TSINGHUA SCIENCE AND TECHNOLOGY
ISSN 1007-0214 14/18 pp228-232
Volume 11, Number 2, April 2006

Spectroscopic Detection of Chiral Aggregation at Liquid-Liquid Interfaces*

Hitoshi Watarai**, Sayaka Wada, Kazuhiko Fujiwara

Department of Chemistry, Osaka University, Osaka, Japan

Abstract: Two new spectroscopic methods to detect the optical activity of liquid-liquid interfaces have been developed. The first one is the centrifugal liquid membrane (CLM) method combined with a conventional circular dichroism (CD) spectropolarimetry and the second one is a more interfacial specific second harmonic generation CD (SHG-CD) spectrometry. In the CLM-CD method, a cylindrical glass cell containing small amounts of organic and aqueous phases was rotated at about 7000 r/min in a sample chamber of a CD spectropolarimeter to generate an interface with a high specific interfacial area between the two-phase liquid membranes. The CD spectra of the J-aggregate of protonated 5,10,15, 20-tetraphenylporphyrin formed at the toluene-sulfuric acid interface have been measured. As for the SHG-CD, a circularly polarized wavelength-variable fs-laser system was constructed to measure the interfacial SHG spectra of a flat liquid-liquid interface. The ion-associated aggregation of a water-soluble anionic porphyrin promoted with a cationic amphiphile at the heptane-water interface was observed by this technique and the observed SHG-CD spectra proved the generation of a characteristic optical activity accompanied by the formation of the interfacial aggregate of inherently achiral porphyrin molecules. These methods will pioneer a new field of interfacial chiral chemistry in the studies of solvent extraction mechanisms.

Key words: chiral aggregation; liquid-liquid interface; spectroscopic detection; centrifugal liquid membrane; second harmonic generation circular dichroism

Introduction

Optical activity is a fundamental property, which provides valuable information on the structure of molecules and the conformation of their assemblies. Therefore, it is widely used in many fields including organic chemistry, coordination chemistry, and biochemistry. However, it has been rarely applied in the field of surface and interfacial chemistry, in spite of great requirement of chiral measurement of such systems. The main problem might be in a very low amount of the interfacial compound and also in a difficulty to match such interfacial amount with conventional measurement methods. This situation clearly required some invention for proper combination of sample system and optical measurement system.

The liquid-liquid interface is a specific reaction field, at which key reactions to determine the solvent extraction kinetics can proceed in most systems[1]. Interfacial compounds behave usually with different manners from those in bulk phases. The liquid-liquid interface is also considered as a model of biomembrane. However, it is quite difficult to measure the optical activity of the interface, although the optical activity is the most important property of biomolecules and biomem-

Received: 2005-10-09

* Supported by the Grant-in-Aid for Scientific Research (No. 16105002) from the Ministry of Education, Science, Sports, and Culture, Japan

** To whom correspondence should be addressed.
E-mail: watarai@chem.sci.osaka-u.ac.jp

branes. There have been no previous reports about the measurement of optical activity of the liquid-liquid interface. We described here two new experimental methods to measure the optical activity of liquid-liquid interfaces, which are very useful for the studies of interfacial phenomena in solvent extraction systems.

The centrifugal liquid membrane (CLM) method, which provides the thin two-phase liquid membrane system in a rotating cell, has been used to measure *in situ* interfacial spectra such as UV-Vis[2], fluorescence[3], and Raman spectra[4]. In the present study, we applied the CLM method to measure the circular dichroism spectra at the liquid-liquid interface in combination with a conventional circular dichroism spectropolarimetry.

Another promising technique to measure the chirality at the interface will be the second harmonic generation-circular dichroism (SHG-CD) spectroscopy, as demonstrated by Hicks and co-workers[5,6]. SHG is advantageous to other spectroscopic techniques in its inherent high interfacial selectivity due to the second order nonlinear susceptibility and in its high sensitivity afforded by the resonance effect in an adsorbed dye molecule. Therefore, SHG spectroscopy can provide specific information on the electronic state and orientation of dye molecules, which is exhibited only at the interface.

