Total Internal Reflection Fluorometric Study of the Ion-Association Adsorption of Ionic Derivatives of Porphyrin at Liquid–Liquid Interface

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(Received August 14, 1996)

Interfacial ion-association adsorption of 5,10,15,20-tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphine (H_2tpps^{4-}) with hexadecyltrimethylammonium ion and 5,10,15,20-tetrakis(1-methyl-4-pyridinio)-21*H*,23*H*-porphine (H_2tmpyp^{4+}) with 1-hexadecanesulfonate was measured by means of a total internal reflection fluorometry at toluene/water systems. The adsorptivity of the porphyrins was negligibly small in the absence of the ionic surfactants, but it was increased by the addition of the surfactants in the 10^{-8}—10^{-7} M concentration. The adsorptivity was governed by the positive or negative charge of the surface-active counter ion and its concentration, which determined the electrostatic potential of the interface. Further, it was found that zinc(II)-1,10-phenanthroline, a hydrophobic complex cation, remarkably enhanced the interfacial adsorption of $[Zntpps]^{4-}$ ion, but showed no positive effect on the adsorption of $[Zntmpyp]^{4+}$ ion.

Adsorption and reaction at the liquid–liquid interfaces have recently become attractive subjects in the fundamental studies of solvent extraction of metal ions and the related separation and detection methods including liquid-membrane separation, ion-selective electrodes, optical sensors and counter current chromatography.$^{1)}$ A series of our investigations which was started by the invention of a high-speed stirring method$^{2)}$ has revealed some significant features of the role of the interface in solvent extraction kinetics. For example, in the chelate extraction of Ni(II) with β-hydroxy oxime, the interfacial adsorptivity of the ligand which was dissolved initially in an organic phase was primarily important for the determination of the location where a rate-controlling reaction took place.$^{3)}$ In the kinetics of ion-association extraction of Fe(II) with 1,10-phenanthroline derivatives, the adsorptivity of the metal complex was important as well as the adsorptivity of the ligand which forms the complex ion.$^{4)}$

Furthermore, in the synergistic extraction rate of Ni(II) dithizonate with 1,10-phenanthroline, it was found that a charged ternary complex was formed at the interface during the course of the extraction, which behaved as an intermediate complex in the extraction reaction.$^{5)}$ These findings were highly helpful for understanding the role of the interfacial reaction in the overall extraction processes. The high-speed stirring spectrometry is reliable for the measurement of the interfacial adsorptivity of a dilute species in organic phase and even in aqueous phase, but it is inherently an indirect method for the identification of interfacial species. This was the reason why we studied direct spectrometric methods to identify the adsorbed species and to determine its amount at the interface.

We developed previously an optical stir cell method and a Teflon capillary plate method as direct means to obtain interfacial absorption spectra under stirred and static conditions, respectively.$^{6)}$ Most recently, a total internal reflection fluorometry (TIRF) method was introduced for the measurement of an interfacially adsorbed water-soluble fluorophore, by taking advantage of the high sensitivity of fluorometry.$^{7)}$ TIRF measurements at liquid–solid or air–solid interfaces were previously reported by other workers,$^{8)}$ while the study on liquid–liquid interface has been less developed.$^{9)}$

The applicability of porphyrins in analytical reagents is now well recognized, owing to its very high molar absorptivity at Soret band or high fluorescence sensitivity, especially in the optical biosensing of O_2, CO, NO, glucose and oxalate.$^{10)}$ Recently, we found out the protonation reaction of tetraphenylporphyrin at liquid–liquid interface$^{11)}$ and revealed the kinetic mechanism by a two-phase stopped-flow spectrometry.$^{12)}$ The spectral change at the interface which was highly sensitive to acid concentration suggested a utility of the interfacial protonation as a pH sensor. Fluorometry using water-soluble porphyrins has already been demonstrated in the detection of trace amounts of Mg^{2+} and Zn^{2+}.$^{13)}$ In the present study, the interfacial adsorption of water-soluble porphyrins: 5,10,15,20-tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphine (H_2tpps^{4-}) and 5,10,15,20-tetrakis(1-methyl-4-pyridinio)-21*H*,23*H*-porphine (H_2tmpyp^{4+}), was investigated in the presence of an ionic surfactant as a counter ion by means of TIRF. The dependence of TIRF intensities on the surfactant and porphyrin concentrations was studied in detail and the mechanism of the interfacial adsorption was discussed based on the electrostatic point of view.

Experimental

Chemicals. 5,10,15,20-Tetrakis(4-sulfonatophenyl)-21*H*,23*H*-porphine (H_2tpps^{4-}) and 5,10,15,20-tetrakis(1-methyl-4-pyridinio)-

21*H*,23*H*-porphine (H_2tmpyp^{4+}) were both purchased from Dojindo Laboratories. Hexadecyltrimethylammonium bromide (CTAB) from Nacalai Tesque Inc., sodium 1-hexadecanesulfonate (SHS) from Tokyo Chemical Industry Co., Ltd., and 1,10-phenanthroline (phen) from Wako Pure Chemical Industries, Ltd. were all analytical reagent grade. Toluene, Wako Pure Chemical Industries, Ltd., G.R., was fractionally distilled after being treated with sulfuric acid and a fuming sulfuric acid mixture. The stock solution of zinc(II) perchlorate was prepared by dissolving metal zinc powder (Wako, G.R.) in perchloric acid (Wako, G.R.). The pH of the aqueous solution was controlled within 6.2—6.4 by 0.0017 M (1 M = 1 mol dm^{-3}) acetate buffer. Other reagents were all analytical reagent grade. Water was purified successively by Yamato Auto Still WAG28 and Milli-QII systems.

