Cationic Porphyrins in Water. ^{1}H NMR and Fluorescence Studies on Dimer and Molecular Complex Formation

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Three kinds of cationic porphyrins have been employed to study intermolecular interaction of these porphyrins in water. Fluorescence behavior of 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis[1-octylpyridinium] tetrachloride (TOPyP) is essentially the same as that of 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis[1-methylpyridinium] tetrachloride (TMPyP), which has been assumed to form a dimer in water even at very low concentrations ($>2 \times 10^{-7}$ M). In contrast with these cationic porphyrins, 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis[trimethylphenylammonium] tetrachloride (TAPP) shows the fluorescence spectrum having well-resolved Q(0–0) and Q(0–1) bands. The self-association of TAPP is detectable at higher TAPP concentrations and/or in the presence of NaCl. ^{1}H NMR clearly indicates the formation of the TAPP aggregates where the monomer–aggregates exchange rates are fast. TMPyP and TOPyP form molecular complexes with proflavin (PFl) that are more stable than the TAPP–PFl complex. The continuous variation method for the absorption spectral change indicates the 2:1 complex of TMPyP and/or TOPyP and PFl and the 1:1 complex of TAPP and PFl. These cationic porphyrins also form molecular complexes with bovine serum albumin (BSA) at pH 5.3, the stoichiometries being 4:1 and 2:1 for the TMPyP- and TAPP–BSA complexes, respectively. All the experimental results cannot provide the direct evidence for the dimer model of TMPyP but can be interpreted reasonably in terms as that both TMPyP and TOPyP form the dimers in water even at very low concentrations while TAPP exists predominantly as its monomer. A dipole-induced dipole interaction seems to play an important role in the formation of the dimers and other molecular complexes of TMPyP and TOPyP.

Introduction

In the previous papers,[1,2] we inferred that 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis[1-methylpyridinium] cation ($TMPyP^{4+}$) forms a dimer in water even at extremely low concentrations ($>2 \times 10^{-7}$ M). Such conclusion has been derived mainly from the results of fluorescence spectroscopy. Although all fluorescence results can be explained by the $TMPyP^{4+}$-dimer formation, it has been very difficult to obtain the direct proof for the dimer formation in the ground state. In order to prove our dimer model, we need to measure the monomer–dimer equilibrium at the $TMPyP^{4+}$ concentrations below 10^{-7} M by using spectroscopy that provides information on the structure in the ground state.

$TMPyP^{4+}$ shows coalesced fluorescence Q bands that tend to separate into two bands upon dilution with water to 1×10^{-8} M. We assumed that the coalescence of the Q bands is due to the dimerization of $TMPyP^{4+}$.[1,2] It is obvious that the excimer formation of $TMPyP^{4+}$ via a dynamic process does not occur in such dilute solution. Although we have found several conditions that cause the resolutionof the Q(0–0) and Q(0–1) fluorescence bands, we cannot eliminate completely the electronic effects of the cationic porphyrin under the specific conditions. For example, the anionic surfactant micelles yield the well-resolved fluorescence Q bands, the increase in the fluorescence quantum yield, and the prolonged fluorescence lifetime. Such micellar effects may be interpreted in terms of either neutralization of the positive charges of $TMPyP^{4+}$ by anionic surfactant molecules or the dissociation of the $TMPyP^{4+}$ dimer to the monomer. The concentration[2] and temperature effects[1] on the $TMPyP^{4+}$ fluorescence spectrum are the only findings that suggest the monomer–dimer equilibrium without worry about the electronic effects. These effects, however, have not been analyzed quantitatively because of the technical difficulty. Brookfield et al.[3] have reexamined our dimer model of $TMPyP^{4+}$ and found the deviation from the Lambert–Beer's law at the TMPyP concentrations below 1×10^{-6} M and the 2-nm red shift of the Soret band upon increase of the $TMPyP^{4+}$ concentration from 1×10^{-7} to 1×10^{-5} M, which have been explained by the monomer–dimer equilibrium in the highly diluted solution and the minimal exciton coupling in the dimer, respectively. Their results strongly support the dimer model of $TMPyP^{4+}$ but are not sufficient to prove the novel dimerization of this cationic porphyrin.

The positive charges of the pyridinium groups at the meso positions of $TMPyP^{4+}$ can be delocalized on the well-extended π-conjugation system of the porphyrin when the porphyrin ring becomes coplanar to the pyridinium ring. If the monomer model of $TMPyP^{4+}$ postulated by Hambright and Fleischer[4] and Pasternack and his co-workers[5,6] is correct, it should be considered that the delocalization of the positive charges enhances the electrostatic repulsion or reduces the van der Waals interaction between the $TMPyP^{4+}$ molecules compared with the system where the positive charges are localized. In spite of this expectation, $TMPyP^{4+}$ associates with a cationic dye, proflavin (PFl), to form a stable π-complex.[7] 4,4′,4″,4‴-(21*H*,23*H*-Porphine-5,10,15,20-tetrayl)tetrakis[trimethylphenylammonium] cation ($TAPP^{4+}$) is a good porphyrin to compare the electronic effects on the porphyrin dimerization with TMPyP because the positive charges on the nitrogen atoms of the ammonium groups of $TAPP^{4+}$ cannot be delocalized on the π-conjugation system. It has been reported that $TAPP^{4+}$ does not dimerize in water up to $[TAPP^{4+}] = 2 \times 10^{-4}$ M.[8] The abilities of $TAPP^{4+}$ to form the self-aggregate(s) and molecular complexes with other π-systems are expected to be similar to those of anionic porphyrins such as 4,4′,4″-(20-phenyl-21*H*,23*H*-porphine-5,10,15-triyl)tris[benzenesulfonic acid] ($TPPS_3$) and 4,4′,4″,4‴-(21*H*,23*H*-porphine-5,10,15,20-tetrayl)tetrakis[benzenesulfonic acid] ($TPPS_4$). $TPPS_3$[5] and $TPPS_4$,[9] whose negative charges are localized on the SO_3^- groups, are known to form a dimer in water containing inorganic salts, the binding constants being reported to be $(3–9.6) \times 10^4$

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M^{-1} at the ionic strengths of 0.05–0.1.

