The Influence of Aggregation of Porphyrins on the Efficiency of Photogeneration of Hydrogen Peroxide in Aqueous Solution

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The pH-dependence of the ability of coproporphyrin (CP) and uroporphyrin (UP) to photogenerate hydrogen peroxide (H_2O_2) in aqueous solution was investigated, with special attention to the structure–activity relationship related to the aggregation of the porphyrins. It was found that the efficiency was strongly dependent on the aggregation of CP and UP mediated by changes in the pH of the solution, and a dimeric form had a weak ability to produce H_2O_2, while a highly aggregated form had a good ability. The increased efficiency of the highly aggregated porphyrin to produce H_2O_2 was further demonstrated using a different type of aggregate formed by the electrostatic interaction of cationic tetrakis-5,10,15,20-(*N*-methyl-4-pyridyl)porphin (TMPyP) with anionic tetrakis-5,10,15,20-(4-sulfonatophenyl)porphin (TSPP). The present results demonstrated the importance of the state of aggregation of porphyrin to photogenerate H_2O_2, and the results may help to develop a new type of medicine for photodynamic therapy.

Key words   coproporphyrin; uroporphyrin; water-soluble porphyrin; highly aggregated form; pH-dependence; hydrogen peroxide photogeneration

Porphyrins belong to an important class of photosensitizers used in photodynamic therapy.[1,2] Upon light irradiation, porphyrins produce singlet oxygen, which causes cytotoxic activity at specific subcellular sites, leading to cell death. The quantum yield of singlet oxygen depends on the porphyrin employed, being significantly lowered by dimerization or aggregation of the porphyrin.[3,4] The aggregation of porphyrins also affects the transfer of electrons from porphyrins to accepter molecules, and the efficiency of a monomer was greater than that of a dimer by a factor of about two.[5] We are interested in examining the influence of the aggregation of porphyrins in terms of the ability to photogenerate hydrogen peroxide (H_2O_2). Although this approach has provided significant information about the photodynamic actions of porphyrins involving the electron transfer process,[6,7] few attempts have been made to clarify structure–activity relationships, partly due to the lack of an appropriate analytical method for determining H_2O_2. Although H_2O_2 has usually been determined with a colorimetric method based on an enzymatic reaction using peroxidase coupled with phenol and 4-aminoantipyrine, there is a large degree of interference from strong absorbance due to porphyrin itself.[8] Thus, we employed a luminol chemiluminescence method making it possible to detect down to 1 μM of H_2O_2 even in the presence of a high concentration of porphyrin.[8] Using this method, we found that highly aggregated porphyrin was much more active in producing H_2O_2 than the dimer and concluded that a highly aggregated form of porphyrin had a semiconductor-like role, causing effective charge separation, leading to a larger amount of H_2O_2.[8]

As a continuation and extension of this work, we examined the effect of pH on the photogeneration of H_2O_2, because porphyrins are known to take different aggregated forms depending on their environment, including the pH of solution.[9—12] Coproporphyrin (CP) and uroporphyrin (UP) were chosen, because these porphyrins are known to photogenerate sufficient amounts of H_2O_2.[6—8] It was found that the efficiency in producing H_2O_2 was strongly dependent on

the aggregation of CP and UP mediated by changes in the pH of the solution, with a dimer suppressing, and a highly aggregated form enhancing, the production of H_2O_2. The increased efficiency with which the highly aggregated porphyrin produced H_2O_2 was further demonstrated using a different type of aggregate[13] whose formation was driven by the electrostatic interaction of cationic tetrakis-5,10,15,20-(*N*-methyl-4-pyridyl)porphin (TMPyP) with anionic tetrakis-5,10,15,20-(4-sulfonatophenyl)porphin (TSPP).

