Lipid Bilayer-Corked Capsule Membranes. Reversible, Signal-Receptive Permeation Control

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Cell membranes are mainly composed from a lipid bilayer matrix of phospholipids, proteins incorporated either on the surface or in the interior, and polysaccharides adsorbed mainly on the outer surface. Complexities of natural membranes have been necessitated the use of simple models for investigations at the molecular level. Liposomes, prepared from naturally occurring phospholipids, have been widely used as a simple closed-membrane analogue.[1-3] Recently, Kunitake and Okahata[4,5] and other investigators[6-13] have reported that lipid bilayer vesicles can be formed from various kinds of synthetic single-chain, double-chain, and triple-chain amphiphiles having cationic, anionic, and nonionic polar head groups.

Liposomes, as well as synthetic bilayer vesicles, can entrap water-soluble substances in the inner aqueous phase, retain them for extended periods, and release by a function of the phase transition of bilayers or other outside effects. Although they are stable under some static conditions, vesicles are liable to fuse with each other and their bilayer walls may be too weak and fragible under the dynamic changes of outside effects such as temperatures near the phase transition, ambient pH, ionic strength, and osmotic pressures. These are serious drawbacks of lipid bilayer vesicles for a kinetic study of permeability and its control. The strength of the bilayer wall and the prevention of the vesicle fusion have been recently improved by various methods of polymerization of lipid bilayers[14-19] or by covering vesicles with synthetic polymers[20] or polysaccharides.[21]

As another type of closed-membrane analogue, microcapsules prepared from polymer membranes have been investigated in designing and constructing sustained-drug-release devices and artificial cells.[22,23] Chang and co-workers have described the uses of a nylon capsule membrane for an artificial cell by trapping enzymes in the inner aqueous phase.[22] Although capsule membranes are physically strong, they are semipermeable and therefore have the disadvantage that they cannot store low molecular weight substances in the inner aqueous core.[24,25] In order to improve this disadvantage, the egg lecithin coated capsule membrane was reported.[26] In spite of their potential usefulness, the detailed characterization or the chemical modification of the capsule membrane has not been fully given.

Yoshio Okahata was born in Wakayama-ken, Japan, and received his B.S. and M.S. degrees from Doshisha University. He received his Ph.D. from Kyushu University in 1977. He was a Research Associate and then Assistant Professor at Kyushu University. He spent the years 1980–1982 in the United States as a Visiting Research Associate at the University of Massachusetts and at the University of California at Irvine. He then became an Associate Professor at the Tokyo Institute of Technology where he remains today. His interests cover polymer catalysts (protease models), catalysis by micelles and polymer micelles, synthetic bilayer membranes, and functional nylon capsule membranes.

To overcome the above problems from both types of closed-membrane analogues such as liposomes and capsule membranes, we recently developed the lipid bilayer-corked capsule membrane.[27-39] The capsule is

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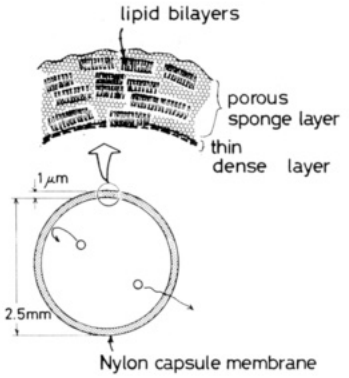

Figure 1. Schematic illustration of the bilayer-corked capsule membrane. A nylon-2,12 membrane has a asymmetrical porous structure such as the dense thin inner layer (pore diameter ca. 1–2 nm) and the macroporous thick part of the outer side (pore diameter 0.1–0.3 μm) in which multiple bilayers are deposited parallel to the membrane plane.

formed by physically strong, ultrathin, porous nylon membranes, which are corked with multiple lamellae of lipid bilayers. A schematic illustration of the capsule is shown in Figure 1. In biological membranes, the lipid bilayer wall is known to be strengthened by glycoproteins or polysaccharides incorporated in lipid bilayer membranes. In our capsule, the lipid bilayer is supported by the physically strong polymer capsule membrane. Permeation through the bilayer-corked capsule membrane of water-soluble substances such as NaCl, glucose, and fluorescent probes preserved in the inner aqueous phase was reversibly controlled by outside effects such as temperature,[27–30] photoirradiation,[31,32] ambient pH,[33,34] interaction with divalent cations,[35–37] and electric field.[38,39] Their signal-receptive permeability control is explained by changes in the physical state of corking bilayers that act as a permeation valve. Liposomes as well as synthetic bilayer vesicles cannot achieve such a reversible permeability control, because they may be fused with each other and their bilayer walls are easily broken under the dynamic outside effect.

