Bilayer Formation in Ethanol from Dialkylammonium Amphiphile Appended with Nitroaniline Moiety

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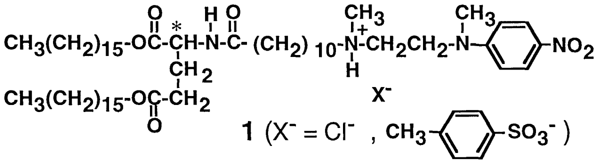
A double-chain ammonium amphiphile with the p-nitroaniline moiety formed ordered bilayer assemblies in ethanol media. p-Toluenesulfonate counterion effectively stabilized the bilayer. Excitonic interaction among highly oriented chromophores at the bilayer surface was probed by absorption and circular dichroism spectra.

Synthetic bilayer membranes are usually formed in water from a large variety of covalent amphiphilic compounds,[1] and from non-covalent amphiphilic networks of complementary hydrogen bonds.[2] They were formed also in hydrocarbon media for Ca^{2+}-complexed phosphate amphiphiles,[3] diacetylenic phospholipids,[4] and a series of fluorocarbon amphiphiles comprised of solvophilic and solvophobic moieties.[5] Though it is known that large amounts of water-miscible organic solvents cause lyses of aqueous bilayers,[6] we recently found that an oriented bilayer membrane was specifically formed in binary water-ethanol media (ethanol ; ca.40 vol%) from chromophoric chiral amphiphile **1** · **Cl** (in the L-glutamate form).[7] However, the regular bilayer structure of **1** · **Cl** was deteriorated at ethanol concentrations beyond 60 vol%.[7] In the present study, we succeeded to maintain the ordered bilayer structure of **1** by replacing the counterion with p-toluenesulfonate (pTS).



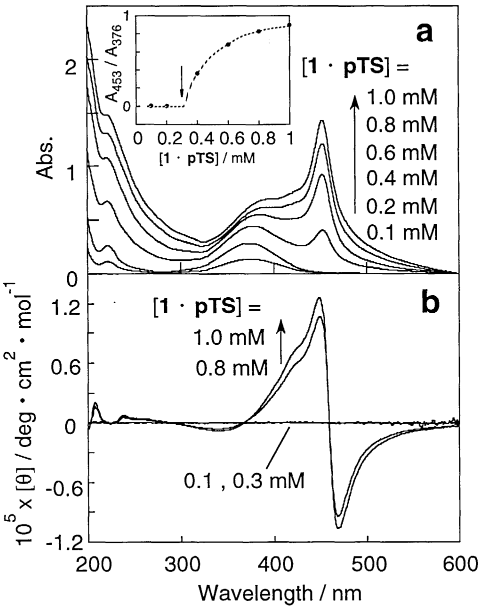


Figure 1. UV-Vis absorption spectra (a) and CD spectra (b) of **1** · **pTS** in ethanol. 20 ℃.

1 (in the free form) and pTS monohydrate[8] were dissolved in spectral grade ethanol (Kishida Chemicals), and the equimolar mixtures were gently ultrasonicated for 10 min to give transparent dispersions (Bransonic Model B1210J-MTH). Figure 1(a) displays the concentration dependence of absorption spectra of **1** · **pTS** at 20 ℃. When **1** · **pTS** was dissolved at 0.1 mM, the absorption maximum (λ_{max}) was observed at 376 nm. This λ_{max} is ascribed to that of a monomerically dispersed **1** as observed for **1** · **Cl** in ethanol.[7] A new absorption peak at 453 nm appeared with increasing **1** · **pTS** concentrations, and its intensity became predominant at concentrations above 0.6 mM. Circular dichroism (CD) spectrum of **1** · **pTS** also showed a remarkable concentration dependence (Figure 1(b)). At lower concentrations of 0.1 mM and 0.3 mM, CD spectra were not detected. In contrast, at a higher concentration range, intense CD spectra with positive and negative peaks were obtained at 446 and 469 nm ($[\theta]_{446} = 1.27 \times 10^5$ deg · cm^2 mol^{-1}, $[\theta]_{469} = -1.05 \times 10^5$ deg · cm^2 mol^{-1} ; [**1** · **pTS**] = 1.0 mM, 20 ℃). Dramatically enhanced CD spectra with exciton coupling have been typically observed for highly oriented chromophores aligned in crystalline bilayers.[7,9] As the concentration dependence of the CD spectral intensity is in agreement with the appearance of the 453 nm - peak in the absorption spectra, the spectral red shift observed is ascribed to excitonic interaction among chirally stacked pNA chromophores. According to Molecular Exciton theory,[10] the observed spectral characteristics are consistent with the presence of tilt orientation (J-aggregate), as in the case of **1** · **Cl** in a binary water-ethanol media (ethanol 40 vol %, λ_{max} ; 462 nm).[7] X-ray diffraction of a powdery sample of **1** · **pTS** gave a long period of 5.81 nm that is larger than a molecular length (4.96 nm by CPK molecular model), but is considerably smaller than the extended bimolecular length.