The aim of the present study is verification of the $TMPyP^{4+}$-dimer formation in water. In spite of various efforts, it was very difficult to obtain the direct evidence. Then, we compared the fluorescence and ^1H NMR spectroscopic behavior of $TMPyP^{4+}$ with that of $TAPP^{4+}$ and determined the stoichiometries of the molecular complexes of $TMPyP^{4+}$ and $TAPP^{4+}$ with PFl and bovine serum albumin (BSA) using a continuous variation method. The results obtained support the dimer model of $TMPyP^{4+}$. 4,4′,4″,4‴-(21*H*,23*H*-Porphine-5,10,15,20-tetrayl)tetrakis(1-octylpyridinium) cation ($TOPyP^{4+}$) was also used as a comparative porphyrin. We expected that long alkyl chains of the peripheral substituents inhibit the dimerization of this porphyrin.

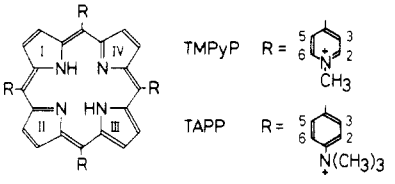
Experimental Section

The tetrachloride salt of $TMPyP^{4+}$ (TMPyP), PFl (3,6-dimethylacridinium hemisulfate), and sodium 9,10-anthraquinone-2-sulfonate (AQS) employed were previously described.[7] The tetraiodide salt of $TOPyP^{4+}$ (TOPyP(I)) was prepared by heating a mixture of 5,10,15,20-tetra-4-pyridyl-21*H*,23*H*-porphine and octyl iodide in *N*,*N*-dimethylformamide at 90 °C and purified by an alumina column chromatography with chloroform (83% yield). TOPyP(I) was converted to the tetrachloride salt (TOPyP) by passing an aqueous methanol solution through an ion-exchange column (Dowex Model 1-X8). Crude TOPyP in water was passed again through an ion-exchange column and recrystallized from a mixture of acetone and methanol (10:1). The aqueous solution of *p*-toluenesulfonate salt of $TAPP^{4+}$ (TAPP(Ts)), which was prepared by the reaction of 5,10,15,20-tetrakis(4-(*N*,*N*-dimethylamino)phenyl)-21*H*,23*H*-porphine with methyl *p*-toluenesulfonate in *N*,*N*-dimethylformamide, was passed through an ion-exchange column (Dowex Model 1-X8) to yield the tetrachloride salt of $TAPP^{4+}$ (TAPP). Crude TAPP was purified by an alumina column chromatography with a mixture of chloroform and methanol (5:1) and recrystallized from methanol. The purity was checked by TLC and ^1H NMR. BSA (Sigma No. A-4378) was purchased and used without further purification.

Measurements of the absorption, fluorescence, and 400-MHz ^1H NMR spectra and of the fluorescence lifetimes were carried out under aerobic conditions by using the instruments described in the previous paper.[7]

Results and Discussion

^1H NMR of TAPP and TMPyP. The concentration effects on the 400-MHz ^1H NMR of TAPP in D_2O are shown in Figure 1. The signals due to the $H_{2,6}$ and $H_{3,5}$ protons of the peripheral aromatic rings of TAPP shift to higher magnetic fields, and the difference in the chemical shifts between these two kinds of protons ($\Delta\delta$) became small with increasing TAPP concentration. The



coalescence of the signals due to the phenyl ring protons occurs at the TAPP concentration of 1×10^{-2} M. In the case of TMPyP, the pyridinium ring protons ($H_{2,6}$ and $H_{3,5}$) are observed at 9.33 and 9.00 ppm at the TMPyP concentrations below 1×10^{-2} M.[2] Above this concentrations, both signals shift to higher magnetic fields and $\Delta\delta$ increases with increasing TMPyP concentration (see Figure 8 in ref 2). The similar observation for TMPyP has been reported by Pasternack et al.[6]

The coalescence of the signals due to the phenyl ring protons of TAPP at higher TAPP concentrations means that the $H_{2,6}$ and $H_{3,5}$ protons of TAPP cannot be distinguished magnetically because of a fast-exchange phenomenon. In other words the self-association of the TAPP molecules occurs and all the protons at the periphery become magnetically equivalent because of the fast exchange between monomer and self-aggregate(s). Assuming

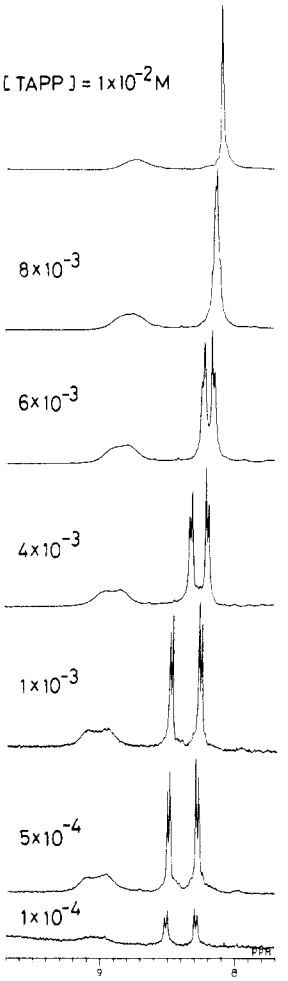


Figure 1. 400-MHz ^1H NMR spectra of TAPP in D_2O at various concentrations at 23 ± 0.5 °C. Sodium 3-(trimethylsilyl)-1-propanesulfonate was used as an external standard.

dimer formation, we tried to determine the association constant (K) using the following equation:[10]

$$\left(\frac{\Delta}{S_t}\right)^{1/2} = \left(\frac{2K}{\Delta_2}\right)^{1/2}(\Delta_2 - \Delta) \qquad (1)$$

where $\Delta = \delta_1 - \delta$, $\Delta_2 = \delta_1 - \delta_2$, and $K = [\text{TAPP dimer}]/[\text{TAPP}]^2$. S_t is the total concentration of TAPP, δ is the observed chemical shift, and δ_1 and δ_2 are the infinite chemical shifts of the monomer and the dimer, respectively. If there is only monomer–dimer equilibrium, a linear relationship should be observed for the plot of $(\Delta/S_t)^{1/2}$ vs Δ. In spite of this expectation, we could not apply eq 1 to the present system, indicating that the aggregates other than the dimer are also formed in the aqueous TAPP solution. Judging from the results shown in Figure 1, it can be concluded that a π–π interaction between the TAPP molecules cannot be neglected even when the TAPP concentrations are 10^{-4}–10^{-3} M.

