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# Phase Transition Behavior and Membrane Structure of Monoalkyl Amphiphiles

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Membrane structure and crystal-liquid crystal phase transition behaviors were investigated for two kinds of artificial amphiphiles with single alkyl chain and *N*-benzylideneaniline moiety. These artificial amphiphiles showed both thermotropic and lyotropic mesomorphisms in a similar manner to biological lipids. Artificial amphiphile with one polar head group formed the multilayer liposome even in a low concentration region of amphiphile/water system. Artificial amphiphile with two polar head groups at both ends of the molecule does not form the multilayer liposome but forms monomolecular lamellae. Addition of cholesterol to the two polar-headed amphiphile changed its aggregated structure drastically from monomolecular lamellae to large single liposomes composed of monomolecular layer. In the case of two polar-headed amphiphile/water system, two endothermic peaks were observed, which were assigned to gel I to gel II and gel II to mesophase transitions. These transitions may correspond to meltings of partial and whole part of hydrophobic portion in amphiphilic molecule, respectively.

Biological membranes are mainly composed of phospholipids which are composed of one polar head group and two alkyl chains. It has been extensively discussed on the reason why phospholipids form the bimolecular-layered membrane in water at a low concentration region, instead of globular micells or two-dimensional lamellae.[1] Many artificial amphiphiles were synthesized by Kunitake *et al.*[2-5] For some of these amphiphiles, membrane structures and permeation properties were investigated on the basis of X-ray diffraction, differential scanning calorimetry and osmosis experiments.[6-9] It was concluded that existence of two alkyl chains played an important role for formation of the stabler bilayer structure. Also, it was confirmed that these artificial amphiphiles with two alkyl chains exhibited similar characteristics with respect to molecular aggregation states, thermal properties and permeation properties of water and metal cations to those of biological phospholipids.

Ordinary amphiphiles with single alkyl chain such as soap or detergent form bimolecular lamellae in water only at a high concentration region, while they do not form bimolecular layers but form cylindrical or globular micelles at a low concentration.[10] A series of ammonium amphiphiles composed of *N*-benzylideneaniline moiety and single alkyl chain were synthesized.[11-13] It is well known that *N*-benzylideneaniline moiety is capable of forming liquid crystalline structures due to its additional intermolecular interaction and rigid character.[14] Therefore, it is interesting to investigate the effect of *N*-benzylideneaniline moiety on the formation of single- or bimolecular-layered membrane in water at a low concentration region.

The purpose of this paper is to investigate the membrane structure and the crystal-liquid crystal phase transition behavior of artificial amphiphiles in water system, which are composed of single alkyl chain, mesogenic group and one or two polar head groups.

## Experimental

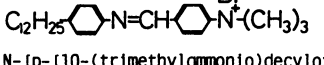
*Materials.* Figure 1 shows the chemical structures of artificial amphiphiles studied in this paper. *N*-[*p*-(trimethylammonio)benzylidene]-*p*-dodecylaniline bromide ($C_{12}BBN^+3C_1$: sample 1) was prepared by reaction of *p*-dodecylaniline and (*p*-formylphenyl)trimethylammonium bromide.[11] *N*-[*p*-[10-(trimethylammonio)decyloxy]]-*p*-[10-(trimethylammonio)-decyloxy]aniline dibromide ($N^+C_{10}BBC_{10}N^+$: sample 2) was prepared by condensation reaction of [10-(*p*-formylphenoxy)-decyl]trimethylammonium bromide and [10-(*p*-aminophenoxy)-decyl]trimethylammonium bromide in refluxing ethanol for 1 h in the presence of a small amount of acetic acid.[13] These artificial amphiphiles are designated as monoalkyl-monocation ($C_{12}BBN^+3C_1$) and monoalkyl-dication ($N^+C_{10}$-$BBC_{10}N^+$).

*Electron Microscopic Observation.* Several mg of amphiphiles were dissolved in deionized water and subjected to ultrasonic treatment. Almost equal volume of 2 wt% aqueous solution of uranyl acetate was added to the resulting suspension. A drop of the solution was placed and dried on a carbon grid for an electron microscopic observation (TEM).

