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CRYSTAL-LIQUID CRYSTAL PHASE TRANSFORMATION AND WATER PERMEABILITY OF ARTIFICIAL AMPHIPHILES AS BIOMEMBRANE MODEL

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Artificial ammonium amphiphiles with monoalkyl and dialkyl groups form the multilamellar liposome composed of the bimolecular layer. Artificial amphiphiles have characteristics of both thermotropic and lyotropic mesomorphisms. The diffusive permeability of the polymer/artificial amphiphiles composite membrane to water exhibits a distinct jump in the vicinity of the crystal-mesomorphic phase transition temperature.

A bimolecular membrane of phospholipids such as lecithin is in a state of liquid crystalline structure capable of reversible structural modifications and its permeability property depends upon such reversible change. Two of authors succeeded in synthesis of totally artificial ammonium amphiphiles which are able to form the bimolecular structure[1]. The purpose of this paper is to establish the structural properties of artificial ammonium amphiphiles as biomembrane model and also, to investigate the water permeability of the composite membrane composed of polymer and artificial ammonium amphiphiles.

1. Phase Transition and Bilayer Structure of Dialkyldimethylammonium bromide

Dialkyldimethylammonium bromide(DAAB) was prepared by step alkylation of dimethylamine. The chemical formula of DAAB is $[CH_3(CH_2)_n]_2N^+(CH_3)_2\cdot B_r^-$ (n=11∿17).

Figure 1 is an enlargement of a favourable area of dioctadecyldimethylammonium bromide(DOAB)(n=17) stained negatively by uranyl acetate and shows the distinct structure of multilamellar liposome(spherulite composed of concentric lamellae). The mean thickness of multilamellar liposome was estimated to be 4.2 nm. The total bimolecular length of DOAB is 4.9∿5.0 nm on the assumption of the planar zigzag conformation of dialkyl groups (Table 1). The average thickness of multilamellar liposome is comparable with two times as long as linear DOAB. The use of uranyl acetate for negative staining offers advantage in the direct visualization of liposome structures because the rigid setting of salts is believed to preserve structures. The external form of multilamellar liposome is confirmed to be

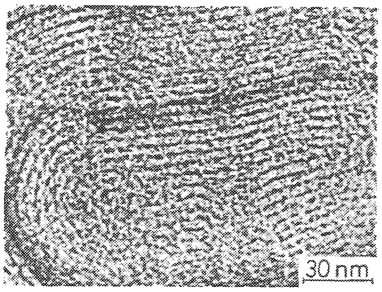

Figure 1. Electron micrograph of DOAB stained negatively by uranyl acetate.

Table 1. Structural characterization of artificial ammonium amphiphiles.

Lipids	$T_c^{\bullet}$(K)	Bimolecular Length(nm)	Long Period (nm)	Width between Dark Striations(nm)	Tilt Angle (°)
$2C_{18}N^+2C_1Br^-$	324	4.9-5.0	3.5-3.7	4.3-4.4	47
$2C16N^+2C_1Br^-$	317	4.4-4.5	2.3-2.4	3.8-4.0	32
$2C_{14}N^+2C_1Br^-$	305	3.9-4.0	2.1-2.2	3.0-3.2	33
$2C_{12}N^+2C_1Br^-$	289	3.4-3.5	1.9-2.0	2.8-2.9	34
$2C_{18}$+Cholesterol		4.9-5.0	4.2-4.3	5.8-6.0	58
$C_{12}BBN^+3C_1$	309	5.9-6.0	2.8-2.9	5.8-6.0	30

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spherical or oval from the feature of a shadow reflected by vapor depositing with Pt-Pd2).

Figure 2 shows the variation of the phase transition temperature, T_c and the long spacing, L with the concentration of DOAB. T_c decreases steadily to a limiting value of 324 K, T_c*. By means of X-ray study, many sharp Debye rings were observed below T_c, while only one diffuse diffraction ring corresponding to the spacing of 0.46 nm was discernible above T_c. This wide angle X-ray diffraction change apparently indicates melting of the hydrocarbon chains of DOAB at T_c. As a consequence that the small angle X-ray scattering (SAXS) spacing, L corresponding to the thickness of bimolecular lamellae was clearly observed in a temperature range above T_c, it is apparent that melting of dialkyl groups is not accompanied by appearance of the isotropic phase but the mesomorphic phase over the temperature range above T_c. Hydrated waters associated with the polar head groups do not form ice on cooling amphiphile/water mixture below 273 K. The critical concentration above which non-bound water appears is determined by extrapolating the magnitude of the heat absorbed in the endothermic transition due to melting of ice at 273 K vs the concentration of DOAB to zero. The critical concentration between gel and gel+water phases is 0.87 and is shown by the broken line in figure 2.

