Formation of Bilayer Structure in Organic Solvents from Amphiphiles Due to Interpeptide Hydrogen Bonding

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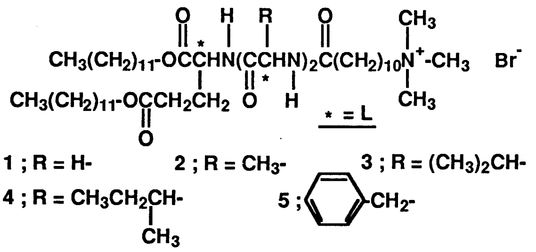
In order to produce molecular organization in a wide variety of media, novel amphiphiles containing an associative tripeptide moiety were prepared. Electron microscopy revealed bilayer formation from the amphiphile not only in water but also in nonpolar organic solvents. ^{1}H-NMR spectroscopy gave direct evidence of interpeptide hydrogen bonding in the aggregate.

The organization of molecules provides a variety of new functions. Therefore, it is of importance to study how to make molecular assemblies. Conventional molecular assemblages such as micelles, bilayer membranes, and Langmuier-Blodgett films have been prepared in bulk water or on the air-water interface. On the other hand, the recent researches report the examples of bilayer formation in some organic solvents using new classes of molecules.[1-3] However, these molecules do not form any aggregates in water, whereas the conventional amphiphiles so far studied form no aggregate in organic media. This work is aimed to synthesize an amphiphile that forms a bilayer membrane without respect to the type of media. Such an ambidexterous molecule will help to develop the chemistry of molecular systems. We thus introduced a tripeptide group into the conventional amphiphile at the center of the molecule. In the present paper, we describe the systematic study of their aggregation behavior in more than thirteen solvents including water, and discuss the role of the tripeptide part.

The tripeptide-containing amphiphiles used in the work are shown below.[4] Possessing two hydrophobic chains and a



$$CH_3(CH_2)_{11}\text{-}OC\overset{*}{C}HN(\overset{O}{C}\overset{*}{C}HN)_2C(CH_2)_{10}N^+(CH_3)_3\ Br^-$$

$$CH_3(CH_2)_{11}\text{-}OCCH_2CH_2 \quad * = L$$

1 ; R = H-   2 ; R = CH_3-   3 ; R = $(CH_3)_2CH$-
4 ; R = $CH_3CH_2CH(CH_3)$-   5 ; $C_6H_5CH_2$-

hydrophilic head group, these amphiphiles formed a bilayer membrane in water. On the other hand, the tripeptide-containing amphiphiles were dissolved in various organic solvents by heating, and gave transparent solutions. The amphiphiles of **2**-**5** yielded transparent gels with 1-day incubation at room temperature, when the dielectric constant[5] of the solvent is in the range of 2.0 to 2.3 (Table I). These transparent gels were stable for several weeks. The amphiphile of **1** gave a translucent solution like the aqueous solutions of bilayer forming amphiphiles, when cyclohexane or CCl_4 were used as a solvent.

We could find bilayer structures in the transparent gels and in the translucent solutions using a transmission electron microscope (TEM; JOEL JEM 100S). Figure 1 is an example of the TEM images of the CCl_4 solution of **2**.[6] In the expanded view, we can see the bundle of fibrils whose diameter corresponds to the bilayer thickness (ca.70 Å). The overview shows that the network structure of the bundle causes gelation.

Table 1. Appearance of 2mM Solution of Tripeptide Containing Amphiphiles after 3 days Incubation

solvent	dielectric constant	1	2	3	4	5
n-hexane	1.8799 (25°C)	■	■	■	■	■
cyclohexane	2.023 (20°C)	▒	▨	▨	▨	▨
carbon tetrachloride	2.238 (25°C)	▒	▨	▨	▨	▨
benzene	2.275 (25°C)	⁙	▨	▨	▨	▨
toluene	2.379 (25°C)	⁙	▨	▨	▨	▨
chloroform	4.806 (20°C)	□	□	□	□	□
ethylacetate	6.02 (25°C)	⁙	⁙	⁙	⁙	⁙
tetrahydrofuran	7.58 (25°C)	⁙	⁙	▨	▨	□
1,1,2,2-tetrachloroethane	8.20 (20°C)	□	□	□	□	□
acetone	20.70 (25°C)	⁙	⁙	⁙	⁙	□
ethanol	24.55 (25°C)	□	□	□	□	□
methanol	32.70 (25°C)	□	□	□	□	□

■ insoluble ▒ translucent solution ⁙ precipitation
▨ transparent gel □ transparent solution

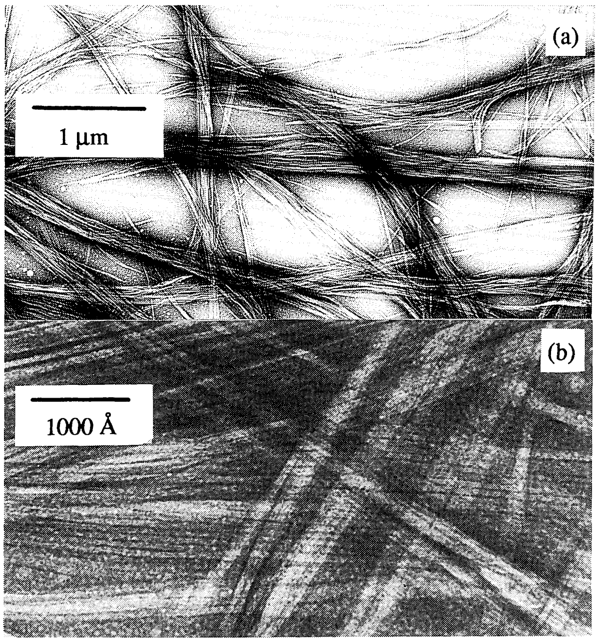


Figure 1. Electron micrographs of bilayer aggregate formed from amphiphile of **2** in CCl_4.[6] (a) overview, (b) expanded view.