Experimental

Chemicals   Coproporphyrin I dihydrochloride (CP) and uroporphyrin I dihydrochloride (UP) were purchased from Frontier Scientific (Logan, UT, U.S.A.). Tetrakis-5,10,15,20-(*N*-methyl-4-pyridyl)porphin tosylate (TMPyP) and tetrakis-5,10,15,20-(4-sulfonatophenyl)porphin sodium salt (TSPP) were purchased from Mid-Century (Chicago, IL, U.S.A.). Luminol was obtained from Tokyo Kasei (Tokyo, Japan) and was used after recrystallization. H_2O_2 (35 wt% in water) was a product of Ishizu Seiyaku (Osaka, Japan). Iron(III) tetrakis-5,10,15,20-(*N*-methyl-4-pyridyl)porphin chloride (Fe(III)TMPyP) was synthesized from TMPyP and $FeCl_2 \cdot 4H_2O$.[14] The purity was checked using the molar extinction coefficient reported previously.[14] All other chemicals were of analytical grade.

Preparation of Porphyrin Stock Solutions   After CP and UP were dissolved in 10 mM NaOH, they were neutralized by adding appropriate amounts of 100 mM HCl, and then pure water was added to make 500 μM porphyrin stock solutions. Stock solutions containing 500 μM TMPyP or TSPP were prepared by dissolving the porphyrins directly in pure water. These solutions were kept in a refrigerator and diluted with the following solutions to make different pHs; 10 mM NaOH for pH 12.0, 10 mM Na_2HPO_4/NaOH for pH 9.5—11.0, 10 mM Na_2HPO_4/NaH_2PO_4 for pH 5.3—9.0, 10 mM NaH_2PO_4/H_3PO_4 for pH 2.3—5.0, and acidic solutions of 0.1 M H_3PO_4, 1 M H_3PO_4, and 1 M HCl for pH 0.1—1.6.

Photo-Irradiation Experiments   Photo-irradiation experiments were performed as reported previously.[8] In brief, a 5-ml volume of a 50 μM porphyrin solution was put in a cylindrical glass vessel and photo-irradiated by a 1 kW tungsten projector lamp (Rikagaku Seiki, Tokyo, Japan) for 10 min with stirring at 25 °C. Subsequently, 0.5 ml of the solution was pipetted into a plastic cell and the amount of H_2O_2 produced was determined by a luminol chemiluminescence method as described below. The rise in temperature of the solution after 10 min of photo-irradiation was at most 0.3 °C, which was negligible. We also verified that the pH of the solution did not change significantly before and after photo-irradiation experiments.

Determination of H_2O_2   A luminol chemiluminescence method was used to determine H_2O_2.[8] As a catalyst, we used Fe(III)TMPyP,[15] which

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produced a strong peak of chemiluminescence within a few seconds.[8] Luminol and Fe(III)TMPyP were dissolved in 0.1 M Na_2CO_3. To a sample solution (0.5 ml) placed in a plastic cell, a 117 μM luminol solution (1.5 ml) and a 11.7 μM Fe(III)TMPyP solution (1.5 ml) were injected simultaneously and mixed. The chemiluminescence arising from the solution was recorded for about 2 min. A Shimadzu multiconvertible spectrophotometer (Double-40) was modified to detect the chemiluminescence. The amounts of H_2O_2 photogenerated were evaluated using calibration graphs measured in the presence of both H_2O_2 and porphyrin, because porphyrin affected significantly the profile of chemiluminescence, as reported previously.[8]

Absorption Spectra and Fractionation In order to obtain information about the state of aggregation of porphyrin, absorption spectra of porphyrin (50 μM) solutions were measured on a Shimadzu UV-1200 spectrometer using a cell with a 1 mm light-path. The pH of the porphyrin solution was changed by diluting a 500 μM porphyrin stock solution with a pH-adjusting solution as described above. We described the final pH after the dilution, because the addition of porphyrin affected greatly the pH of the solution. The spectra were measured at 1 min after the pH was adjusted, because CP and UP rapidly aggregated especially in the acidic range, i.e., pH 1—5.