Characterization of Capsule Membranes

Large nylon-2,12 capsules were obtained from ethylenediamine and 1,10-bis(chlorocarbonyl)decane by interfacial polycondensation described previously.[21,27–39] 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

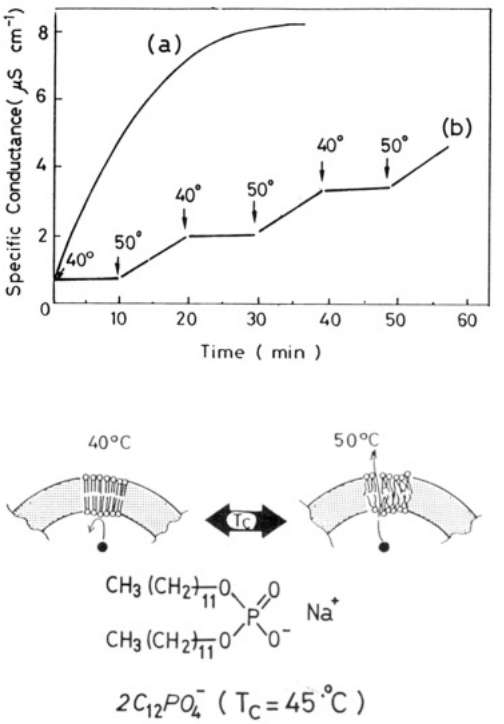

Figure 2. Temperature-sensitive permeation of NaCl from (a) the uncorked capsule membrane and (b) the $2C_{12}PO_4^-$-corked capsule membrane. The capsule was soaked alternatingly in 40 or 50 °C water at arrows.

the asymmetrical structure that is broken by a large number of pores connecting the outside to the inside of the capsule:[40] the highly dense and very thin inner layer (thickness ca. 0.1 μm, pore diameter 1–2 nm) and the macroporous, thick part of the outer side (thickness 0.9 μm, pore diameter 0.1–0.3 μm), schematically shown in Figure 1. These pores arise naturally during the formation of the capsules.

The obtained, semipermeable capsules were dialyzed against aqueous solution of permeants to give capsules containing the probe molecule. They were transferred to a hot dodecane solution of dialkyl amphiphiles and cooled. Amphiphiles were precipitated spontaneously as multiple bilayers at the outer sponge layer of the capsule between the inner aqueous and outer dodecane solution. The amphiphile content on the capsule was 10 ± 2 μg/capsule, and dodecane was confirmed not to be an impurity in the corking amphiphiles. From the extensive studies of X-ray diffraction analyses in which the incident beam was exposed perpendicular to the intersection of the capsule membrane and transmission electron micrographs of the ultrathin section of the capsule membrane stained negatively with uranyl acetate, the corking amphiphiles were proved to exist as well-oriented, multilamellar bilayers that almost pile up parallel to the sponge layer of the capsule membrane plane, as illustrated in Figure 1.[29,34,36]

The liquid crystalline property is one of the fundamental physicochemical properties of lipid bilayers. In differential scanning calorimetry (DSC), all amphiphile-corked capsule membranes showed a sharp endothermic peak that indicates a phase transition from solid to liquid crystalline state, similar to that in aqueous dispersions of bilayer vesicles.[41]

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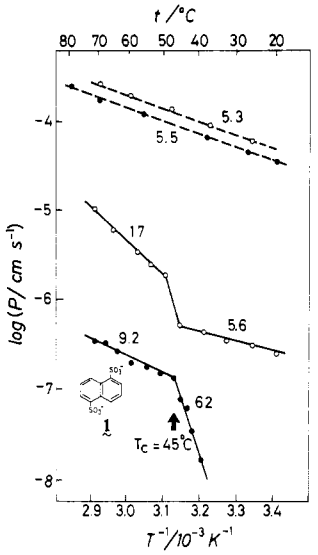

Figure 3. Arrhenius plots of permeation of small NaCl (○) and large naphthalene-1,5-disulphonate 1 (●) from the capsule membrane corked with $2C_{12}PO_4^-$ bilayers (T_c = 45 °C). Broken lines show the permeation from the uncorked capsule membrane. Numbers indicate the activation energies (kcal mol^{-1}) obtained from Arrhenius slopes.