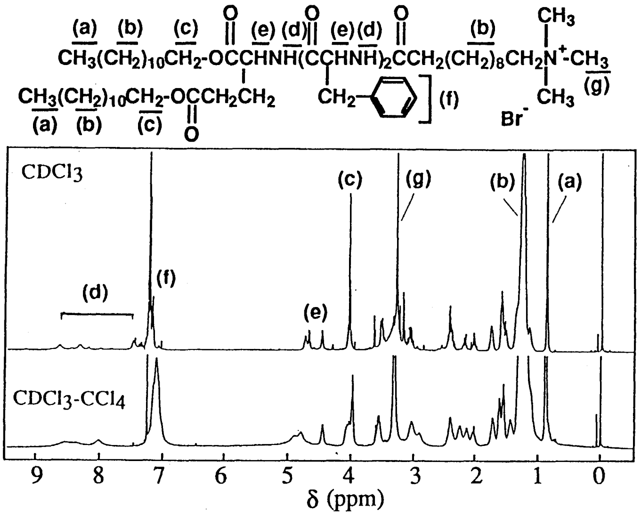


Figure 2. ^{1}H-NMR spectra of amphiphile of **5** in $CDCl_3$ and in 1:4 mixture of $CDCl_3$-CCl_4 (vs. TMS). Spectral measurements were carried out using 4 mM of solutions after 1 d incubation.

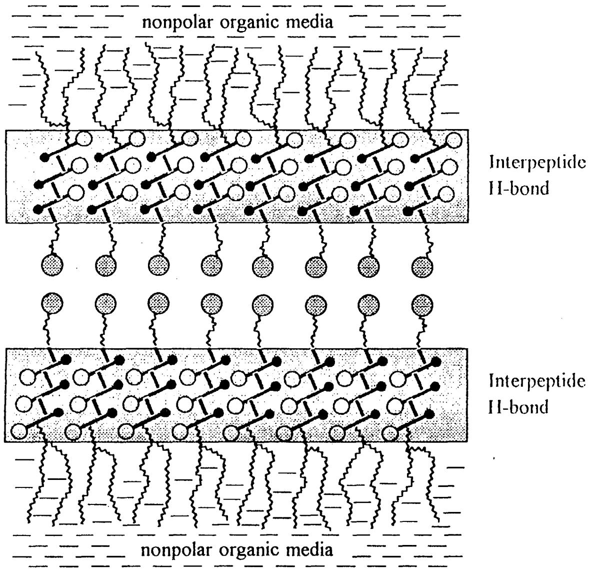


Figure 3. Schematic illustration of bilayer structure in organic solvent.

The same fibrous bilayer structure was also observed in the other organic gels and in the translucent solutions. Therefore, a bilayer structure is formed in nonpolar organic solvents whose dielectric constant is in the range of 2.0 to 2.3. The gelation also occurs in polar solvents such as $CHCl_3$ when the dielectric constant is reduced by the addition of a nonpolar solvent. These results will determine what kind of organic solvent is suitable for aggregate formation.

Figure 2 displays the ^{1}H-NMR (JEOL JNM-GSX500) spectra of **5** in $CDCl_3$ and in a $CDCl_3$-CCl_4 mixture. As previously described, gelation did not occur in the $CDCl_3$ solution, while the solution gelled by reducing dielectric constant using nonpolar solvent, CCl_4. The NMR peaks shifted and broadened with gelation. The broadening was marked at the peaks assigned to the tripeptide part. However, the gelation did not affect the contour of the peaks attributed to protons far from the tripeptide part. For example, the triplet peak of the terminal methyl protons did not show any change with the gelation. These results imply that the amphiphiles rigidly bind with each other at the tripeptide part, and that the alkyl chains are in fluid state (Figure 3). On the other hand, peaks attributed to the amide protons immediately disappeared with the addition of a trace amount of D_2O in the $CDCl_3$ solution because of the fast H-D exchange. However, in the $CDCl_3$-CCl_4 mixture, the amide proton peaks remained for several hours after the addition of D_2O, which meant that the strong hydrogen bonding is resisting the H-D exchange. Thus, the interpeptide hydrogen bonding must cause the bilayer formation.

In conclusion, we showed that the tripeptide-containing amphiphiles formed bilayer structure not only in water but also in organic solvents. This is the first example of an ambidexterous amphiphile. Interpeptide hydrogen bonding is crucial in this case. The bilayer formation in organic media can be widely achieved by selecting an organic solvent of adequate value of dielectric constant or regulating it by mixing the solvents. Ambidexterity of the amphiphile makes it possible to compare the characteristics of the assembly in water and in organic solvents using the same molecule.

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References and Notes

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4 Peptide-containing amphiphiles were prepared as follows. Didodecyl-L-glutamate was condensed with BOC-dipeptide or BOC-amino acid in the presence of diethylphosphorocyanidate at room temperature. After the protection group (BOC) was removed using HBr/Acetic Acid, the resulting compound was allowed to react with 11-bromoundecanoyl chloride in the presence of Et_3N with ice cooling. Alkyl bromide, thus obtained, was quaternized by Me_3N.

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6 Staining of aggregates in organic solvents was carried out by the same manner described in reference 1; Sample solution (2mM) was dropped onto a carbon-coated TEM grid. Excess liquid was blotted off, and dried in vacuo. Aqueous uranyl acetate (2wt%) was then dropped onto the dried specimen. Excess solution was blotted off, and dried in vacuo.