Figure 2 shows a transmission electron micrograph of **1** · **pTS** dispersed in ethanol (0.8 mM). The sample was post-stained with uranyl acetate. These aggregates are made of short tubes (length ; 250 - 750 nm, width ; 150 - 200 nm, thickness ; 12 - 63 nm) and some of them display closed-end surfaces. The observed thickness of tubes indicate that they are comprised of several layers of tilt bilayers.

The critical micelle concentration, CMC, of bilayer **1** · **pTS** in ethanol was determined by plotting the ratio of absorbances at 453 nm and at 376 nm (A_{453} / A_{376}) against concentration of **1** · **pTS** (Figure 1a, inset). The A_{453} / A_{376} value is increased beyond the concentration of ca. 0.3 mM, and this corresponds to the CMC of J-aggregated bilayer **1** · **pTS**. The observed CMC is much lower than those observed for micellar aggregates formed

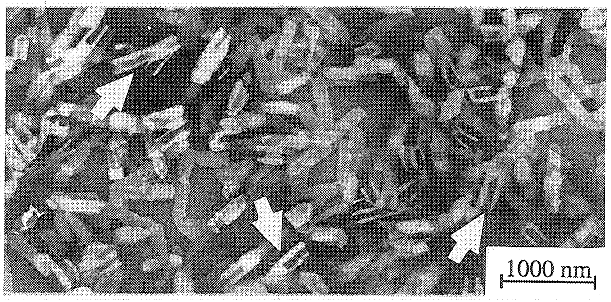


Figure 2. Transmission electron micrograph of **1 · pTS** in ethanol. Sample is post-stained by uranyl acetate.

in polar organic media,[11] and comparable to those reported for fluorocarbon bilayers in chlorocyclohexane.[5]

Figure 3 illustrates a spectral change of ethanolic dispersions of **1 · pTS**. As the temperature is raised, the absorbance at 453 nm due to the J-aggregated bilayer is decreased and there appears a peak at 373 nm (at 35 ℃) with an isosbestic point. The 453-nm peak appeared again by cooling the dispersion from 35 ℃ to 20 ℃. Thus, formation of the J-aggregated bilayer in ethanol is thermally reversible. Differential scanning calorimetry (DSC) conducted for **1 · pTS** in ethanol (2 mM) afforded two endothermic peaks at 36.4 ℃ ($\triangle H$; 48.8 kJ mol^{-1}, $\triangle S$; 157.6 J $K^{-1}mol^{-1}$) and 44.5 ℃ ($\triangle H$; 26.5 kJ mol^{-1}, $\triangle S$; 83.4 J $K^{-1}mol^{-1}$). The $\triangle S$ value observed for the former peak is in the range of the ordinary gel-to-liquid crystal phase transition of aqueous bilayers,[12] while the sum of the two $\triangle S$ values exceed that accompanied by the phase transition. Therefore, it is likely that the endothermic peak at 44.5 ℃corresponds to a dissolution process of the liquid crystalline bilayer to the monomeric dispersion.

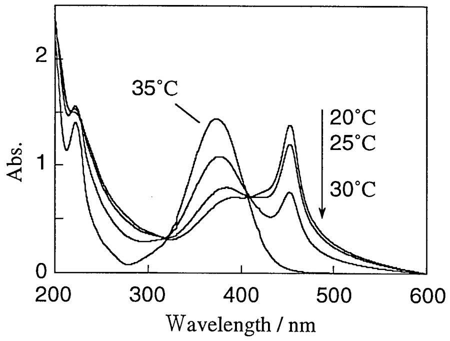


Figure 3. Temperature dependence of UV-Vis spectra in ethanol. [**1 · pTS**] = 1 mM.

It is noteworthy that the pTS counterion effectively stabilized the bilayer structure of **1** in ethanol. Other counterions - chloride and bromide - had no stabilization effects. Therefore, pTS would be bound tightly at the bilayer surface via electrostatic, van der Waals, and possibly dipole-dipole interaction. Stacking of pNA chromophores and binding of pTS molecules at the bilayer surface must make crucial contributions to the enhanced stability of the bilayer organization, since the dialkyl glutamate amphiphiles with the trimethylammonium head group is lysed at ethanol contents of 25 - 30 vol%.[7]

In conclusion, an ordered bilayer membrane is formed in 100% ethanol from the pNA-appended ammonium amphiphile with pTS counterion. Together with bilayer formation in aqueous, binary aqueous/organic,[7] and nonpolar hydrocarbon media,[3,5] the present result is a satisfactory indication of bilayer formation in a large variety of media.

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