TIRF Measurements. TIRF measurements were carried out on a Hitachi 650-40 fluorescence spectrophotometer. Two right-angled glass (BK-7) prisms (10×10×10 mm) were attached on each side of the cell holder for excitation and detection, so that the incident excitation beam introduced through the toluene phase struck the interface with the incident angle of 72°, which was enough greater than the critical total reflection angle, $\theta_c = 63°$, in the toluene/water pair calculated by the next equation:

$$\theta_c = \sin^{-1}(n_2/n_1), \qquad (1)$$

where n_1 and n_2 were the refractive indices of toluene (1.494) and water (1.333), respectively. The fluorescence from the interface could be measured from the perpendicular direction through the glass prism. The details of the optical arrangement were described elsewhere.[7] The TIRF intensity depended on the vertical position of the interface in the cell holder. Therefore, the best position of the interface which gave a maximum intensity was carefully adjusted in each measurement. The penetration depth of the evanescent wave into the aqueous phase, d_p, could be estimated by the next equation as 136 nm for the excitation wavelength at λ =420 nm,

$$d_p = (\lambda/2\pi)(n_1{}^2 \sin^2\theta - n_2{}^2)^{1/2}, \qquad (2)$$

where θ=72°. The upper inside wall of the optical cell (10×10 mm) was made hydrophobic by treating with dichlorodimethylsilane in benzene so as to afford a flat toluene/water interface. The volume of each phase was 1.2 ml in most cases. All measurements were made at 25±0.1 °C, which was kept by circulating thermostated water. Fluorescence polarization measurements were carried out by using a excitation beam polarizer and a emission beam polarizer. Absorption spectra were measured by JASCO Uvidec-430 spectrophotometer and pH's by Horiba F-14 pH meter.

Interfacial Tension. Interfacial tension in toluene/water systems was measured by means of a drop volume method employing a water-jacketed solution cell containing the organic phase and a flat-ended glass capillary which was immersed in the organic phase and connected to a micrometer syringe Gilmont GS-1200, pressing out the aqueous phase from the capillary end. The measurement was carried out at 25±0.1°C. Lando-Oakley correction was used in the calculation of the interfacial tension.

Distribution Measurements. The distribution constants of phen in toluene/water system was determined by a batch method employing centrifugal tubes and a mechanical shaker. One hour shaking was enough for the two-phase equilibration. From the initial concentration of phen (3.17×10^{-5}—9.50×10^{-5} M) and the aqueous phase concentration determined spectrophotometrically, the distribution constant K_D was determined as 0.903.

Results and Discussion

Effect of CTAB on TIRF Intensities of H_2tpps^{4-}. Neither H_2tpps^{4-} nor H_2tmpyp^{4+} were extracted, regardless of the presence or absence of the ionic surfactants. The TIRF was observed only when the oppositely-charged surfactant to the porphyrin was added into the aqueous phase. The intensity of the fluorescence and excitation spectra were greatly enhanced by the amount of the surfactant as a counter ion for the porphyrins. Figure 1 shows the effect of CTAB on the intensities of TIRF and aqueous phase fluorescence of H_2tpps^{4-}. The addition of CTAB from 2.0×10^{-8} M (pointed as A in Fig. 1) to 2.0×10^{-7} M (pointed as B in Fig. 1) enhanced linearly the TIRF in a logarithmic scale, whereas the aqueous phase fluorescence was hardly affected by the CTAB in the concentration region, because hexadecyltrimethylammonium ion (CTA^+) was adsorbed at the interface and an excess of H_2tpps^{4-} was in the aqueous phase. In the region of A—B, the ion-association adsorption of H_2tpps^{4-} and CTA^+ is caused by an electrostatic attraction between

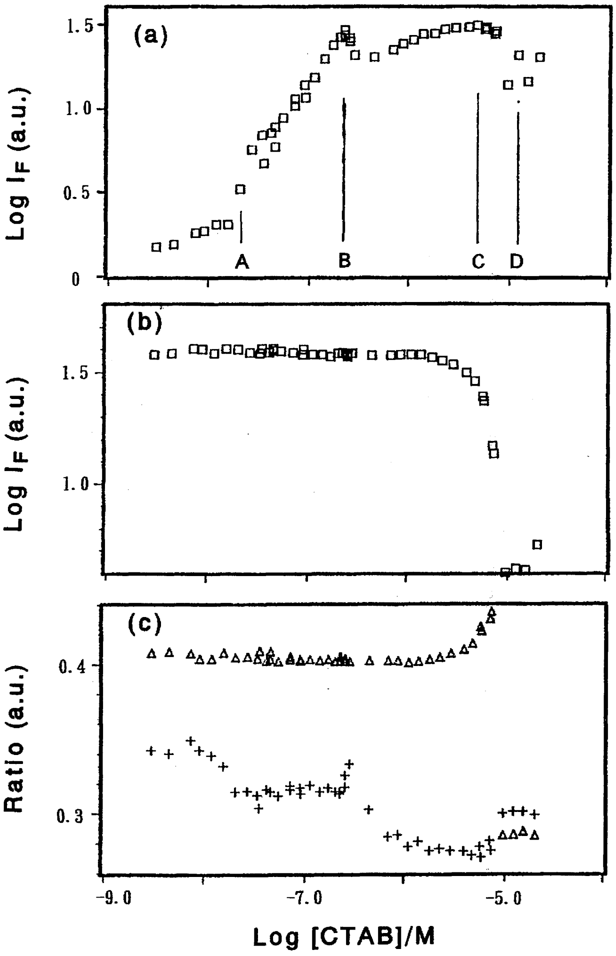

Fig. 1. Effect of CTAB concentration on the H_2tpps^{4-} fluorescence in toluene/water; (a) TIRF, (b) the aqueous phase fluorescence intensity, and (c) the ratio of two peaks, (+) interface and (△) aqueous phase; $[H_2tpps^{4-}]=1.89\times10^{-6}$ M, pH=6.2—6.4 by acetate buffer (0.0017 M). A—D refer to the spectra in Fig. 2.

the interfacially adsorbed CTA^+ and anionic H_2tpps^{4-}. The result shown in Fig. 1 suggested that the trace amount of H_2tpps^{4-} was concentrated at the 1 cm^2 interfacial area as a counter ion of the preferentially adsorbed CTA^+. The ratio of fluorescence intensity at the longer Q(0,1) band to that at the shorter Q(0,0) band depends on the molecular structure and microenvironment of porphyrins.[14] Therefore, the fluorescence intensity ratio of Q(0,1)/Q(0,0) can be used as a measure of the change of the microscopic location of a porphyrin. The ratio in TIRF of H_2tpps^{4-} was almost constant in the region of A—B, as shown in Fig. 1(c). However, when the CTAB concentration exceeded B, TIRF intensity somewhat decreased and the maximum wavelength shifted to longer wavelength. The intensity ratio in the TIRF decreased (Fig. 1(c)) and in the interfacial excitation spectra C (Fig. 2) a shoulder at 411 nm was observed, suggesting the H-aggregation of H_2tpps^{4-} as reported in hematoporphyrin.[15] These results indicate that the feature of TIRF of this system can be divided into two CTAB concentration regions: a monolayer ion-association adsorption in A—B region and a mutilayer or aggregation adsorption above B region. In the CTAB concentration around 10^{-5} M (D in Fig. 1(a)), H_2tpps^{4-} was essentially accumulated at the interface. Furthermore, the ratio of the aqueous phase fluorescence peaks at 663 and 726 nm was close to those at the interface, as shown in Fig. 1(c), suggesting that H_2tpps^{4-} in the aqueous phase was in a similar situation with the interface by the adsorption on CTA^+ micelles or toluene-swelling microemulsions.