It should be noted that no coalescence of the signals due to the pyridinium ring protons is seen for TMPyP.[2] The fact that $\Delta\delta$ increases with increasing TMPyP concentration without broadening of the signals[2,6] cannot be explained by the self-association with a fast exchange rate. Pasternack et al.,[6] who claim the monomer model of TMPyP, analyzed the ^1H NMR data by using eq 1 and obtained the K value of 5.4 M^{-1} for the TMPyP association. It is very hard, however, to imagine the TMPyP self-aggregate having a small K value but an extremely slow exchange rate.

As Figure 1 shows, the 5×10^{-4} M TAPP solution in D_2O shows broad signals at 8.96 and 9.10 ppm, which are assigned to the protons on the pyrrole rings I and III, respectively. As the TAPP

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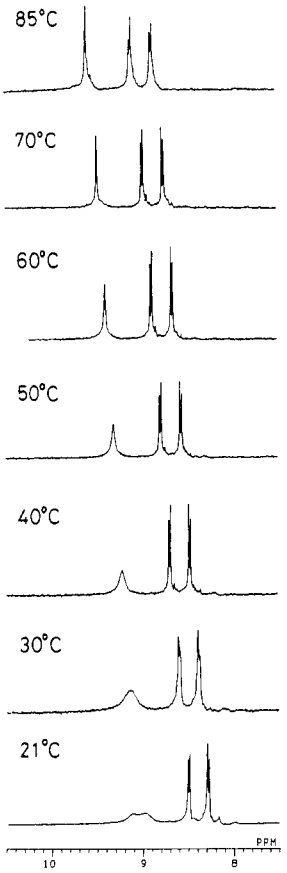

Figure 2. ^1H NMR spectra of TAPP (5×10^{-4} M) in D_2O at various temperatures.

concentration increases, these signals shift to the higher magnetic fields and coalesce at [TAPP] = 1×10^{-2} M. This coalescence should also be ascribed to the fast exchange between the monomer and the self-aggregate(s) of TAPP. Figure 2 shows the effects of temperature on ^1H NMR of TAPP (5×10^{-4} M) in D_2O. Both signals of the pyrrole and phenyl ring protons shift to lower magnetic fields with increasing temperature. The signals of the pyrrole rings coalesce at 30 °C, and the broad coalesced signal at lower temperature becomes sharp at higher temperature. Such ^1H NMR spectroscopic behavior of the pyrrole-ring protons can be explained by the N–D tautomerism with a relatively slow rate at room temperature.[11,12]

The problem is the downfield shifts of the TAPP proton signals at higher temperatures. In the case of 5,10,15,20-tetraphenylporphine (TPP) in CS_2, the coalescence of the pyrrole-ring protons due to the N–H tautomerism occurs at −40 °C and the coalesced signal appears at an average magnetic field of the chemical shifts of the protons on rings I and III at −80 °C, where a very slow equilibrium is established, and does not shift at 30 °C.[11] The magnetic resonance observed for TPP is common for the exchange process where the electronic structure of the sample is not affected by the temperature. Since the positive charges on the trimethylammonium groups of TAPP are localized, the electronic structure of the porphyrin ring of TAPP seems to be similar to that of TPP. Therefore, it may be reasonable to assume that, as discussed above, there is the π–π interaction between the TAPP molecules even in the 5×10^{-4} M solution at room temperature while the main species is the monomer. TMPyP also shows the same temperature-dependent ^1H NMR (Figure 3). In the present stage, however, we should not discuss impatiently the self-aggregation of TMPyP by using the ^1H NMR data obtained in the previous[2] and present studies because the ^1H NMR spectroscopic behavior of TMPyP differs from that for typical monomer–dimer

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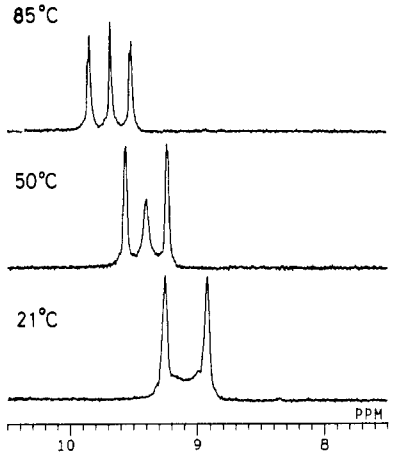

Figure 3. ^1H NMR spectra of TMPyP (5×10^{-4} M) in D_2O at 21, 50, and 85 °C.

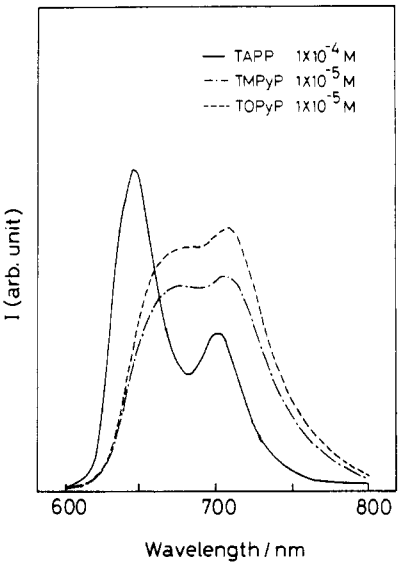

Figure 4. Fluorescence spectra of TAPP, TMPyP, and TOPyP in water at room temperature. A Hitachi Model 650-60 spectrofluorometer (excitation and emission bandwidths = 5 nm) was used. TAPP, TMPyP, and TOPyP were excited at 573, 423, and 423 nm, respectively.

