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# Bilayer Membranes of Four-Chained Ammonium Amphiphiles[1]

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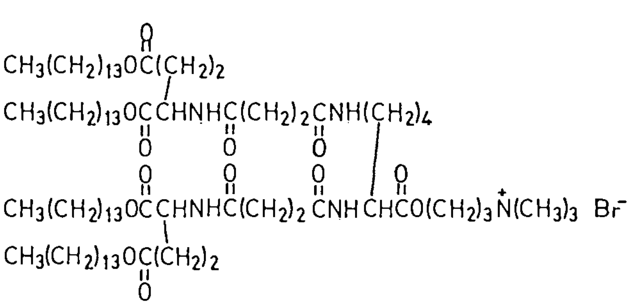
Novel amphiphiles in which four long alkyl chains are connected to the single trimethylammonium head group via amino acid residues gave clear dispersions upon sonication. Formation of bilayer membranes was confirmed by electron microscopy, differential scanning calorimetry, and fluorescence depolarization.

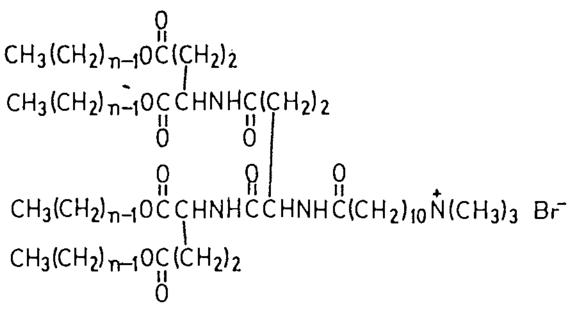
It has been reported by us and others that stable bilayer membranes are spontaneously formed in water from single-chain, double-chain, and triple-chain amphiphiles.[2] These results endorse our notion that bilayer formation phenomena can be considerably extended if the molecular design of component amphiphiles is appropriate. In particular, the use of proper connector groups between the hydrophilic and hydrophobic units was essential in order to attain bilayer formation from triple-chain ammonium amphiphiles.[2,3]

In the present study, we intended to enrich the variety of bilayer-forming amphiphiles by designing novel four-chain compounds. The conventional theory that is based on the hydrophile-lipophile balance never predicts formation of stable bilayer membranes from these compounds.

Novel four-chain amphiphiles 1 and 2 were synthesized by the stepwise combination of alkyl chains through the glutamic acid residue or the lysine residue, which was followed by quaternization with trimethylamine.[4] They are combinations of glutamic acid-based double chain amphiphiles which we described before.



1 2(2$C_{14}$-L-Glu-$C_4$)-L-Lys-$C_3N^+$



2 2(2$C_n$-L-Glu)-L-Glu-$C_{11}N^+$
n = 12, 14, 16

These amphiphiles gave transparent dispersions (20 mM) in water by sonication with a Bransonic Sonifier 185 (microtip, sonic power 30) for several minutes. Transmission electron micrographs (instrument, Hitachi H-600) of the aqueous dispersions[5] are given in Fig. 1. Amphiphile **1** forms vesicular and tubular aggregates. The vesicles are not smoothly curved and the layer thickness is ca. 130 Å. The tubular aggregates have similar layer thicknesses. The extended molecular length of **1** as estimated from the CPK molecular model is 38 Å; therefore, the observed layer thickness corresponds to multilayers. Figure 1b shows the formation of tape-like aggregates from **2**(n = 12). Their width is 125 to 250 Å and the thickness is ca. 75 Å. Twisting of the tape is also observed. Since the extended molecular length of **2**(n = 12) is 41 Å, the observed thickness corresponds to that of a bilayer. Well-organized aggregates cannot be found in electron micrographs when the alkyl chain is longer. Irregular rods are found for **2**(n = 14), and coagulated particles are found in **2**(n = 16) (Fig. 1c).

