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Bilayer Membranes of Amphiphilic Crown Ethers with Amino Acid Residue

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An amphiphilic crown ether (5) with amino acid residues and two long hydrocarbon chains($2C_{12}$) was synthesized and the membrane(vesicle) properties of 5 were examined. 5 spontaneously formed a stable bilayer membrane and gave uniform single unilamellar vesicles(SUV). The resulting SUV showed strong retaining power related to the release of a trapped fluorescent marker and a highly selective complexing ability for specific metal cations.

Membrane-forming dialkyl crown ethers have recently been noted as model compounds for receptor molecules in biological membranes.[1-5] However, some of them have no additional hydrophilic groups besides the crown ring, and it appears that they have difficulty in forming well-ordered aggregates due to their low hydrophilicity. Shinkai et al. first described the formation of lamellar and rod-like aggregates using dialkyl diaza-18-crown-6 with strongly hydrophilic anion-cap($-SO_3^-$).[4] Okahara et al. reported the synthesis of dialkyl crown ether containing oligooxyethylene groups introduced between two dodecyl chains and 18-crown-6 ring, and presented the electron micrographs of myelin-like fingerprint structures and single to multilayered membranes of the crown ether dispersed in water.[5]

It is known that the stability of lamellar aggregates and the hydrophilicity of ionic[6] and nonionic[7] surfactants in water are significantly improved by introducing hydrogen bond-forming polar groups such as amino acid residues between long alkyl chain and main hydrophilic group in the surfactant molecules. Furthermore, Murakami et al. reported the formation of stable single vesicles from dialkyl ionic surfactants involving amino acid residues.[8]

Under these circumstances, we prepared a series of dialkyl crownethers containing amino acid residues and examined them for the structural effect of the side arm group on the solution properties, membrane stability and complexing ability with metal cation.[9] Here, we report the synthesis and membrane (vesicle) porperties of 6-[bis-(dodecyloxymethyl)methoxyethanoylamino]hexanoylaminomethyl-18-crown-6 (5 in Fig.1), which is found to form the most stable bilayer membrane among the compounds we prepared.

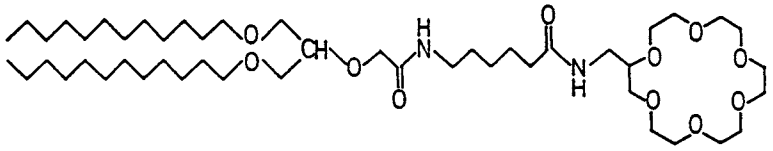

Fig.1. Dialkyl crown ether 5.

The synthetic route for dialkyl crown ether 5 is as follows. The intermediate compound 4 was prepared by the following process from the starting material, 1,3-dialkoxy-2-propanol(1) which was available from earlier research.[10] The crown ether 5 was prepared from 4 and amino-methyl-18-crown-6 in the same manner as monoalkyl crown ethers bearing amino acid residue described before.[11]

$$(ROCH_2)_2CHOH \xrightarrow[K,\ t\text{-}BuOH]{BrCH_2COOH} (ROCH_2)_2CHOCH_2COOH \xrightarrow[CHCl_3]{SOCl_2} (ROCH_2)_2CHOCH_2COCl$$

1 R:n-$C_{12}H_{25}$ 2 3

$$\xrightarrow[(CH_3)_2CO\ :\ H_2O]{H_2N(CH_2)_5COOH,\ NaOH} (ROCH_2)_2CHOCH_2CONH(CH_2)_5COOH$$

4

$$4 + H_2NCH_2\text{-18-CROWN-6} \xrightarrow[THF]{DCC} (ROCH_2)_2CHOCH_2CONH(CH_2)_5CONHCH_2\text{-18-CROWN-6}$$

Dialkyl Crown Ether 5

The product 5 was isolated as a colorless viscous liquid in a yield of 30% based on the intermediate 4. The structure and purity of 5 were verified by spectral and elemental analyses. ^{1}H NMR(CCl_4) 0.87(t,6H), 1.28(m,46H), 1.95-2.20(m,4H), 3.25-3.90(m,36H), 7.10-7.40(t,2H). IR(liquid film) 3300, 2955, 1668, 1115 cm^{-1}. Anal.Found: C,65.84; H,10.69; N,3.10 %. Calcd for $C_{48}H_{94}N_2O_{11}$: C,65.87; H,10.83; N,3.20%.