The absorption spectrum of CP or UP measured at each pH was fractionated into several pure components in a computer analysis, and the relative concentrations of the pure forms at individual pHs were estimated according to a procedure similar to that described previously.[16] In this analysis, several standard spectra of pure forms of porphyrins must first be selected. This was done by referring to typical standard spectra assigned previously to a monomeric form, another type of monomeric form protonated at the inner nitrogen atoms of monomeric porphyrin (abbreviated as a N-protonated monomer), and a dimeric form.[8—12] Relative concentrations of individual species were calculated with a computer program developed in our laboratory by fitting the sum of the fractions of the standard spectra of the pure forms to the spectrum observed.

Results

pH-Dependence of Absorption Spectra of CP We have recently reported that the efficiency with which porphyrins photogenerate H_2O_2 was strongly dependent on the degree of aggregation, and a dimeric form had a weak ability to produce H_2O_2, while monomeric and highly aggregated forms had a good ability.[8] In the present study, we investigated the influence of the aggregation from the viewpoint of pH-dependence, since porphyrins are known to self-associate, depending on pH.[9,10,12] First, we used CP, because this porphyrin was in a monomer–dimer equilibrium at around a neutral pH,[8,10,11] and expected to show strong pH-dependence. To obtain information on the state of the aggregation of CP, we measured absorption spectra at various pHs. Typical spectra are shown in Fig. 1a. At pH 11.76, a Soret band showing a peak at 390 nm was observed, which was previously assigned to a monomer;[10,11] however, the spectrum showed a discernible shoulder at a shorter wavelength to the side of the Soret band. When the pH decreased to acidic values such as pH 5.67, this shoulder afforded a clear peak at 368 nm, which was previously assigned to a dimer with face-to-face stacking.[10—12] When the pH decreased further such as to pH 4.30, the CP solution became slightly turbid, and the intensity of the Soret band decreased markedly, showing a feature of a highly aggregated form, as has been observed with many porphyrins.[8—10] We measured the spectrum at 1 min after adjusting the pH to 4.30. At pH 0.17, the porphyrin solution again became clear and gave a sharp peak of a Soret band at 398 nm, showing the presence of another type of monomeric species which was protonated at the inner nitrogen atoms of a monomeric CP (abbreviated as a N-protonated monomer).[12]

By analyzing these absorption spectra measured at various pHs, we examined the pH-dependence of the distribution of the monomer, N-protonated monomer, dimer, and highly aggregated species. As standard spectra of the dimer, the highly aggregated species, and the N-protonated species, we used recordings made at pH 5.67, 4.30, and 0.17, respectively, as shown in Fig. 1a. A standard spectrum of the monomer was made by subtracting the contribution of the dimer from the spectrum measured at pH 11.76. Figure 1b shows the pH-dependence of the distribution of these different CP species determined by fractionating the spectra measured at various pHs. The N-protonated monomer appeared also at around pH 5. This species was identified as a component in which one proton was bound to the inner nitrogen atoms of CP, assuming that one or two protons bound to the inner nitrogen atoms of CP gave similar spectra, as in the case of deuteroporphyrin.[12] However, the amount of the N-protonated monomer that appeared at around pH 5 was very small, probably because CP was liable to form the dimer and highly aggregated species at this pH. When the concentration of CP was diluted to 5 μM, the contribution of the N-protonated monomer significantly increased (data not shown), showing that this component was apparently present at around pH 5. Such a N-protonated monomer was more clearly observed with UP, as will be described later. Among the pH dependent distribution of the discriminated CP species, the concentration of the highly aggregated species fluctuated markedly, as can be seen from Fig. 1b. Such a fluctuation was attributable to the difficulty in matching the observed spectrum with a single standard spectrum of the highly aggregated species and further involvement of precipitated species.