Under the constant CTAB concentrations of 2.27×10^{-8} M and 2.27×10^{-7} M which correspond to A and B in Figs. 1 and 2, H_2tpps^{4-} concentration was varied and the TIRF and aqueous phase fluorescence intensities were measured (Fig. 3). In the lower CTAB concentration, the interfacial adsorption of H_2tpps^{4-} was very low. The fluorescence intensities in aqueous phase was consistent with those in the aqueous H_2tpps^{4-} solution without CTAB, and then the decrement in the aqueous phase concentration was negligible. In the higher CTAB concentration, TIRF intensity increased with the increase of H_2tpps^{4-} concentration until $[H_2tpps^{4-}]=1.56\times10^{-7}$ M, where TIRF intensity showed a maximum value. At this concentration, the interface must be saturated with H_2tpps^{4-}. The decrement of the aqueous phase intensity in Fig. 3(b) was linearly proportional to the increment of TIRF intensity in Fig. 3(a) in the concentration region $[H_2tpps^{4-}]=10^{-8}$—10^{-7} M. The decrement in aque-

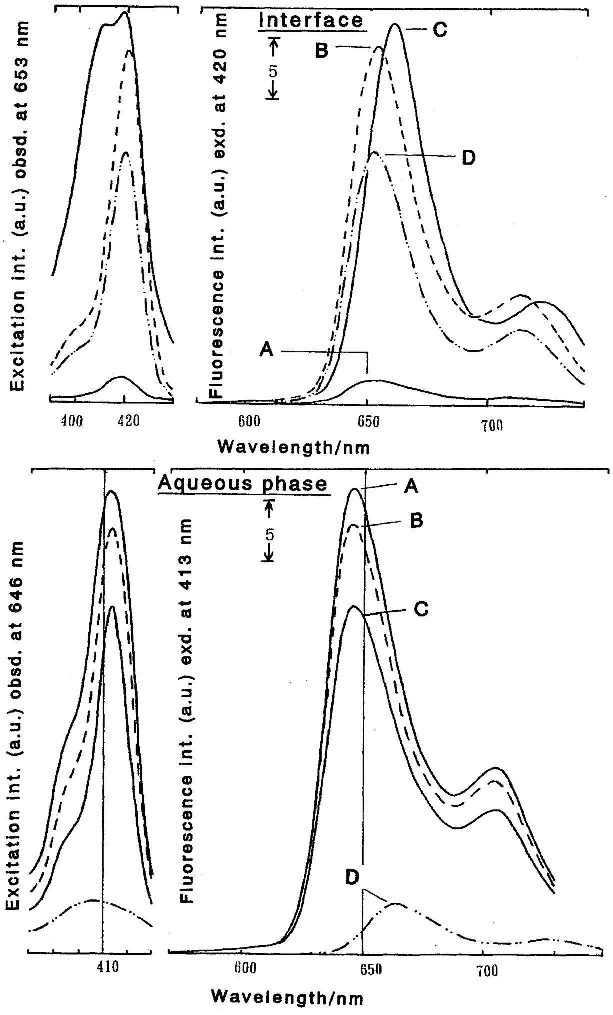

Fig. 2. Variation of the excitation and fluorescence spectra of H_2tpps^{4-} at toluene/water interface and the aqueous phase under the various concentration of CTAB; A—D are corresponding to those in Fig. 1.

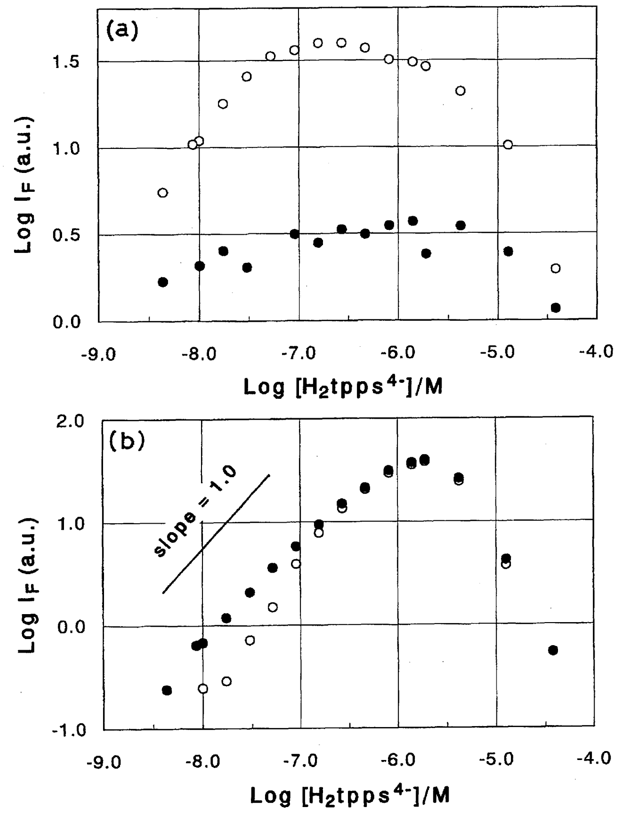

Fig. 3. Dependence of H_2tpps^{4-} concentration on the TIRF (a) and the aqueous phase fluorescence (b) intensities in toluene/water at constant CTAB concentrations; (●) [CTAB]= 2.27×10^{-8} M, (○) [CTAB]=2.27×10^{-7} M.

