Polymerizable Lipid-Corked Capsule Membranes. Polymerization at Different Positions of Corking Lipid Bilayers on the Capsule and Effect of Polymerization on Permeation Behavior[1]

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Abstract: Polymerizable lipid bilayers were corked in the spongy layer of a nylon capsule membrane and then polymerized on the capsule. The capsule was also corked with prepolymerized lipid bilayers. Three different types of polymerizable lipids were used as corking bilayers: (i) lipids **1**–**3** having butadiene or methacrylate groups in the hydrophobic dialkyl chain, (ii) lipid **4** having the polymerizable group in the hydrophilic part with a long spacer chain, and (iii) lipid **5** having the polymerizable group as a counteranion. The effect of polymerization of corking bilayers on NaCl permeation from the capsule was studied at temperatures below and above the phase transition of the bilayers. Only the capsule corked with lipid bilayers having the polymerizable group as a counteranion (type iii) could give stable, nonreleased corking bilayers without losing bilayer properties. This study describes the construction of stable, lipid-corked capsule membranes as well as the effect of polymerizing lipids on these matrices.

We recently developed the lipid-bilayer-corked capsule membrane, in which multiple bilayers are immobilized in the ultrathin, spongy nylon capsule membrane (see Figure 1).[2] The capsule has advantages of both lipid bilayer vesicles and polymer capsules: liposomes can entrap water-soluble substances in the inner aqueous phase but their bilayer walls are too weak and fragile under the dynamic changes of outside effects necessary to control their permeability, whereas polymeric capsule membranes are physically strong but their porous semipermeable membrane cannot store substances in the inner aqueous phase. The bilayer-corked capsule membrane can reversibly control the permeability of water-soluble substances preserved in the inner aqueous phase responding to various physical and chemical stimuli from the outside such as temperature changes,[3–6] photoirradiation,[7] ultrasonic power,[8] electric field,[9] ambient pH changes,[10,11] interaction with divalent cations,[11–13] and antigen–antibody interaction.[1] Their signal-receptive permeation control is explained by changes in the physical state of corking bilayers that act as a permeation valve. The bilayer-corked capsules are stable and reused under mild experimental conditions in the range 5–60 °C. However, the capsule has the disadvantage that the corking bilayers may release from the matrix when the capsule is exposed to harsh conditions such as hot water (above 70 °C) for a long time.

In this study, we prepared capsules corked with polymerizable lipid bilayers in order to obtain stable bilayer-corked capsules without releasing the corking lipids and studied the effect of polymerization on the stability and bilayer properties by using NaCl permeation measurements at temperatures below and above the phase transition of bilayers. As shown in Figure 1, three different types of polymerizable lipids were chosen for corking bilayers: (i) phosphatidylcholine having one or two butadiene groups in the hydrophobic alkyl chains (**1** and **2**) and the dialkylammonium amphiphile with a methacrylate group at the terminus of one hydrophobic alkyl chain (**3**), (ii) the dialkylammonium amphiphile having an acrylate group at the hydrophilic head group with a long spacer alkyl chain (**4**), and (iii) the dialkylammonium amphiphile having a styrenesulfonate group as a counteranion (**5**). These amphiphiles were corked in the spongy layer of the nylon capsule membrane and then polymerized on the capsule. The capsule was also corked with prepolymerized lipid bilayers. It is of interest to evaluate the effect of polymerizing the corking bilayers in the polymer matrix both on the fluidity of bilayers and on the formation of defects caused by polymer shrinking, depending on the position of polymerization in lipids.

In order to obtain stable aqueous dispersions of liposomes, several laboratories studied various types of polymerized liposomes.[14–21] The strength of the bilayer wall and prevention of vesicle fusion were largely improved and permeability of polym-

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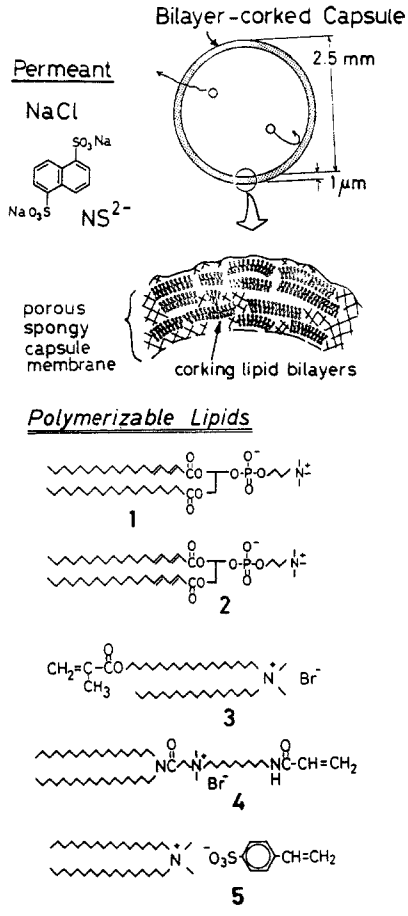

Figure 1. Schematic illustration of the polymerizable lipid-corked capsule membrane and structures of the permeants and polymerizable lipids.

erized vesicles is reduced to about half that of unpolymerized vesicles.[17b] The bilayer-corked capsule membrane offers certain advantages for the study of kinetic permeation measurements compared with monomeric or polymeric liposomes because the capsule is physically strong, is easy to handle, and has a large inner aqueous phase, although the corking lipid barrier is composed of fragmented multibilayer structures.

