Total Internal Reflection Fluorescence Measurements of Ion-Association Adsorption of Water-Soluble Porphyrins at Liquid/Liquid Interface

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(Received December 8, 1994)

Ion-association adsorption of water-soluble, surface-inactive anionic and cationic porphyrins with the oppositely charged surfactants was observed at toluene/water interface by means of a total internal reflection fluorometry. Water-soluble zinc(II)-porphyrin was also adsorved at the liquid/liquid interface with a hydrophobic zinc(II)-1,10-phenanthroline complex ion.

Adsorption of ionic species at the liquid/liquid interface is a significant elementary process in the solvent extraction and the related separation systems such as liquid-membrane separation, ion-selective liquid-membrane electrodes and counter current chromatography. Our previous investigations revealed that the adsorptioin and dissociation of hydrophobic extractants at the interface are primary factors governing the rate of extraction of metal complexes.[1] The adsorption equilibria of the organic extractants have mainly been studied by means of a spectrophotometry under highly stirred conditions and an interfacial tension measurement.[2] The high-speed stirring method developed in our previous studies is powerful for the spectrophotometric measurement of the interfacial adsorption of solutes in organic phase even at very low concentration.[3] These methods have been successfully used for the study of the role of the liquid/liquid interface in various extraction systems, while for the purpose of *in situ* measurements of the liquid/liquid interfaces other spectroscopic techniques are required.

In the present study, we will report successful applications of a total internal reflection fluorometry (TIRF) for the *in situ* measurements of interfacial ion-association adsorption of water-soluble porphyrins. Since TIRF was first introduced for selective surface illumination at liquid/solid interfaces by Hirschfeld in 1965,[4] a number of applications have been reported mostly on water/glass interfaces, wherease the applications on liquid/liquid interfaces have extremely been limited.[5]

The porphyrins used in this study were 5,10,15,20-tetraphenyl-21H,23H-porphinetetrasulfonic acid (TPPS) and 5,10,15, 20-tetrakis(N-methylpyridinium-4-yl)-21H,23H-porphine (TMPyP), both purchased from Dojindo. Cetyltrimethylammonium bromide (CTAB) and sodium hexadecane sulfonate (SHS) were used as ionic surfactants, which could control both sign and density of the charge at the interface. Toluene was used as an organic solvent and pH of the aqueous phase was controlled within 6.2-6.4 by 0.0017 M (1M = 1mol/dm^3) acetate buffer. The optical arrangement for the total internal reflection fluorometry is illustrated in Figure 1. Hitachi 650-40 fluorescence spectrophotometer was used for the fluorescence measurements. On both sides of the cell holder which faced toward the excitation light source and the fluorescence detector, two right-angle glass prisms were set so that the incident excitation beam struck the interface with the incident angle of about 72° , which was enough greater than the critical total reflection angle of 63° in the toluene/water system. The penetration depth of the evanescent wave in the aqueous phase side of the interface was estimated as 136nm at 420nm excitation. The upper inside of an optical quartz cell (10mm) was treated with dichlorodimethylsilane in benzene so as to make the inside wall hydrophobic and afford a flat toluene/water interface. All measurements were carried out at 25 ± 0.1° C.

TPPS (-4 charged) and TMPyP (+4 charged) were both well soluble in aqueous phase in neutral pH and could not be extracted

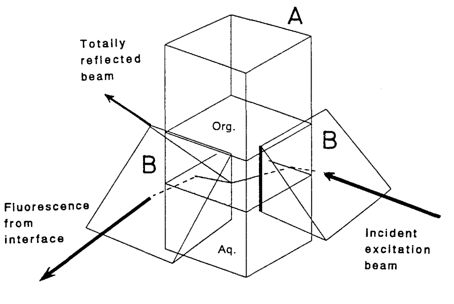

Figure 1. Schematic drawing of the optical cell arrangement for the TIRF measurements; (A) 10 mm optical cell, (B) right angle glass prism.

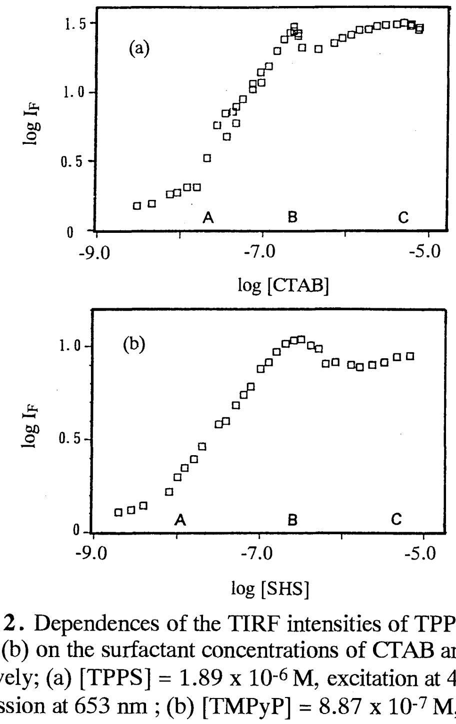

Figure 2. Dependences of the TIRF intensities of TPPS (a) and TMPyP (b) on the surfactant concentrations of CTAB and SHS, respectively; (a) [TPPS] = 1.89 x 10^{-6} M, excitation at 420 nm and emission at 653 nm ; (b) [TMPyP] = 8.87 x 10^{-7} M, excitation at 430 nm and emission at 663 nm.