The aqueous dispersions were subjected to differential scanning calorimetry (instrument, Seiko Electronics, SSC 560) under the conventional conditions : sample, 20 mM; heating rate, 1 °C/min. The DSC thermograms are given in Fig. 2. Amphiphile **1** gives a sharp peak at 47 °C, but amphiphiles **2** produce broader peaks. The peak temperature in the latter series does not necessarly rise with alkyl chain lengths.

That these DSC peaks really arise from the gel-to-liquid crystal phase transition of bilayers was confirmed by the fluorescence depolarization experiment[6]: probe, 1,6-diphenyl-1,3,5-hexatriene (DPH, Tokyo Kasei); instrument, Hitachi 650-10S spectrofluorimeter. The results are included in Fig. 2. Polarization parameter P which is determined according to the published procedure[6] sharply changes in the case of **1** from 0.4 to 0.1 due to increased fluidity at the temperature region of the DSC peak. These changes in P were comparable to the observed value for bilayers of double chain amphiphiles[6] and triple chain amphiphiles[2] Similar decreases in the P value are observed for **2**, but the changes are less sharp, consistent with broader DSC peaks. The lower cooperativities of the transition observed for **2**(n = 12-16) are indicative of lessened regularity of these assemblages.

The phase transition data are summarized and compared with related bilayers in Table 1. The phase transition of bilayer **1** occurs sharply, as inferred from DSC and fluorescence polarization data. This indicates highly cooperative melting of aligned side chains. The $\Delta H$ and $\Delta S$ values for bilayer **1** are greater than the corresponding bilayer of double-chain amphiphile **3** in consistent with our previous supposition that $\Delta H$ of the phase transition increases with the number of alkyl chains in an amphiphilic molecule.[7] Regular chain packing is also favarable in the CPK model building. On the other hand, the $\Delta H$ and $\Delta S$ values of **2** are much smaller than those of **1**, without regard to the length of the alkyl chain. As mentioned above, this result must be related to inferior alignments of the alkyl chain in the crystalline state. The lysine residue appears particularly effective for alignment of two pairs of the double chain.

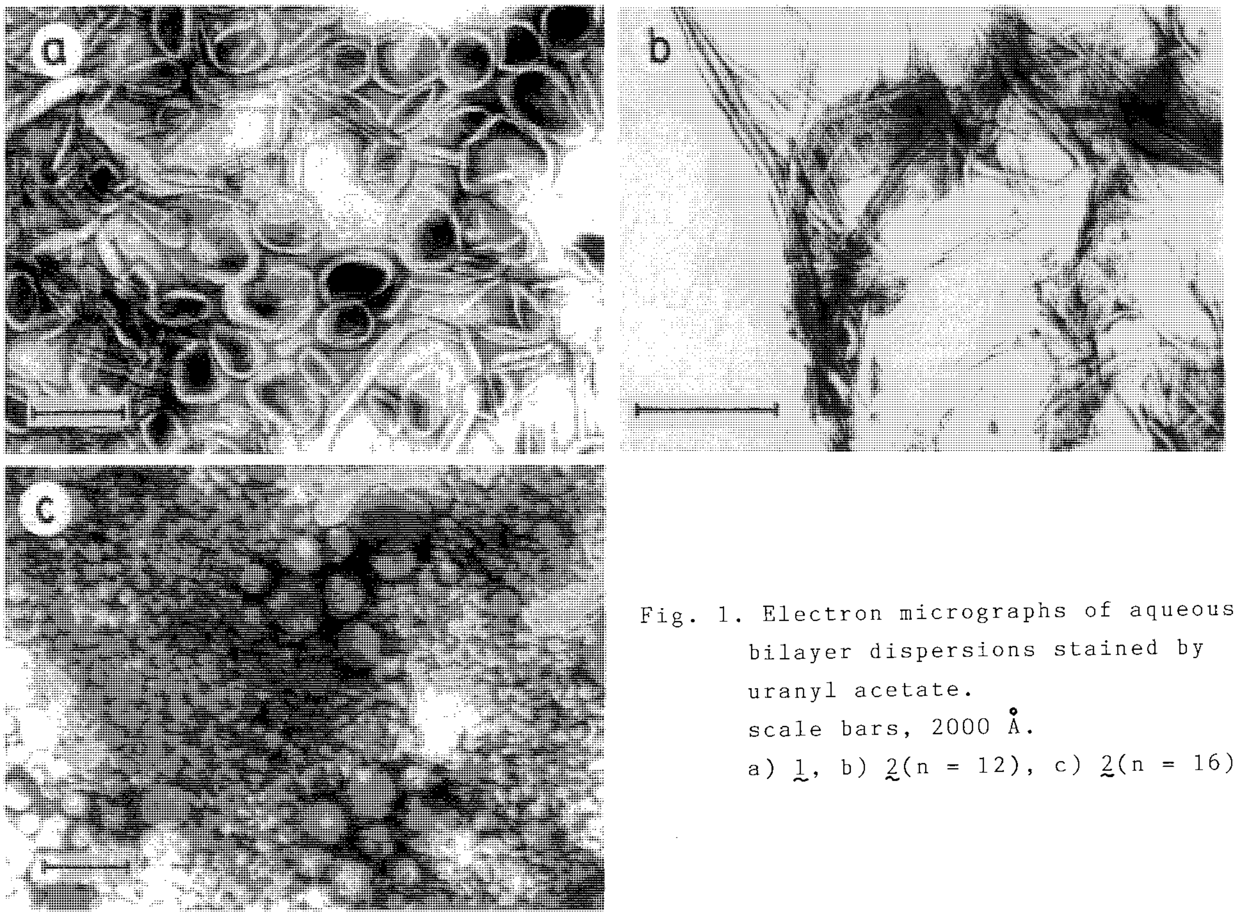
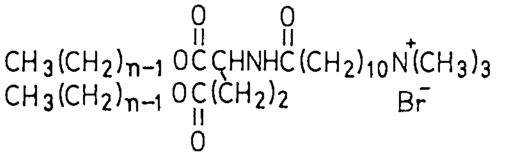


