ μs , described in latter):

$$Ef_q = \frac{k_q[NaN_3]}{k_q[NaN_3] + 1/\tau_\Delta} \quad (3)$$

where [$NaN_3$] is the concentration of sodium azide. In the presence of 10 mM sodium azide, the $Ef_q$ becomes 0.996. The roughly estimated contributions of the HSA damage through the ET mechanism for 120 min irradiation were 0.57 and 0.44 for FEtPP and EtPP, respectively. Therefore, the contributions of the $^1O_2$ mechanism are 0.43 and 0.56 for FEtPP and EtPP, respectively. The ET mechanism was slightly enhanced in the case of fluorinated P(V) porphyrin.

The HSA damage was not observed under anaerobic conditions. The formed radical cation of the amino acid residue through the ET should undergo a reaction with the surrounding elements, such as molecular oxygen or water. Furthermore, re-oxidation of the reduced photosensitizer, which is formed thorugh ET from the amino acid residue to the photoexcited photosensitizer, is important. *In vivo*, oxidative agents, such as metal ions, might oxidize the reduced photosentizer. In *in vitro* experiments, molecular oxygen is an important oxidative agent to remove the electron from the reduced photosensitizer. The rapid reverse-ET should inhibit the following reactions in simple aqueous solution without oxygen. These results showed that the following reaction with molecular oxygen is necessary for protein oxidation through ET in this experimental condition. Formed superoxide through re-oxidation of the reduced photosensitizer should be dismutated into hydrogen peroxide and decomposed into water and molecular oxygen. The electron, which is removed from the photosensitizer, should be used to form hydroxide ion or final product of decomposed amino acid.

### Singlet oxygen generation by the photosensitized reaction of P(V)porphyrins

The photosensitized $^1O_2$ generation by these P(V) porphyrins was confirmed by the detection of near-infrared emission around 1270 nm (Fig. 6), which is assigned to the $^1O_2(^1\Delta_g)$–$^3O_2(^3\Sigma_g^-)$ transition. The estimated $\Phi_\Delta$ values for FEtPP and EtPP were 0.68 and 0.59, respectively. The fluorination of this porphyrin slightly improved the $^1O_2$ generating ability. These relatively large values of $\Phi_\Delta$ indicate that the $^1O_2$ mechanism is also important for photosensitized biomolecule damage in the presence of a sufficient concentration of molecular oxygen.

### Time profile of $^1O_2$ emission and estimated lifetime of the triplet excited state

The lifetime of the $^1O_2$ ($\tau_\Delta$) and the triplet excited state ($T_1$) of these photosensitizers ($\tau_T$) was estimated from the time-resolved emission of $^1O_2$ (data not shown). The emission intensity of $^1O_2$ as a function of time, $I(t)$, can be expressed with the following equation [21]:

$$I(t) = I_0 \left\{ \exp\left( -\frac{1}{\tau_d} \right) - \exp\left( -\frac{t}{\tau_r} \right) \right\} \qquad (4)$$

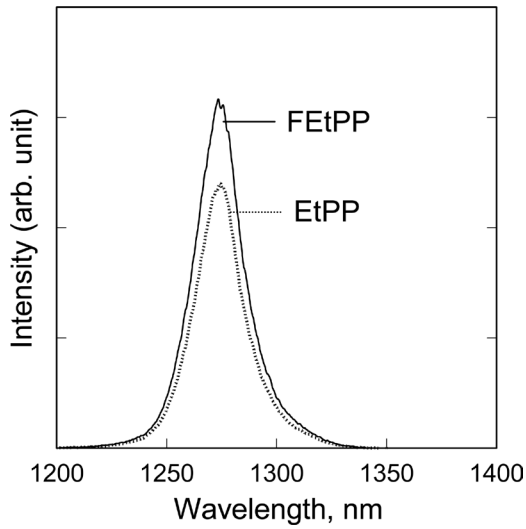


**Fig. 6.** Near-infrared emission spectra of $^1O_2$ generated by the photosensitization of FEtPP and EtPP. The sample solution contained 8 μM FEtPP or EtPP in a 10 mM sodium phosphate buffer (pH 7.6)