into toluene phase at all, even after full one day shaking of the two phases and even under the presence of the ionic surfactants. Both porphyrins showed intense fluorescence in the aqueous phases, wherease no fluorescence was observed at the interface, unless the ionic surfactants were added. The addition of SHS or CTAB even below 10^{-7} M enhanced remarkably the TIRF of TMPyP or TPPS, respectively, as shown in Figure 2. In the region A - B, the TIRF intensity increased with the surfactant concentration without any change in the spectral shape. In the region B - C, a significant shift appeared in the excitation and fluorescence spectra, suggesting an interfacial aggregation[6] and some quenching by energy transfer.[7] Throughout the concentration region A - C, there appeared no fluorescence in the organic phase. The surfactants in the cell were suggested to be adsorbed at the interface from the interfacial tension lowering measured by means of the drop volume method. [3] The observed interfacial fluorescence enhancement can be interpreted in terms of ion-association reaction at the liquid/liquid interface. If one can postulate stoichiometry for the interfacial adsorption, the ion-association reaction and the apparent formation constants can be given by,

$$TPPS^{4-} + n\,CTA^{+} \rightleftarrows TPPS \cdot nCTA^{(4-n)-}{}_{i}$$

$$K = [TPPS \cdot nCTA^{(4-n)-}]_{i} / [TPPS^{4-}][CTA^{+}]^{n} \quad (1)$$

and

$$TMPyP^{4+} + n\,HS^{-} \rightleftarrows TMPyP \cdot nHS^{(4-n)+}{}_{i}$$

$$K = [TMPyP \cdot nHS^{(4-n)+}]_{i} / [TMPyP^{4+}][HS^{-}]^{n} \quad (2)$$

where the subscript i refers to the interface. The saturated interfacial concentrations of TPPS • $nCTA^{(4-n)-}{}_{i}$ and TMPyP • $nHS^{(4-n)+}{}_{i}$ could be estimated from the decrements in the aqueous phase concentrations of the porphyrins as $\Gamma_i^* = 3.1 \times 10^{-11}$ mol/cm^2 and $\Gamma_i^* = 3.2 \times 10^{-11}$ mol/cm^2, respectively, provided that the concentrations of TPPS and TMPyP were in excess of those of CTAB and SHS, respectively. Hence, the interfacial concentrations of TPPS • $nCTA^{(4-n)-}$ and TMPyP • $nHS^{(4-n)+}$ at a given surfactant concentration were determined by $\Gamma_i^*(I_F/I_F^*)$, where I_F and I_F^* are the TIRF intensities before and at the saturation. The log-log plots of the interfacial concentrations against the total concentrations of the surfactants, according to the equations (1) and (2), indicated n = 1 for both systems, and gave the values of K = 3.7×10^4 mol/M^{2}cm^2 for TPPS-CTAB system and K = 1.0×10^2 mol/M^{2}cm^2 for TMPyP-SHS system under the conditions described in the caption of Figure 2. Polarized TIRF measurements showed that the I_F was strongest when the polarizers for the excitation and detection were both set for s-polarization, therefore the transition dipole moments of the adsorved porphyrins was suggested to be in parallel to the interface.

The ion-association adsorption of TPPS-CTAB and TMPyP-SHS is more clearly demonstrated by the interfacial separation of TPPS or TMPyP by the use of CTAB or SHS. Figure 3 shows exactly this situation: CTA^+ and HS^- could selectively attract the oppositely charged porphyrins. The interfacial ion-association adsorption observed in the present study is invaluable not only as a direct proof of the microscopic separation of the ions within the diffuse layer, but also a direct evidence for the formation of the electrical double layer in the visinity of the liquid/liquid interface.

The interfacial adsorption of zinc(II)-TPPS complex with CTA- was also observed by means of TIRF measurement. Zinc(II)-TPPS complex was formed in the aqueous mixture of TPPS (2.0×10^{-6} M) and zinc(II) perchlorate (1.0×10^{-3} M) at pH 6.1-6.3, after standing for over night. The interface between toluene and the aqueous solution did not show any TIRF. However, an addition of 1,10-phenanthroline (phen) to the aqueous phase induced an intense TIRF. These phenomena were interpreted in terms of the ion-association adsorption of Zn-TPPS anion with $Zn(phen)_3{}^{2+}$ at the interface, since $Zn(phen)_3{}^{2+}$ was adsorbable at the liquid/liquid interface.[8]

The present study clearly demonstrated the ion-association discrimination of a trace amount of ionic species at the interface. This means that the ionic species can be separated and determined at the liquid/liquid interface by the use of the TIRF with an ion-association reagent, regardless of the extraction into the organic phase.

The authers wish to thank Dr. S. Hamai and Mrs. F. Funaki for their kind suggestions. This work was supported by Grant-in-Aids for General Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 05453066 and No. 05303004).

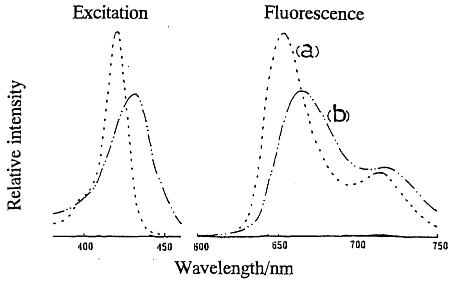

Figure 3. Interfacial ion-association separation of TPPS and TMPyP from their mixture in aqueous phase with the ionic surfactants of CTAB and SHS, respectively;(a) [TPPS] = 8.52×10^{-7} M, [CTAB] = 2.26×10^{-7} M, (b) [TMPyP] = 8.87×10^{-7} M, [SHS] = 2.00×10^{-7} M.

References and Notes

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