pH-Dependence of Photogeneration of H_2O_2 Induced by CP Then, we examined the pH-dependence of the photogeneration of H_2O_2 caused by CP. The result is shown in Fig. 2. We confirmed that H_2O_2 did not photogenerate at all in the absence of porphyrin. A remarkable drop in efficiency to produce H_2O_2 was observed at around pH 5, where CP formed a dimer. This result was consistent with a previous finding that the dimer of porphyrins generally suppressed the production of H_2O_2.[8] On either side of this pH, H_2O_2 was effectively produced. These pH regions, stimulating the production of H_2O_2, corresponded to the ranges of the monomeric component, increasing at pH >5, and of the highly aggregated species, increasing at pH <5. This strongly suggested that the monomer and highly aggregated species provided effective structures for the photogeneration of H_2O_2, which again supported the previous conclusion.[8] The determination of H_2O_2 by measuring the luminol chemiluminescence at pH <3 was difficult in the presence of CP, due to unusual increases in the intensity of the chemiluminescence.

pH-Dependence of Absorption Spectra of UP The same experiments were performed using UP. Figure 3a shows typical absorption spectra. At pH 11.65, a Soret band was observed at 396 nm, which was assigned to a monomer, as has been reported previously.[9,11] At pH 4.72, the intensity of the band decreased markedly and two peaks were observed at 380 and 403 nm, respectively. One of the peaks, 380 nm, giving a rather broad peak with a marked decrease in the intensity of the Soret band, being blue-shifted, corresponded to an aggregated species like a dimer as in CP. However, the degree of the decrease in intensity was much greater than in the cases of the dimer reported previously[10,11] and of the CP
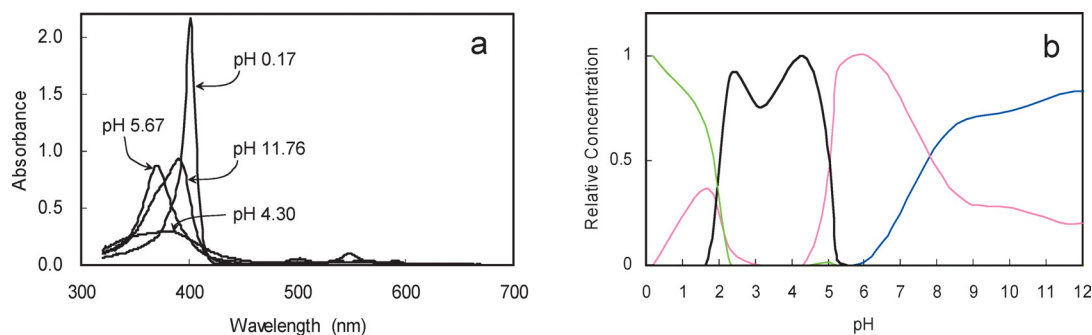
Fig. 1. Absorption Spectra of CP Measured at Various pHs (a) and the Distribution of the Discriminated CP Species, Depending on pH (b)

The spectra were measured at 50 μM using a cell with a 1 mm light-path. Blue: monomer, pink: dimer, black: highly aggregated form, green: N-protonated monomer.
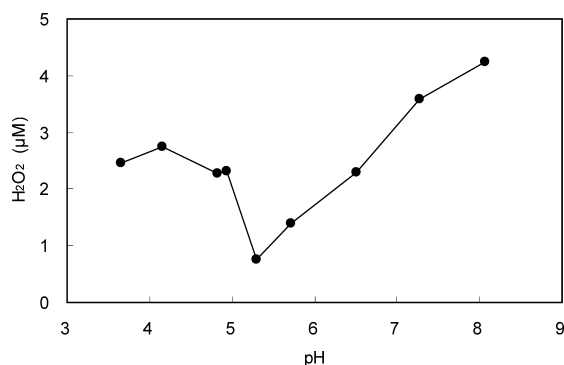

Fig. 2. pH-Dependence of CP-Induced Photogeneration of H_2O_2

The concentration of CP was 50 μM. The photo-irradiation lasted 10 min.