SHG spectroscopy has been utilized to study solvation and aggregation of dye molecules at the electrochemical liquid-liquid interfaces. Recently, the nonlinear optical activity of originally chiral molecules adsorbed at various interfaces has been observed by means of SHG-CD[7,8]. However, the nonlinear optical activity of the liquid-liquid interface adsorbed by an aggregate of achiral molecules has never been reported. The present study reports the nonlinear chirality of J-aggregate formed from the achiral tetrakis(4-sulfonatephenyl)porphyrin diacid (H_4TPPS^{2-}) and cetyltrimethylammonium (CTA^+) surfactant at the heptane-water interface by the SHG-CD spectroscopy.

Porphyrin has been investigated extensively because of its spectroscopically unique properties, including a large molar absorptivity and biological activity. Optical activity of porphyrin derivatives has also been studied[9]. Ohno et al. reported that a water-soluble achiral porphyrin, tetrakis(4-sulfonatophenyl) porphyrin (H_2TPPS^{4-}), showed optical activity, once it formed J-aggregate in an acidic aqueous solution or in a high ionic-strength solution, suggesting an exciton coupling of the extend dipoles in the porphyrin aggregate[10]. Porphyrin aggregates in bulk solutions have been characterized by various spectroscopic techniques. However, no report has been known on the chirality of porphyrin aggregate at the liquid-liquid interface. In the present study, we used H_2TPP as a hydrophobic porphyrin and H_2TPPS^{4-} as a water-soluble porphyrin, both of which were expected to form J-aggregate at the interface in proper solution conditions.

1 Centrifugal Liquid Membrane/ Circular Dichroism Spectrometry

1.1 Materials

5,10,15,20-Tetraphenylporphyrin (Aldrich, H_2TPP) was dissolved in 4.0×10^{-5} mol/L toluene and diluted to 1.0×10^{-5} mol/L in the CLM experiment. Toluene (Nakarai Tesque, G.R.) was purified by distillation after the treatment with a mixture of fuming sulfuric acid and sulfuric acid. Sulfuric acid (Nakarai Tesque, G.R.) for the CD experiments was diluted to the concentration of 4 mol/L. The aqueous solutions were prepared with water distilled and deionized through a Milli-Q system (Millipore, Milli-Q SP. TOC.).

1.2 CLM/CD measurement

CD spectra were measured by a CLM cell installed in a circular dichroism spectropolarimeter (JASCO, J-820) in the configuration shown in Fig. 1. CLM cell, which has 3.3 cm height and 2.1 cm outer diameter with a sample inlet hole of 2 mm in diameter at the bottom was fixed horizontally in the sample chamber of the CD spectropolarimeter. A portion of 0.500 cm^3 of 4 mol/L H_2SO_4 and 0.300 cm^3 toluene were put into the cylindrical cell thorough the sample inlet hole. Then, the cylindrical cell was rotated at about 7000 r/min by the high-speed motor (Nakanishi Inc., NK-260) fixed on *XZ*-stage, connected to a speed controller (Nakanishi Inc., NE-22E). The toluene phase was spread as an inner liquid membrane and the aqueous phase as an outer liquid membrane by a centrifugal force. The shape and size of the cross section of the light beam of the spectropolarimeter were controlled by a round hole slit with the diameter of 9 mm, to adjust the light beam

center to the center of the liquid membranes cell.

The sum of the CD spectra of the interface, the bulk organic phase, and the bulk aqueous phase can be measured by this method. After the blank was measured, H_2TPP toluene solution (0.100 cm^3) was injected rapidly by a micro-syringe from the sample inlet hole to initiate the protonation of H_2TPP and the aggregation of H_4TPP^{2+}. Normal absorption spectra of the interface were also measured by the CLM cell with a photodiode array spectrophotometer (Agilent Technologies, Agilent 8453), by the same manner with the previous studies[11]. All measurements were carried out at (298±2) K.

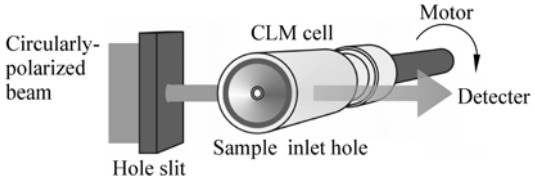

Fig. 1 Centrifugal liquid membrane cell.

