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Polymorphism in Bilayer Membranes of Novel Double-Chain Ammonium Amphiphiles[1)]

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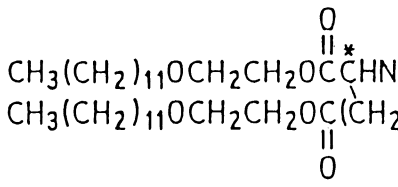
New double-chain ammonium amphiphiles which possess the ether linkage in the alkyl tails form bilayer membranes with regular side-chain packing in the crystalline state and display remarkable myelin figures in the liquid crystalline state.

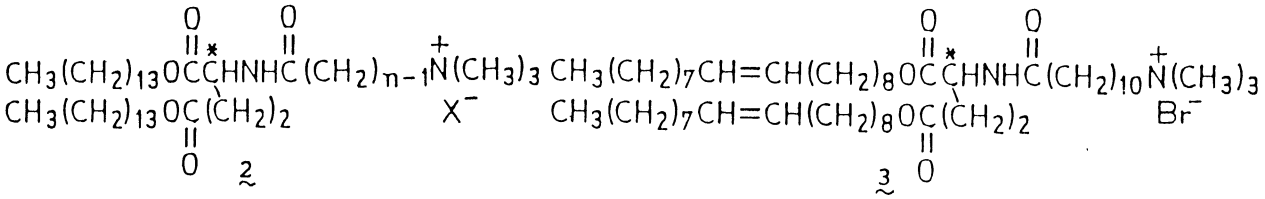
Dynamic morphological changes of biomembranes are closely related to fundamental functions of the biological cell such as fusion and fission. As models for these processes, dynamic structural changes of lecithin liposomes[2,3)] and the formation of myelin figures from lecithin have been studied extensively.[4-7)] The lipids used in these studies are limited to a rather narrow class of lecithin and related compounds. In order to elucidate the nature of molecular organization in relation to these dynamic membrane behaviors, it is essential to examine the correlation between the molecular property of bilayer membranes and their dynamic behavior. We and others have shown that a large variety of synthetic amphiphiles produce stable bilayer membranes. Some of the simple double-chain ammonium amphiphiles display versatile morphology changes in motion,[8-9)] and helical superstructures are formed from crystalline bilayer membranes of chiral amphiphiles.[10-15)] These versatilities in chemical structure and aggregate morphology render synthetic bilayers superior to lecithin in studying the structure-morphology relationship.

In this communication, we describe the thermal and morphological behavior of novel double-chain ammonium amphiphiles that possess the ether linkage in the hydrophobic tails. These results are compared with those of the corresponding amphiphiles which either lack in the ether linkage as in 2 or possess the oleyl chain as in 3. These amphiphiles are different only in the structure of the hydrophobic tail. The bilayer characteristics of amphiphiles related to 2 were extensively investigated by us[16)] and the oleyl unit in 3 is known to enhance the membrane fluidity.



1 2($C_{12}OC_2$)-L-Glu-C_nN^+

n = 2 (X = Cl), 11 (X = Br)



n = 2 (X = Cl), 11(X = Br)

Compounds 1 were sonicated in deionized water (Bransonic Cell Disruptor 185, 30 s, room temperature) to give transparent 20 mM dispersions, which were then placed on slide glasses and observed by a dark-field optical microscope (Olympus BHF with Ushio 200-W high pressure Hg lamp).[12] Differential scanning calorimetry (DSC) was conducted for the same dispersions with Seiko Instruments Inc. SSC/560 instrument.

Figure 1 demonstrates DSC thermograms. Compound 1 (n = 11) gives pretransition at 22 °C in addition to a sharp main peak at 33 °C (half-peak width, 1.2 °C; ΔH = 67 kJ/mol; ΔS = 218 J/K mol), whereas 2(n = 11) gives pretransition at 35 °C and a main transition peak at 45 °C (half-peak width, 1.3 °C; ΔH = 41 kJ/mol; ΔS = 128 J/K mol). In spite of the identical carbon number in the chain, the transition temperature of the latter is higher by 12 °C than that of the former. When the longer (C_{18}) oleyl chain is introduced, a broad transition centered at 32 °C is found. Thus, the ether linkage lowers T_c substantially without disturbing the chain packing in the crystalline state, as indicated by the sharp DSC peak. In contrast, the cis olefinic unit causes T_c lowering accompanied by lower cooperativity of chain melting as evidenced by the broad DSC peak. The same difference is found among the corresponding amphiphiles with a shorter spacer methylene: 1(n = 2), T_c 7 °C; 2(n = 2), T_c 24 °C. Lowering of T_c with shorter spacer methylene lengths was generally observed for bilayers of glutamate-based double-chain amphiphiles.[16] The ΔH and ΔS values obtained for 1(n = 11) are much larger than those for 1(n = 2) and 2(n = 11). This implies that disordering of aligned alkyl chains during the phase transition is most outstanding in the case of 1(n = 11)