Experimental Section

Materials. Butadiene lipids, *rac*-1-stearoyl-2-(octadeca-2,4-*trans*,-*trans*-dienoyl)glycero-3-phosphorylcholine (**1**), and *rac*-1,2-bis(octadeca-2,4-*trans*,*trans*-dienoyl)glycero-3-phosphorylcholine (**2**) were kindly supplied by Dr. Akimoto of Toyo Soda Co. Ltd. (Tokyo). Disodium naphthalene-1,5-disulfonate (NS^{2-}) as a permeant was purchased from Tokyo Kasei Co. Ltd. The structures and purities of the prepared polymerizable amphiphiles were confirmed by TLC with a flame ionization detector, NMR and IR spectroscopy, and elemental analyses (C, H, and N, within ±0.2% errors).

Octadecyl[20-(methacryloyloxy)eicosyl]dimethylammonium Bromide (3). *N,N*-Dimethyloctadecylamine was quaternized with 20-(methacryloyloxy)eicosyl bromide in ethanol in the presence of a small amount of radical inhibitor under a reflux condition for 3 days. The obtained white powder was recrystallized from *n*-hexane twice: mp 65–67 °C; yield 4.1 g (70%); TLC (9:1 ether/NH_3) showed one spot (R_f (0.7); NMR ($CDCl_3$) δ 0.9 (t, 3 H, CH_3), 1.3–1.7 (m, 68 H, CH_2), 1.9 (t, 3 H, CH_3), 3.4 (s, 6 H, CH_3N^+), 3.5–3.7 (m, 4 H, CH_2N^+), 4.1 (t, 2 H, OCH_2), 5.5 (t, 1 H, CH=C), 6.1 (s, H, CH=C).

[(Dioctadecylcarbamoyl)methyl](ω-acrylamidodecyl)dimethylammonium Bromide (4). *N,N*-Dioctadecylamine was allowed to react with chloroacetyl chloride in chloroform in the presence of triethylamine at 0 °C. The obtained (dioctadecylcarbamoyl)methyl chloride was allowed to react with dimethylamine in ethanol at room temperature and then quaternized with ω-(acrylamido)decyl bromide in ethanol in the presence of a small amount of radical inhibitor under a reflux condition for 3 days. The obtained white powder was recrystallized from ethyl acetate and then from ethanol/hexane (4:1): mp 100 → 120 °C (liquid crystalline behavior); yield 5.9 g (34%); R_f (9:1 chloroform/methanol) 0.7; NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.3–1.7 (m, 80 H, CH_2), 3.2 (s, 6 H, CH_3N^+), 3.3 (t, 2 H, CH_2N^+), 3.5 (s, 2 H, N^+CH_2CO), 5.4 (b, 2 H, CH_2=C), 6.1 (t, 1 H, CH=C).

Dioctadecyldimethylammonium *p*-Styrenesulfonate (5). An aqueous vesicular dispersion (60 mL) of dioctadecyldimethylammonium bromide (3.1 g, 49 mmol) prepared by sonication and an aqueous solution (100 mL) of sodium *p*-styrenesulfonate (2.0 g, 9.7 mmol) were mixed at 70 °C, and the white precipitates were collected and recrystallized from ethyl acetate twice: mp 90 → 128 °C (liquid crystalline behavior); yield 3.0 g (95%); R_f (9:1 chloroform/methanol) 0.6; NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.3–1.7 (m, 64 H, CH_2), 3.3 (s, 6 H, CH_3N^+), 3.4–3.5 (t, 4 H, CH_2N^+), 5.1–5.9, (m, 2 H, CH_2=C), 6.5–7.0 (m, 1 H, CH=C), 7.3–8.0 (m, 4 H, aromatic H).

Lipid-Bilayer-Corked Capsules. Large, ultrathin nylon-2,12 capsule membranes were prepared from ethylenediamine and 1,10-bis(chlorocarbonyl)decane by an interfacial polycondensation described previously.[2-13] Nylon capsules with an ultrathin membrane thickness of 1.0 ± 0.2 μm and a large diameter of 2.5 ± 0.5 mm were obtained. The nylon membrane was found to have a porous structure that is broken by a large number of pores (0.1–0.3-μm diameter) connecting the outside to the inside of the capsule.[22] These pores arise naturally during the formation of capsules.

