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Chiral Analysis of Amino Acids by Synergistic Heteroaggregation with Porphyrins at Liquid–Liquid Interface

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At the interface of the toluene/water system, H_4TPyP^{4+} and $CuTPPS^{4-}$ formed heteroaggregates, which could recognize the chirality of D- and L-phenylalanines (Phe) and other amino acids, though neither H_4TPyP^{4+} nor $CuTPPS^{4-}$ could indicate the chirality of the amino acids. The composition of H_4TPyP^{4+}:$CuTPPS^{4-}$ in the aggregate was estimated as 1:1 as reported in an aqueous solution. But, CD spectra of the interfacial heteroaggregates were different from that in the aqueous solution, suggesting different chiral interaction. The present results suggest a new mode of the interfacial chiral reaction exhibited by the synergistic effect of the heteroaggregation of the inversely charged porphyrins.

The liquid–liquid interface has attractive functions such as the selective concentration of surface active species from bulk phases and the catalytic role in the kinetics of solvent extraction of metal ions. A two-dimensional monomolecular layer is ready to be generated at the interface with the saturation concentration in the order of 10^{-10} mol cm^{-2}. Therefore, at the liquid–liquid interface, supramolecular aggregates tend to be formed even under a low bulk concentration of the monomer. These features of liquid–liquid interfaces are thought to be advantageous for the chiral analysis of bulk phase species, combined with an effective technique to observe optical chirality at the interface. In our laboratory, centrifugal liquid membrane-circular dichroism (CLM-CD) and second-harmonic generation-circular dichroism (SHG-CD) methods have been developed, and the chiral aggregation of porphyrins and phthalocyanines formed at liquid–liquid interfaces have been investigated.[1–3] Many researchers have reported the chiral aggregation in a bulk phase.[4–6] However, chiral aggregation mechanisms have not been fully understood. Purrello et al. have reported that the protonated form (H_4TPyP^{4+}, Figure 1) of 5,10,15,20-tetra(4-pyridyl)-21*H*,23*H*-porphine (TPyP, Figure 1), the deprotonated form ($CuTPPS^{4-}$, Figure 1) of Cu(II)–*meso*-tetrakis(4-sulfophenyl)porphine (CuTPPS, Figure 1) and phenylalanine formed chiral heteroaggregates in acidic aqueous solutions showing an induced circular dichroism (ICD),[7] though the reaction mechanism was not clarified. In the present study, the feasibility of H_4TPyP^{4+}–$CuTPPS^{4-}$ heteroaggregates for the chiral analysis of amino acids at the liquid–liquid interface has been demonstrated by using CLM-CD. TPyP was dissolved in an organic phase, and $CuTPPS^{4-}$ and phenylalanine were dissolved in an acid aqueous phase (pH 2.3) at first.

The formation of chiral heteroaggregates of H_4TPyP^{4+}, $CuTPPS^{4-}$ and phenylalanine in the toluene/water system was directly measured using centrifugal liquid membrane-absorption spectrometry (CLM-Abs),[8] centrifugal liquid membrane-circular dichroism spectroscopy (CLM-CD),[9,10] and centrifugal liquid membrane-linear dichroism spectroscopy (CLM-LD).[10] In the CLM measurements, a cylindrical glass cell, having 1.9 cm diameter and 3.4 cm length, was placed horizontally, attached by an electric motor in the sample chamber of the CD spectropolarimeter (J-820E, JASCO, Japan). The procedure for the CLM measurements was essentially the same with that reported previously.[9,10] The cylindrical cell was rotated at 7000 rpm by a speed-controlled electric motor (NE-22E, Nakanishi Inc., Japan). At first, a blank spectrum was measured by introducing 0.480 mL of an aqueous solution of 0.01 M phenylalanine and 0.1 M (H^+, Na^+)ClO_4^- (pH 2.3) and 0.480 mL toluene with a microsyringe into the cylindrical cell through a sample injection hole. Then, 0.020 mL of aqueous solution of $CuTPPS^{4-}$ and 0.020 mL of a chloroform solution of TPyP were added to initiate the interfacial reaction. The sum of the spectra of the bulk liquid membrane phases and the interface was measured by this method. The calculated values of the thickness of the organic and aqueous phases were 0.26 and 0.25 mm, respectively. The interfacial area between two phases was 20 cm^2.