Fig. 1. Electron micrographs of aqueous bilayer dispersions stained by uranyl acetate.
scale bars, 2000 Å.
a) 1, b) 2(n = 12), c) 2(n = 16)

Table 1. Aggregation behavior of four-chained amphiphiles

| Amphiphile | Tc/ °C | ΔH/ kJ·mol$^{-1}$ | ΔS/ J·K$^{-1}$ mol$^{-1}$ | Electron micrograph |
|---|---|---|---|---|
| 1 | 47<br>(49) | 73<br>(100) | 229<br>(310) | vesicle,tube |
| 2(n=12) | 52<br>(54,70) | 27<br>(44,35) | 83<br>(136,101) | tape,helix |
| 2(n=14) | 48,55<br>(57,77) | 28,16<br>(77,19) | 88,47<br>(233,56) | tape |
| 2(n=16) | 52,57<br>(52,58) | 21,14<br>(26,56) | 63,43<br>(80,169) | globule |
| 3(n=12) | 33 | 45 | 148 | vesicle,helix |
| 3(n=14) | 37,47 | 4,37 | 13,115 | helix |

( ) ; frozen sample



$$\begin{array}{l} CH_3(CH_2)_{n-1}\,O\overset{O}{\overset{\|}{C}}\,CHNH\overset{O}{\overset{\|}{C}}(CH_2)_{10}\overset{+}{N}(CH_3)_3 \\ CH_3(CH_2)_{n-1}\,O\underset{O}{\underset{\|}{C}}(CH_2)_2 \qquad\qquad Br^- \end{array}$$