The semipermeable capsules were dialyzed against an aqueous solution of the permeant (0.2 M NaCl or 1×10^{-3} M disodium naphthalenedisulfonate (NS^{2-})) to give capsules containing the probe molecule in the inner aqueous core. They were transferred to a hot dodecane solution of polymerizable lipid (10–20 μg/1 mL) and cooled to room temperature.[2,4-6] Amphiphiles were precipitated spontaneously as multiple bilayers in the spongy capsule membrane between the inner aqueous and the outer dodecane solution. The prepolymerized lipids were also corked in the capsule membrane in a similar manner. The amount of corked lipids was 30 ± 5 μg per capsule, and dodecane was confirmed not to be an impurity in the corkings.[5,12] Extensive X-ray diffraction analyses of the cross section of the membrane and transmission electron micrographs of the capsule membrane stained negatively with uranyl acetate showed that the polymerizable lipids were present as well-oriented, multilamellar bilayers that organize parallel to the spongy layer of the capsule membrane plane, as well as other nonpolymerizable lipid-corked capsule membranes[4,10,12] (see Figure 1).

The phase transition behavior of corking bilayers on the capsule was obtained from differential scanning calorimetry (DSC).[2-6] Five crushed capsules corked with monomeric bilayers or polymerized bilayers were sealed with 50 μL of water in an Al sample pan and heated from 5 to 90 °C at 2 °C per min with an SSC-575 instrument (Seiko Electric Co. Ltd., Tokyo). The phase transition temperature (T_c) and the enthalpy change at T_c (ΔH, kcal mol^{-1}) of the monomeric and polymeric lipid bilayers on the capsule are summarized in Table I.

Table I. DSC Data of Polymerizable Lipid Bilayers

	monomeric form		polymeric form	
lipid	T_c/°C	ΔH/(kcal mol^{-1})	T_c/°C	ΔH/(kcal mol^{-1})
1	29	6.5	32	3.5
2	22	5.9	14	1.7
3	44	14	52	4.2
4	47	13	44	11
5	53	6.3	53	6.5

Polymerization of Lipids on the Capsule. Lipid bilayers corked in a capsule membrane were polymerized by irradiation with UV light (Ushio high-pressure mercury lamp, 500 W) at 5 or 50 °C in an aqueous solution containing 0.2 M NaCl or 1×10^{-3} M NS^{2-} in order to prevent the release of interior permeants during polymerization.

The extent of polymerization of corking bilayers was determined as follows. In the case of lipids **1**, **2**, and **5**, the amount of nonreacted monomers was estimated from the absorption spectra at 260 nm of the chloroform solution of lipids extracted from the bilayer-corked capsule membrane (polymeric lipids have no strong absorption near 260 nm). These lipids could be polymerized to ca. 50% and 100% under photoirradiation for 5 and 20 min, respectively. In the case of lipids **3** and **4**, the extract solution of amphiphiles was analyzed by thin-layer chromatography, since these amphiphiles have no strong absorption in the UV range. It took longer to polymerize these lipids under photoirradiation: 50 min for ca. 50% polymerization and 180 min for 100% polymerization.

The polymerized lipids extracted from the capsule were analyzed by gel permeation chromatography (GPC) (instrument: Toyo Soda HLC-802A; column: TSK-GMH 6; eluent: tetrahydrofuran; standard: polystyrene). The average molecular weight and the degree of polym-

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Table II. Average Molecular Weight of Polymerized Lipids[a]

lipid	5-min irradiation[b]	20-min irradiation[c]
1	1×10^4 (20)[d]	2×10^5 (260)[d]
2	2×10^4 (27)[d]	1×10^5 (130)[d]
5	1×10^4 (14)[d]	7×10^5 (950)[d]

[a] Obtained by GPC measurements of the extract from the bilayer-corked capsule after UV irradiation for the respective time. [b] Ca. 50% polymerization. [c] Ca. 100% polymerization. [d] Average degree of polymerization (D_p).

Table III. Effect of Polymerization of Corking Lipids on NaCl Permeation

lipid	permeant	temp of polymerization	temp of permeation	permeation rate[a]/(10^{-6} cm s^{-1}) $P_{0\%}$	$P_{50\%}$	$P_{100\%}$	P_{100}/P_0
1	NaCl	50	33	0.194	2.50	3.50	18
	NaCl	5	33	0.194	2.64	14.1	73
	NS^{2-}	50	33	0.104	0.182	0.31	2.9
	NS^{2-}	5	33	0.104	0.192	0.51	4.9
2	NaCl	5	10	2.54	3.92	6.06	2.4
3	NaCl	5	37	4.22	7.21	15.0	3.6
4	NaCl	5	37	2.38	4.37	4.91	2.1
5	NaCl	50	48	0.35	0.365	0.397	1.1

[a] Average of three data points, which contains an experimental error of ±5%.

erization of lipids **1**, **2**, and **5** in 50% and 100% polymerization are summarized in Table II.