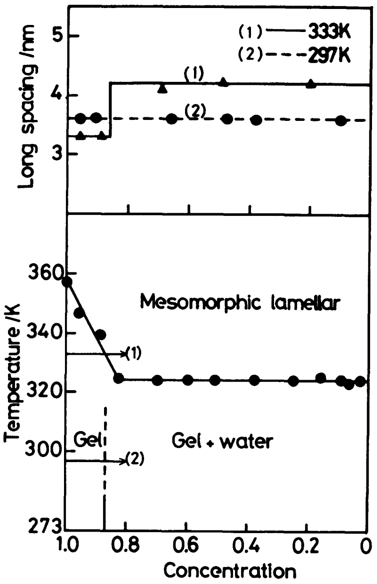

Figure 2. Variation of T_c and L with concentration of DOAB.

Above T_c line, the DOAB/water system exists in the form of mesomorphic lamellae in which the long hydrocarbon chains of DOAB are in fluid state. The phase diagram of figure 2 exhibits that DOAB has both characteristics of thermotropic and lyotropic mesomorphisms, just in the same manner of phospholipids in the biomembrane3).

At 297 K (below T_c*), the spacing of biomolecular lamellae, L remains constant at 3.6 nm over all range of the DOAB/water concentration. This result draws reasonable speculation that water molecules penetrate into the space existing around the polar head groups, and water molecules associate with the polar head groups up to C=0.87 without spraying apart the surfaces of bimolecular layers. On the other hand, at 333 K (above T_c*) L remains constant at 3.3 nm up to about C=0.87 at which there is a sharp increase by some 0.9 nm, followed by the constant long spacing of 0.42 nm over the range of $0 < C < 0.87$. L of 0.36 nm determined by SAXS for gel state is comparable with the mean thickness of the bimolecular structure evaluated by the electron micrograph. The temperature or concentration dependences of L for the DOAB/water system are strikingly different from those for phospholipids in biomembranes such as lecithin, which showed the sharp decrease of L at T_c upon heating the lipid/water mixture from 273 K3). The remarkable difference on the concentration dependence of L between DOAB and lecithin above T_c* may arise from the geometrical and structural requirements of the polar head groups as discussed elsewhere2).

On the assumption of the planar zigzag conformation for the dioctadecylalkyl chains and also, the ordered packing arranged normal to the lamellar surface, the thickness of the bimolecular lamella should be around 4.9∿5.0 nm. This magnitude is larger than the observed lamellar thickness. If the hydrocarbon chains are tilted to the lamellar plane, it is possible to elucidate that the average thickness of the bimolecular structure is shorter than the magnitude of two times as long as the DOAB molecule. Tilting of the alkyl chains may take place so that the repulsive effect induced by the voluminal requirement of the expanded polar head groups is reduced on the lamellar surface. The remarkable increase of L for the mesomorphic phase at around C=0.87 may be caused by the release of repulsive force among the polar head groups, due to decrease of the volume ratio of hydrophilic group/hydrophobic group. As shown in Table 1, addition of cholesterol to the DOAB/water system may make a similar contribution to a fluid state of hydrocarbon chain with respect to the aggregation state caused by geometrical implications.

2. Water Permeation of Composite Membrane Composed of Polymer and DOAB

Polymer/DOAB blend films were cast from a tetrahydrofuran solution of poly (vinyl chloride) (PVC) and DOAB in which the weight fraction of DOAB was 15%. PVC does not exhibit any thermal transition or the prominent mechanical absorption due to the thermal molecular motion in the temperature range studied here. The water transport properties were measured in normal osmosis experiment. Distilled water and aqueous solution of KCl of 10 wt% were placed in each of the two chambers of the osmosis apparatus, separated by the composite membrane.