TABLE I: Fluorescence Lifetimes of TAPP in Water under Various Conditions

concn, M	temp, °C	τ_f, ns	concn, M	temp, °C	τ_f, ns
2×10^{-5}	23	7.4	5×10^{-4}	11	7.2
5×10^{-4}	25	7.0	5×10^{-4}	25	7.0
1×10^{-2}	25	7.7	5×10^{-4}	42	7.2
2×10^{-5}	4	8.4	5×10^{-4}	75	7.1
2×10^{-5}	11	7.3	5×10^{-4}	94	6.9
2×10^{-5}	25	7.0	1×10^{-2}	4	6.8
2×10^{-5}	42	7.2	1×10^{-2}	25	7.7
2×10^{-5}	75	7.2	1×10^{-2}	85	7.6
2×10^{-5}	95	6.6	2×10^{-5} [a]	24	8.0
5×10^{-4}	4	8.1	2×10^{-5} [b]	24	6.6

[a] The fluorescence lifetime of TAPP in D_2O. [b] The fluorescence lifetime of TAPP in H_2O containing 3.5 M NaCl.

equilibrium and does not provide strong information to judge the structure of TMPyP in water.

Fluorescence of TAPP, TMPyP, and TOPyP. Fluorescence spectra of TAPP, TMPyP, and TOPyP in water are shown in Figure 4. As we reported,[1,2] the Q(0–0) fluorescence band of TMPyP shifts abnormally to a longer wavelength in water, leading to partial overlap with the Q(0–1) fluorescence band. The spectrum of TOPyP was essentially the same as that of TMPyP. The coalescence of the fluorescence Q bands upon dimerization has been reported for the potassium complex of porphyrin having benzo-15-crown-5 at the meso positions in CH_3Cl–CH_3OH (1:1)[13]

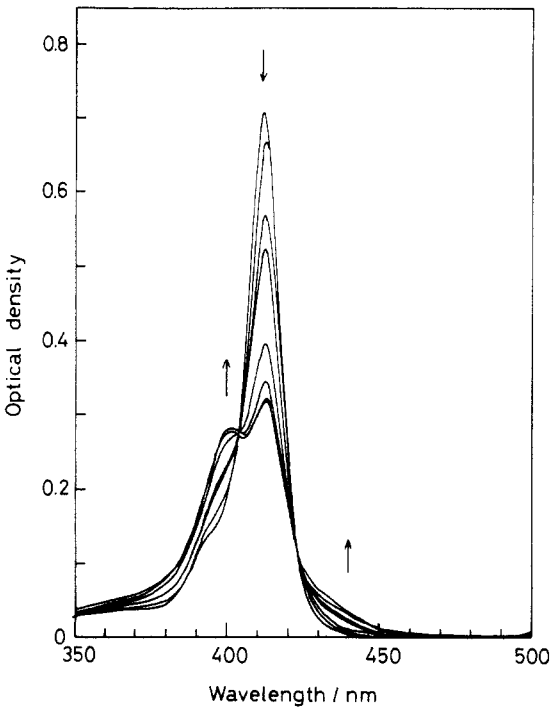

Figure 5. Absorption spectral change of TAPP (2×10^{-5} M) in water upon addition of NaCl at room temperature. A quartz cell having a 1-mm optical length was used. [NaCl] = 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 M.

and $TPPS_4$ in water containing the potassium complex of 18-crown-6.[14] On the other hand, TAPP shows the well-separated fluorescence Q bands at 645 and 702 nm. No change in the spectral shape was observed upon increase of the TAPP concentration up to 1×10^{-2} M.

The fluorescence lifetimes (τ_f) of TAPP were measured under various conditions and the results are summarized in Table I. Although ^1H NMR indicates the formation of the self-aggregates at higher TAPP concentrations, the fluorescence lifetime does not depend on the TAPP concentration. In addition, no marked effect of temperature is found over the concentration range 2×10^{-5} to 1×10^{-2} M. These results suggest that the self-aggregates of TAPP are nonfluorescent or have very low fluroescence yields. As suggested by ^1H NMR, the self-aggregates of TAPP have considerably loose structures. Such loose aggregates may show weak exciton coupling interactions leading to reduction of the fluorescence quantum yields. On the contrary, the TMPyP molecules may form a tight face-to-face dimer having a D_2 symmetry. Such a dimer in the excited state may show the strong exciton coupling and fluoresce.

Effects of Inorganic Salt. It has been known that the self-association of water-soluble porphyrins is facilitated by inorganic salts.[5,9,15-17] The effect of NaCl on the absorption spectrum of TAPP (2×10^{-5} M) in water is shown in Figure 5. A new absorption band appears at 404 nm along with a shoulder band at around 435 nm at the expense of the Soret band at 412 nm upon addition of NaCl. Similar spectral changes have been observed in the course of dimerization of hematoporphyrin[18] and the porphyrin having crown ether at the meso positions.[13] These

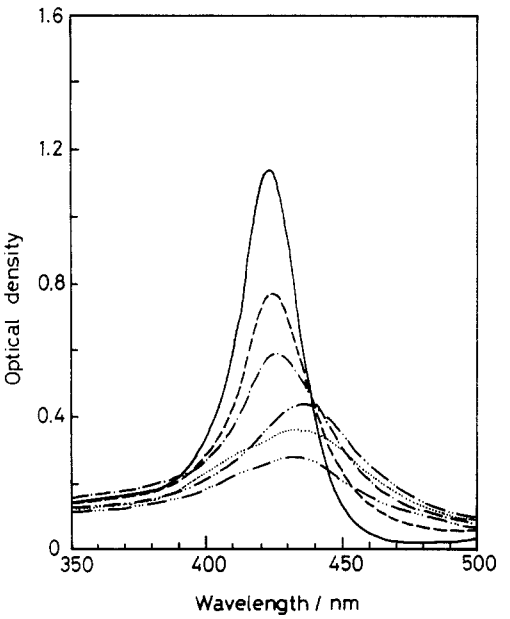

Figure 6. Absorption spectral change of TOPyP (5×10^{-6} M) in water upon addition of NaCl at room temperature. A quartz cell having a 1-cm optical length was used. [NaCl] = 0 (—), 0.2 (- -), 0.3 (-·-), 0.5 (-··-), 1.0 (-···-), 2.0 M (···).