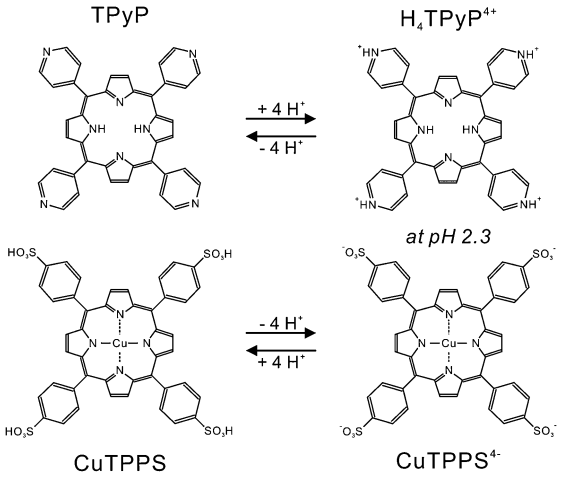

Figure 1. Molecular structures of TPyP and CuTPPS.

The interfacial tension at the toluene/water (20.8 mL/8.0 mL) interface was measured using the Wilhelmy plate technique with a 10.0×10.0 mm^2 filter paper.[11] An interfacial pressure sensor (USI-32, Hybrid Instruments Co., Japan) was used. All the measurements were conducted at 27 ± 2 °C, using a UC-65 cryostat (EYELA, Japan). In the interfacial tension experiments, H_4TPyP^{4+} stock solution was prepared in an acidic solution of pH 2.3. The pH values of the aqueous phases were measured using a F-14 pH meter (HORIBA, Japan) equipped with a 6366-10D glass electrode.

Figure 2 shows the CLM-CD spectra of the H_4TPyP^{4+}–$CuTPPS^{4-}$ chiral heteroaggregates including D- or L-Phe, which

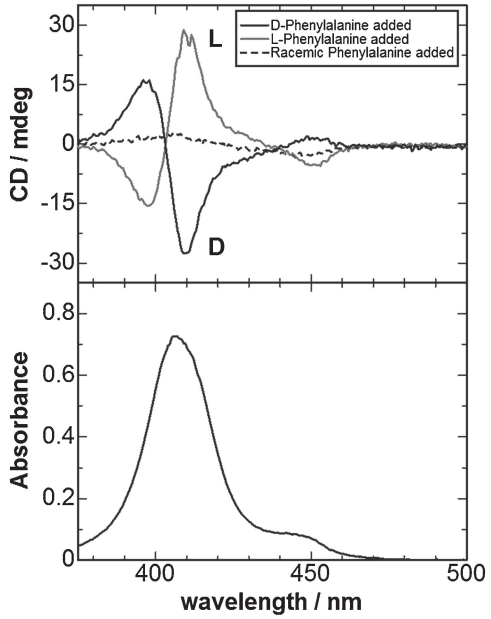

Figure 2. CD and absorption spectra of chiral heteroaggregates at the liquid–liquid interface measured by CLM-CD. $[TPyP]_{org} = 8.0 \times 10^{-6}$ M, $[CuTPPS^{4-}]_{aq} = 7.2 \times 10^{-5}$ M, $[Phe]_{aq} = 0.01$ M, $[HClO_4]_{aq} + [NaClO_4]_{aq} = 0.1$ M, pH 2.3, toluene:chloroform = 96:4, v/v, $t = 30$ min.

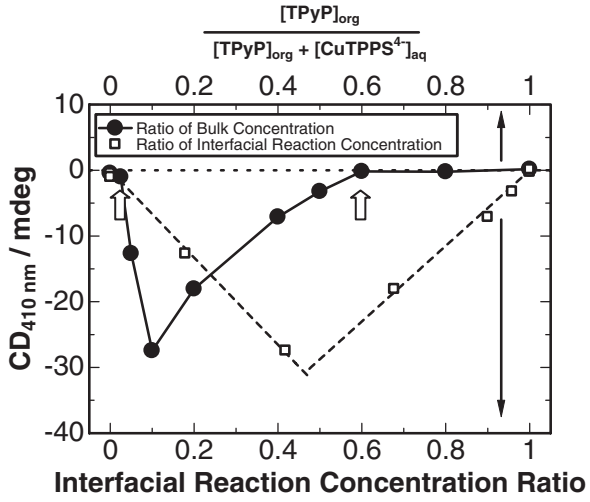

Figure 3. Job plot of H_4TPyP^{4+}–$CuTPPS^{4-}$ heteroaggregate measured by CLM-CD. $[TPyP]_{org} + [CuTPPS^{4-}]_{aq} = 8.0 \times 10^{-5}$ M, $[D\text{-Phe}]_{aq} = 0.01$ M, $[HClO_4]_{aq} + [NaClO_4]_{aq} = 0.1$ M, pH 2.3, toluene:chloroform = 96:4, v/v, $t = 30$ min. Interfacial reaction concentration ratios were calculated by eq 1. Linear dashed lines were fitted lines of interfacial reaction concentration ratio data. The open arrows show the critical concentrations which generate the aggregation.