ous H_2tpps^{4-} concentration was calculated from the aqueous phase fluorescence decrement between the two CTAB concentrations. Thus, the maximum interfacial concentration was calculated as $[H_2tpps^{4-}]_i = 3.11\times10^{-11}$ mol cm^{-2}. The decrease in TIRF and aqueous phase fluorescence intensities in higher H_2tpps^{4-} concentration may be attributable to the concentration-quenching due to energy transfer, as previously observed in 4,4′-bis(phenylamino)-1,1′-binaphthalene-5,5′-disulfonate at decalin/water interface[8] and chlorophyll monolayer.[16]

In the H_2tpps^{4-}–CTAB systems, polarization TIRF spectra were also measured. Both excitation beam and emitted fluorescence were polarized at parallel or perpendicular to the interface and the TIRF intensities were measured. When both polarizers were set parallel to the interface, the strongest TIRF intensity was observed, suggesting a parallel adsorption of H_2tpps^{4-} ring at the interface. Two maxima at 411 and 420 nm in the excitation spectrum observed in $[H_2tpps^{4-}] = 1.89\times10^{-6}$ M and $[\mathrm{CTAB}] = 3.70\times10^{-6}$ M, corresponding to the situation C in Fig. 1(a), depended on the combination of the polarizers. When the excitation beam was polarized perpendicular to the interface, TIRF intensity at 411 nm became greater than that at 420 nm, and when polarized parallel to the interface, the TIRF intensities were inverted. This result suggested that the 411 nm peak could be attributed to the H_2tpps^{4-} aggregate, in which the porphyrin ring was adsorbed perpendicular to the interface, being stacked like a H-aggregate.[17]

Effect of SHS on TIRF Intensities of H_2tmpyp^{4+}. TIRF intensity of H_2tmpyp^{4+} varied with the SHS concentration, as shown in Fig. 4(a). In the region A—B, TIRF intensity increased linearly with the SHS concentration and reached a maximum at the concentration B. In an excess SHS over B, TIRF was not drastically changed, but the fluorescence intensity of the aqueous phase was steeply lowered. In the concentration around D, H_2tmpyp^{4+} was thought to be accumulated at the liquid–liquid interface and toluene-swollen micelles, from the agreement of Q(0,1)/Q(0,0) ratios in the TIRF and aqueous phase spectra, as shown in Fig. 4(c), and the change in the excitation spectrum D in Fig. 5. The H_2tmpyp^{4+} concentration dependence of TIRF and aqueous-phase fluorescence intensities was examined at $[\mathrm{SHS}] = 2.43\times10^{-7}$ M. The TIRF intensity in the region of $[H_2tmpyp^{4+}] = 10^{-7}$—$10^{-6}$ M was constant, while the aqueous phase fluorescence intensity linearly increased with the H_2tmpyp^{4+} concentration. From the TIRF intensity and the decrement of the H_2tmpyp^{4+} concentration in Fig. 4, the saturated interfacial concentration of H_2tmpyp^{4+} was calculated as $[H_2tmpyp^{4+}]_i = 3.36\times10^{-11}$ mol cm^{-2}.

Interfacial Tension Lowering. The interfacial tension in toluene/water system was remarkably lowered by the addition of CTAB or SHS under the presence or absence of H_2tpps^{4-} or H_2tmpyp^{4+}, as shown in Fig. 6. Comparison between Fig. 1 or 4 and Fig. 6 indicates that in the region A—B, where the TIRF intensity increased with the surfactant concentration, the interfacial tension was not lowered. This means that TIRF measurement is detecting the adsorption of very dilute porphyrin far from the saturation. Over the concentration B, the interfacial tension was appreciably lowered and the interface was saturated with the surfactant. The presence of H_2tpps^{4-} or H_2tmpyp^{4+} shifted the interfacial tension curves toward somewhat higher surfactant concentration (right direction in Fig. 6). This means that the activity of the surfactants in the interface was lowered by the ion-association with the porphyrins.

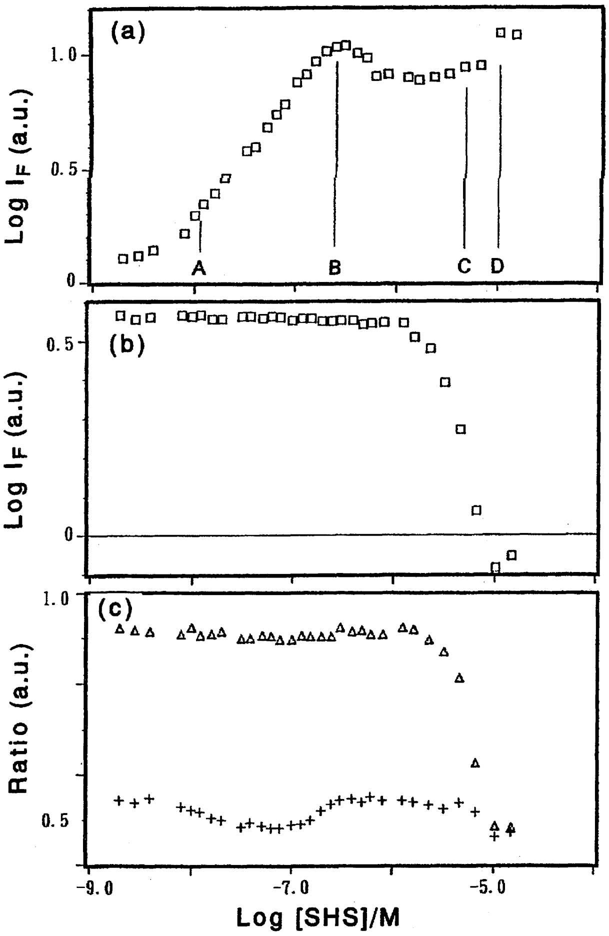

Fig. 4. Effect of SHS concentration on the H_2tmpyp^{4+} fluorescence in toluene/water; (a) TIRF, (b) the aqueous phase fluorescence intensity, and (c) the ratio of two peaks, (+) interface and (△) aqueous phase; $[H_2tmpyp^{4+}] = 8.78\times10^{-7}$ M, pH=6.2—6.4 by acetate buffer (0.0017 M). A—D refer to the spectra in Fig. 5.