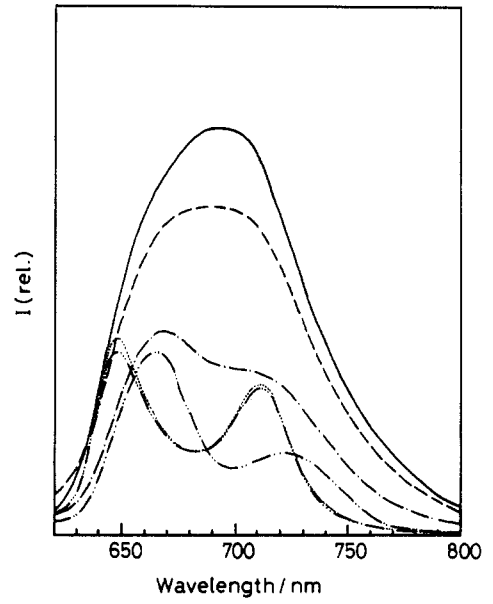

Figure 7. Fluorescence spectral change of TOPyP (5×10^{-6} M) in water upon addition of NaCl at room temperature. [NaCl] = 0 (—), λ_{ex} = 423 nm), 0.2 (- -, λ_{ex} = 424 nm), 0.3 (-·-, λ_{ex} = 426 nm), 0.5 (-··-, λ_{ex} = 435 nm), 1.0 (-···-, λ_{ex} = 433 nm), 2.0 M (···, λ_{ex} = 435 nm).

absorption spectral data clearly indicate that there are, at least, three kinds of TAPP in aqueous NaCl solution. It may be reasonable to assign that the absorption maxima at 415 and 404 nm are ascribed to the monomer and the dimer of TAPP, respectively. The species having the absorption maximum at around 435 nm may be the higher aggregates of TAPP. The NaCl-induced self-association of TAPP (1×10^{-3} M) was supported by ^1H NMR. The signals due to the phenyl-ring protons at 8.45 and 8.25 ppm and the porphyrin-ring protons at around 9 ppm were broadened and shifted to higher magnetic fields upon addition of 0.5 M NaCl, the chemical shifts of the phenyl- and porphyrin-ring protons in the presence of NaCl being 7.86 and 7.51 ppm and ca. 8.3 ppm, respectively. These results clearly indicate that NaCl promotes the formation of self-aggregates of TAPP with relatively slow exchange rates.

In contrast with TAPP, no marked change in the Soret band was observed for TMPyP (1×10^{-5} M) when 3 M NaCl was

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added, λ_{max} being 422 nm in the absence of NaCl and 423 nm in the presence of 3 M NaCl. No marked effect of NaCl on ^1H NMR of TMPyP was observed. Addition of 0.5 M NaCl to the 1×10^{-3} M TMPyP solution caused the 0.01- and 0.19-ppm upfield shifts of the $H_{2,6}$ and $H_{3,5}$ protons, respectively.

Complex absorption and fluorescence spectral changes were observed for TOPyP (Figures 6 and 7). Addition of NaCl causes the red shift of the Soret band at 424 nm along with the reduction of absorbancy (Figure 6). It should be noted that the well-separated Q(0–0) and Q(0–1) fluorescence bands appear at 648 and 711 nm, respectively, in concentrated NaCl solution (Figure 7). Judging from the results of the NaCl effects on the TAPP association, the red-shifted broad absorption band (λ_{max} = 435 nm) seems to be ascribed to the higher aggregates of TOPyP.

The effects of NaCl on the electronic spectra can be explained reasonably as follows. The TAPP molecules associate spontaneously in the concentrated NaCl solution to form the dimer and small amounts of the higher aggregates. A main component of the higher aggregates seems to be a trimer having twelve positive charges that are partially neutralized by added inorganic salt. Since TMPyP is in the dimer form even at low concentration and the TMPyP dimer has eight positive charges, further spontaneous association hardly occurs because of a strong electrostatic repulsion between the TMPyP dimers even when large amounts of NaCl are added to the system. In the case of TOPyP, the dimer is the main species in water without inorganic salt and the addition of NaCl enhances the interaction between hydrophobic alkyl chains yielding the higher aggregates or the small micelles that show broad Soret bands at longer wavelengths. It has been well established that the critical micelle concentrations of amphiphiles are lowered by addition of inorganic salts.[19] Within the small micelles, some porphyrin chromophores can be located at the less hydrophilic micelle–water interface, resulting in the dissociation of the dimer to the monomer. Small amounts of the TOPyP monomer thus obtained may exhibit the well-separated fluorescence Q bands. Most of the TOPyP higher aggregates may be nonfluorescent and/or have very low fluorescence quantum yields. The spectroscopic results are hardly explained by the monomer model for TMPyP and TOPyP.