Temperature-Sensitive Permeation[27-30]

Figure 2 shows typical time courses of NaCl permeation. Permeation of NaCl stored in the inner aqueous phase from the capsule membrane was followed by detecting increases in electrical conductance in the outer aqueous phase, after dropping one capsule into deionized water. Apparent permeation rates, P (cm s^{-1}), were calculated from an initial slope of the increases of the electrical conductance as described in previous papers.[27-39]

When the uncorked nylon-2,12 capsule membrane was employed, the permeation of NaCl was very fast [P = (1.2–2.5) × 10^{-4} cm s^{-1} at 40–50 °C]. In the case of the capsule membrane corked with anionic $2C_{12}PO_4^-$ bilayers (T_c = 45 °C), the permeability was markedly reduced 50–100 times relative to the uncorked capsule at 40 °C. When the capsule was immersed alternatingly in hot water at 40 or 50 °C, the permeability was reversibly changed by a factor of 10 between 40 and 50 °C. This drastic permeability change due to temperatures could be repeated without damaging either the capsule or corking bilayers. Since the $2C_{12}PO_4^-$ bilayers have the phase transition temperature (T_c) at 45 °C from the rigid solid to fluid liquid crystalline state, the drastic permeation changes between 40 and 50 °C may be due to the phase transition of corking bilayers.[29] In Figure 2 and the following figures, the corking bilayers are illustrated as a simple form although they exist actually as blocks of multiple bilayers in the sponge layer of the capsule membrane. The similar thermosensitive permeation change was observed for the permeation of glucose and the large, water-soluble naphthalene probes.[30]

Permeation rates of a small NaCl and a large naphthalene-1,5-disulfonate probe 1 from the $2C_{12}PO_4^-$ bilayer-corked capsule membrane were obtained at temperatures below and above T_c, and Arrhenius plots are shown in Figure 3. Numbers in the graph show activation energies (kcal mol^{-1}) obtained from Arrhenius slopes.[30] The permeation of NaCl and the probe 1 through the uncorked, semipermeable capsule membrane was very fast, and Arrhenius plots gave a simple straight line with E_a = 5.3–5.5 kcal mol^{-1}, in which the permation mainly proceeds by a diffusion process. In the permeation of small molecules such as NaCl through the bilayer-corked capsule membrane, Arrhenius plot gave a discontinuous inflection at T_c. At temperatures above T_c, highly hydrated NaCl permeates through the fluid but hydrophobic bilayer matrix with a relatively high activation energy (E_a = 17 kcal mol^{-1}). When the bilayer is in the rigid solidlike state below T_c, permeation through the bilayer matrix becomes difficult, and NaCl permeates slowly through defective pores in the multilamellar structures instead. The E_a value below T_c (5.6 kcal mol^{-1}) then becomes similar to that of the uncorked capsule membrane (E_a = 5.5 kcal mol^{-1}).

For the permeation of the relatively bulky molecule such as the probe 1, the Arrhenius plot sharply inflected and the slope below T_c was steaper than above T_c. In the rigid solidlike bilayers below T_c, the permeant cannot pass through the small deffective pores in lamellae and must diffuse, disturbing or melting a large number of boundary lipids in bilayers with a very high E_a value (62 kcal mol^{-1}). At temperatures above T_c, the probe 1 can permeate through the fluid liquid crystalline bilayers with a relatively small E_a value (9.2 kcal mol^{-1}).

The difference in Arrhenius curves near T_c between NaCl and the probe 1 can be explained as follows.[30] Dehydration of the permeant should be important in NaCl permeation, and the discontinuity in the Arrhenius plot at T_c is observed, because the decrease in entropy for the penetration step is different between temperatures below and above T_c. In the permeation of the relatively hydrophobic and large probe 1, the perturbation of boundary lipids becomes important relative to the dehydration process, and the resulting very high activation energy must be exactly compensated by the melting entropy; hence, the Arrhenius plots are continuously inflected at T_c. Similarly, two types of Arrhenius plots depending on permeants have also been reported in liposomal membranes:[42-45] the permeation of smaller molecules such as sugar or metal ions gives a disruption at the phase transition in Arrhenius plots, and in the case of larger molecules such as a dye the Arrhenius plot only breaks at T_c.

