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FORMATION OF DISK-LIKE AGGREGATES FROM SINGLE-CHAIN PHOSPHOCHOLINE AMPHIPHILES IN WATER[1)]

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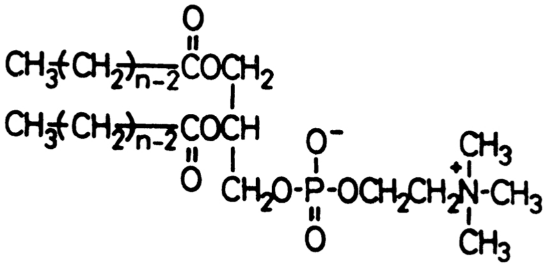
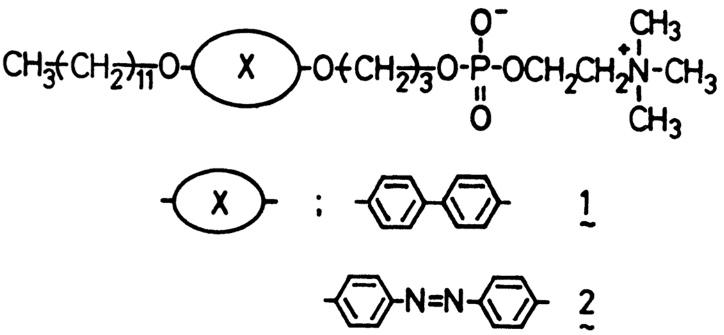
Single-chain phosphocholine amphiphiles which possess rigid segments in the hydrophobic portion formed disk-like aggregates, which are different from lecithin vesicles. When the rigid segment was azobenzene unit, *trans*-to-*cis* isomerization changed some membrane characteristics.

1,2-Diacyl-*sn*-glycerol-3-phosphocholine(lecithin) is an important lipid component of the biomembrane. Lecithin by itself can form bilayer vesicles(liposome) in water, and the liposome has been examined extensively as a model biomembrane and practically as a drug carrier.[3)]



lecithin



The structural characteristics of lecithin have been discussed in connection with the formation of stable bilayers. The phosphocholine group is characterized by strong hydrophilicity and zwitterionic nature. The attraction between the neighboring phosphocholine groups was considered to contribute to the membrane stability.[4)]

It has been reported from our laboratory that a variety of dialkyl(double-chain) and single-chain amphiphiles form stable bilayer membranes.5) The hydrophilic head group of these amphiphiles was in most cases simple ones such as ammonium, phosphate and polyoxyethylene. It is interesting to study the behavior of synthetic amphiphiles with a zwitterionic phosphocholine group as hydrophilic moiety, since this particular head group is especially important in the bioligical system.

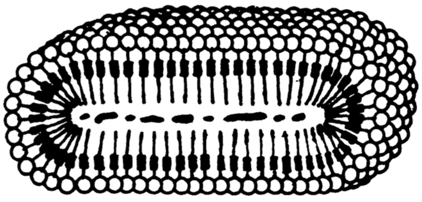
We report herein the synthesis and aggregation behavior of single-chain phosphocholine amphiphiles **1** and **2**.

Amphiphiles **1** and **2** were prepared by condensation of the corresponding single-chain alcohols and β-bromoethylphosphoryl dichloride, followed by the quaternization by trimethylamine, according to the procedure of Eibl and others.6,7) The products were purified by recrystallization and identified by elemental analysis, NMR and IR spectroscopies and thin layer chromatography.8) Liquid crystalline textures were observed in the melting point measurement by a polarizing microscope.

The amphiphiles produce clear solutions(5 mg/ml, *ca* 10 mM) upon sonication (Branson Sonifier 185) for several minutes in water or in 0.02 M borate buffer(pH 8). The solutions were stained negatively by aqueous 2 % phosphotungstic acid(pH 8), sonicated for 30 seconds and subjected to electron microscopic examination(Hitachi-H500).

Figures 1a and 1b are electron micrographs for **1** and **2**, respectively. Stacked disks are clearly seen in Figure 1a, and also in Figure 1b. The morphology did not change significantly with other staining reagents(uranyl acetate and ammonium molybdate).

The disk thickness is 60 - 70 Å and corresponds roughly to two times of the length of the extended molecules(40 Å for **1** and 43 Å for **2**, as estimated from the CPK molecular model). Dark streaks running through the middle of the layer is attributable to deposition of the staining reagent.9) These observations suggest the formation of the disk-like aggregate illustrated as **3**.



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The aggregate weight determined by the small-angle light scattering technique(Toyo Soda Co., Model LS-8) was 1.5 x 10^6 and 3.8 x 10^6 for **1** and **2**(sample concentration, 5 x 10^{-4} M), respectively. They are consistent with the aggregate geometry observed by electron microscopy. In differential scanning calorimetry(Daini-Seikosha Co., Model SSC-560), an endothermic peak was observed at 292 K(ΔH = 1.7 kJ/mol) for a 1 wt% solution of **2**. This peak is attributable to the phase transition from crystalline to liquid crystalline states. The ΔH value is only 3 - 10 % of those for lecithin(ΔH = 20 - 60 kJ/mol for n = 14 - 22). It is possible that the change in the molecular motion during the phase transition becomes less drastic when the dialkyl chain is replaced by the partially rigid single chain.10) For **1**, the endothermic peak was not detected, or ΔH was not more than 0.5 kJ/mol.