Permeation Measurements. Permeation of NaCl or a water-soluble dye, NS^{2-}, from inside the capsule was followed by detecting increases in the electrical conductance or the fluorescence intensity at 340 nm (excited at 280 nm) in the outer aqueous phase, respectively, after dropping one capsule into the distilled water of the permeation cell at various temperatures.[3-13]

Apparent permeation rates, P (cm s^{-1}), were calculated from the initial slope of the increase of the electrical conductance or the fluorescence intensity using the following equation:[3-13]

$$P = \frac{1}{6}\frac{kd}{C_0} \quad (1)$$

where k, d, and C_0 are the initial slope of a permeant release, the capsule diameter (2.5 mm), and the initial concentration of permeant stored in the inner aqueous phase, respectively. Permeation measurements were carried out at least in triplicate under the individual conditions and gave good reproducibility. The P values in the tables and figures are the average of three data points and contain an experimental error of 5%.

Results and Discussion

Polymerization at Alkyl Chains. When the capsule membrane was corked with phosphocholine lipids **1** having a polymerizable butadiene group at an alkyl chain, the permeation rate of NaCl from the capsule unexpectedly increased as polymerization proceeded as shown in Figure 2. The permeation rates in the monomeric form (0% polymerization) and at 50% and 100% polymerization are summarized in Table III. The capsules corked with bilayers of **1** were irradiated with UV light at 5 and 50 °C in aqueous solution for the respective time (0–20 min) and the extent of polymerization was obtained by extraction of the residual monomeric **1**. The degree of polymerization of lipid **1** at 50% and 100% polymerization was 20 and 260, respectively (see Table II). The NaCl permeation from the capsule was studied at 33 °C near the phase transition temperature of the monomeric and polymeric bilayers (see Table I). The permeability increased gradually as a function of the lipid polymerization on the capsule. The extent of permeation enhancement depended on the polymerization temperature: the ratio of permeation rates at 100% polymerization and the monomeric form (0% polymerization), P_{100}/P_0, was 73 and 18 when the lipids were polymerized at 5 °C (in the solid state below T_c) and at 50 °C (in the fluid liquid crystalline state above T_c), respectively. Thus, when the lipids on the capsule were polymerized in the solid state below T_c, the barrier effect for NaCl permeation was largely decreased relative to that polymerized in the fluid state. The same trend of permeation enhancement caused by polymerization was observed when the relatively large permeant

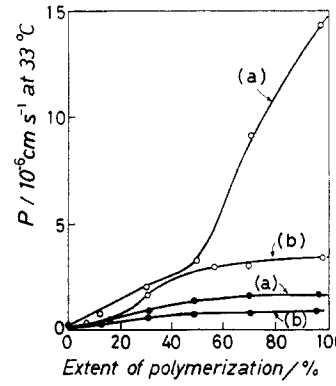

Figure 2. Effect of polymerization on permeation of NaCl (○) and the large NS^{2-} dye (●) from the capsule corked with **1** at 33 °C. Lipids were polymerized at (a) 5 °C and (b) 50 °C on the capsule membrane under photoirradiation for 0–20 min. The extent of polymerization was controlled by the irradiation time of the UV light and determined from the extracted monomers.

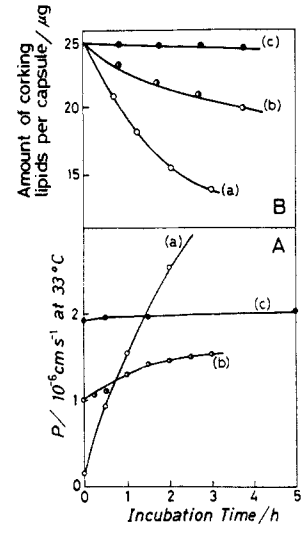

Figure 3. (A) NaCl permeation at 33 °C from the capsule corked with lipid **1** and (B) the amount of nonreleased lipids on the capsule after incubation in hot water at 75 °C. The lipids on the capsule were polymerized in (a) 0% (the monomeric form), (b) 50%, and (c) 100%.

NS^{2-} was used instead of NaCl, although the extent of rate enhancement was small relative to that for NaCl (P_{100}/P_0 = 4.9 and 2.9 for the polymerization at 5 and 50 °C, respectively). When capsules corked with other lipids having two butadiene groups at two alkyl chains (**2**) and with a methacrylate group at the terminus of an alkyl chain (**3**) were employed, similar permeation enhancement was observed as shown in Table III.

Although the barrier effect of corking bilayers for NaCl permeation was decreased by polymerization, the physical stability of corking bilayers was increased. Figure 3 shows both the permeation rate of NaCl and the amount of corking lipids on the capsule after the capsule was soaked in hot aqueous solution at 75 °C for the respective time. The soaking solution contains 0.2 M NaCl in order to prevent the release of interior permeants during the incubation process. The amount of nonreleased lipids on the capsule was determined by weighing the dried capsule after incubation. For the capsule corked with **1** in the monomeric form, NaCl permeation was dramatically increased as a function of the incubation time at 75 °C. On the contrary, for the capsule corked with **1** polymerized at 100%, NaCl permeation was very fast at the beginning but unchanged during the incubation at 75 °C even after 5 h. The permeability increased incrementally when the incubation time was increased in the case of the 50% polymerized capsule.