Ion-Association Adsorption Equilibria. A stoichiometric model for the ion-association adsorption of H_2tpps^{4-} and CTA^+ can be represented by

$$H_2tpps^{4-} + nCTA_i^{+} \rightleftarrows H_2tpps\cdot nCTA_i^{(4-n)-} \qquad (3)$$

$$K = [H_2tpps\cdot nCTA^{(4-n)-}]_i/[H_2tpps^{4-}][CTA^{+}]_i^{\,n} \qquad (4)$$

where [ ] and [ ]$_i$ refer to the formal concentrations in aqueous phase (M) and the interface (mol cm^{-2}). The total concentration of CTAB is given by the next equation, assuming that all CTA^+ ions are practically adsorbed:

$$[\mathrm{CTAB}]_T V = [CTA^{+}]_i A_i + n[H_2tpps\cdot nCTA^{(4-n)-}]_i A_i \qquad (5)$$

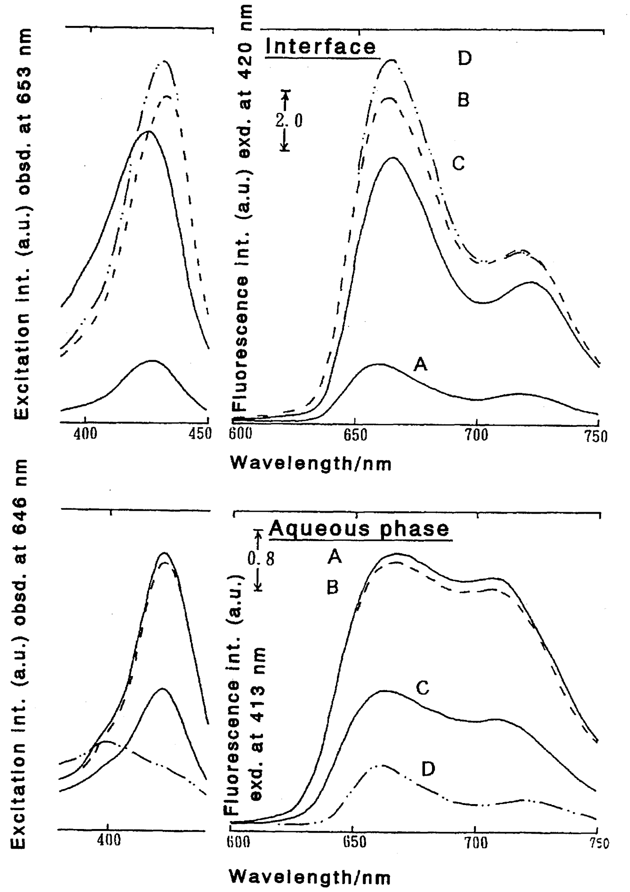

Fig. 5. Variation of the excitation and fluorescence spectra of H_2tmpyp^{4+} at toluene/water interface and the aqueous phase with the concentration of SHS; A—D are corresponding to those in Fig. 4.

where V and A_i are the volume of aqueous phase (1.2 cm^3) and the interfacial area (1.0 cm^2). Provided that $[H_2tpps^{4-}]$ is approximated by $[H_2tpps^{4-}]_T$ (i.e., $[H_2tpps^{4-}]_T \gg n[H_2tpps{\cdot}nCTA^{(4-n)-}]_i A_i/V$) and $n=1$, the next equation is derived:

$$\log [H_2tpps{\cdot}CTA^{3-}]_i = \log (K[H_2tpps^{4-}]_T/(1+K[H_2tpps^{4-}]_T)) + \log ([CTAB]_T V). \quad (6)$$

The interfacial concentrations of $[H_2tpps{\cdot}CTA^{(4-n)-}]_i$ were calculated from $[H_2tpps{\cdot}CTA^{(4-n)-}]_i = \Gamma_i I_F/I_{Fsat}$ where I_F was the TIRF intensity in Fig. 1, I_{Fsat} the maximum TIRF intensity in Fig. 3 and Γ_i the maximum interfacial concentration of $[H_2tpps^{4-}]_i = 3.11\times10^{-11}$ mol cm^{-2}. The plots of $\log [H_2tpps{\cdot}CTA^{(4-n)-}]_i$ against $\log ([CTAB]_T V)$ showed a straight line with slope 1.10 and the intercept gave the value for K as 3.06×10^6 M^{-1} (Figure is not shown). For the case of H_2tmpyp^{4+}, an analogous analysis could be applied by defining the equations:

$$H_2tmpyp^{4+} + nHS_i^- \rightleftarrows H_2tmpyp{\cdot}nHS_i^{(4-n)+} \quad (7)$$

$$K = [H_2tmpyp{\cdot}nHS^{(4-n)+}]_i/[H_2tmpyp^{4+}][HS^-]_i^n. \quad (8)$$

where HS^- is 1-hexadecanesulfonate. The plots of $\log [H_2tmpyp{\cdot}HS^{3+}]_i$ and $\log ([SHS]_T V)$ gave a straight line with the slope of 0.96 and the value of $K=8.68\times10^4$ M^{-1} was obtained (Figure is not shown). These results indicate that the ion-association adsorption can be treated formally as 1 : 1 reaction between the porphyrin and surfactant, regardless of the difference in the charge number. The results does not mean the formation of a contact ion pair of a porphyrin and a surfactant molecule at the interface, because the concentration of interfacial porphyrins measured by the TIRF method includes those in a Stern layer and diffuse layer just outside the Stern layer. Therefore, the present result only suggests that the concentration of the porphyrins in the totally internal reflection region (ca. 136 nm) is linearly proportional to the surfactant concentration.