3 n = 12, 14

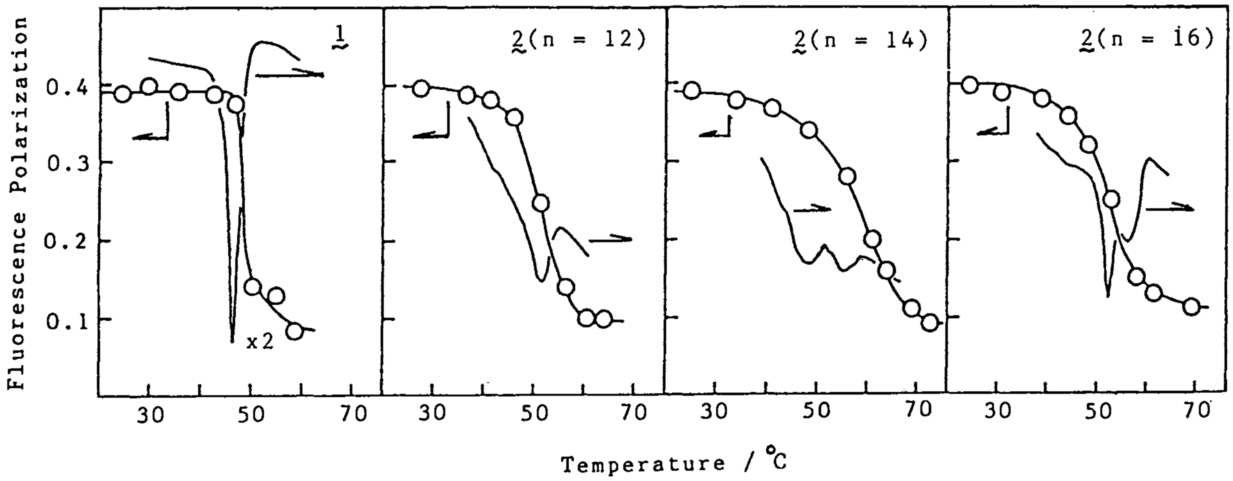


Fig. 2. DSC thermograms and temperature dependence of fluorescence polarization. DPH, 0.1 mol% of the bilayer.

In conclusion, we demonstrated that well-designed four chain amphiphiles produced typical bilayer membranes in water. This is a direct contradiction of the above-mentioned presumption based on the hydrophile-lipophile balance.[8) ] Apparently, the favorable side-chain packing can compensate energetically unfavorable exposure of some hydrophobic portions to water. The multiple chiral carbons involved in the bilayer component would create chirally specific binding sites.

We are grateful to Mr. M. Yamamoto for his help in preparing amphiphile 1.

References

1) Contribution No. 918 from Department of Organic Synthesis.
2) for a summary, see T. Kunitake, N. Kimizuka, N. Higashi, and N. Nakashima, J. Am. Chem. Soc., 106, 1978 (1984)
3) N. Higashi and T. Kunitake, J. Am. Chem. Soc., 107, 692 (1985).
4) Synthesis of these four-chained amphiphiles will be reported elsewhere.
elemental analysis. 1, Found : C, 64.03; H, 10.07; N, 4.44%.
Calcd for $C_{86}H_{162}O_{14}N_5Br$ $2.5H_2O$ : C, 63.95; H, 10.42; N, 4.34%.
2(n = 12), Found : C, 65.69; H, 10.63; N, 4.04%.
Calcd for $C_{77}H_{147}O_{11}N_4Br$ $1.5H_2O$ : C, 65.50; H, 10.71; N, 3.97%.
2(n = 14), Found : C, 66.29; H, 10.77; N, 3.82%.
Calcd for $C_{85}H_{163}O_{11}N_4Br$ $2.5H_2O$ : C, 66.20; H, 10.98; N, 3.63%.
2(n = 16), Found : C, 68.76; H, 11.26; N, 3.35%.
Calcd for $C_{93}H_{179}O_{11}N_4Br$ $H_2O$ : C, 68.64; H, 11.21; N, 3.44%.
5) Negatively stained samples (1 mM) were prepared by mixing aqueous bilayer dispersions with aqueous uranyl acetate without sonication.
6) T. Nagamura, S. Mihara, Y. Okahata, T. Kunitake, and T. Matsuo, Ber. Busenges. Phys. Chem., 82, 1093 (1978).
7) T. Kunitake, R. Ando, and Y. Ishikawa, Memoirs of the Faculty of Engineering Kyushu University, 46, 221 (1986).
8) C. Tanford, "The Hydrophobic Effect. Formation of Micelles and Biological Membranes," Wiley, New York (1973), Chap.9.

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