The K^+-complexing stability constant(log $K_1^!$) of 5 was evaluated to be 4.84 in methanol at 25 °C with Frensdorff's method.[12]

The vesicles composed totally of compound 5 were prepared in $1x10^{-4}$ Mol KCl aqueous solution according to the usual sonication method. As seen in Fig.2, the electron microphotographic measurements with staining by uranyl acetate, clearly showed the formation of single unilamellar vesicles(SUV). The same unilamellar structure could be seen

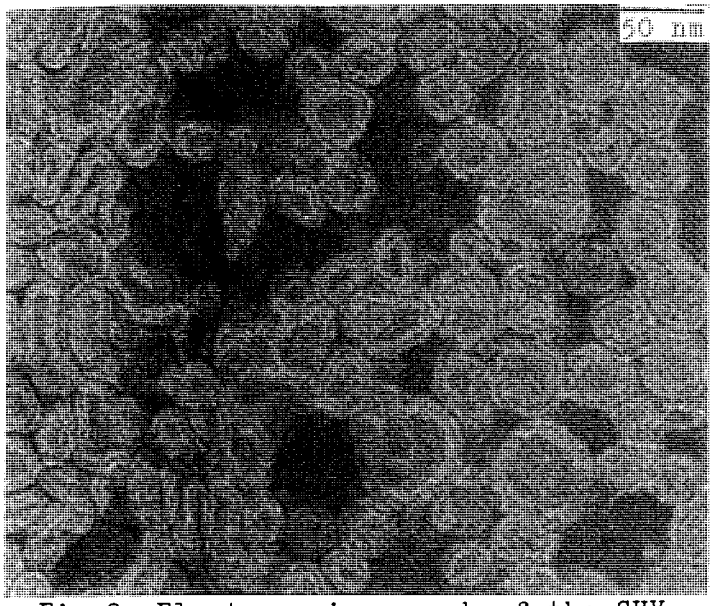

Fig.2. Electron micrograph of the SUV stained by uranyl acetate.

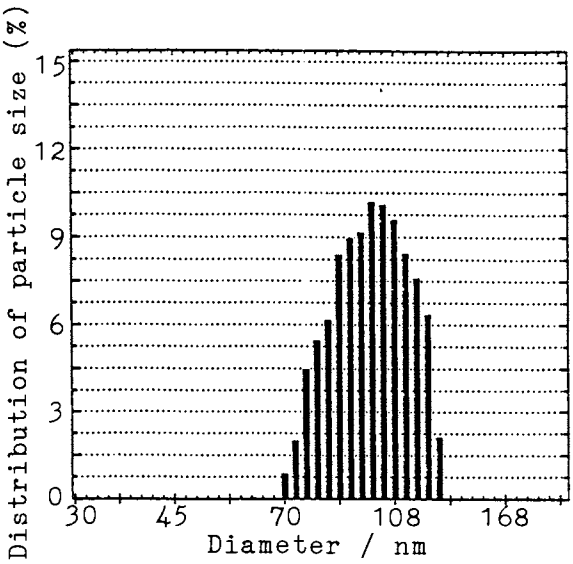

Fig.3. Distribution curve of particle sizes of LUV.

on large unilamellar vesicles(LUV) which were prepared by the extrusion technique, i.e., the aqueous suspension of 5 was freeze-thawed five times and then extruded through a polycarbonate filter(Nuclepore Corp.; poresize 100 nm). As can be seen in Fig.3, the distribution curve of LUV indicates a sharp single peak with an average diameter of 90±10 nm.