shown in Fig. 1a (the spectrum at pH 5.67). Thus, we assigned the broadened spectrum showing the peak at 380 nm to an oligomer whose degree of aggregation exceeded that of a dimer, but was smaller than that of a highly aggregated form. Similar spectra between the oligomer and the dimer suggested that the oligomer also formed a cofacial stacking structure. The other peak, being rather sharp and appearing at 403 nm, corresponded to a N-protonated monomer (one proton bound to the inner nitrogen atom of porphyrin), which was similar to ones observed previously with deuteroporphyrin[12] and appeared in CP. At pH 2.27, the UP solution became turbid and then precipitated. Such precipitates formed within a few minutes. We measured the spectrum at 1 min after adjusting the pH to 2.27. At pH 0.11, the solution containing UP became clear again and gave a peak of a Soret band at 403 nm. This corresponded to the species in which two protons were bound to the inner nitrogen atoms of porphyrin, enhancing the solubility. We observed that one and two protons bound to the inner nitrogen atoms of UP gave similar peaks at pHs 0.11 and 4.72, similar to the cases of deuteroporphyrin[12] and CP in this study.

Then, we examined the pH-dependence of the distribution of UP's components, as in the case of CP. The spectra obtained at pHs 11.65, 2.27, and 0.11, shown in Fig. 3a, were used as standard spectra of the monomer, the highly aggregated species, and the N-protonated monomer, respectively. A standard spectrum of the oligomer was made by subtracting the contribution of the N-protonated monomer from the spectrum measured at pH 4.72. Figure 3b shows the pH-dependence of the distribution of these different UP species determined by fractionating the spectra measured at various pHs. As in the case of CP, the concentration of the highly aggregated species of UP was an apparent one.

pH-Dependence of Photogeneration of H_2O_2 Induced by UP Figure 4 shows the pH-dependence of the photoproduction of H_2O_2 by UP. As in the case of CP, the determination of H_2O_2 in the presence of UP was difficult at pH $<$3, due to unusual increases in the intensity of chemiluminescence. Strong activity by UP to produce H_2O_2 was observed at pH $>$6, which coincided with the pH at which a monomeric form was predominant. The efficiency decreased at around pH 6, corresponding to a decrease in the monomeric component as well as the appearance of a N-protonated monomer. This indicated that the N-protonated monomer was not a preferred structure to produce H_2O_2. Probably, the presence of a cationic charge weakened the transfer of electrons from porphyrin to oxygen molecules, which is essential to produce H_2O_2.[8] At around pH 4.5, UP formed mainly an oligomer when the spectrum was measured at 1 min after adjusting the pH, but it rapidly adopted an aggregated form (data not shown). Thus, the small increase in the activity to produce H_2O_2 at this pH seemed to be attributable to the contribution of both the oligomeric and the highly aggregated forms, which were more capable than the dimeric form. Efficiency decreased greatly below pH 4, where UP initially gave the spectrum, showing a highly aggregated form, but precipitated within a few minutes. The rate of precipitation of UP arising in the acidic pH region looked to be more rapid than in the case of CP. It is reasonable to consider that the precipitated UP was excluded from the photo-reaction system, because the absorption spectrum of such species was no longer observed, resulting in a lack of ability to absorb light.