Ground-State Complexes with PFl and BSA. In the previous paper,[7] we reported the complexation of TMPyP with PFl, the formation constant, K, being 2161 ± 55 M^{-1} at 25 °C. According to the same procedures described previously,[7] the K values were determined for the TAPP– and TOPyP–PFl systems. The absorption spectral changes of TAPP and TOPyP upon addition of PFl were essentially the same as that of the TMPyP–PFl system.[7] Determination of the K values for complexation of TAPP and TOPyP with PFl was performed by measuring fluorescence quenching of these cationic porphyrins by PFl by exciting the porphyrins at the isosbestic points. A linear Stern–Volmer plot was observed for TAPP up to [PFl] = 1×10^{-3} M. Above this concentrations, the plots were saturated. The deviation from the Stern–Volmer linear relationship seems to be ascribed to the dimerization of PFl itself.[20] No change in the fluorescence lifetime of TAPP was detected in the presence of various amounts of PFl while fluorescence quenching took place, indicating the formation of a nonfluorescent TAPP–PFl ground-state complex. From a linear Stern–Volmer plot, the K value was determined to be 800 ± 50 M^{-1}. The same procedures provided the K value of 2100 ± 100 M^{-1} for the TOPyP–PFl complex, which is almost the same as K for the TMPyP–PFl complex. It is noteworthy that the K value for the TAPP–PFl complex is much smaller than those for the TMPyP– and TOPyP–PFl complexes. In spite of the expectation of electrostatic repulsion, both TMPyP and TOPyP, whose positive charges at the peripheral substituents can be delocalized on the porphyrin rings, form considerably stable complexes with PFl. The ability of TAPP, whose positive charges are localized on the trimethylammonium groups of the peripheral substituents, to form the PFl complex is weaker than those of TMPyP and TOPyP.

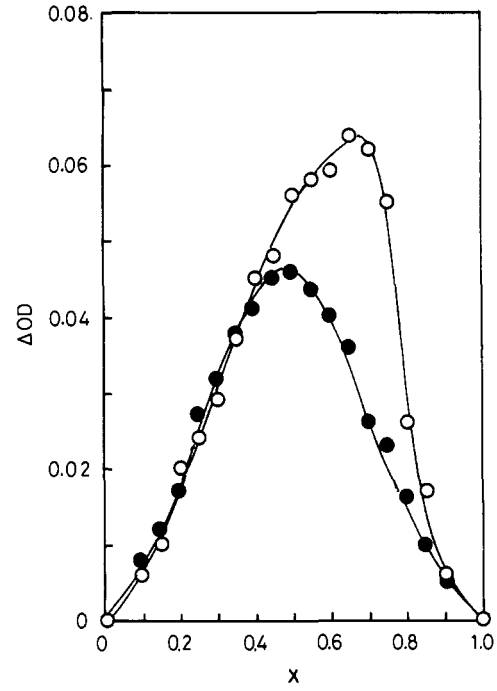

Figure 8. Continuous variation plots for complexation of TMPyP (○) and TAPP (●) with PFl in water at room temperature. The changes in the optical densities at 515 and 520 nm were monitored for TMPyP and TAPP, respectively. The total concentrations were adjusted to 1×10^{-4} M.

In order to know the stoichiometries of the molecular complexes with PFl, a continuous variation method was employed for absorption spectral changes.[21] The total concentration was adjusted to 1×10^{-4} M. Under the experimental conditions, the dimerization of PFl can be neglected because a Lambert–Beer's linear relationship was observed up to [PFl] = 3×10^{-4} M. The Job plots for the TMPyP– and TAPP–PFl systems are shown in Figure 8. The discontinuities were observed at x = 0.67 and 0.50 for the TMPyP– and TAPP–PFl systems, respectively, where x = [porphyrin]/{[porphyrin] + [PFl]}. It can be concluded, therefore, that the 2:1 TMPyP–PFl and 1:1 TAPP–PFl complexes are formed. The Job plot also indicates the formation of the 2:1 complex of TOPyP and PFl. It is very difficult to consider that a PFl molecule gathers two TMPyP molecules that originally exist in the monomer forms. The most reasonable explanation is as follows: the extremely stable dimers of TMPyP and TOPyP associate with PFl to form the 2:1 molecular complexes while TAPP in the monomer form yields the 1:1 complex. We confirmed that the discontinuity appears at x = 0.5 when the total concentration of the TMPyP dimer and PFl is adjusted to 1×10^{-4} M by assuming that only the TMPyP dimer exists in water. The van der Waals interaction between the TMPyP or TOPyP dimer and PFl seems to be stronger than the electrostatic repulsive force. The electrostatic repulsion is not so significant because of the delocalization of a positive charge of PFl on the acridinium ring.

The same procedures provided clearly the stoichiometries of the TMPyP– and TAPP–BSA complexes to be 4:1 and 2:1, respectively (Figure 9). The measurements were carried out at pH 5.3 (without inorganic salt), which corresponds to the isoionic point of BSA. Although our study on the interaction between the cationic porphyrins and BSA is preliminary, the present results suggest that there are two binding sites in BSA for the cationic porphyrins and the TMPyP dimer and the TAPP monomer are bound to each binding site to form the 4:1 and 2:1 complexes, respectively. The detailed study is now in progress.

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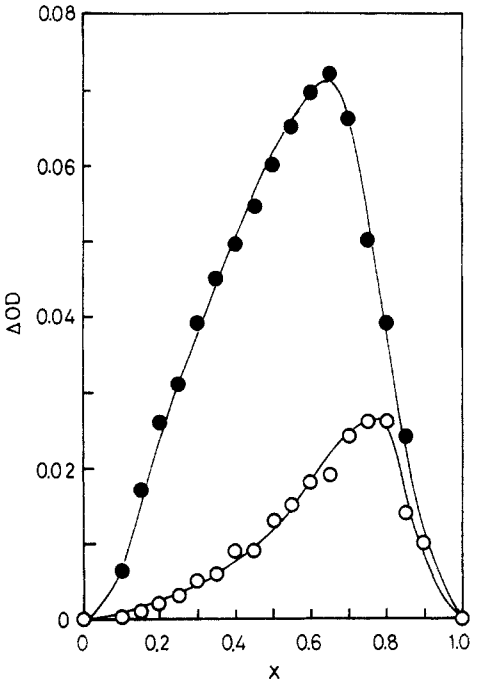


Figure 9. Continuous variation plots for complexation of TMPyP (○) and TAPP (●) with BSA in water (pH 5.3) at room temperature. The changes in the optical densities at 520 and 515 nm were monitored for TMPyP and TAPP, respectively. The total concentrations were adjusted to 1×10^{-4} M.