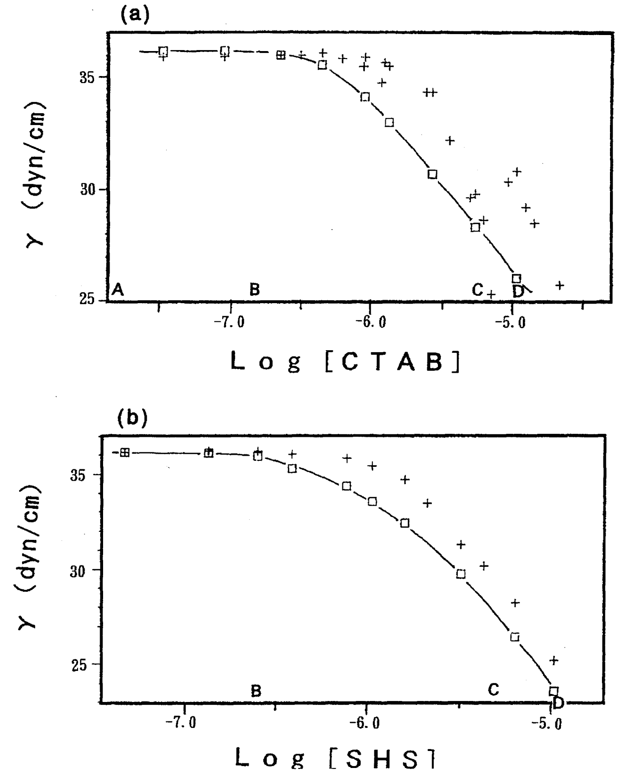

Fig. 6. Interfacial tension lowering in toluene/water with the concentrations of CTAB (a) and SHS (b): (a) (□) $[H_2tpps^{4-}]=0$ M, (+) $[H_2tpps^{4-}]=1.96\times10^{-6}$ M; (b) (□) $[H_2tmpyp^{4+}]=0$ M, (+) $[H_2tmpyp^{4+}]=1.01\times10^{-6}$ M. A—D refer to the situations in Fig. 1 or 4.

Electrical Double Layer Consideration. It will also be possible to interpret the ion-association reaction from electrostatic point of view. The adsorption of the ionic-surfactants at toluene/water interface produces a two-dimensionally charged surface onto which opposite-charged ions can adsorb from the bulk aqueous phase. The distribution of the opposite-charged ions, i.e., counter ions for the surfactant monolayer, is governed by the electrical potential at the charged surface ϕ_0 which is related to the interfacial charge density and the bulk electrolyte concentration in the aqueous phase by the Gouy–Chapman equation:

$$\phi_0 = \frac{2kT}{ze} \sinh^{-1} \left\{ \frac{\sigma}{(8\varepsilon\varepsilon_v nkT)^{1/2}} \right\}, \quad (9)$$

where σ is the charge density at the interface ($\mathrm{C\,cm^{-2}}$), e is the elementary charge (1.602×10^{-19} C), ε is the relative dielectric permittivity of the bulk water (78.30 at 25 °C), ε_v is the permittivity of the vacuum ($8.8542\times10^{-14}\ \mathrm{C^2\,J^{-1}\,cm^{-1}}$), n is here the concentration of acetate ion ($\mathrm{number\,cm^{-3}}$), and z is the charge of acetate ion.[18] The charge density of the surfactant monolayer σ was estimated from the interfacial concentration of $\mathrm{CTA^+}$ or $\mathrm{SH^-}$, multiplied by e and the Avogadro number, assuming quantitative adsorption of these ions. The relation between the electrical potential at the interface and the interfacial concentration of the ionic derivatives of porphyrin at equilibrium can be represented by

$$\log C_\mathrm{i} = \log C_\mathrm{o} - (ze/2.303RT)\phi_0 \qquad (10)$$

where C_i and C_o refer to the interfacial and bulk phase concentration of the porphyrin, respectively, and z to the charge of the porphyrin. In Fig. 7, the interfacial concentrations determined by TIRF were plotted against ϕ_0. Both plots for $\mathrm{H_2tpps^{4-}}$ and $\mathrm{H_2tmpyp^{4+}}$ showed good linearity with the slopes of 33.7 V^{-1} and −36.0 V^{-1} for $\mathrm{H_2tpps^{4-}}$ and $\mathrm{H_2tmpyp^{4+}}$ systems, respectively. The absolute values of slopes were smaller than the ideal one of 67.6 V^{-1} expected from Eq. 10; 49.9 and 53.3% of the expected one for $\mathrm{H_2tpps^{4-}}$ and $\mathrm{H_2tmpyp^{4+}}$ systems, respectively. This means that the interfacial concentration at the equilibrium determined by TIRF was apparently reduced to about one half of the expected one from the original interfacial potential ϕ_0. This may be attributable to the lowering of the interfacial potential by the adsorption of the opposite-charged porphyrins as counter ions at the Stern layer.

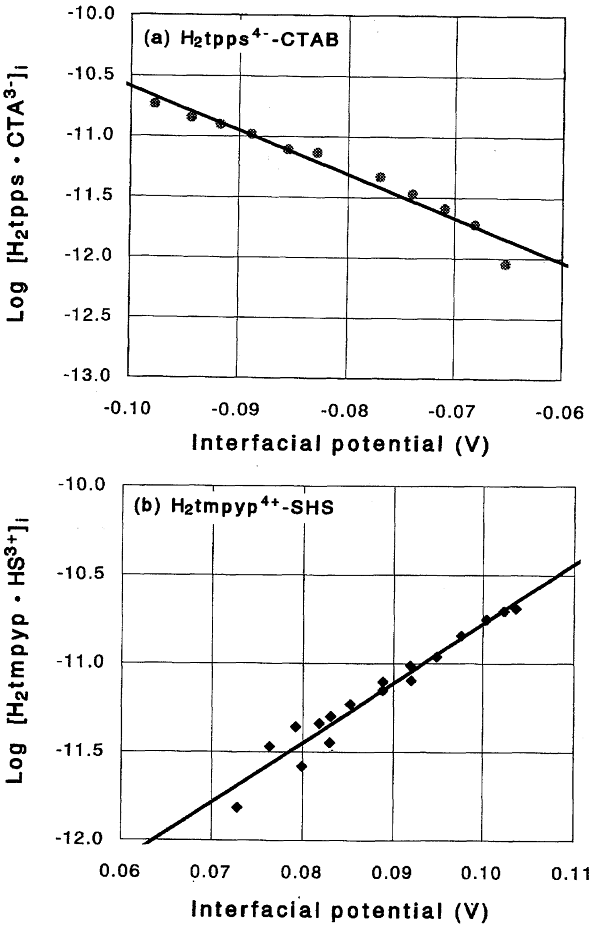

Fig. 7. Linear correlation between the interfacial concentration of the porphyrins and the interfacial potential; (a) $\mathrm{H_2tpps^{4-}}$–CTAB system, (b) $\mathrm{H_2tmpyp^{4+}}$–SHS system.