The morphology shown in Figure 1 is in sharp contrast with those of lecithin vesicles. Since the hydrophilic moiety is common in these amphiphiles, the morphological difference is produced by the change in the hydrophobic portion(from dialkyl to single-chain). Different phase transition behaviors similarly arise from the structural difference. We have shown that the aggregate morphology of single-chain amphiphiles can be controlled by the chemical structure of amphiphiles.11) Thus, it will be now possible to prepare aggregates of varied morphologies which possess phosphocholine head groups. The new amphiphiles would also be advantageously used for functionalization of lecithin bilayer membranes, since their hydrophilic portion would be compatible with that of lecithin and a variety of functionality can be introduced with ease to the rigid segment.

An example of the functionalization is provided by photoisomerization of **2**. When aqueous dispersion of **2** was irradiated at 366 nm with a 500-W Hg lamp, the absorptions due to the *trans*-azobenzene unit at 247 and 343 nm disappeared, and a new peak of the *cis* isomer appeared at *ca.* 450 nm, in consistence with the literature data.12,13) The content of the *cis* isomer was about 50 % at the photostationary state. The endothermic peak which appeared at 292 K in DSC for the all-*trans* aggregate was not detected for the photostationary mixture. Electron microscopy indicated partial development of the lamellar structure upon irradiation. The reverse(*cis*-to-*trans*) isomerization could be induced thermally or by irradiation at 450 nm, and the original morphology was restored.

In conclusion, single-chain amphiphiles with rigid segments in the hydrophobic group and with phosphocholine head group were shown to form disk-like aggregates. This class of amphiphiles will be useful in the synthetic membrane research, because the phosphocholine head group has strong hydrophilicity and is influenced relatively little by the change in ionic strength. Furthermore, these amphiphiles would be particularly prospective as a modifier of the lecithin bilayer.

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electron microscope and an irradiation apparatus, respectively.

References and Notes

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8) **1**: mp 160 → 271°C(the liquid crystalline region indicated by the arrow); tlc with a flame ionization detector R_f = 0.23($CHCl_3$:CH_3OH:H_2O = 65:25:4); elemental analysis, found C 61.84, H 8.60, N 2.35 %. Calcd for $C_{32}H_{52}NO_6P\cdot 0.5HBr$, C 62.22, H 8.49, N 2.27 %. **2**: mp 130 → 235°C; tlc, R_f = 0.43; elemental analysis, found C 58.84, H 8.14, N 6.31 %. Calcd for $C_{32}H_{52}N_3O_6P\cdot 0.6HBr$, C 58.80, H 8.11, N 6.43 %.

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10) Similarly small ΔH values were obtained with other single-chain aggregates.[5)]

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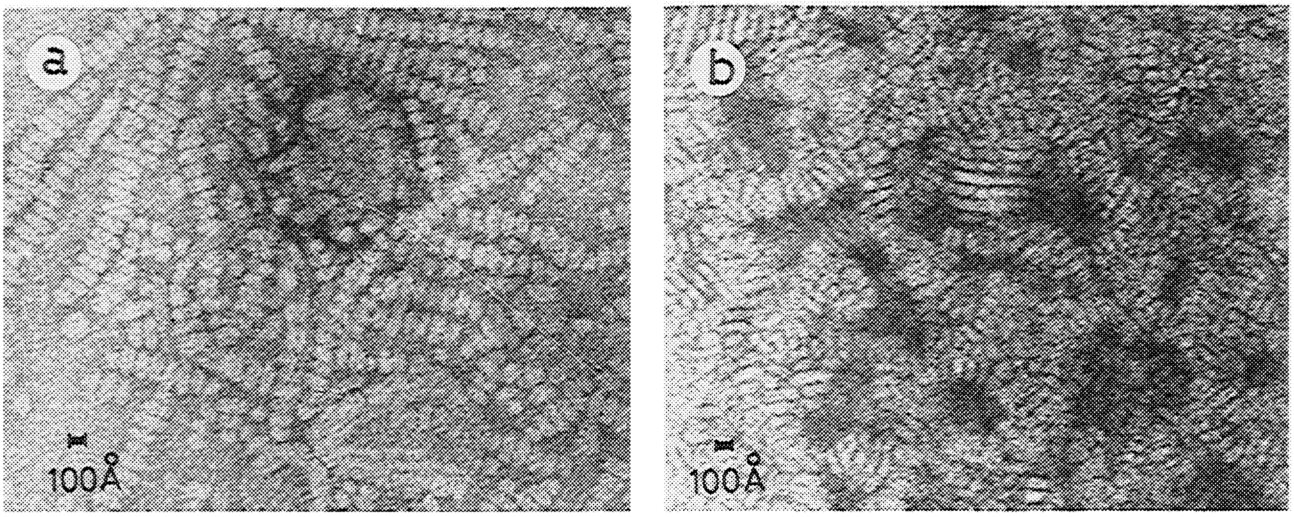

Figure 1: Electron micrograph. Stained by phosphotungstic acid(pH 8).
a. **1**, magnification x300,000. b. **2**, magnification x300,000.

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