1.3 CD spectra of interface

UV-Vis absorption spectra of the J-aggregate of H_4TPP^{2+} adsorbed at the toluene-4 mol/L H_2SO_4 interface and H_2TPP monomer in the bulk toluene solution are shown in Fig. 2. H_2TPP monomer in toluene has the Soret band at 419 nm and Q band around 515 nm. Two red-shifted bands of 473 nm and 720 nm in Fig. 2a were assigned to the J-aggregate of H_4TPP^{2+} formed at the interface. It took about 5 min for the completion of the J-aggregate formation after the addition of H_2TPP toluene solution. The interfacial adsorption equilibrium in the toluene-4 mol/L H_2SO_4 system can be represented by

$$H_2TPP_0 + 2H^+ \rightleftharpoons H_4TPP_i^{2+} \quad (1)$$

$$nH_4TPP_i^{2+} \rightarrow (H_4TPP^{2+})_{ni} \quad (2)$$

where the subscript "i" shows the species adsorbed at the interface. The monomer of the protonated H_2TPP (H_4TPP^{2+}), whose absorption maximum is at 438 nm, was not clearly noted in Fig. 2a, because most of it formed the aggregate by the reaction (2) under the present H_2TPP concentration.

Figure 2b shows the CD spectrum of the interfacial aggregate of H_4TPP^{2+}. We could observe the circular dichroism band similar to positive and negative Cotton effect centered at 473 nm, which corresponds to the

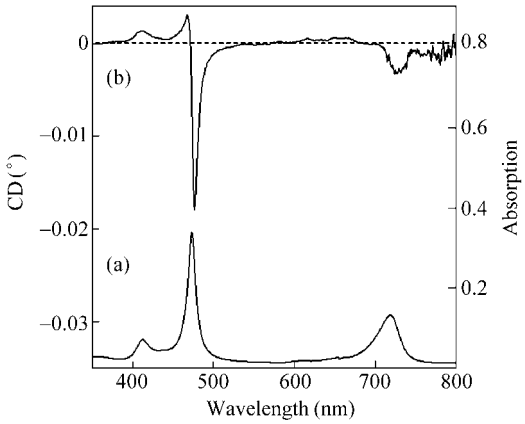

Fig. 2 Absorption spectrum (a) and CD spectrum (b) of the J-aggregate of H_4TPP^{2+} in the toluene-4 mol/L sulfuric acid system measured by the CLM method. Initial H_2TPP concentration in toluene was 1.0×10^{-5} mol/L.

absorption maximum wavelength of the J-aggregate. The CD signal in the J-band increased with time and reached its maximum value after about 5 min. The absorbance at 473 nm also showed a similar increase. These results indicated that the observed optical activity was assigned to the J-aggregate formed at the interface. The CD measurement has been made also for the non-aggregated system of 1.0×10^{-5} mol/L H_2TPP toluene/water. As expected, it did not show any signal in CD spectrum. Therefore, it was confirmed that the bulk H_2TPP monomer in toluene had no optical activity and only the optical activity of the interfacial J-aggregate was measured in the present study.

In the case of H_4TPPS^{2-}, the exciton-coupled circular dichroism of the J-aggregate has been assigned to the helical alignment of the porphyrin in J-aggregate[10]. It can be thought that the CD spectrum of the J-aggregate of H_4TPP^{2+} observed in the present study has a similar helical alignment. Ohno et al. assigned the CD dispersions at 491 nm and 420 nm of the J-aggregate of H_4TPPS^{2-} to the transition dipoles of long axis of rod-shaped J-aggregate of H_4TPPS^{2-} and to the short axis perpendicularly to the long axis, respectively[10]. Therefore, the CD band of H_4TPP^{2+} aggregate centered at 473 nm may be assigned to the linear oscillator transitions polarized in the long axis of the J-aggregate. The CD band centered at 412.5 nm shown in Fig. 2a may correspond to the electric transition of short axis of H_4TPP^{2+} in the J-aggregate. The CD signal at 412.5 nm of the H_4TPP^{2+} aggregate showed only positive one. Optically inactive H_2TPP monomer in

toluene, having the Soret band at 419 nm, gives neither CD signals in the measurements using an ordinary quartz cell nor CLM cell. Hence, it was confirmed that the free H_2TPP monomer in toluene had no CD band at 412.5 nm. We also observed the CD signal of the J-aggregate in the Q band region. In the wavelength region, the CD signal was broader and weaker than that for the Soret band.