*Differential Scanning Calorimetry.* The thermal behavior of the crystal-liquid crystal phase transition in the amphiphile/water system was studied with differential scanning calorimeter (DSC).

*X-Ray Analysis.* The membrane structure in the amphiphile/water system was studied by wide and small angle X-ray measurements. X-Ray diffraction photographs

1. N-[p-(trimethylammonio)benzylidene]-p-dodecylaniline bromide($C_{12}BBN^+3C_1$)



2. N-[p-[10-(trimethylammonio)decyloxy]]-p-[10-(trimethylammonio)decyloxy]aniline dibromide($N^+C_{10}BBC_{10}N^+$)

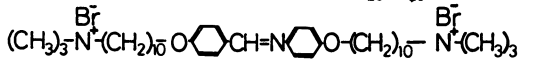


Fig. 1. Chemical structures of totally artificial lipids and their abbreviations.

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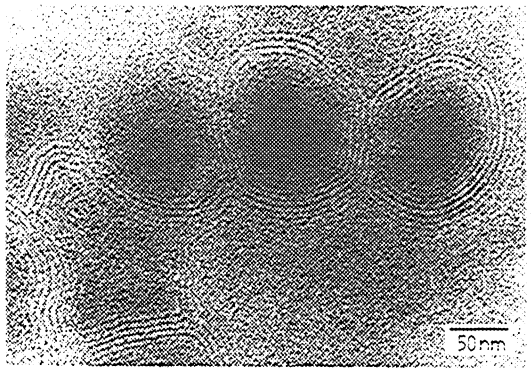


Fig. 2. Electron micrograph of $C_{12}BBN^{+}3C_1$ negatively stained with uranyl acetate.

were obtained with Ni-filtered Cu $K\alpha$ radiation.

## Results and Discussion

*Membrane Structure and Phase Transition of $C_{12}BBN^{+}3C_1$.* Figure 2 shows the electron micrograph of $C_{12}BBN^{+}3C_1$ negatively stained with uranyl acetate. $C_{12}BBN^{+}3C_1$ favorably forms multilayer liposomes in a similar fashion to those of biological lipids. An apparent mean distance between dark striations was 5.8—6.0 nm which nearly corresponds to the bimolecular length of $C_{12}BBN^{+}3C_1$ (5.9—6.0 nm) calculated by the CPK molecular model. Since the deposition of uranyl acetate for negative staining amounts to 1—2 nm thick,[8,9] the exact repeating distance was evaluated from the small angle X-ray scattering (SAXS) measurement as mentioned later.

The multilamellar liposome was observed even in a fairly dilute aqueous solution of $C$=0.001 (weight fraction of amphiphile in water system) by means of an electron microscopy. This concentration is quite lower in comparison with the case for ordinary surfactants with monoalkyl chain which form the bimolecular lamellar structure at a higher concentration range above $C\simeq0.6$.[10] The stable liposome formation at an extremely low concentration in the case of $C_{12}BBN^{+}3C_1$ must be due to introduction of the rigid segment of *N*-benzylideneaniline moiety which enhances the intermolecular interaction.

Figure 3 shows the phase diagram and the concentration, (C: weight fraction) dependence of the long spacing, ($L$) for $C_{12}BBN^{+}3C_1$. Thermal analytical studies of $C_{12}BBN^{+}3C_1$ were carried out over the concentration range of $0.1<C\leq1.0$. The endothermic transition due to melting of alkyl chains was observed over whole concentration range studied here. The endothermic peak due to melting of ice was observed at 273 K in the concentration range lower than about $C$=0.83. In the concentration range of $0.83<C\leq1.0$, no endothermic transition due to melting of ice was observed in heating curve. Water molecules hydrated to the polar head groups did not form ice on cooling the $C_{12}BBN^{+}3C_1$/water mixture below 273 K.[6] The critical concentration at which nonhydrated (free) water appeared was determined by extrapolating the

