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Formation of Stable Bilayer Membranes in Binary Aqueous−Organic Media from a Dialkyl Amphiphile with a Highly Dipolar Head Group[1]

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We describe herein how an ordered bilayer assembly is formed spontaneously in binary aqueous−organic solvent media, when component amphiphiles possess a highly dipolar head group.

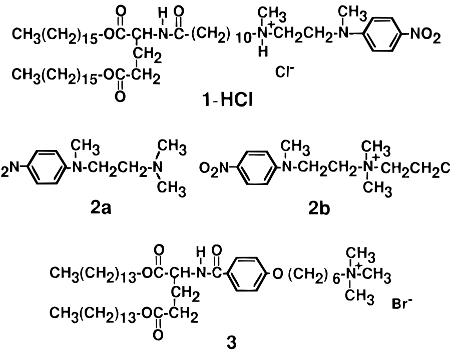
Synthetic bilayer membranes are formed from a large variety of synthetic amphiphiles[2] and hydrogen-bonded amphiphilic pairs[3] in water. In addition, they are formed in aprotic organic solvents from Ca^{2+}-complexed phosphate amphiphiles[4] and fluorocarbon amphiphiles comprised of solvophilic and solvophobic moieties.[5] The importance of designed "amphiphilicity" as a key strategy to generate soluble supramolecular assemblies in organic media has been further manifested by the complementary hydrogen bond networks of alkylated melamines with naphthalene−diimide[6] and the JANUS molecule.[7] These assemblies are obtained as transparent dispersions, distinct from organo-gels formed from peptide-lipids.[8]

In order to develop a general strategy to construct ordered self-assemblies in media other than water and aprotic organic solvents, additional assembling force is undoubtedly required. In the present study, we prepared a double-chain amphiphile with a dipolar head group that forms bilayer membranes in water as well as in water−organic solvent mixtures.

As a highly dipolar chromophore, *N*-methyl-*p*-nitroaniline (pNA) was incorporated in the head group of L-glutamate-based amphiphile **1**.[9,10] pNA is known as a solvatochromic probe and is used as a nonlinear optical chromophore.[11]

An equimolar amount of hydrochloric acid was added to **1**, and the mixture was dispersed in ethanol or in water by ultrasonication ([**1-HCl**] = 1.2 mM, Branson Sonifier Model 185, sonic power 45 W). For spectroscopic measurements, the mixture was diluted with appropriate amounts of ethanol and deionized water. These dispersions were gently sonicated for 10 min (Bransonic Model B1210J-MTH). **1-HCl** in water gives electron micrographs that indicate formation of globular aggregates with diameters of 300−2000 Å. In 40 vol % ethanol−water, however, tape-like structures are found (Figure 1a). Some of the tapes are unmistakably twisted, and are typical of chiral crystalline bilayers.[12]

Chart 1



Absorption and circular dichroism (CD) spectra of these dispersions show remarkable medium dependency. Figure 2A compares absorption spectra of **1-HCl** in water, in ethanol, and in ethanol−water mixtures at 20 °C. The absorption spectrum in ethanol (λ_{max} at 384 nm) is essentially identical to that of non-associating **2a**, indicating that **1-HCl** in ethanol is molecularly dispersed. On the other hand, the bilayer dispersion of **1-HCl** in pure water possesses an absorption peak centered at 400 nm with significant decrease in absorption intensity (a). As a water-soluble **2b** gives an almost identical absorption peak in water, the pNA chromophore in the bilayer must be exposed to the typical aqueous environment. In contrast, a remarkably red-shifted peak is found for **1-HCl** at 462 nm in ethanol−water (Figure 2A: b and c), with its intensity attaining a maximum at the ethanol content of 40−60 vol %. These red shifts are originated from exciton interaction among aligned pNA chromophores, since exciton coupled CD spectra are found only in this particular ethanol−water media (Figure 2B). Molecular exciton theory[13] predicts the presence of the head-to-tail orientation (J-aggregate) of the pNA chromophores. This orientation allows pNA head groups to be placed more closely to the adjacent chiral carbons.

In 100% water, no CD peak was observed. This is partly because the chromophores are fairly apart from the chiral carbon. The molecular orientation of glutamate-based double-chain amphiphilies is known to be fixed at a tilt angle of ca. 55° against the bilayer plane.[14] This angle is close to 54.7° where exciton splitting becomes zero in spite of close proximity of aligned transition moments.[13] If this situation applies to the pure water medium, the exciton coupling would be much diminished.

The observed ethanol effect is interesting. It is reported that added ethanol deteriorates the molecular order in lipid bilayers,[15a] and that the molecular area of phosphatidylcholine hydrated with

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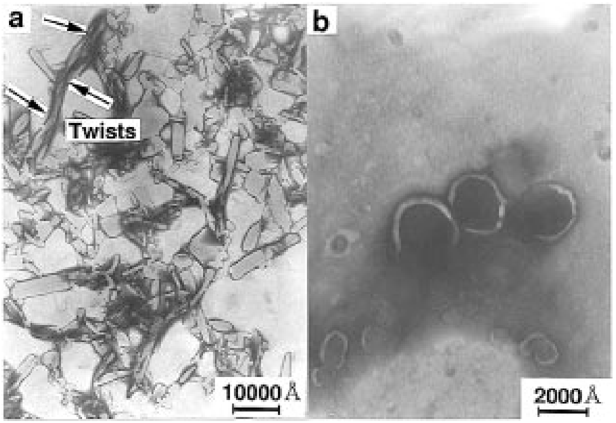

Figure 1. Transmission electron micrographs of **1** in 40 vol% ethanol−water sample prepared at room temperature (a) and at 55 °C (b). Samples are stained by uranyl acetate.