2 SHG-CD Spectrometry

2.1 SHG experiments

The apparatus of SHG measurements included a Ti:sapphire laser (Spectra-physics Maitai; 80 MHz, 100 fs, tuning range of 780-920 nm) and a photon counter (Stanford research systems, SR400). An angle of the linearly polarized fundamental beam was adjusted by an achromatic half waveplate and a circularly polarized fundamental beam was generated using an achromatic quarter waveplate (Newport, 10RP44-3). All SHG spectra were acquired in total internal reflection condition with the incident angle of 74.1°, and the beam spot of the fundamental beam at the interface was about 2.0 mm. For the SHG measurements, the heptane-water interface was formed in a rectangular optical quartz cell with a flat interfacial area of 2.0× 1.0 cm^2, which was thermostated at (300±0.5) K. UV/Vis and ordinary CD spectra of bulk solutions were measured by Jasco V-550 spectrophotometer and Jasco J-710 circular dichroism spectropolarimeters, respectively.

2.2 Interfacial aggregation of TPPS

The aqueous phase contained initially 5.35×10^{-7} mol/L TPPS and 3.36×10^{-6} mol/L cetyltrimethylammonium bromide (CTAB) at pH 3.0 controlled by 10^{-3} mol/L hydrochloric acid. An interfacial SHG spectrum measured by an irradiation of p-polarized fundamental beam showed a maximum at 420 nm. UV/Vis spectra of the aqueous solution including TPPS and CTAB gave an absorption maximum at 434 nm, assigned to H_4TPPS^{2-} monomer, which was blue-shifted to 420 nm as the CTAB concentration was increased. The absorption maximum of 420 nm was assigned to the J-aggregate of H_4TPPS^{2-}. Thus, the formation of J-aggregate of H_4TPPS^{2-} at the heptane-water interface was confirmed. Furthermore, it was concluded that the J-aggregation of H_4TPPS^{2-} was promoted preferentially at the interface by the ion-association adsorption with CTA^+ molecules.

2.3 SHG-CD of interfacial TPPS aggregate

We studied the SHG-CD of the interfacial aggregate by using a circularly polarized fundamental beam. s-Polarization detected SH spectra of the interface were measured, irradiated by left- and right-circularly polarized incident beams. The left circularly polarized SH intensity was larger than the right circularly polarized one around the resonant SH response maximum at 420 nm. The difference between left- and right-circularly polarized SH spectra is represented as SHG-CD spectrum defined by

$$I_{\text{SHG-CD}} = \frac{I_{\text{lcp}} - I_{\text{rcp}}}{0.5(I_{\text{rcp}} + I_{\text{lcp}})} = \frac{4\left[(f_{\text{Im}} - g_{\text{Im}})h_{\text{Re}} - (f_{\text{Re}} - g_{\text{Re}})h_{\text{Im}}\right]}{|f|^2 + |g|^2 + |h|^2 - 2(f_{\text{Re}}g_{\text{Re}} + f_{\text{Im}}g_{\text{Im}})} \quad (3)$$

where subscripts "lcp" and "rcp" are the left-circularly polarized and right-circularly polarized fundamental beams, respectively. The coefficients *f*, *g*, and *h* are linear combinations of various electric-dipole and magnetic-dipole susceptibility components and the relative weights of these components depend on s- and p-polarized components of the second harmonic field[12,13]. Subscripts "Re" and "Im" represent real and imaginary parts of each coefficient. Figure 3 shows the SHG-CD spectrum of the aggregate of H_4TPPS^{2-} observed by a circularly-polarized fundamental beam, in which the intensity was zero at the absorption

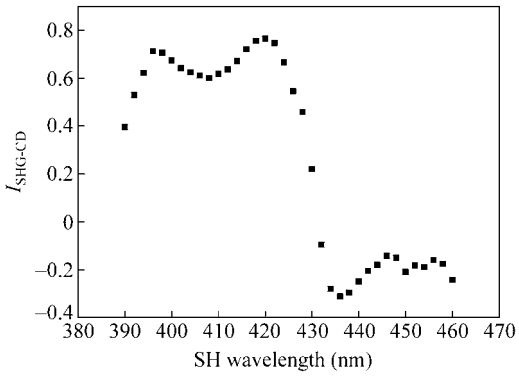

Fig. 3 SHG-CD spectrum of adsorbed TPPS aggregate at the heptane-water interface. Aqueous phase: 5.1×10^{-7} mol/L TPPS, 1.15×10^{-6} mol/L CTAB, 0.01 mol/L HCl (pH 2.0).

maximum of the monomer, 430 nm, and positive and negative peaks were observed at 420 nm and 438 nm, respectively.