Efficiency of Porphyrin Aggregates Made by Electrostatic Interaction to Photogenerate H_2O_2 In order to further investigate the effect of aggregation, we used a different type of aggregate formed by the electrostatic interaction of cationic and anionic porphyrins. We chose TMPyP and TSPP, because these mixtures are known to make electrostatic aggregates at around neutral pH.[13] As shown in Fig. 5, TMPyP and TSPP alone showed typical absorption spectra, corresponding to monomers.[13] Although TSPP (50 μM) was reported to make a dimer in a solution of high ionic strength,[13] the ionic strength here was much lower and TSPP gave a monomer. When TMPyP and TSPP were mixed, the absorption spectra broadened markedly, showing that TMPyP and TSPP formed a face-to-face aggregate through
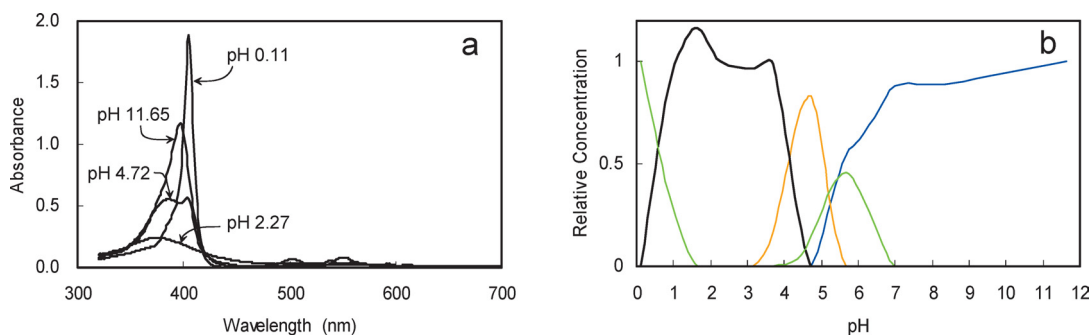
Fig. 3. Absorption Spectra of UP Measured at Various pHs (a) and the Distribution of the Discriminated UP Species, Depending on pH (b)

The spectra were measured at 50 μM using a cell with a 1 mm light-path. Blue: monomer, yellow: oligomer, black: highly aggregated form, green: N-protonated monomer.

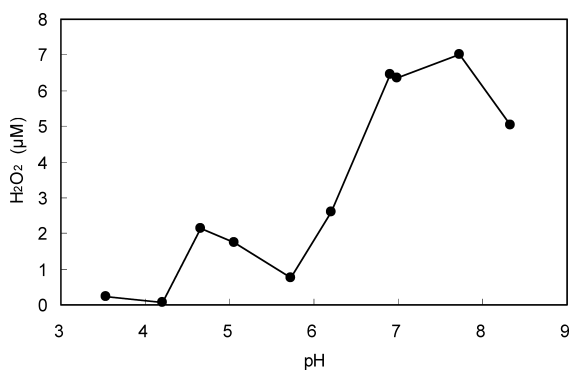Fig. 4. pH-Dependence of UP-Induced Photogeneration of H_2O_2

The concentration of UP was 50 μM. The photo-irradiation lasted 10 min.

Table 1. Amounts of Photogenerated-H_2O_2 in the Presence of TMPyP and TSPP Mixed at Various Concentrations[a]

TMPyP (μM)	TSPP (μM)	H_2O_2 (μM)
50	0	0.6
40	10	1.6
25	25	2.4
10	40	0.8
0	50	0
Filtrate[b]		0.3

a) Measurements were performed at 25 °C in 10 mM sodium phosphate buffer (pH 7.0). The photo-irradiation lasted 10 min. *b*) Obtained from the equimolar (25 μM) mixture of TMPyP and TSPP using a membrane filter of pore size 0.45 μm.
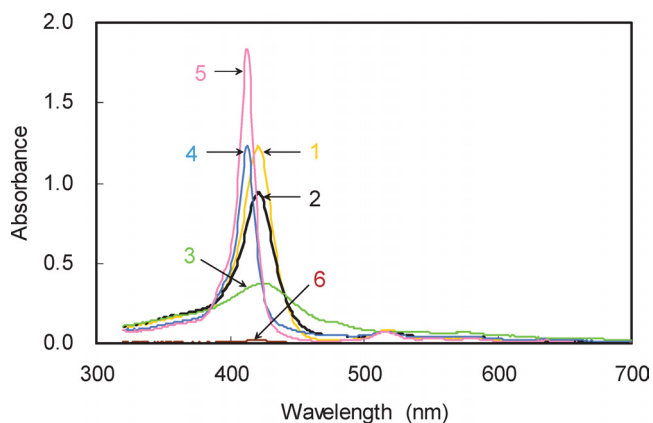