Permeation rates of inorganic salts and dyes through the bilayer-corked capsule membrane were in the range of 10^{-5}–10^{-8} cm s^{-1}, which was much larger than the NaCl permeability (10^{-12} cm s^{-1}) through liposomal membranes. In this study, porous capsule membranes are corked with blocks of multiple bilayers having some defective pores in lamellar structures. This may be one of the causes of somewhat high permeability relative to liposomes.

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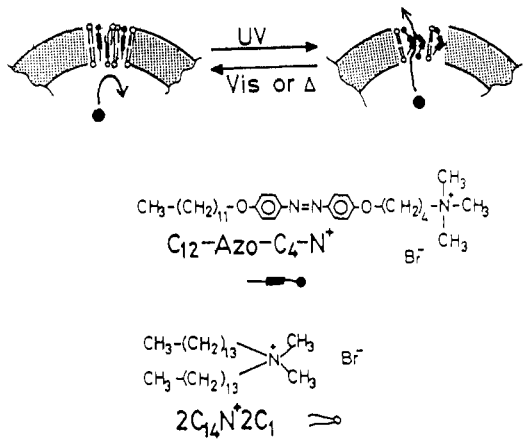

Figure 4. Schematic illustration of photoresponsible permeation control of the capsule membrane corked with mixed bilayers of C_{12}-azo-C_4-N^+ and $2C_{14}N^+2C_1$ (5:1 by weight).

Photoresponsive Permeation[31,32]

When the capsule membrane was corked with photoisomerizable amphiphile, C_{12}-azo-C_4-N^+, and dialkylammonium bilayers ($2C_{14}N^+2C_1$) (5:1 by weight), the permeation through the capsule membrane is expected to be changed by photoirradiation.

When the capsule corked with the trans C_{12}-azo-C_4-N^+ and $2C_{14}N^+2C_1$ bilayers was irradiated at 360 nm with a 500-W Hg lamp, the absorption peaks at 239 and 358 nm due to thr *trans*-azobenzene unit disappeared and a new peak of the cis isomer appeared at 450 nm, and the permeability of NaCl was increased by a factor of 3.2 ($P = 6.0 \times 10^{-6}$ cm s^{-1}) relative to that from the capsule corked with trans isomers ($P = 1.9 \times 10^{-6}$ cm s^{-1}). Upon irradiation with light of wavelengths >400 nm, the *cis* C_{12}-azo-C_4-N^+ in the corking bilayers reverted more than 90% to the trans isomer and the permeability was reduced again to the original slow rate ($P = 2.0 \times 10^{-6}$ cm s^{-1}). This permeability control based on trans–cis photoisomerization was reproduced more than 5 times. The increase of the NaCl permeation by UV light irradiation is due to the formation of the distorted cis configuration in the corking bilayers, as illustrated in Figure 4.

Liposomes, in which rhodopsin[46] or C_{12}-azo-C_4-N^+ amphiphile[47] is embedded in bilayers, have been reported as a model of photoreceptor cell membranes. Permeation of cations and water molecules across the lipid bilayers increases upon photoirradiation; however, the morphology of the liposome seems to be chemically and physically damaged by photoirradiation, and therefore there is no responsive permeability regulation.

Ambient pH-Responsive Permeation[33,34]

When the bilayer-forming amphiphile having dissociative head groups ($-NH_3^+$, $-COO^-$, PO_4^-) is employed for corking bilayers, permeability is expected to be affected by ambient pH changes.

Figure 5 shows typical time courses of permeation of the cationic fluorescent probe 2 at different pHs in the outer medium. Permeation was followed by detecting increases in the fluorescence intensity of the outer water phase after dropping one capsule into 3 mL of aqueous solution (pH 7) in a quartz cell. pH values of the outer