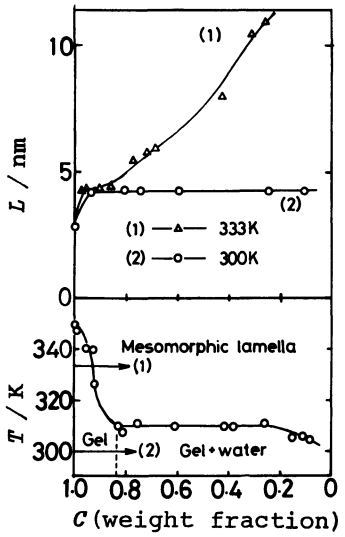


Fig. 3. Variation of the phase transition temperature (lower) and the long spacing (upper) with the concentration of $C_{12}BBN^{+}3C_1$.

magnitude of the heat of fusion of ice at 273 K *vs.* the concentration of $C_{12}BBN^{+}3C_1$ to zero. The critical concentration between gel and gel+water phases was determined to be 0.83, where the gel phase means the crystalline phase with bound water hydrated to the hydrophilic polar head groups. This value of the critical concentration indicates that on an average, five water molecules hydrate to one polar head group of $C_{12}BBN^{+}3C_1$ and these bound water molecules do not form ice on cooling even to 220 K.[8] The gel to mesophase transition temperature decreases steadily with a decrease of $C_{12}BBN^{+}3C_1$ concentration to the limiting value of 310 K over the concentration range of $0.83<C\leq1.0$. The phase diagram of the $C_{12}BBN^{+}3C_1$/water system has a very similar behavior to those of biological lipids[15] and artificial amphiphiles with two alkyl chains.[6,7,9] It is apparent from the phase transition behavior that $C_{12}BBN^{+}3C_1$ has characteristics of both thermotropic and lyotropic liquid crystals in a similar fashion to phospholipids or artificial lipids with two alkyl chains. $L$ measured at 300 K increases with decreasing the $C_{12}BBN^{+}3C_1$ concentration down to $C$=0.83, where free water appears in the $C_{12}BBN^{+}3C_1$/water mixture. As mentioned before, the hydrophilic polar head group can incorporate limited amount of water and therefore, the bimolecular lamellae of $C_{12}BBN^{+}3C_1$ were gradually spread with an increase in the water concentration up to $C$=0.83.

The formation of lamellar structure was confirmed by the result that the ratio of reciprocal of long spacing for higher order scatterings were 1:2:3. The long spacing of the lamellar layer in a gel state is approximately one-half of the bimolecular length estimated from the CPK molecular model on the assumption of planar zigzag conformation of hydrocarbon chains. This result may be explained by the following two possible models for the assembly of $C_{12}BBN^{+}3C_1$

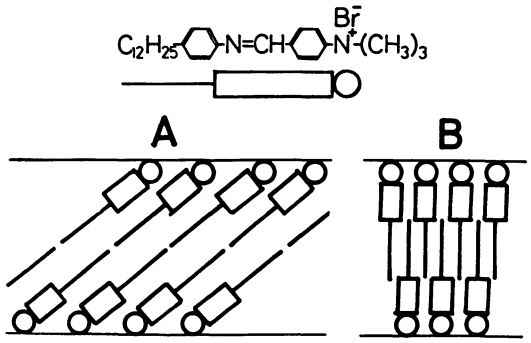


Fig. 4. Schematic assembly models for $C_{12}BBN^+3C_1$ bilayer.

molecules as shown in Fig. 4. Figure 4A is the model that hydrocarbon chains tilt with an angle of 30° to the lamellar surface. Figure 4B shows the mutual insertion of hydrocarbon chains from the opposite directions. Structure amplitudes were calculated for the two models of Figs. 4A and 4B and compared with an observed relative structure amplitude. The most plausible packing model for the $C_{12}BBN^+3C_1$/water system was determined to be the latter one (the mutual insertion of hydrocarbon chains) on the basis of the electron density distribution obtained by means of SAXS measurement.[16,17] In this case, the alkyl chains are almost perpendicular to the membrane surface. The detailed results will be reported elsewhere.[17]