**Table 1.** Singlet oxygen quantum yields and the related time constants

| Porphyrin | $\Phi_\Delta$ | $\tau_\Delta$ (μs) | $\tau_T$ (μs) |
|---|---|---|---|
| FEtPP | 0.68 | 3.5 | 1.8 |
| EtPP | 0.59 | 3.5 | 1.9 |

The sample solution contained 8 μM P(V)porphyrins in a 10 mM sodium phosphate buffer (pH 7.6).

where $I_0$ is the pre-exponential factor, $\tau_d$ is the decay time constant of the emission, and $\tau_r$ is the rise time constant of this emission. When the $\tau_\Delta$ is longer than $\tau_r$, $\tau_d$ corresponds to $\tau_\Delta$. In general, $\tau_r$ equals to $\tau_T$ because the $T_1$ is dominantly quenched by $O_2$ molecules. In contrast, $\tau_r$ indicates $\tau_\Delta$, if $\tau_T$ is longer than $\tau_\Delta$. The analysis of the time-resolved $^1O_2$ emission gave the kinetic parameters, as shown in Table 1. The observed value of $\tau_d$, around 3.5 μs, almost coincided with the typical lifetime of $^1O_2$ ($\tau_\Delta$) in $H_2O$ (2 ~ 4 μs) [20]. The values of $\tau_r$ should correspond to the $\tau_T$ in $H_2O$. The estimated $\tau_T$ values indicate that $^1O_2$ is generated within about two μs in the photochemical process of P(V)porphyrin in a phosphate buffer.

### Redox potentials of P(V)porphyrins

The reversible reduction peak was observed for both P(V)porphyrins (FEtPP: -0.40 V *vs* SCE; EtPP: -0.30 V *vs* SCE) (Table 2). The oxidation potentials of P(V)porphyrin ($E_{ox}$) were roughly estimated from the reduction potential ($E_{red}$) and the energy of the singlet excited state ($S_1$) of P(V)porphyrins ($E_{S1}$). The $E_{S1}$ was calculated from the fluorescence maximum of P(V) porphyrins. The redox potential of EtPP was smaller

**Table 2.** Redox potential and the parameter about the electron transfer oxidation

| Porphyrin | $E_{red}$ (V) | $E_{ox}$ (V) | $-\Delta G$ (eV) |
|---|---|---|---|
| FEtPP | -0.40 | 1.63 | 0.98 |
| EtPP | -0.30 | 1.73 | 1.05 |

The $E_{ox}$ was calculated from the value of $E_{red}$ and the wavelength of fluorescence maximum. The $-\Delta G$ was estimated from the excitation energy of P(V)porphyrins and the redox potentials of P(V) porphyrins and tryptophan.

than that of FEtPP, suggesting that the axial ligand fluorination does not increase the electron affinity of the P(V)porphyrin ring.

The free energy change ($-\Delta G$) for the ET oxidation of the tryptophan residue by the photoexcited P(V) porphyrins was roughly calculated from the following equation [22]:

$$-\Delta G = E_{S1} - e(E_{ox}' - E_{red}) \quad (5)$$

where $e$ is the electronic charge and $E_{ox}'$ is the oxidation potential of the amino acid. The oxidation potential of tryptophan is almost 0.65 V *vs*. SCE under the similar conditions of this study [23]. Because the charge of the P(V) porphyrin is neutralized by the ET, the factor of the distance between the ET donor and acceptor is negligible [8]. The estimated values of $-\Delta G$ (Table 2) suggest that the oxidation of the tryptophan residue of HSA through the ET by the photoexcited FEtPP and EtPP is possible.