Fig. 5. Absorption Spectra of TMPyP and TSPP Mixed at Various Concentrations

Measurements were performed in 10 mM sodium phosphate buffer (pH 7.0). The total concentration of TMPyP and TSPP was constant at 50 μM. Each number corresponds to the following composition: 1 (yellow), TMPyP (50 μM); 2 (black), TMPyP (40 μM) and TSPP (10 μM); 3 (green), TMPyP (25 μM) and TSPP (25 μM); 4 (blue), TMPyP (10 μM) and TSPP (40 μM); 5 (pink), TSPP (50 μM); 6 (brown), filtrate obtained with the equimolar concentrations (25 μM) of TMPyP and TSPP.

an electrostatic effect.[13] When equimolar concentrations (25 μM) of TMPyP and TSPP were mixed, the solution became turbid within seconds, but did not make precipitates for at least one day. The efficiency to photogenerate H_2O_2 was strongly dependent on the ratio of TMPyP to TSPP as summarized in Table 1, with a ratio of 1 : 1 being most effective. We also found that the monomeric forms of TMPyP and TSPP suppressed markedly the photo-production of H_2O_2. This finding suggested that the ability of monomeric TMPyP and TSPP to transfer electrons to oxygen molecules was low,

though these porphyrins are known to produce singlet oxygen.[17,18] The decrease in the activity of TMPyP seemed to be attributable to the cationic environment of TMPyP, which prevented the transfer of electrons, similar to the action of the N-protonated species of UP. The decrease in the activity of TSPP might be related to the greater oxidation potential of TSPP (1.10 V *vs.* SCE)[19]; however, further study is required to clarify this point. The size of the aggregates produced at a ratio of TMPyP to TSPP of 1 : 1 was >500 nm, because both the intensity of the absorption spectrum and the photo-activity decreased remarkably after the solution was filtrated using a membrane filter (size: 0.45 μm), as shown in Fig. 5 and Table 1.

Discussion

Photodynamic therapy for tumor treatment has become a major topic in porphyrin chemistry as well as photobiology in recent years.[1,2] In this therapy, singlet oxygen ($^1O_2$) produced by the energy transfer from porphyrin to oxygen molecules is widely accepted as a key intermediate.[20,21] On the other hand, photosensitizers, in general, are also known to produce superoxide anion (O_2^-) or H_2O_2 by electron transfer. For example, rose bengal produced not only $^1O_2$ *via* an energy-transfer reaction but also O_2^- and H_2O_2 *via* an electron transfer reaction, depending on the state (solidified or solubilized) of the chromophore.[22] A recent paper reported that fullerenes as photosensitizers produced O_2^- more effectively than $^1O_2$ especially in polar solvents such as water.[23]

In the present study, we detected H_2O_2 produced by the photosensitization of porphyrin in aqueous solution. The reaction was considered to proceed *via* the transfer of electrons from triplet excited porphyrin molecules to oxygen molecules to produce O_2^-, leading to the formation of H_2O_2.[8]

Thus, the porphyrin cations (Por$^+$) may form concurrently with O$_2^-$ by the following reactions:

Por+$h\nu$→Por*   (excitation)

Por*+O$_2$→Por$^+$+O$_2^-$ (electron transfer)
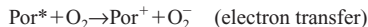
where Por and Por* show porphyrin and triplet excited porphyrin molecules, respectively. We recently observed that porphyrin molecules decomposed with the production of H$_2$O$_2$.[8] It is probable that Por$^+$, which formed concurrently with O$_2^-$, easily decomposed by itself, because of the lack of an electron donor in the present system. In aqueous solution, O$_2^-$ is always in equilibrium with its conjugate acid (HO$_2\cdot$) and, in the absence of other reactants, it disproportionates spontaneously to oxygen and H$_2$O$_2$ by the following reactions[24]:

HO$_2\cdot$+HO$_2\cdot$→H$_2$O$_2$+O$_2$

HO$_2\cdot$+O$_2^-$+H$^+$→H$_2$O$_2$+O$_2$
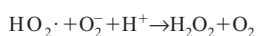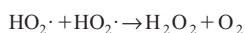
An analysis of the overall disproportionation reaction as a function of pH revealed that the radicals disappeared most rapidly at the pK_a of HO$_2\cdot$ (corresponding to pH 4.7) and, above pH 6, the rate of disproportionation decreases by one order of magnitude per pH unit.[24] This indicates that H$_2$O$_2$ was produced most effectively at around pH 5. However, in the present study, CP reduced remarkably the production of H$_2$O$_2$ at around pH 5 (Fig. 2). Such a decrease corresponded to the appearance of a dimeric form, as can be seen from the pH-dependent distribution of CP species shown in Fig. 1b. This result was consistent with a previous finding that dimer-forming porphyrins generated small amounts of H$_2$O$_2$.[8] Moreover, the dimer of porphyrin is generally known to suppress energy transfer and/or charge separation more strongly than the monomer.[3—5] Hence, we concluded that the dimeric form of CP was not a preferred structure for the transfer of electrons from porphyrin molecules to oxygen molecules, leading to the generation of H$_2$O$_2$. At pH <5, the photogeneration of H$_2$O$_2$ caused by CP was greatly enhanced. We concluded that the highly aggregated form of CP was involved in this effect from the result of a spectral analysis. The participation of the aggregated species was also observed with UP, which increased the production of H$_2$O$_2$ at around pH 5. In the latter case of UP, an oligomer initially formed (Fig. 3b), but it rapidly changed to an aggregated form, as mentioned in the Result section. Such an enhanced efficiency in the production of H$_2$O$_2$ was further demonstrated using a different type of aggregate formed by the electrostatic interaction of TMPyP with TSPP. In general, H$_2$O$_2$ is effectively photogenerated on the surface of solid-phase porphyrins.[25—27] It is likely that a highly aggregated form of porphyrin adopted a semiconductor-like role, causing effective charge separation, leading to a larger amount of H$_2$O$_2$, as has been discussed previously.[8]

As for the ability of monomeric porphyrins to produce H$_2$O$_2$, both the CP and UP monomers were highly efficient. We have recently reported that H$_2$O$_2$ was more effectively generated with increasing numbers of dissociable carboxyl groups in porphyrins, and hence, CP and UP having four and eight dissociable carboxyl groups, respectively, had a marked ability to produce H$_2$O$_2$.[8] This was simply explained by the notion that the transfer of electrons from CP and UP to oxy-

gen molecules might be accelerated by the highly anionic environment needed to stabilize the porphyrin cations. However, this explanation was insufficient, because a different kind of anionic porphyrin, TSPP, did not produce any H$_2$O$_2$. Further studies are required to clarify this point. It should be pointed out that the efficiency of the monomers of CP and UP to photogenerate H$_2$O$_2$ was higher than that of the corresponding highly aggregated forms. One reason making such a difference may be ascribed to the higher efficiency of the monomer than the highly aggregated form to absorb light, as can be seen from the absorption spectra of monomers and the highly aggregated forms of porphyrins shown in Figs. 1a and 3a.

To date, the photochemistry of aggregated compounds has not drawn much attention. Several authors have discussed the effects of porphyrin aggregates on the formation of singlet oxygen.[28—30] In the present study, we found that the aggregated form of porphyrin enhanced the production of H$_2$O$_2$ probably *via* the formation of O$_2^-$. Such a process has not been considered previously. Therefore, the present study may help to develop a new type of photo-medicine for photodynamic therapy.

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