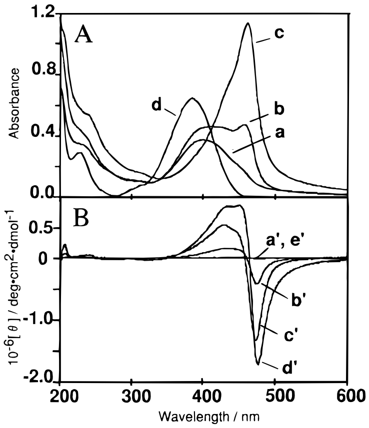

Figure 2. (A) UV−vis spectra of **1** in ethanol−water at 20 °C: ethanol content (a) 0%, (b) 20 vol %, (c) 50 vol %, and (d) 100 vol %. (B) CD spectra of **1** in ethanol−water at 20 °C: ethanol content (a′) 0%, (b′) 20 vol %, (c′) 40 vol %, (d′) 60 vol %, and (e′) 100%.

ca. 10 water molecules was expanded by 6% with addition of one ethanol molecule per lipid.[15b] Although a small content of ethanol (6−7 vol %) induced interdigitation of phosphatidylcholine vesicles,[15c,d] larger amounts of water-miscible organic solvents caused lyses of aqueous bilayer membranes.[16]

In contrast to the exciton-coupled CD spectra of **1-HCl** which is most enhanced at ethanol contents of 40−60%, the CD peak of the excition-coupled phenyl chromophore in aqueous **3** disappeared at ethanol contents of 25−30 vol %, implying deterioration in the bilayer order. Therefore, the lysis of bilayer **3** that possesses the conventional ammonium head group is readily promoted by ethanol addition. It is apparent that the dipolar chromophore in **1** is unique in bringing enhanced stability against aqueous ethanol, though the stabilization is lost completely at 90 vol % ethanol. Similar J-aggregate spectra (λ_{max} 462 nm) were observed for **1-HCl** in 40 vol % mixtures of water−2-propanol, and water−acetonitrile (25 °C). On the other hand, its absorption spectrum is broadened in water−methanol (40 vol %), with its peak intensity at 462 nm being decreased about two-thirds of the other media. It has been reported that ethanol−water and acetonitrile−water mixtures contain specific molecular clusters rather than giving molecularly homogeneous (ideal) mixtures.[17] The content of the hydrated ethanol cluster is greatest at 42 vol % ethanol−water. This unique feature may be related to the particular bilayer organization observed.

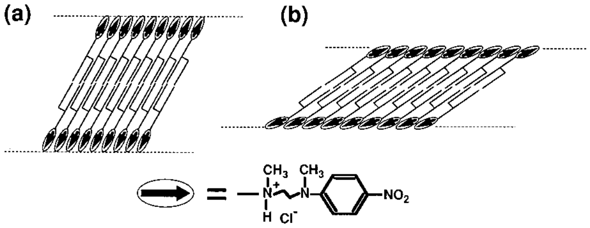

Figure 3. Schematic illustration of bilayer structures: (a) in water and (b) J-aggregate bilayer in water−organic solvent mixtures.

When the medium temperature is raised, the 462 nm peak in 40% ethanol−water starts to decrease at 45 °C, and a new broad absorption centered at 405 nm emerges at 55 °C. The J-aggregate absorption was regenerated upon cooling. In differential scanning calorimetry (DSC), an endothermic peak was observed at 54.7 °C (ΔH = 47.5 kJ mol^{-1}) for this dispersion. These spectral and thermal observations indicate that the bilayer of **1-HCl** undergoes reversible gel-to-liquid crystal phase transition. Maintenance of the bilayer structure above the phase transition temperature was also confirmed by electron microscopy. An electron micrograph of **1-HCl** (sample prepared from 40 vol % ethanol−water at 55 °C) displays formation of large vesicles with diameters of 1000 to 4000 Å (Figure 1b).

Figure 3 schematically represents the molecular orientation of **1-HCl** in pure water (a) and in aqueous organic solvent mixtures (b). Dipolar head groups undergo specific orientational changes in the presence of organic cosolvents, and the oriented pNA chromophores enhance bilayer stability in these media.

Formation of micellar aggregates in non-aqueous media[18] and precipitation of tubular aggregates from ethanol−water solution have been reported for diacetylenic phosphatidylcholines.[19] However, formation of a soluble molecular assembly that expresses specific molecular orientational changes as well as gel-to-liquid crystalline phase transition characteristics in water−organic solvent media is unprecedented. Together with the bilayer formation in aprotic organic media we previously reported, we can draw a general conclusion that spontaneous bilayer assembly can be designed in a very wide solvent system range.

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