Interfacial Electrostatic Separation between $\mathrm{H_2tpps^{4-}}$ and $\mathrm{H_2tmpyp^{4+}}$. The interfacial charge served by the adsorption of ionic surfactants can discriminate a positive or negative porphyrin as a counter ion. When the aqueous phase was containing both of $\mathrm{H_2tpps^{4-}}$ (8.5×10^{-7} M) and $\mathrm{H_2tmpyp^{4+}}$ (8.8×10^{-7} M), the addition of $\mathrm{CTA^+}$ at toluene/water interface caused the TIRF spectra of only $\mathrm{H_2tpps^{4-}}$, while the addition of $\mathrm{HS^-}$ showed only $\mathrm{H_2tmpyp^{4+}}$ spectra. No TIRF spectra were observed without the surfactants, indicating that there was no ion-association adsorption between $\mathrm{H_2tpps^{4-}}$ and $\mathrm{H_2tmpyp^{4+}}$ measurable by TIRF. These results proved definitely that the electrostatic discrimination and concentration of the trace amount of porphyrins could be accomplished within the interfacial region of at most 100 nm thickness and detected by TIRF.

Adsorption of Zn(II)–$\mathrm{tpps^{4-}}$ with Zn(II)–phen Complex. The reaction between $\mathrm{H_2tpps^{4-}}$ and excess $\mathrm{Zn^{2+}}$ in aqueous solution was very slow, but yielded a fluorescent $\mathrm{[Zn(tpps)]^{4-}}$ complex quantitatively after one day. The aqueous solution of $\mathrm{[Zn(tpps)]^{4-}}$ and toluene was contacted in a quartz cell and then the effects of CTAB, phen, pyridine, trioctylamine and trioctylphosphine oxide on the TIRF of $\mathrm{[Zn(tpps)]^{4-}}$ were examined under the presence of excess $\mathrm{Zn^{2+}}$ ions. Among these additives, phen enhanced most effectively the TIRF intensity. Therefore, the effect of phen on the TIRF was studied in detail. Under the constant concentrations of $\mathrm{[Zn(tpps)]^{4-}}=1.93\times10^{-6}$ M and $\mathrm{[Zn^{2+}]}=9.8\times10^{-4}$ M, TIRF intensity was measured with various concentrations of phen. In Fig. 8, the phen concentration dependence of TIRF intensity at 607 nm is shown together with the aqueous phase fluorescence intensity and the intensity ratios at the peaks of 607 and 662 nm. The TIRF intensity increased in proportion to about the third power of phen concentration. The TIRF peak ratio increased with the increase in the TIRF intensity. The maximum in the total internal reflection excitation spectrum in Fig. 9 shifted from 430 to 440 nm, suggesting an interfacial aggregation (probably forming J-aggregate[19]) of the $\mathrm{[Zn(tpps)]^{4-}}$ complex or an incorporation of $\mathrm{[Zn(tpps)]^{4-}}$ into a hydrophobic environment of the interface with the excess Zn(II)–phen complex.

The effect of phen on the adsorption of $\mathrm{[Zn(tpps)]^{4-}}$ in the presence of excess $\mathrm{Zn^{2+}}$ was interpreted by considering the formation of $\mathrm{[Zn(tpps)]^{4-}}$, the distribution of phen, the formation of $\mathrm{Zn^{2+}}$–phen complexes and the ion-association adsorption:

$$\mathrm{Zn^{2+} + H_2tpps^{4-} \rightleftarrows [Zn(tpps)]^{4-} + 2H^+}, \qquad (11)$$

$$\mathrm{phen \rightleftarrows phen_o}, \quad K_\mathrm{D} \qquad (12)$$

$$\mathrm{Zn^{2+} + phen \rightleftarrows [Znphen]^{2+}}, \quad \beta_1 \qquad (13)$$

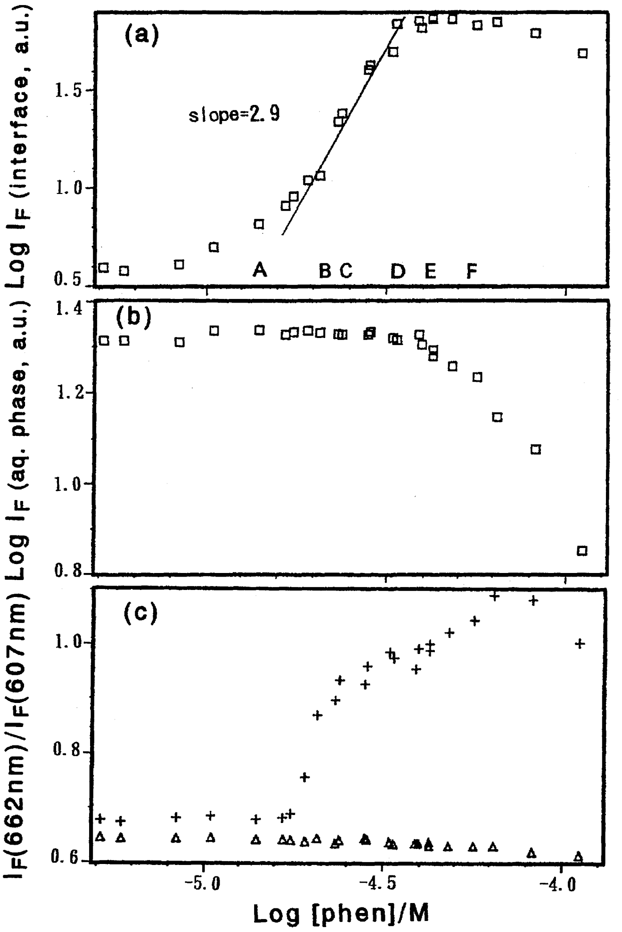

Fig. 8. Enhancement of (a) TIRF intensity, (b) lowering of aqueous phase fluorescence intensity and (c) the intensity ratio at 662 nm and 607 nm in the TIRF (+) and aqueous phase spectra (△) of $[\mathrm{Zn(tpps)}^{4-}]$ in toluene/water system with phen concentration in the presence of excess Zn^{2+}; $[\mathrm{Zn(tpps)}^{4-}]=1.93\times10^{-6}$ M, $[\mathrm{Zn}^{2+}]=9.8\times10^{-4}$ M, $[\mathrm{NaClO_4}]=0.01$ M, pH=6.1—6.2 by acetate buffer (0.0017 M). A—F refer to the spectra in Fig. 9.