As shown in the upper portion of Figure 3, the corking lipids in the monomeric form were easily released from the capsule as a function of the incubation time. The 100% polymerized lipids,

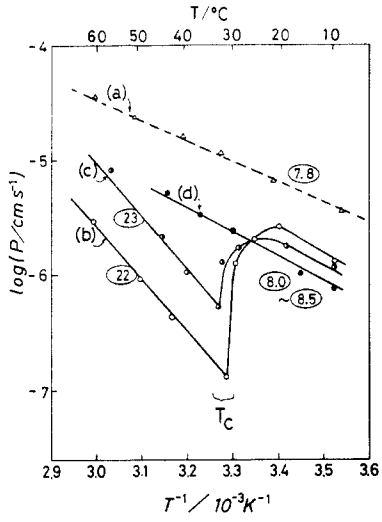

Figure 4. Arrhenius plots of NaCl permeation from the capsule corked with lipid **1**: (a) uncorked capsule; (b) capsule corked with the monomeric lipid; (c) capsule corked with lipids in 50% polymerization; (d) capsule corked with lipids in 100% polymerization. The circled numbers are the activation energies (kcal mol^{-1}) obtained from the slope.

however, hardly released from the capsule even after an incubation time of 5 h at 75 °C. Thus, when the lipids are not polymerized on the capsule, the corking bilayers are easily leached from the capsule and the barrier effect is lost during incubation under more harsh conditions. On the contrary, when the corking lipid is polymerized to 50% ($M_w = 1 \times 10^4$) and 100% ($M_w = 2 \times 10^5$), the barrier effect is not decreased upon increasing the incubation time because of the physical stability of the polymerized lipids on the capsule, although their original permeability is very large. This suggests that the decrease in the barrier effect by polymerization is not due to the release of corking lipids from the capsule, but may be due to a change of bilayer structures by polymerization.

When capsules corked with **2** having two butadiene groups in each alkyl chain and corked with **3** having a methacrylate group at the terminus of an alkyl chain were employed, similar effects of polymerization were observed (see Table III).

The phase transition from the solid state to the fluid liquid crystalline state is known to change the permeability of the bilayer-corked capsule membrane as well as that of liposomes in aqueous solution.[2-6] Polymerization of lipids is expected to affect the phase transition property of corking bilayers. DSC data of corking bilayers in both monomeric and polymeric forms are summarized in Table I. Figure 4 shows Arrhenius plots of NaCl permeation from the capsule corked with **1** at 0%, 50%, and 100% polymerization. Activation energies (E_a, kcal mol^{-1}) obtained from slopes of Arrhenius plots are also shown in the figure. The permeation of NaCl through the uncorked capsule was very fast, and the Arrhenius plot gave a simple straight line, in which NaCl diffuses freely through the spongy layer of the capsule membrane. When the capsule corked with **1** in the monomeric form (0% polymerization) was employed, NaCl permeation dramatically decreased at temperatures near T_c of the corking bilayers and increased with rising temperatures. When the extent of polymerization increased (50% and 100%), the permeability at temperatures above T_c was increased and the inflection at T_c of the Arrhenius plots became small; a straight Arrhenius plot was obtained in the 100% polymerized capsule.

As reported previously, two different types of Arrhenius plots for NaCl permeation through the bilayer-corked capsule membrane have been observed:[4-6] (i) the permeability is *increased* in the fluid liquid crystalline state of corking bilayers above T_c compared with the solid state below T_c, as well as that observed usually in liposomal membranes (type I), and (ii) the permeability is dramatically *decreased* in the liquid crystalline state above T_c relative to the solid state below T_c (type II). An Arrhenius plot of type I is observed for capsules corked with ionic (cationic or anionic) bilayers, and a type II plot is often observed for capsules corked with neutral charged (zwitterionic or nonionic) bilayers.[4-6] Therefore, for capsules corked with polymerizable lipids, the cationic bilayers of **3** or **4** and the zwitterionic bilayers of **1**, **2**, and **5** are expected to give Arrhenius plots of type I and type II, respectively.

The permeation enhancement and the disappearance of the inflection at T_c as a function of polymerization can be explained by using the activation energy data as follows. When the capsule membrane is corked with zwitterionic **1**, an Arrhenius plot of type II is obtained, in which the permeability in the solid state below T_c is not largely reduced because of defective pores in the lamellae of the corking bilayers.[4-6] When the bilayer is in the solid state below T_c, permeation through the rigid bilayers becomes difficult, and NaCl may be released through many defective pores in multiple lamella bilayers. E_a below T_c (8.2 kcal mol^{-1}) then becomes similar to that of the uncorked capsule (7.8 kcal mol^{-1}), in which NaCl permeation simply proceeds by a diffusion process (E_a = 3–10 kcal mol^{-1}). Since these pores may disappear in the fluid liquid crystalline state at temperatures above T_c, NaCl may permeate and diffuse through the fluid hydrophobic matrix instead of defective pores, and the permeation rate was dramatically decreased above T_c. E_a above T_c (22 kcal mol^{-1}) is higher than that below T_c because a hydrated electrolyte such as NaCl permeates or diffuses in the fluid but the hydrophobic bilayer matrix. When the corking lipid was polymerized to 100%, the NaCl permeation above T_c was increased and E_a above T_c became equal to that below T_c (8.0 kcal mol^{-1}). This means that polymerization at the hydrophobic alkyl chains of **1** may produce many porous defects in the bilayers and NaCl permeates smoothly through these pores with a small activation energy.