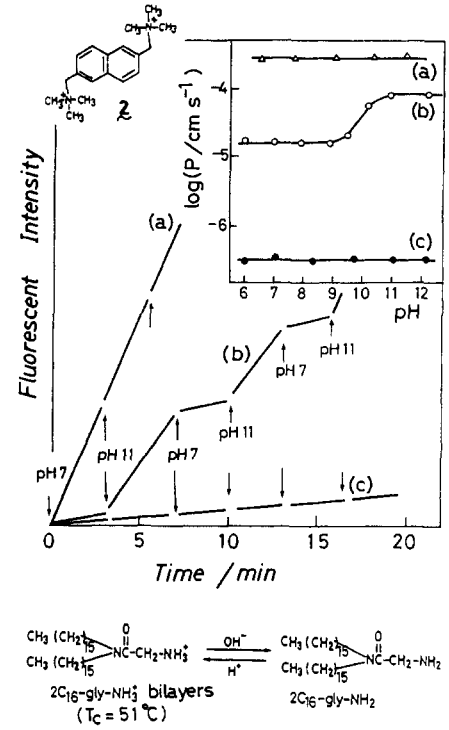

Figure 5. pH-sensitive permeation of a fluorescent probe **2** from nylon capsules by pH changes of the outer medium. pH values were controlled by the addition of 0.1 M HCl or NaOH at arrows. The inset shows pH–rate profiles of permeation: (a) the uncorked capsule at 60 °C; (b) the $2C_{16}$-Gly-NH_3^+-corked capsule at 60 °C (above T_c); (c) the $2C_{16}$-Gly-NH_3^+-corked capsule at 25 °C (below T_c).

medium were changed by adding aliquots of aqueous 0.1 M HCl or NaOH solution. In the case of the uncorked, semipermeable capsule, the permeation was very fast and not affected by the ambient pH 7 or 11. When the capsule corked with $2C_{16}$-Gly-NH_3^+ bilayers having the dissociative NH_3^+ head group (T_c = 51 °C) was employed, the probe permeatin was remarkably reduced at ambient pH 7 at both 60 and 25 °C, which means the cationic $2C_{16}$-Gly-NH_3^+ bilayers are impermeable to the cationic probe. When the outer medium was changed from pH 7 to 11 at 60 °C, the permeability was immediately enhanced by a factor of 7–8 and reduced again nearly to the original slow rate by returning to the ambient pH 7. This permeability regulation by ambient pH changes could be reproduced repeatedly until most of the probe permeated. It is obvious from the inserted pH–rate profile (curve b) in Figure 5 that the permeability enhancement in the basic medium at 60 °C is due to the neutralization of ammonium head groups of $2C_{16}$-Gly-NH_3^+ bilayers (pK_a = 10). Thus, the $2C_{16}$-Gly-NH_3^+ bilayers are impermeable to the probe **2** in the cationic bilayer form below pH 9, but permeable in the neutral form ($2C_{16}$-Gly-NH_2) above pH 11. The electrostatic repulsion between cationic bilayers and cationic permeants is not important for the observed permeability change, because the similar result was obtained when nonionic and zwitterionic probes were employed.

In contrast to the results at 60 °C, only a minimum effect of the ambient pH on the permeation was observed at 25 °C. The cationic $2C_{16}$-Gly-NH_3^+ bilayers on the capsule was confirmed to have T_c at 51 °C by DSC measurements. Thus, the permeability was hardly affected by ambient pH in the rigid solid state at 25 °C

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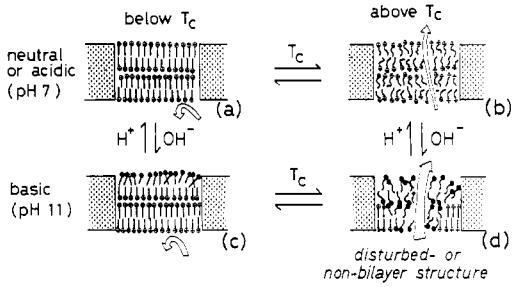
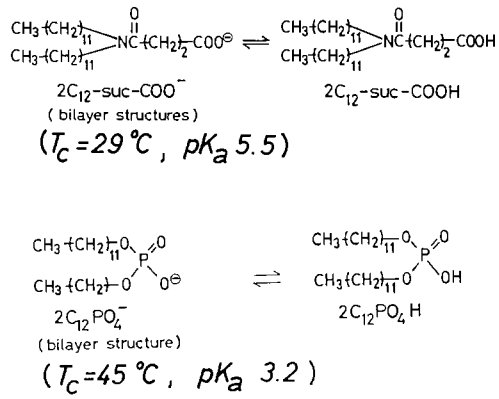


Figure 6. Supposed permeation mechanism of the cationic $2C_{16}$-Gly-NH_3^+ corked capsule membrane, depending on both ambient pH and the phase transition temperature of bilayers. ⊕ and ● show the cationic and neutralized head groups of amphiphiles, respectively.