The membrane stability formed with 5 was examined by measuring the release of a trapped fluorescent marker(Fluorescein-5(and-6)-sulfonic acid; F-1130). It was realized that a suspension of vesicles containing 2 mM F-1130, fluoresces only slightly by their self-quenching, but the suspension fluoresces more than 30-fold when the dye is released and diluted into the entire solution volume.[13] Self-quenching thus allows materials remaining in the vesicles to be distinguished from those released into the much larger medium. Thus, the kinetics of fluorescence-increase in a suspension of vesicles containing 2 mM F-1130 becomes an important measure of the membrane stability(or membrane retaining power).

Figure 4 shows the time dependence curves of the fluorescence-increase during incubation at 25 °C with the egg PC and 5 vesicles containing 2 mM F-1130. As can be seen, bilayer membranes composed totally of compound 5, showed more moderate differences in fluorescence intensity than those in the egg PC membrane,

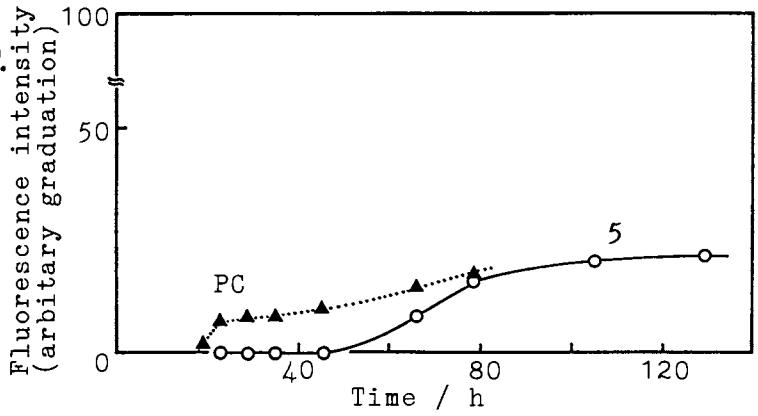

Fig.4. Kinetics of fluorescence increase during incubation at 25 °C with SUV of 5 and PC vesicles containing 2 mM F-1130.

indicating that the membrane of 5 prevented release more completely than did the PC vesicles, thouth the release were expected to be suppressed, to some extent, even in 5 vesicles due to the electrostatic attraction between the dye molecules and the cations in the crown rings. Such high membrane stability in the present crown vesicles is based on the existence of the amino acid residue in the crown compound, and the existence will strengthen the membrane stability by their inter and intra molecule hydrogenbonding interaction.

Figure 5 shows the electrophoretic mobilities of the crown vesicles bathed in various concentrated solutions of different metal ions. It can be seen that the shift tendency in the phoretic mobilities in the positive direction, is strongly dependent on the species of metal cation and has the following order, $Ba^{2+} > K^{+} > Li^{+} > Ca^{2+}$, which is the same order with the binding affinity of each ion to the crown ring. This electrophoretic behaviour directly indicates that the new crown vesicles are covered with the crown rings at their outer layer and that the crown rings hold their high selective complexing ability with a specific cation.

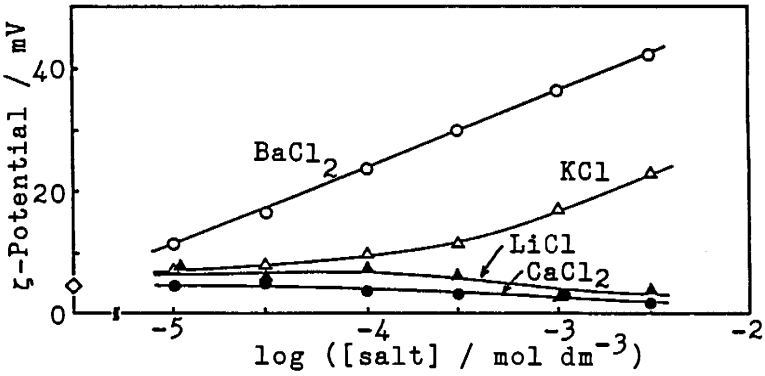

Fig.5. Zetapotential of totally 5 vesicles bathed in various concentrations of aqueous salt solution containg each cation.

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