An Arrhenius plot similar to type II membranes, shown in Figure 4, was observed when a capsule corked with zwitterionic **2** was used. For the cationic corking bilayers of **3**, an Arrhenius plot similar to type I was obtained. Although not shown in the figure, in each case wherein lipids possessed polymerizable alkyl chains, NaCl permeation was largely increased and the inflection of Arrhenius plots at T_c was not observed as a function of lipid polymerization. From the DSC data of Table I, the enthalpy changes (ΔH) at T_c of **1**, **2**, and **3** were largely decreased after polymerization: ΔH values of the monomeric and polymeric forms of these lipids were 5.9–14 and 1.7–4.2 kcal mol^{-1}, respectively. This clearly indicates that polymerization at the hydrophobic alkyl chains reduces the fluidity of the dialkyl chains in bilayers. A similar result is known from studies in polymerized vesicles having functional groups in the alkyl chains.[16c]

The following explanation can be concluded from the results of Figures 2–4 and Tables I and III. If corking bilayers on the capsule were polymerized in the hydrophobic alkyl chains, the barrier effect for NaCl permeation decreased with increasing extent of polymerization because of the formation of porous defects in corking bilayers, although their physical stability was largely increased and corking bilayers were not leached from the capsule compared with the monomeric state. The formation of porous defects or the distortion of bilayer structures by polymerization can be explained by shrinkage of the bilayers on the capsule. Hupfer et al. have reported[23] that the area per molecule of the monolayer on the water/air interface of a polymerizable lipid is decreased from 1 to 0.6 mm^2 by polymerization. Benz et al. reported[24] that the electric resistance of the planar lipid bilayer membrane covered on a small pore of a Teflon sheet (BLM) was largely decreased from 10^6 to 10^2 Ω cm^2 by polymerization. On the contrary, in aqueous dispersion of liposomes, the polymerization at the alkyl chain of lipid bilayers increased the barrier effect for permeation compared with the monomeric state.[25-27] The bilayer

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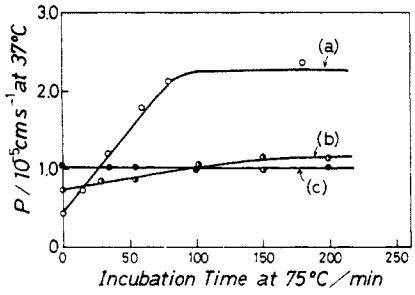

Figure 5. NaCl permeation from the capsule corked with lipid **4** at 37 °C after incubation in hot water at 75 °C. The lipids on the capsule were polymerized at (a) 0% (the monomeric form), (b) 50%, and (c) 100%.

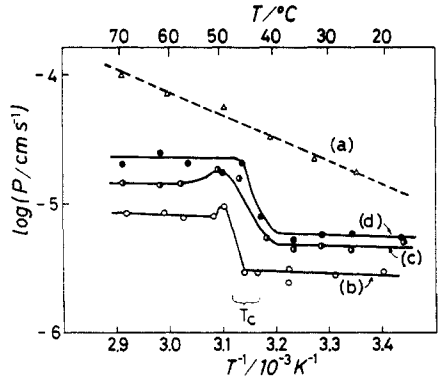

Figure 6. Arrhenius plots of NaCl permeation from the capsule corked with lipid **4**: (a) uncorked capsule; (b) capsule corked with the monomeric lipid (0% polymerization); (c) capsule corked with lipids in 50% polymerization; (d) capsule corked with lipids in 100% polymerization.

wall in vesicles seems not to form these pores if the lipid matrix is shrunk under polymerization. Thus, when the lipid bilayers are buried in the matrix, polymerization at alkyl chains may form defect pores due to molecular packing derived shrinkage of the lipids.

Polymerization at the Hydrophilic Group. Kunitake and co-workers demonstrated that the long spacer chain between the polymeric group and the bilayer-forming dialkyl moiety in lipids is important in forming stable polymerized vesicles.[15,28] Recently, Ringsdorf and co-workers reported that polymerizable lipids with a long hydrophilic spacer group can form stable polymerized monolayers, vesicles, and multibilayers both from the polymerization of preformed monomeric aggregates and from the prepolymerized lipid.[29] In our capsule membrane study, the spacer alkyl group is expected to decrease the antagonism between polymer-enhanced stability and polymer-reduced barrier effect.