Chart I



(below T_c), in contrast to that at 60 °C (above T_c). This different effect of the ambient pH below and above T_c may be explained as follows (Figure 6). At temperatures below T_c, the cationic $2C_{16}$-Gly-NH_3^+ bilayers exist as the rigid solid state (having a strong barrier to the permeation) in the neutral (or acidic) medium [state a]. In the basic medium (pH 11), the rigid solid state of bilayers below T_c seems to neutralize only the surface of multiple bilayers and the inner part of the bilayer still exists in the cationic form which has the strong barrier to the permeation [state c]. At temperatures above T_c, the nonbilayer or disturbed-bilayer cationic bilayers are immediately neutralized in the basic medium, and the permeability is drastically enhanced probably because of the nonbilayer or disturbed-bilayer structure of the neutral $2C_{16}$-Gly-NH_2 amphiphiles [state d].

The capsule corked with $2C_{16}$-Gly-NH_3^+ bilayers (T_c = 51 °C), which was immersed in the basic aqueous solution (pH 11) above T_c, showed an endothermic peak at 33 °C (melting point of $2C_{16}$-Gly-NH_2 amphiphiles), instead of at 51 °C (T_c of cationic bilayers) by DSC measurements. X-ray diffraction patterns of bilayer spacings were hardly observed in this neutralized capsule. When the capsule was immersed in the basic solution below T_c, corked amphiphiles still showed the original bilayer characteristics. These results strongly suggest that the $2C_{16}$-Gly-NH_3^+ bilayers are neutralized and permeability is increased only in the fluid state above T_c.

When the $2C_{12}$-suc-COO^- or $2C_{12}PO_4^-$ amphiphiles having anionic head groups were used for the corking bilayers, the permeability was increased in the acidic region below their pK_a values owing to the neutralization of the head group, only at temperatures above their T_c values.[34]

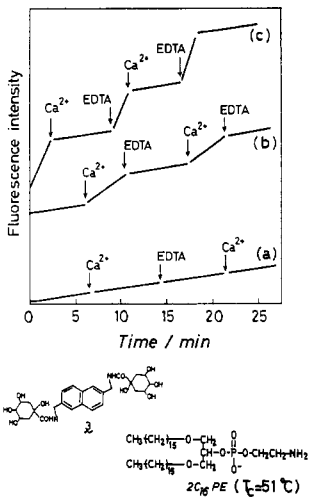


Figure 7. Ca^{2+}/EDTA-sensitive permeation of a nonionic fluorescent probe **3** from the $2C_{16}$PE-corked capsule membrane in the fluid state of bilayers at 60 °C (above T_c), depending on both the ambient pH and the Ca^{2+} concentration: (a) pH 2, [Ca^{2+}] = (2.0×10^{-3})–0.15 M; (b) pH 7, [Ca^{2+}] = 0.15 M; (c) pH 12, [Ca^{2+}] = 2.0×10^{-3} M.

It is reported[48,49] that pH-sensitive liposomes in which dissociative amphiphiles are incorporated would be useful for clinical implications; they would release encapsulated drug when passing around tumor cells that have a considerably lower pH than that of normal tissues. It is difficult, however, to realize the reversible permeation control in liposomal membranes, because they are easily damaged and fused with each other by continuous changes of ambient pH.[49]

Ca^{2+}-Sensitive Permeation[35-37]

It is well-known that phospholipids having an acidic phosphate head group can interact with divalent cations and change their bilayer structures.[50,51] In this section, we prepared the capsule membrane corked with synthetic 1,3-dihexadecylglycero-2-phosphoethanolamine ($2C_{16}$PE) bilayers and studied the effects of Ca^{2+} ions, an ambient pH, and temperatures on the permeability of the capsule membrane.[37]