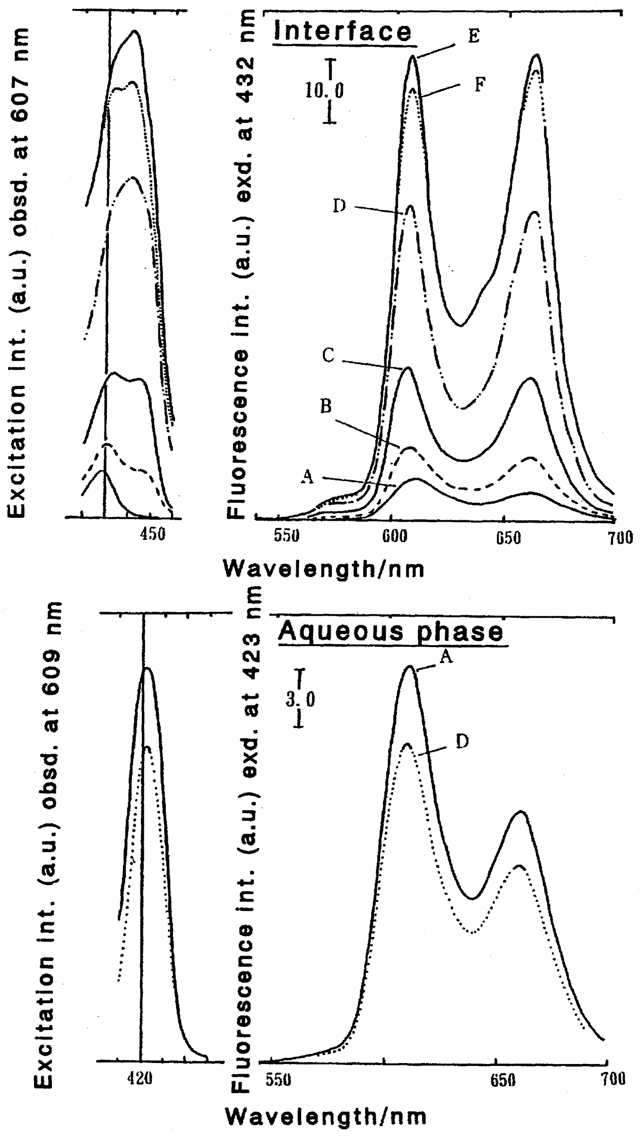

Fig. 9. Variation of fluorescence and excitation spectra in toluene/water interface and aqueous phase under the various phen concentrations in the presence of excess Zn^{2+}; $[\mathrm{Zn(tpps)}^{4-}]=1.93\times10^{-6}$ M, $[\mathrm{Zn}^{2+}]=9.8\times10^{-4}$ M, $[\mathrm{NaClO_4}]=0.01$ M, pH=6.1—6.2 by acetate buffer (0.0017 M): A—F are corresponding to those in Fig. 8.

$$\mathrm{Zn}^{2+} + 2\mathrm{phen} \rightleftarrows [\mathrm{Zn(phen)_2}]^{2+}, \quad \beta_2 \tag{14}$$

$$\mathrm{Zn}^{2+} + 3\mathrm{phen} \rightleftarrows [\mathrm{Zn(phen)_3}]^{2+}, \quad \beta_3 \tag{15}$$

$$[\mathrm{Zn(phen)}_n]^{2+} + [\mathrm{Zn(tpps)}]^{4-} \rightleftarrows [\mathrm{Zn(phen)}_n]^{2+}\cdot[\mathrm{Zn(tpps)}]_{\mathrm{i}}^{4-} \quad K_{\mathrm{i}}. \tag{16}$$

The TIRF intensity of $[\mathrm{Zn(tpps)}]^{4-}$ must be dependent on the concentration of $[\mathrm{Zn(phen)}_n]^{2+}$ (n=1, 2 or 3) which adsorbs at the toluene/water interface and works as a counter ion of $[\mathrm{Zn(tpps)}]^{4-}$. Hence, the TIRF intensity of $[\mathrm{Zn(tpps)}]^{4-}$ is represented by

$$\begin{aligned} I_{\mathrm{F}} &= \phi[\mathrm{Zn(phen)}_n{}^{2+}\cdot\mathrm{Zn(tpps)}^{4-}]_{\mathrm{i}} \\ &= \phi K_{\mathrm{i}}\beta_n[\mathrm{Zn(tpps)}^{4-}][\mathrm{Zn}^{2+}]([\mathrm{phen}]_{\mathrm{o}}/K_{\mathrm{D}})^n, \end{aligned} \tag{17}$$

where ϕ is a conditional TIRF efficiency which is estimated as 7.6×10^{11} $\mathrm{cm^2\,mol^{-1}}\cdot$(unit I_{F})$^{-1}$ under the present experimental conditions, assuming that the saturated interfacial concentration is 10^{-10} mol cm^{-2}. The slope of 2.9 in Fig. 8(a) strongly suggested that the ion-association adsorption of $[\mathrm{Zn(tpps)}]^{4-}$ was caused by the adsorption of $\mathrm{Zn(phen)_3}^{2+}$ as the predominant species. As the interface was saturated at the phen concentration of 3.5×10^{-5} M, as can be seen in Fig. 8, one can estimate the ion-association adsorption constant as $\log K_{\mathrm{i}}=-5.3$ by using $\log\beta_3=17.33$[20] and by assuming the saturated concentration of $[\mathrm{Zn(phen)_3}^{2+}\cdot\mathrm{Zn(tpps)}^{4-}]_{\mathrm{i}}=10^{-10}$ mol cm^{-2}.

The effect of Zn(II)–phen complex on the adsorption of $[\mathrm{Zn(tmpyp)}]^{4+}$ was examined also, but there was no enhancement effect on the TIRF intensity; this absence was expected from the unfavorable electrostatic interaction between them. This result also supports the ion-association adsorption mechanism discussed above.

It was concluded in the present study that the water-soluble porphyrins and its Zn(II) complex could be adsorbed at the liquid–liquid interface, being assisted by the adsorption of interfacially active ions or complexes. The phenomena of ion-association adsorption are generally important in the separation processes of ionic species, e.g., ion-chromatography, ion flotation, liquid-membrane and solid phase extraction as well as liquid–liquid extraction. Though the interfacial structure and orientation of the ion-association complex is still equivocal, future study employing time-resolved fluorescence depolarization technique will provide a more definitive picture.

The authors thank Dr. S. Hamai of Akita University for his kind suggestions. This work was supported by a Grant-in-Aid for Scientific Research (A) No. 07404042 from the Ministry of Education, Science, Sports and Culture.

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