### Fluorescence quenching of P(V)porphyrins by HSA

The fluorescence lifetime ($\tau_f$) of P(V)porphyrins with or without HSA was summarized in Table 3. The time-resolved fluorescence intensity could be fitted by a single exponential function in the FEtPP case. The double exponential function was well-fitted in the case of EtPP, suggesting the conformation difference. In the presence of HSA, the decay curves could be fitted by the double exponential function for the cases of both P(V) porphyrins, indicating that the microenvironment of porphyrins is affected through the interaction of HSA. The value of $\tau_f$ was decreased by the interaction with HSA, supporting the ET reaction between amino acid and the $S_1$ of P(V)porphyrins.

### Photostability of P(V)porphyrins

The stability of these P(V)porphyrins during the photosensitized reaction was checked by UV-vis absorption measurements. The Soret band absorbance of P(V)porphyrins was decreased by photo-irradiation ($\lambda_{max}$ = 519 nm, 1 mW.cm$^{-2}$, 120 min) in the presence of 10 μM HSA as follows: 5% and 8% for FEtPP and EtPP,

**Table 3.** Fluorescence lifetime of P(V)porphyrins with or without HSA

| Porphyrin | HSA | $\tau_f$ (ns) [fraction] | |
|---|---|---|---|
| FEtPP | without | 4.43 [1.000] | |
| | + 5 μM | 4.08 [0.449] | 0.81 [0.551] |
| | + 10 μM | 4.07 [0.414] | 0.90 [0.586] |
| | + 20 μM | 4.02 [0.392] | 0.94 [0.608] |
| EtPP | without | 4.95 [0.785] | 2.37 [0.215] |
| | + 5 μM | 4.68 [0.634] | 1.43 [0.366] |
| | + 10 μM | 4.61 [0.579] | 1.39 [0.421] |
| | + 20 μM | 4.48 [0.556] | 1.33 [0.444] |

The sample solution contained 8 μM P(V)porphyrins with or without HSA in a 10 mM sodium phosphate buffer (pH 7.6). Ex = 410 nm. Em = 630 nm.

respectively. These results indicated that a part of P(V) porphyrin itself is decomposed through photosensitized reaction.

## CONCLUSION

In conclusion, P(V)porphyrins, FEtPP and EtPP, could induce protein photodamage through $^1O_2$ generation and the ET mechanism. $^1O_2$ generation is a well-known mechanism for porphyrin photosensitization [24, 25]. The ET mechanism is hardly observed in the case of protein or DNA damage by a visible-light photosensitizer [5]. The time-resolved fluorescence study suggests that the electron abstraction from the tyrptophan residue to the $S_1$ of P(V)porphyrins contributes to the ET mechanism of HSA photodamage. The radical cation of the tryptophan residue formed through ET should undergo further reaction with the surrounding elements, such as water and oxygen. An oxidized product, such as *N*-formylkynurenine, should be finally formed [26]. Since HSA damage was not observed under anaerobic conditions, the final protein damage depends on the oxygen under this experimental condition. The fluorination of the axial ligand of P(V)porphyrin slightly improved the $\Phi_\Delta$ value. The total quantum yield of the protein photodamage was slightly enhanced through this fluorination of the axial ligand of P(V)porphyrin. FEtPP and EtPP should have ability to induce DNA photodamage. The ET and $^1O_2$ generation selectively cause guanine-specific oxidation. Indeed, we have previously reported the guanine-specific damage by other P(V)porphyrin, dihydroxoP(V)tetraphenylporphyrin (OHPP) [9]. The values of $\Phi_\Delta$ of FEtPP and EtPP are lager than that of OHPP (0.28). Furthermore, the values of $E_{red}$ of FEtPP and EtPP are higher than that of OHPP (-0.5 V). Therefore, we can speculate that FEtPP and EtPP induce severe DNA photodamage at guanine residues compared with the previous reported P(V)porphyrin.

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