Furthermore, we obtained the stoichiometries for the molecular complexes of zinc complex of TMPyP (ZnTMPyP) with PFl and BSA to be 1:1 and 2:1, respectively. ZnTMPyP, therefore, seems to exist predominatly as an monomer in water.

Ground-State Complexes with AQS. AQS is bound to TMPyP to form a nonfluorescent complex via both electrostatic and van der Waals interactions.[7] AQS quenches the fluorescence of TMPyP without a change in the fluorescence spectral shape. In the case of TOPyP (5×10^{-6} M), however, the effects of AQS are quite different from those for TMPyP as shown in Figure 10. The fluorescence of TOPyP (5×10^{-6} M) is quenched by AQS up to [AQS] = 5×10^{-6} M. Above this concentration, the well-separated fluorescence Q bands appear and the fluorescence intensities of these Q bands drastically increase with increasing AQS concentration. In order to explain this novel fluorescence behavior, we have to employ the dimer model for TOPyP (Scheme I). We postulated a stacking-type complex for the nonfluorescent species formed by complexation of TMPyP with AQS.[7] At lower AQS concentrations, a stacking-type 1:1 complex of the TOPyP dimer and AQS should be formed predominantly. This 1:1 complex is nonfluorescent. An additional AQS can be bound to the 1:1 complex of the TOPyP dimer and AQS to form the 1:2 complex, which is also nonfluorescent. The third AQS molecule may bind with the 1:2 complex where a AQS molecule should be located in the vicinity of the peripheral substituent. Such a 1:3 complex of the TOPyP dimer and AQS should be unstable because of the electrostatic repulsion between the AQS molecules, resulting in the dissociation to the 1:2 complex of the TOPyP monomer and AQS where two AQS molecules may be located at the peripheral substituents to minimize the electrostatic repulsive force. The electrostatic binding of AQS with the cationic periphery seems to be enhanced by the neighboring hydrophobic alkyl chain. Under such circumstances, the 1:2 complex of the TOPyP monomer and AQS may show the well-separated fluorescence Q bands.

The fluorescence of TAPP was also quenched by AQS in a manner observed similar to TMPyP but in lower efficiency (Figure 11). The approach of an AQS molecule to the TAPP porphyrin ring may be restrained to a larger extent because the positive charges are localized on the trimethylammonium groups of the peripheral substituents.

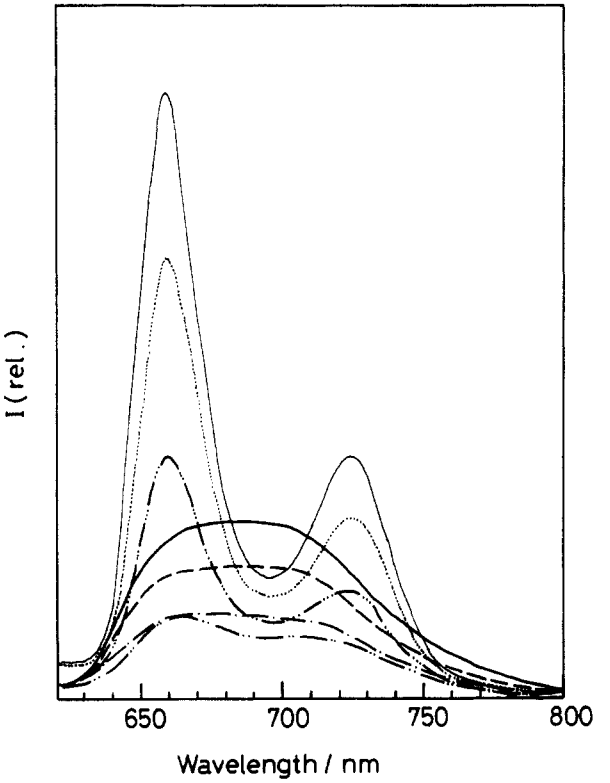
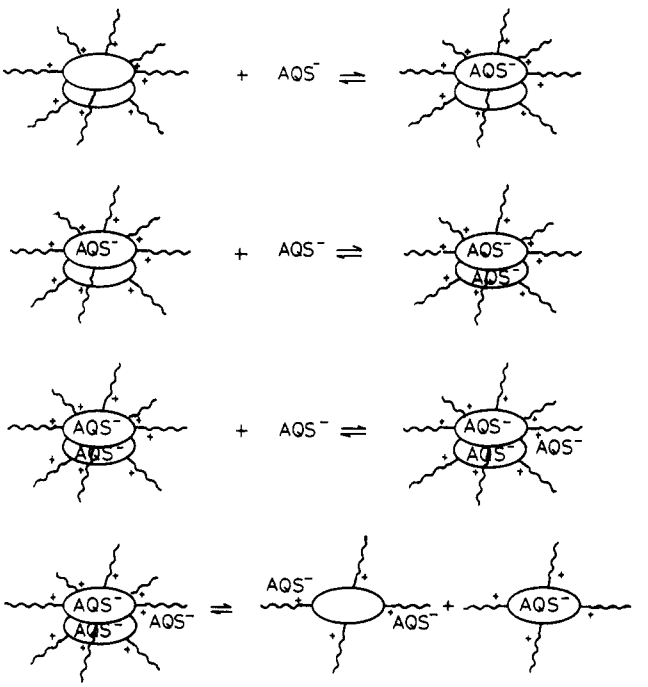


Figure 10. Fluorescence spectral change of TOPyP (5×10^{-6} M) in water upon addition of AQS at room temperature. A Shimadzu Model RF-500 spectrofluorometer (excitation and emission bandwidths = 10 nm) was used. TOPyP was excited at 595 nm. 10^5[AQS] = 0 (—), 0.2 (– –), 0.4 (–·–), 0.5 (–··–), 1.0 (–···–), 1.4 (···), 2.0 M (–).