Figure 7 shows the typical time courses of permeations of a nonionic fluorescent probe **3** through the $2C_{16}$PE-corked capsule membrane by the alternate addition of Ca^{2+} ions and EDTA to the outer solution in different ambient pH at 60 °C (above T_c of corking bilayers). The Ca^{2+}-induced permeability control greatly depended on both an ambient pH and the Ca^{2+} concentration. When the outer medium was pH 2, the permeability was hardly affected by the alternate addition of $CaCl_2$ and EDTA to the outer medium (curve a). On the addition of a higher concentration (0.15 M) of Ca^{2+} ions at pH 7, the permeability increased 2.5 times and reverted to the original slow rate by the addition of twofold amounts of EDTA (0.30 M) (curve b). The permeability was hardly changed by the addition of the low concentration ($<3 \times 10^{-2}$ M) of Ca^{2+} ions. At pH 12, the permeation of the probe **3** was fast in the absence of Ca^{2+} ions and reduced markedly by the ad-

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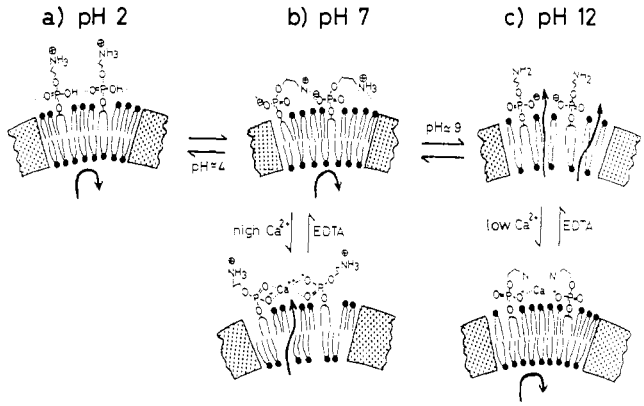

Figure 8. Supposed permeation mechanism of the $2C_{16}PE$-corked capsule membrane responding to both concentration of Ca^{2+} ions and ambient pH only in the fluid state of bilayers above T_c.

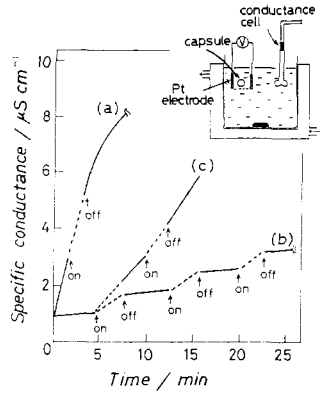

Figure 9. Electrically induced permeability control of NaCl from nylon capsules at 25 °C: (a) the uncorked capsule under 0–100-V field; (b) the $2C_{12}PO_4^-$-corked capsule under a 60-V field; (c) the $2C_{12}PO_4^-$-corked capsule under a 100-V field. The external voltage source was applied at on and ceased at off.

dition of only 2×10^{-3} M of Ca^{2+} ions. On the addition of twofold amounts of EDTA, the permeability enhanced again to the original fast rate (curve c). Those permeability changes by additions of Ca^{2+}/EDTA could be reproduced repeatedly at both pH 7 and 12 without damaging the corking bilayers. The Ca^{2+}-induced permeability change occurred only in the fluid state of bilayers at temperatures above T_c = 51 °C of $2C_{16}PE$ bilayers, but not in the rigid bilayers below T_c.

We proposed the mechanism of the Ca^{2+}-induced permeability control depending on the ambient pH, as shown in Figure 8. In the acidic medium, the phosphoric head groups of the $2C_{16}PE$ bilayers interact strongly with each other by hydrogen bonding[52,53] and the Ca^{2+} ions have difficulty in coordinating with head groups, because the phosphoric groups are undissociated and the Ca^{2+} ion can hardly approach the cationic head groups because of the electrostatic repulsion. Therefore, the permeability was not affected at all even by the addition of a high concentration of Ca^{2+} ions in the acidic medium (pH 2).

Since zwitterionic head groups are also stabilized through intermolecular hydrogen bonds between NH_3^+ and PO_4^- groups in the neutral pH region,[52,53] divalent cations cannot easily coordinate to the head group at low concentration (<0.03 M). At the high concentration of Ca^{2+} ions (0.05–0.3 M), however, divalent cations can coordinate with the zwitterionic head groups to disturb the bilayer structure and the permeability consequently increases. Upon removal of Ca^{2+} ions by the addition of excess EDTA, the permeability can revert to the original slow rate by re-forming the hydrogen-bonded, stable bilayers. Of course, by the addition of excess Ca^{2+} ions over 0.3 M, the increased permeability is not always regained by addition of the EDTA solution.