There observed no CD spectrum by an ordinary CD spectrometer for the same aqueous solution used in SHG experiments. Although an SHG-CD spectrum is not directly comparable to an ordinary CD spectrum, it is found that the SHG-CD spectrum of the interfacial J-aggregate indicates two components with opposite signs, similar to the exciton-coupled circular dichroism of ordinary linear CD spectrum of J-aggregate in bulk solution.

When the SHG-CD measurements were repeated in the same conditions, we obtained the completely opposite signed SHG-CD spectra to that of Fig. 3 with even probability, having negative and positive peaks at 420 nm and 438 nm, respectively. This means that the liquid-liquid interface itself has no chiral selectivity in the ion-associated aggregation of TPPS diacid with CTA cation.

The measurements of SHG-CD spectra were also carried out in the absence of CTAB in the acidic aqueous phase (pH 3.0) and in the presence of CTAB in the neutral condition (pH 8.0). Under neither condition, however, significant SHG-CD signals were observed.

3 Conclusions

It was demonstrated that the CD spectra of the interfacially adsorbed species were successfully measured by the use of CLM method with a conventional CD spectropolarimeter. The circular dichroism of the J-aggregate of H_4TPP^{2+} formed at the toluene-sulfuric acid could be observed directly. Its CD spectrum suggested a rod-like shape of H_4TPP^{2+} J-aggregate twisted to one direction. This method can be applied to any optical active species adsorbed at liquid-liquid interfaces, provided that it has a large molar absorptivity. Especially, it will become a promising tool for the study of biological processes including enzymatic reaction of protein at the liquid-liquid interface.

The SHG-CD spectrometry was demonstrated to be a more interfacial specific and more sensitive method to observe the optical activity of the liquid-liquid interface. The SHG-CD method found out the chirality of the ion-associated aggregate of TPPS diacid with CTA cation at the heptane-water interface. The analysis of SHG polarization spectra revealed the contribution of a magnetic dipole effect to the spectra, which could be assigned to the helical structure of the aggregate.

It is believed that CLM/CD and SHG-CD methods developed by our studies will become powerful techniques to explore the optical activity of interfacial species including self-assembled compounds, amino-acid, proteins, and DNA adsorbed at the liquid-liquid interfaces of solvent extraction processes.

References

[1] Watarai H, Tsukahara S, Nagatani H, Ohashi A. *Bull. Chem. Soc. Jpn.*, 2003, **76**: 1471.

[2] Nagatani H, Watarai H. *Anal. Chem.*, 1998, **70**: 2860.

[3] Nagatani H, Watarai H. *Chem. Lett.*, 1999, **28**: 701-702.

[4] Ohashi A, Watarai H. *Langmuir*, 2002, **18**: 10292.

[5] Petralli-Mallow T, Wong T, Byers J D, Yee H I, Hicks J M. *J. Phys. Chem*., 1993, **97**: 1383.

[6] Hicks J M, Petralli-Mallow M, Byers J D. *Faraday Discuss*, 1994, **99**: 341.

[7] Verbiest T, Kauranen M, Persoons A. *J. Mater. Chem.* 1999, **9**: 2005.

[8] Crawford M J, Haslam S, Probert J M, Gruzdkov Y A, Frey J G. *Chem. Phys. Lett*., 1994, **229**: 260.

[9] Huang X, Nakanishi K, Berova N. *Chirality*, 2000, **12**: 237.

[10] Ohno O, Kaizu Y, Kobayashi H. *J. Chem. Phys.*, 1993, **99**: 4128.

[11] Yulizar Y, Watarai H. *Bull. Chem. Soc. Jpn.*, 2003, **76**: 1379.

[12] Maki J J, Verbiest T, Kauranen M, Elshocht S V, Persoons A. *J. Chem. Phys*., 1996, **105**: 767.

[13] Schianne-Klein M C, Hache F, Roy A, Flyzanis C, Payrastre C. *J. Chem. Phys*., 1998, **108**: 9436.