When an aqueous dispersion of 1(n = 2) is kept on the microscope stage at room temperature (above T_c) for ca. 30 min, flexible aggregates such as large vesicles (diameter, 1-10 μm), tubes and fibers (length, several hundred μm) and beads in a string are observed as shown by Fig. 2a. They are subject to rapid Brownian motion and the shear force due to water flow aligns the fibers in one direction. We have made very similar observations for aqueous dispersions of simple dialkylammonium salts.[8] What is unique in the present case is that a variety of myelin figures are seen near the edge of the slide glass (Figs. 2b, 2c, and 2d). Figure 2b displays a folded myelin figure of simple tubes that has been discussed in the case of lecithin by other workers in detail.[17-20] The diameter of tubes is ca. 1.0 μm. Flexible double-helical rods are also seen, as shown in Figs. 2c and 2d. The double-helix (diameter, 3-5 μm) grows with simultaneous twisting. Their shapes appear to be more flexible than the double-helix of egg york lecithin reported by Sakurai et al.[18-20] Related amphiphiles 2 and 3 also produce fluid vesicles and fibers in the liquid-crystalline state; however, highly-developed, flexible structures have

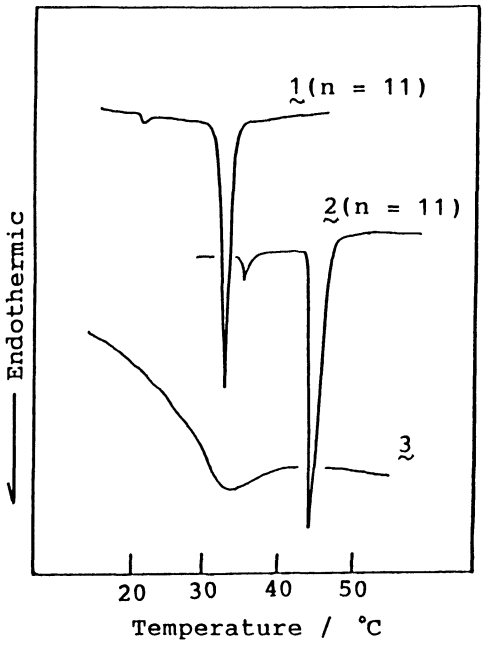


Fig. 1. DSC Isotherms of aqueous dispersion of 1(n = 11), 2(n = 11), 3.

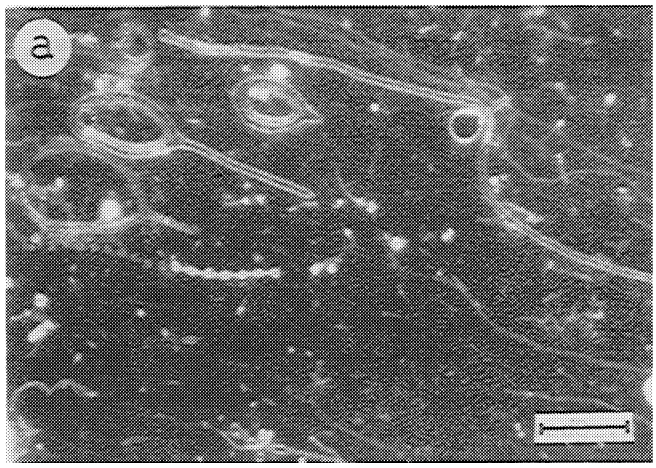
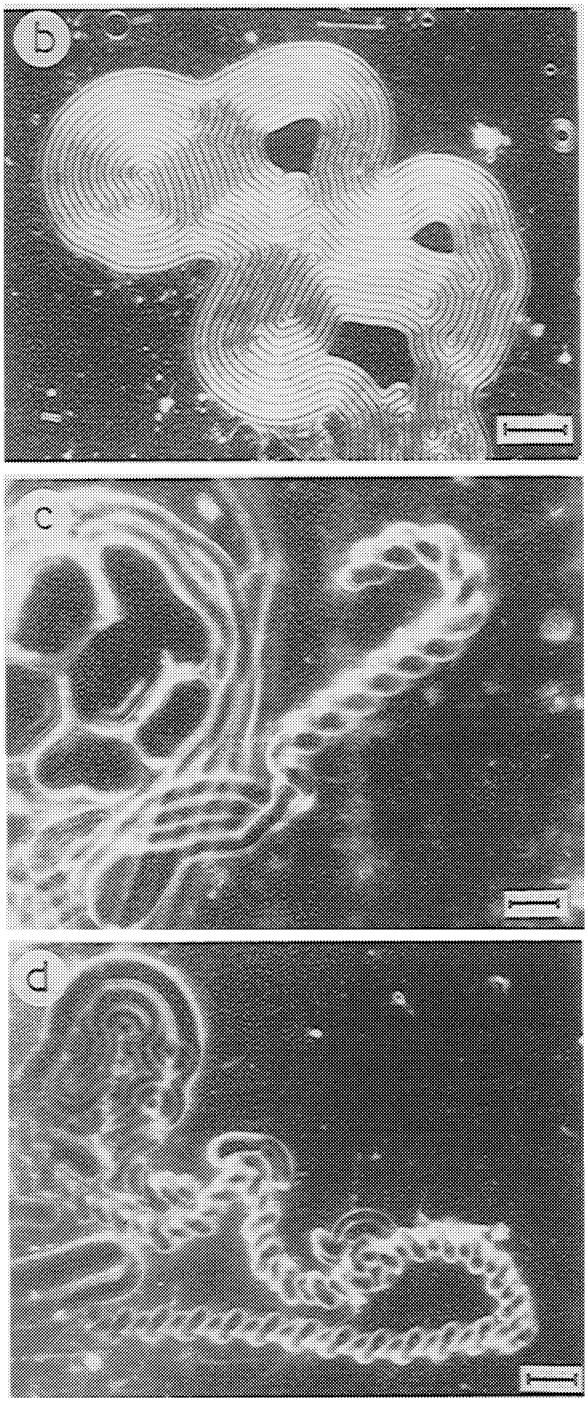