Figure 5 shows the permeability change of NaCl after incubation at 75 °C for 1–5 h, when the capsule was corked with cationic lipid **4** having a long spacer alkyl chain (C_{10}) between the polymerizable group in the hydrophilic part and the dialkyl part. When the corking bilayers were not polymerized on the capsule, the NaCl permeation increased with increasing incubation time in hot water (75 °C), probably due to loss of corking monomeric bilayers from the capsule during the incubation. On the contrary, when the corking lipids were polymerized to 50–100%, NaCl permeation hardly increased even after the 5-h incubation because of increased stability of the polymerized lipids. The permeability enhancement due to polymerization was very small relative to that of the capsule corked with lipids **1–3** having the reactive group at the hydrophobic alkyl chains (see Figure 3 and Table III).

Figure 6 shows Arrhenius plots of NaCl permeation through the capsule corked with the lipid **4** polymerized to 0%, 50%, and

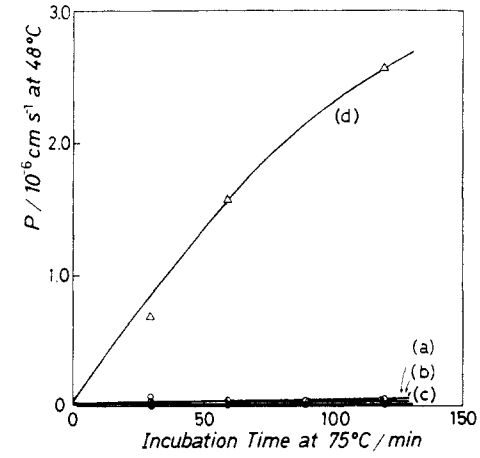

Figure 7. NaCl permeation from the capsule at 48 °C after incubation in hot water at 75 °C: (a) capsule corked with **5** and then polymerized (method A); (b) capsule corked with prepolymerized **5** (method B); (c) capsule corked with dioctadecyldimethylammonium bromide and then encased with poly(styrenesulfonate) (method C); (d) capsule corked with monomeric **5**.

100%. When the capsule was corked with cationic amphiphiles **4**, the NaCl permeation gave expectedly an Arrhenius plot of type I:[4-6] the permeability increases in the fluid liquid crystalline state above T_c relative to the rigid solid state below T_c with a discontinuous inflection. Similarly to the capsule corked with **4** in a monomeric form (0% polymerization), the capsule corked with **4** (50% and 100% polymerized) showed a sharp inflection near T_c, although their permeability increased slightly relative to the monomeric corkings. The polymerized bilayers of **4** still have an enthalpy change at T_c as large as the monomeric form (see DSC data, Table I). These results indicate that lipid **4** having a polymerizable group in the hydrophilic group with a long spacer chain still has a fluid bilayer property and a high barrier effect for NaCl permeation even after polymerization. Since NaCl permeability of the 100% polymerized bilayers was enhanced by a factor of 2.1 compared with that of the monomeric form, the polymerized bilayer may be disturbed slightly due to the shrinkage of lipid matrix. This means that the resulting polymer chain still affects the barrier effect because it is covalently bonded with bilayer-forming dialkyl part although having a long spacer chain.

Polymerization at the Counteranion of the Lipid. Recently, Regen et al.[30] and Ringsdorf et al.[31] reported a new type of polymerized bilayer vesicle in which the polymerizable group exists as a counterion and is not covalently linked with the bilayer-forming amphiphiles. The encasing polymer chains can stabilize the bilayer vesicles without disordering bilayer structures or reducing the bilayer mobility during polymerization. The polymer-encased, bilayer-corked capsule membrane is expected to establish a polymer-enhanced stability without reducing the barrier effect for permeation. We examined three different types of polymer-encased, bilayer-corked capsules using dioctadecyldimethylammonium *p*-styrenesulfonate (**5**): (A) the capsule was corked with monomeric **5** and then polymerized on the capsule, (B) the capsule was corked with prepolymerized **5**, and (C) the capsule was corked with dioctadecyldimethylammonium bromide ($2C_{18}N^+2C_1Br^-$)[3] and then encased with sodium poly(*p*-styrenesulfonate) by the counterion exchange method.

Figure 7 shows the permeability change of NaCl through the capsule corked with three types of polymerized lipids at the counterion after incubation in hot aqueous solution at 75 °C. The capsule corked with monomeric **5** showed a remarkable loss of barrier effect because of the release of corking bilayers from the capsule. In contrast, capsules corked with polymerized bilayers

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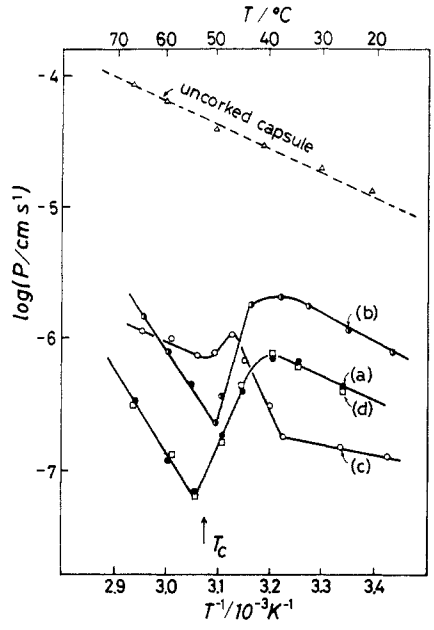

Figure 8. Arrhenius plots of NaCl permeation from the capsule: (a) capsule corked with **5** and then polymerized (method A); (b) capsule corked with prepolymerized **5** (method B); (c) capsule corked with dioctadecyldimethylammonium bromide and then encased with poly(styrenesulfonate) (method C); (d) capsule corked with monomeric **5**.

derived from **5**, exhibited dramatically reduced NaCl permeations, and the high barrier effect was maintained even after a 3-h incubation period.