SCHEME I



Summary

TMPyP is a very important porphyrin because of its wide use for a photosensitizer in the model systems of photosynthesis[22,23] and for an intercalater of DNA.[5,24-27] It is fundamental, therefore,

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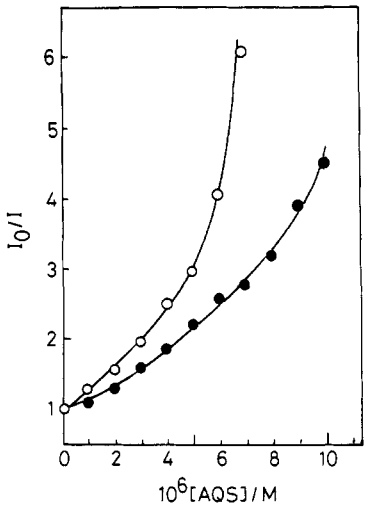

Figure 11. Plots of I_0/I vs [AQS] for the fluorescence quenching of TMPyP (○) and TAPP (●) by AQS at room temperature. The porphyrin concentrations were 5×10^{-6} M. The fluorescence intensities were followed at 704 nm.

to know the abilities of TMPyP to form self-aggregate(s) and molecular complexes in aqueous media. In spite of various attempts to clarify the self-association phenomenon of TMPyP in water,[1-7] the direct evidence for dimerization of this porphyrin has not been reported as yet. The present study provides several experimental aspects that support the dimer model of TMPyP.

^{1}H NMR clearly indicates the formation of the self-aggregates of TAPP having fast exchange rates in concentrated aqueous solution. Since a Lambert-Beer's law can be applied for the Soret band of TAPP in the concentration range 2×10^{-8} to 5×10^{-5} M, the monomer should be the main species in dilute aqueous solution.

Partial neutralization of the positive charges localized on the trimethylammonium groups by added NaCl promotes the association of the TAPP molecules. This behavior of TAPP is similar to that for the anionic water-soluble porphyrins,[5,9,16,17] whose negative charges are localized on the substituents at the meso positions. The formation of the 1:1 complex of TAPP and PFl also supports the weak ability of TAPP to associate spontaneously. Meanwhile, most experimental results obtained for TMPyP and TOPyP can be understood only by the dimer model for these cationic porphyrins. The stoichiometries determined for the TMPyP- and TOPyP-PFl complexes (2:1) as well as that for the TMPyP-BSA complex (4:1) strongly suggest the extremely stable dimers of these pyridinium porphyrins. Since the pyridinium substituent rotates around the C-C single bond at the meso position, the positive charge on the substituent can pour onto the porphyrin ring when the pyridinium ring becomes coplanar to the porphyrin ring. The dipole thus generated seems to induce the dipole of another porphyrin having a well-extended conjugation leading to the strong π-π interaction and reduction in the electrostatic repulsion between these two porphyrin rings. The fact that the association constants for complexation of TMPyP and TOPyP with an cationic dye, PFl, are much larger than that of TAPP with PFl supports this assumption. Appearance of the well-separated fluorescence Q bands of TOPyP upon addition of NaCl and/or AQS is also explained by the dimer model for this porphyrin. If the coalescence of the fluorescence Q bands of TMPyP and TOPyP is ascribed to some electronic effect, the separation of the Q bands should also be observed for TMPyP when NaCl and/or AQS is added, because the electronic structure of the porphyrin ring of TMPyP is the same as that of TOPyP. The novel behavior of TOPyP in water containing NaCl and/or AQS can be interpreted in terms of the dissociation of the TOPyP dimer to the monomer due to the effects of the hydrophobic octyl groups.

Although we could not derive the direct evidence for the TMPyP dimer model from the present study, all results suggest strongly that TMPyP in water exists in its dimer form even at very low concentrations.

Acknowledgment. The present study was supported by a Grant-in-Aid for Science Research No. 62470082 from the Ministry of Education, Science and Culture. We are indebted to H. Abe and H. Takagi for their experimental assistance.

Registry No. TOPyP, 124891-59-4; TOPyP(I), 104207-96-7; TMPyP, 92739-63-4; TAPP, 92739-64-5; PFl, 92-62-6; AQS, 131-08-8; ZnTMPyP, 28850-44-4.

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Evaluation of Group Electronegativity by Pauling's Thermochemical Method

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Pauling's bond energy equation, $D(\text{H-G}) = [D(\text{H-H})\ D(\text{G-G})]^{1/2} + K(\Delta\chi)^2$ (1), is applied to some 28 HG molecules, where H is hydrogen and G any group of atoms, to calculate the electronegativity (χ_G) of a group. In eq 1 D(H-G), D(H-H), and D(G-G) are the energies of the H-G, H-H, and G-G bonds, respectively, $\Delta\chi = |\chi_H - \chi_G|$, and K is a constant. The value of K is optimized to 25 in order to obtain maximum amount of correlation ($r = 0.960$) of the χ_G values for some 19 groups/atoms with the $^1J_{CC}$(ortho-ipso) coupling constants in monosubstituted benzenes. These $^1J_{CC}$ constants form a very good experimental scale for group electronegativity. With Inamoto's "i" scale, derived experimentally, the χ_G values for some 20 groups/atoms correlate quite satisfactorily ($r = 0.936$). However, $\Delta\chi$ for two groups, $CH(OH)CH_3$ and $Si(CH_3)_3$, out of the 28 groups considered are found to be imaginary, for which the bond energy data are held partially responsible. The effects of various bond-determining factors on the χ_G values evaluated by eq 1 are examined critically. It is concluded that, when the order of the G-G bond is 1 as in H-H or H-G, the method yields a reasonable value for the electronegativity of a group.

The electronegativity of an atom was first defined by Pauling.[1] Subsequently, extension of this concept to a group of atoms gave rise to a term "group electronegativity" and various approaches to evaluate this parameter with various degrees of success (for

0022-3654/90/2094-2187$02.50/0