Fig. 2. Dark-field optical micrographs of aqueous dispersion of 1(n = 2). 20-25 °C, scale bars, 10 μm.



not been observed.[21]

Aqueous dispersions of 2(n = 11) form helical superstructures at temperatures below T_c of the bilayer.[10,12] Aqueous dispersions of 1(n = 11) similarly form crystalline helices at room temperature in spite of the added ether linkage, as shown in Fig. 3a. At temperatures above T_c, these helices turn to flexible fibers and separate into smaller vesicles (Fig. 3b), as in the case of 2(n = 11). Myelin figures of folded tubes which are analogous to those of Fig. 2b are also seen in some parts of the view. These observations are distinct from those of amphiphile 3 that contains the oleyl unit. The latter amphiphile does not form helical superstructures at room temperature (e.g. 20 °C)

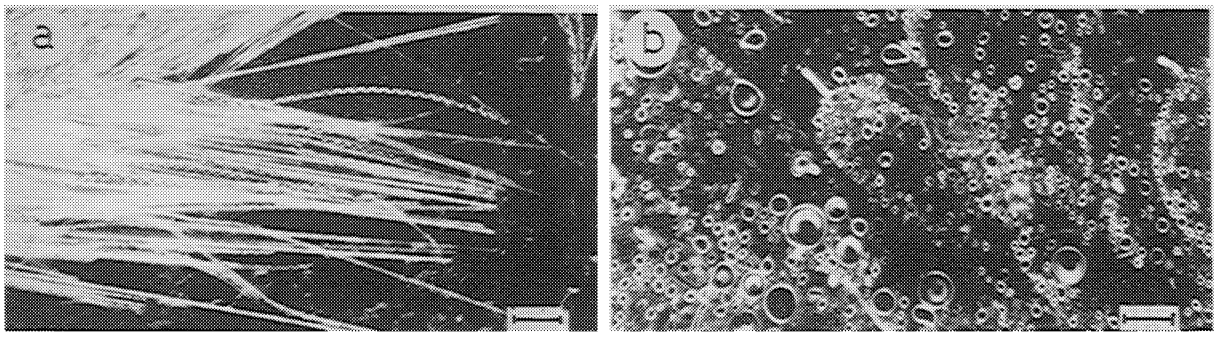


Fig. 3. Dark-field optical micrograph of aqueous dispersion of 1(n = 11). (a) below T_c, (b) above T_c, scale bars, 10 μm.

that is lower than its T_c. We showed already that the helical superstructure is made of chiral bilayers of very high regularity. Apparently, the regular side-chain packing required for helix formation is not available in 3, due to insufficient packing of the oleyl groups, as implied by its broad DSC peak.

In conclusion, we demonstrated that extensive polymorphism is observable for double-chain ammonium amphiphiles with the ether linkage in the hydrophobic tails. The polymorphism appears to require strong cooperativity among the membrane component even at tempratures above T_c. This may be achieved by eliminating net charges of the head group as in lecithin or by incorporating the ether linkage in the tail as in the present study. The ether linkage can make bilayers more fluid (T_c lowering) without loosing component cooperativity (as exemplified by sharp DSC peaks). We can see now why we could not obtain a satisfactory polymorphism with oleyl-unit containing amphiphile 3.

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