*Membrane Structure and Phase Transition of $N^+C_{10}BBC_{10}N^+$.* $N^+C_{10}BBC_{10}N^+$ has the two polar head groups at both ends and *N*-benzylideneaniline moiety of a rigid segment in hydrocarbon chains at the para-position. In general, chemical substances containing a rigid segment such as *N*-benzylideneaniline moiety can form liquid crystalline phase. Therefore, amphiphile of monoalkyl-dication type makes us expect that the monomolecular membrane can be easily formed in a dilute aqueous solution owing to existence of the two polar head groups at both ends of molecule.

Figure 5 (a) shows the electron micrograph of $N^+C_{10}BBC_{10}N^+$ negatively stained with uranyl acetate. $N^+C_{10}BBC_{10}N^+$ amphiphile could not form multilayer liposome, but the parallel aggregation of lamellae. Taking into account the deposition of uranyl acetate, the thickness of parallel aggregated lamellae (5.3—6.4 nm) is considered to correspond to the one molecular length of $N^+C_{10}BBC_{10}N^+$ (4.4—4.5 nm) calculated by use of the CPK model. Figure 5(b) exhibits that incorporation of cholesterol to $N^+C_{10}BBC_{10}N^+$ induces a transformation from monomolecular lamellae to single- or multi-layer liposome. The diameter of vesicles are 100—200 nm and their membrane thickness is 5—20 nm. It is reasonably expected that cholesterol molecules mostly locate in the outer half of the layer, creating suitable curvature for the vesicle formation as shown in Fig. 5 (c). Figure 5(c) represents schematic speculations with respect to formation of monolayer liposome for $N^+C_{10}BBC_{10}N^+$ with

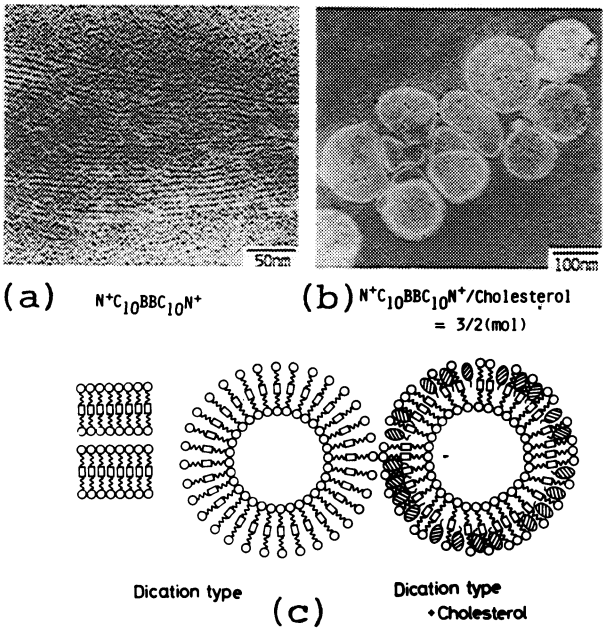


Fig. 5. (a) Electron micrograph of $N^+C_{10}BBC_{10}N^+$ negatively stained with uranyl acetate. (b) Electron micrograph of $N^+C_{10}BBC_{10}N^+$+cholesterol (3:2 molar mixture). (c) Schematic packing model of $N^+C_{10}BBC_{10}N^+$ and possible formation of monolayer liposome.

and without cholesterol. In the case of bimolecular membrane of dialkyl- or monoalkyl-monocation artificial amphiphiles, then number of molecules must be different in the outer and the inner layers because of the different spherical surface area for the inner and the outer shells. On the other hand, $N^+C_{10}BBC_{10}N^+$ forms monomolecular layer due to existence of dication groups at the both ends of the molecule as shown in the left assembly model of Fig. 5 (c). Considering the liposome formation of $N^+C_{10}BBC_{10}N^+$ in the case of this assembly, however, a large amount of vacancy or defect is produced especially in the outer half of spherical shell as shown by the middle aggregation model of Fig. 5 (c). These vacancies or defects may be reduced by addition of the second components such as cholesterol as shown in the right assembly model of Fig. 5 (c). Therefore, the monomolecular liposome of $N^+C_{10}BBC_{10}N^+$ was stably formed by addition of cholesterol.