Figure 8 shows Arrhenius plots of NaCl permeation through a capsule corked with monomeric **5** and three types of polymerized lipids (methods A–C). When the capsule was corked with the lipid **5** resembling the zwitterionic amphiphile, an Arrhenius plot of type II similar to Figure 4 was obtained in which the permeability was dramatically reduced in the fluid state of bilayers above T_c (curve d in Figure 8). After polymerization of lipid **5** on the capsule membrane, the NaCl permeation was not increased by the polymerization and an Arrhenius plot similar to the monomeric one was obtained (curve a in Figure 8). This means that polymerization at the counterion can enhance the stability of corking bilayers without losing the bilayer property such as the phase transition. A similar Arrhenius plot was obtained when the capsule was corked with prepolymerized lipid **5** (method B), although the permeability was slightly increased throughout the temperature range.

When the capsule was corked with dioctadecyldimethylammonium bromide and then encased with poly(styrenesulfonate) by soaking the capsule in an aqueous solution of polyanions, a different Arrhenius plot more like type I was obtained: the NaCl permeation increased in the fluid liquid crystalline state above T_c. In method C, the polymer can interact only with the surface of multilamellar bilayers on the capsule and most of the inner lipid of multibilayers exist as bromide salts. Therefore, the Arrhenius plot of the capsule corked by method C showed type I, similar to that of the capsule corked with cationic $2C_{18}N^+2C_1$ Br^- bilayers.[3] In other words, the polymer net covering only the surface of the lipid-corked capsule is effective in enhancing the stability of corking bilayers without reducing the bilayer property. It should be noted that when the bilayer-corked capsule is treated with polyanions in the aqueous solution, the treatment should be carried out at temperatures below T_c of corking bilayers, since corking bilayers in the fluid state above T_c causes release from the capsule upon treatment with polyanions.

Table IV. Summary of Properties of Polymerized Bilayer-Corked Capsule Membranes[a]

corking bilayers	barrier effect to NaCl	bilayer properties (phase transition)	stability (release from a capsule)
monomeric lipids	○	○	×
polymerized at alkyl chains (lipids of **1**, **2**, and **3**)	×	×	○
polymerized at a head group (lipid of **4**)	×	○	○
polymerized at a counterion (lipid of **5**)	○	○	○

[a] ○ and × indicate "good" and "no good", respectively.

Summary

In order to enhance the physical stability of corking bilayers on the capsule membrane, various types of polymerized lipid were examined for the corking bilayers. The results are schematically summarized in Table IV. For the capsule corked with monomeric lipid bilayers, the monomeric bilayers showed a high barrier effect and clear permeation changes at T_c under the mild condition. However, a serious drawback is that they are liable to release from the capsule under harsh conditions at high temperature. When the capsule membrane was corked with polymerizable lipids at the hydrophobic alkyl chains, the polymerization enhanced the stability of corking lipid but largely decreased both the barrier effect and the bilayer property because of shrinking and disordering of polymerized bilayers. When the corking bilayers were polymerized in the hydrophilic group with a long spacer chain, the distortion of bilayers by polymerization was much improved but the barrier effect was not improved so much. In the case of corking bilayers polymerized at the counterion, the bilayer-corked capsule showed a high stability and a high barrier effect without losing the bilayer property. The capsule could be corked with three methods: (A) the monomeric corking bilayers were polymerized on the capsule, (B) the capsule was corked with prepolymerized bilayers, and (C) the monomeric corking bilayers were encased with polyanions.

In this paper, we could propose important information about not only how to prepare the stable lipid-corked capsule membrane but also how to polymerize the lipids on the polymer matrix. In order to utilize the polymerized bilayers as a stable separation membrane, the bilayer should be immobilized and polymerized in the porous matrix. This work gives an example to evaluate the effect of polymerization of lipid bilayers in the polymer matrix by means of permeation experiments.

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Registry No. **1**, 113056-01-2; **1** (homopolymer), 113056-02-3; **2**, 113055-97-3; **2** (homopolymer), 113056-03-4; **3**, 113055-98-4; **3** (homopolymer), 113056-04-5; **4**, 113055-99-5; **4** (homopolymer), 113056-05-6; **5**, 113056-00-1; **5** (homopolymer), 113056-06-7; NS^{2-}, 1655-29-4; NaCl, 7647-14-5; (ethylenediamine)(1,10-bis(chlorocarbonyl)decane)(copolymer), 41510-72-9; (ethylenediamine)(1,10-bis(chlorocarbonyl)decane)(SRU), 41724-60-1.