had three maxima at ca. 400, 410, and 450 nm (small) indicating the chirality of added amino acid. The formation rate of the chiral heteroaggregates observed by the CLM-CD spectra depended remarkably on the concentrations of TPyP, $CuTPPS^{4-}$, and phenylalanine. The CD intensity increased continuously with time at first, then saturated, and began to decrease finally, suggesting agglomeration of the aggregate. In the shortest case, the saturation time was ca. 30 min. In the conditions of Figure 2, the saturation time was ca. 6 h. In Figure 2, the CD intensity at 30 min was used for the composition analysis of the aggregate to avoid the slow agglomeration of the aggregate.

After the CLM-CD measurement, no CD signal was observed in the organic phase and about 2% of the CLM-CD intensity was observed in the aqueous phase, but it was negligible. Hence, it was concluded that the CD signal observed by the CLM measurements came from the interface. The CD spectra of the heteroaggregate formed at the interface were different from those in the aqueous solution,[7] suggesting the difference in the chiral interaction. In addition, Purrello et al. reported that H_4TPyP^{4+} and $CuTPPS^{4-}$ reacted in a 1:1 ratio in aqueous solution.[7] From the Job plot of Figure 3, the largest CD intensity of heteroaggregates at the interface was apparently observed at 1:9 for the initial concentration ratio of TPyP and $CuTPPS^{4-}$ in the toluene/water system.

The interfacial tension of toluene/water systems including H_4TPyP^{4+}, $CuTPPS^{4-}$, and phenylalanine was observed by the Wilhelmy plate method to evaluate the adsorptivity of these reactants. The results are summarized in Table 1, in which the interfacial adsorption constants from the aqueous phase to the liquid–liquid interface decreased in the order of $H_4TPyP^{4+} > CuTPPS^{4-} \gg$ phenylalanine. Here, the zero CD intensity at both sides of the Job plot of Figure 3 suggest that the chiral

Table 1. Interfacial adsorption constants, K', of TPyP(org), H_4TPyP^{4+}(aq), $CuTPPS^{4-}$(aq), and phenylalanine(aq)

Chemical species	K'/dm
TPyP(org)[a]	5.0×10^{-5} [12]
H_4TPyP^{4+}(aq)[b]	2.2×10^{-2}
$CuTPPS^{4-}$(aq)[b]	4.6×10^{-3}
Phe(aq)[b]	9.1×10^{-7}

[a]Measured by high-speed stirring method,[12] $[NaClO_4] = 0.1$ M, pH 5.5, toluene:chloroform = 95:5, v/v. [b]Measured by the Wilhelmy balance technique, $[HClO_4] + [NaClO_4] = 0.1$ M, pH 2.3, toluene:chloroform = 96:4, v/v.

heteroaggregates could be formed only when the bulk concentrations of the porphyrins were higher than a critical concentration. The critical concentration of the porphyrin seems to be low, when the interfacial adsorption constant is large. Hence, it was thought that H_4TPyP^{4+} and $CuTPPS^{4-}$ adsorbed to the liquid–liquid interface competitively, and the chiral heteroaggregates could be generated when the sum of the interfacial concentration of H_4TPyP^{4+} and $CuTPPS^{4-}$ attained the critical concentration. To simplify the analysis, we assumed that the bulk concentration exceeding the critical bulk concentration can participate in the formation of the chiral interfacial aggregate, and therefore the product of the interfacial adsorption constant and the bulk excess concentration corresponds to the liquid–liquid interfacial reaction concentration. The interfacial reaction concentration ratio was calculated by

$$\frac{K_1'([TPyP]_{org} - [TPyP]_c)}{K_1'([TPyP]_{org} - [TPyP]_c) + K_2'([CuTPPS^{4-}]_{aq} - [CuTPPS^{4-}]_c)} \quad (1)$$