Figure 6 shows the variation of the phase transition temperature with the weight fraction of $N^+C_{10}BBC_{10}N^+$. This phase diagram shows that $N^+C_{10}BBC_{10}N^+$ has characteristics of both thermotropic and lyotropic liquid crystals in a similar manner to those of phospholipids in biomembrane or other artificial amphiphiles. In the case of $N^+C_{10}BBC_{10}N^+$, the two endothermic peaks were recognized by DSC measurement. These were assigned to the gel I to gel II and the gel II to mesophase transitions with consideration of the wide angle X-ray diffraction as discussed later. Also, the critical concentration of $C$=0.84 between gel I and gel I+water (shown by the brokenline in Fig. 6)

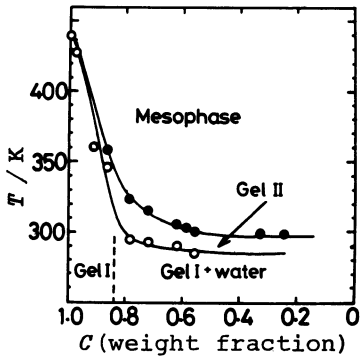


Fig. 6. Variation of the phase transition temperature with the concentration of $N^+C_{10}BBC_{10}N^+$.

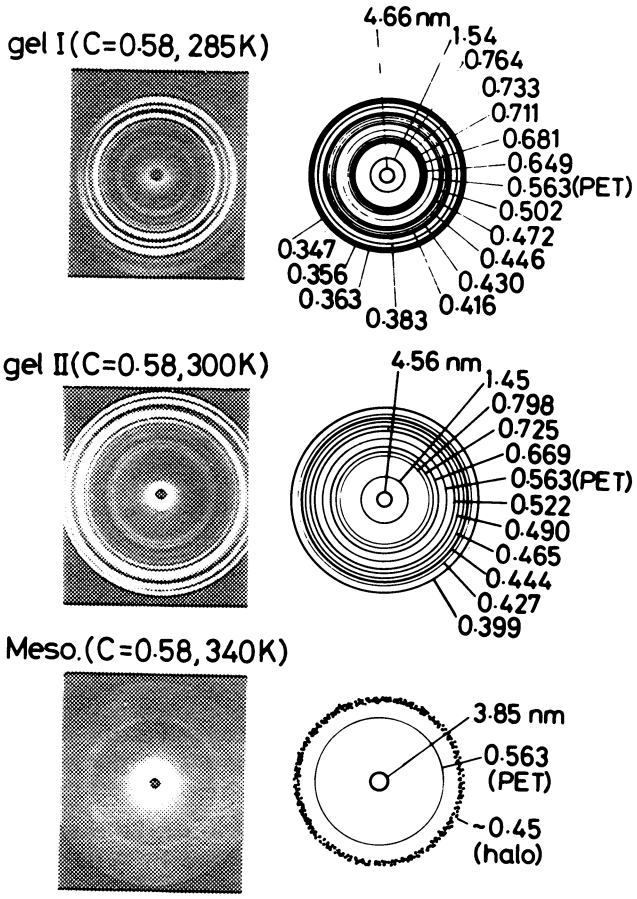


Fig. 7. Wide angle X-ray diffraction patterns for gel I, gel II and mesophase of $N^+C_{10}BBC_{10}N^+$.

was decided in a similar manner to that for $C_{12}BBN^+3C_1$ as mentioned before.