Figure 3 shows the Arrhenius plot of the diffusive permeability (P) to water for the PVC and the PVC/DOAB composite membranes. The log p-1/T plot for the composite membrane reveals a distinct jump at about 312∿315 K corresponding to the crystalline-mesomorphic phase transition temperature of DOAB, though this temperature range is slightly lower than that shown in Figure 2. This striking increase of P may be caused by activation of the thermal molecular motion of the constituents within the composite membrane and/or increase of vacancy fraction around the domain boundary of DOAB. Due to discontinuities at the boundaries of liquid and solid domains within DOAB phase (coexistence of gel and mesomorphic phases), the probability of pore formation may be enhanced at these sites. The author have reported that the diffusive permeability is closely related to the primary relaxation process of the disordered region of membrane polymer$^{4)}$. PVC membrane does not exhibit a discontinuous variation on P. The temperature dependences of P for the PVC and the PVC/DOAB composite membranes apparently indicate that P is in close relation to the thermal molecular motion or the structural transition of the membrane materials.

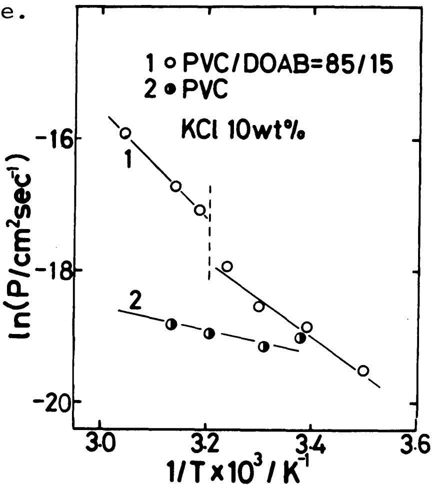

Figure 3. Arrhenius plot of P to water for composite membrane.

3. Phase Transition of p-trimethylammoniumbenzylidene-p'-dodecylaniline bromide

The chemical formula of p-trimethylammoniumbenzylidene-p'-dodecylaniline bromide ($C_{12}BBN^+3C_1$) is $[C_{12}H_{25}$⌬$N=CH$⌬$N^+-(CH_3)_3]\cdot B_r^-$. Ammonium amphiphiles with monoalkyl group have not been found in animals and plants. Artificial ammonium amphiphiles of $C_{12}BBN^{+}3C_{1}$ favorably form a multilamellar liposome in a similar fashion to those with dialkyl group (DAAB) as shown in figure 12). Figure 4 shows the phase diagram and the concentration dependence of the long spacing of $C_{12}BBN^{+}3C_{1}$. The phase diagram of $C_{12}BBN^{+}3C_{1}$/water system has very similar tendency on the concentration dependence to that of DOAB shown in figure 2. $C_{12}BBN^{+}3C_{1}$ exhibits characteristics of thermotropic and lyotropic liquid crystals. The long spacing measured at 300 K increased with the water concentration until $C \simeq 0.85$, where free water starts to appear in $C_{12}BBN^{+}3C_{1}$/water mixture. The hydrophilic polar head group can incorporate limited amount of water and the interlamellar region of $C_{12}BBN^{+}3C_{1}$ were gradually spreaded with increasing the water content up to $C \simeq 0.85$. $C_{12}BBN^{+}3C_{1}$ can exist in a form of the bimolecular lamella in the very diluent aqueous solution ($C \simeq 0.1$), in comparison with usual surfactants which can form the bimolecular lamellar structure only in the higher concentration range above $C \simeq 0.6$5). This ability of the bimolecular lamellar formation must be due to introduction of the rigid conformation of diphenylimino group.

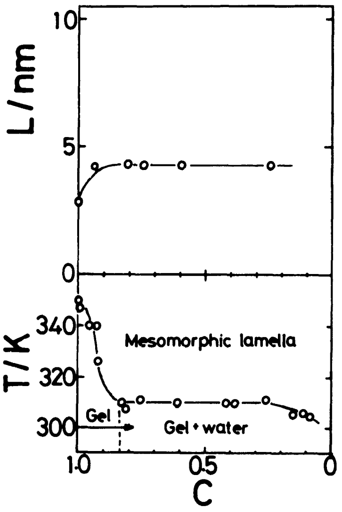

Figure 4. Variation of T_c and L with concentration of $C_{12}BBN^{+}3C_{1}$.

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