where K_1' and K_2' are the interfacial adsorption constants of H_4TPyP^{4+} and $CuTPPS^{4-}$, respectively. $[TPyP]_c$ and $[CuTPPS^{4-}]_c$ are the critical bulk concentrations of TPyP and $CuTPPS^{4-}$, respectively. A Job plot using the interfacial reaction concentration ratio is also shown with square plots in Figure 3. When the interfacial reaction concentration ratio of H_4TPyP^{4+}:$CuTPPS^{4-}$ is around 1:1, the CD intensity reaches maximum. This ratio is the same as the reaction ratio in the aqueous solution.[7] However, the reported CD spectrum of the aqueous heteroaggregates showed maxima at 380 and 450 nm,[7] which were different from ca. 400, 410, and 450 nm of the interfacial heteroaggregates. Both absorption spectra suggested H-aggregate structure, but the CD spectra suggested different interaction of positively charged Phe in the aggregate. In the aqueous solution, a positive Phe ion will associate with H_4TPyP^{4+}–$CuTPPS^{4-}$ complex by hydrophobic interaction with phenyl groups. However, at the interface it might be possible that a positive Phe ion will interact with electrostatic interaction to the complex orienting the phenyl group outside, since the liquid–liquid interface has a lower dielectric constant than the aqueous phase.[13] At the interface, perchlorate ion may behave as a counter ion for the positive Phe ion. Eventually, the distance between the asymmetric carbon in Phe and the chromophore of the porphyrins will be different between the heteroaggregates formed in the aqueous solution and at the interface. Some have reported that the structure of chiral aggregates changed due to solvent effect.[14,15]

The sign of the CD spectra of H_4TPyP^{4+}–$CuTPPS^{4-}$ heteroaggregates at the liquid–liquid interface reversed depending on the chirality of D-phenylalanine and L-phenylalanine, and there was no CD signal in the racemic phenylalanine solution as shown in Figure 2. A linear correlation between the CD intensity at 410 nm and the chiral excess of phenylalanine was confirmed as shown in Figure 4. The observed fitted linear line of the induced CD intensity was CD = 28.7 e.e., where e.e. was defined by e.e. = ([D-Phe] − [L-Phe])/([D-Phe] + [L-Phe]). This relationship can be used for the determination of the chiral purity of the phenylalanine, under the conditions of [Phe] = 0.01 M and t = 30 min. In our previous study, it was a problem that the interfacial CLM-CD signal contained the false signal which came from the linear anisotropy of the interfacial aggregate like linear dichroism (LD).[10] Therefore, we measured CLM-LD spectra of H_4TPyP^{4+}–$CuTPPS^{4-}$ heteroaggregates in the present study. However, the LD intensity was weak and there was no correlation between CD and LD intensities. Thus, CD signals of the present interfacial heteroaggregates were thought to be really chiral signals.

The heteroaggregates of H_4TPyP^{4+} and $CuTPPS^{4-}$ could also be used to analyze the chirality of other amino acids like tyrosine (Tyr) and tryptophan (Trp) at the toluene/water interface, though the CD intensity was smaller than that of Phe heteroaggregate. Neither H_4TPyP^{4+} nor $CuTPPS^{4-}$ could form interfacial aggregate and indicate the chirality of the amino acid, so it was concluded that H_4TPyP^{4+} and $CuTPPS^{4-}$ are working synergistically for the chiral recognition of amino acids. There is a possibility that the H_4TPyP^{4+}–$CuTPPS^{4-}$ heteroaggregate at the liquid–liquid interface can be used for the chiral analysis of various kinds of chiral molecules adsorbable at the liquid–liquid interface, especially for compounds having no chromophore like alchohols, amines, and carboxylic acids. Also, it can be concluded that the present CLM-CD method is a promising tool to investigate the chiral structure and chiral function of heteroaggregates of porphyrins formed at the liquid–liquid interface.

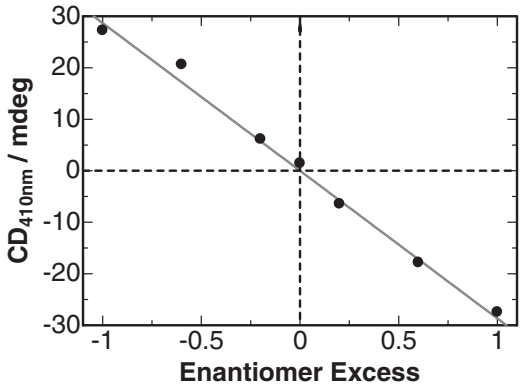

Figure 4. Correlations of enantiomeric excess of phenylalanine, e.e. = ([D-Phe] − [L-Phe])/([D-Phe] + [L-Phe]), and CD, LD signals of TPyP–CuTPPS heteroaggregates measured by CLM-CD. $[TPyP]_{org} = 8.0 \times 10^{-6}$ M, $[CuTPPS]_{aq} = 7.2 \times 10^{-5}$ M, $[Phe]_{aq} = 0.01$ M, $[HClO_4]_{aq} + [NaClO_4]_{aq} = 0.1$ M, pH 2.3, toluene:chloroform = 96:4, v/v, t = 30 min.

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