In the case of gel I ($C$=0.58 at 285 K), many sharp Debye rings were observed as shown in Fig. 7. A spacing of 0.563 nm is due to poly(ethylene terephthalate) (PET) film used as a window of the cell for X-ray measurements. The most inner ring corresponds to the long spacing of $N^+C_{10}BBC_{10}N^+$ (4.66 nm). This value is nearly equal to the single molecular length. In the intermediate range of gel II ($C$=0.58 at 300 K), the almost all diffraction rings became broader in comparison with those of gel I and their spacings were slightly changed. Also, some diffraction rings corresponding to the short spacing between alkyl chains disappeared, for example, 0.711, 0.649 and 0.416 nm spacings in gel I. Furthermore, the long spacing in gel II became slightly shorter (4.56 nm) than that in gel I. The crystallographic analysis of $N^+C_{10}BBC_{10}N^+$ will be reported in detail elsewhere. The variation of the X-ray diffraction patterns indicates the partial melting of hydrocarbon chains which locate between the polar head and the *N*-benzylideneaniline moiety. Since many fairly sharp diffraction rings were yet remained, it seems that the lateral order of the hydrophobic chains in a gel II state still remained considerably. In the case of mesophase ($C$=0.58 at 340 K), the sharp Debye rings corresponding to the interchain distance among the hydrophobic chains changed into diffuse ones but the scattering ring in small angle region still retained (3.85 nm), indicating the existence of the layer structure in the mesomorphic phase. These results apparently indicate that the lateral order in the interchain distance among the hydrophobic chains, was lost by melting of the whole part of the hydrophobic portion in $N^+C_{10}BBC_{10}N^+$ molecule without disappearance of layered structure of dication artificial amphiphile in a mesomorphic state as shown in Fig. 8.

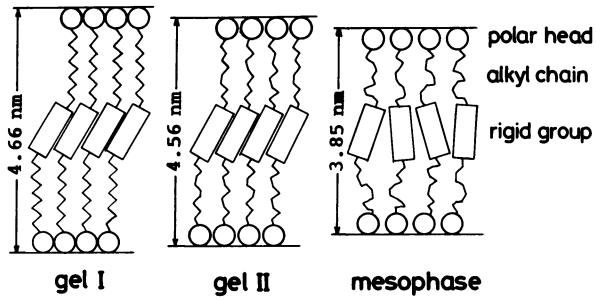


Fig. 8. Schematic representation of molecular membrane structures of $N^+C_{10}BBC_{10}N^+$ for gel I, gel II, and mesophase.

The membrane structures or the aggregation states of $N^+C_{10}BBC_{10}N^+$ for gel I, gel II, and mesomorphic phases were schematically represented in Fig. 8, although they are not clarified yet by the detailed structural analysis. In gel I phase, the alkyl chain has all trans conformation in a crystalline state. In gel II phase, the alkyl chain partially melts, but the lateral order of polar head and rigid *N*-benzylideneaniline moiety remains. In the mesophase, the alkyl chain and the rigid group are in a disordered state but the layer structure are retained.

## Conclusion

The artificial amphiphiles which have one or two polar head groups and *N*-benzylideneaniline moiety as a rigid segment in hydrocarbon chains at the para-position formed bimolecular or monomolecular liposomes in an extremely low concentration, depending on the number of the polar heads. The rigid segment is required for inducing an ordered packing

of amphiphilic molecules in a dilute solution. The aggregated structure of the two polar-headed amphiphile (monoalkyl-dication type) is varied drastically from parallel aggregation of monomolecular lamellae to monomolecular or multilayered liposome by adding cholesterol.

The phase transition temperatures of $C_{12}BBN^+3C_1$ and $N^+C_{10}BBC_{10}N^+$ in water system decreased to the limiting value with an increase in water concentration in a concentration range of $C$=1.0—0.85. Since the long spacing corresponding to the thickness of lamellae was clearly observed above phase transition temperature, it is apparent that melting of hydrocarbon chains is not accompanied by appearance of isotropic phase but the mesomorphic one. The phase diagrams of the amphiphile/water system exhibited that the amphiphiles had characteristics of both thermotropic and lyotropic mesomorphisms.

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