In the basic medium (pH 12), the anionic form of the $2C_{16}PE$ bilayers provides only a weak barrier to the permeation because of the disturbed bilyaer structures by the lack of hydrogen bonds and the electrostatic repulsion between head groups.[54] Divalent cations can easily coordinate in the very low concentration (2.0×10^{-3} M), and Ca^{2+}-coordinated bilayers provide a strong barrier to permeation. The chelation with divalent cations seems to re-form the stable bilayers, eliminating the electrostatic repulsions between head groups. By the removal of Ca^{2+} ions with EDTA, the $2C_{16}PE$ bilayers reversibly revert to the original leaky bilayers.

It is interesting that divalent cations can enhance and reduce the permeability in pH 7 and 12, respectively, depending on the dissociation state of the bilayer head groups and on the concentration of the added Ca^{2+} ions. Both pH-sensitive and Ca^{2+}-sensitive permeation changes occurred only at the high temperature (60 °C) above T_c of the corking $2C_{16}PE$ bilayers but not below T_c. Since $2C_{16}PE$ bilayers exist in the multilamellar structures on the capsule membrane, it seems to be impossible for H^+/OH^- or Ca^{2+} ions to penetrate into the rigid multilamellar bilayers below T_c and to change the bilayer structures. The similar permeation control could be observed by the interaction with other divalent cations such as Ba^{2+}, Cu^{2+}, and Mg^{2+}, but not at all with alkali-metal ions (Na^+, K^+).

It is difficult for liposomal membranes containing negatively charged phospholipids to realize such reversible permeation control, because they are easily fused with each other and precipitated by the addition of divalent cations or pH changes.[52-54]

Electric Field Sensitive Permeation[38,39]

Transmembrane potentials are believed to play a major role in biological processes. When the membrane potential exceeds a threshold value, the lipid bilayer (a poor conducting capacitor) seems to produce transient pores, allowing rapid passage of large substances and particles that cannot normally permeate through the membrane.[55] Extensive pulsation experiments of transmembrane potentials have been conducted on suspensions of liposomes[56] or planar bilayers.[57] In contrast to biological membranes, however, it has been impossible for these lipid membranes to induce stable transient pores that have not been accompanied by mechanical rupture of the membrane for any long duration (>1 s) by an electric field.

We expected that the permeability could be reversibly controlled by the formation of electrically induced transient pores, when the physically strong, bilayer-corked capsule membrane was employed. Figure 9 shows the effect of the intermittent electric field on the NaCl leakage.[38] The capsule membrane was placed

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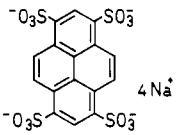
between two platinum electrodes (area 1 cm × 1 cm; separation between the two electrodes 1 cm) connected to a high-voltage power supply (dc 0–300 V) in deionized water. In the case of the uncorked, semipermeable capsule, the permeation was very fast and not affected by external voltages ranging between 0 and 100 V. On the contrary, when the anionic $2C_{12}PO_4^-$ bilayer-corked capsule membrane was employed, the slow permeation of NaCl was enhanced 15 times ($P = 9.0 \times 10^{-6}$ cm s^{-1}) under 60-V fields relative to that in the absence of the electric field ($P = 5.5 \times 10^{-7}$ cm s^{-1}). When the electric field ceased even after a long charging duration (3 min), the permeability reduced to the original slow rate ($P = 5.7 \times 10^{-7}$ cm s^{-1}). The permeation control could be repeated many times without damaging corking bilayers.

The electrically induced permeability enhancement was proportionally increased with increased electric field in the range of 30–80 V. When the voltage was increased above 80 V or the charging duration beyond 5 min, the NaCl leakage did not revert to the original slow rate, probably because of rupture of the bilayer structures, as shown in curve c of Figure 9. The 60-V field corresponds to a voltage of 6 V across the capsule membrane (1 μm thick) (60 kV cm^{-1}). The corking bilayers exist as a multiple structure so that the transmembrane potential per one bilayer (5 nm thick) can be estimated to be 30 mV. This value is relatively smaller than the transmembrane potential (100–800 mV per lipid bilayer) to cause the electrical breakdown in liposomal membranes under the pulse experiments.

When a neutral glucose and a divalent $CaCl_2$ were employed for permeants, the same extent of rate enhancements was obtained as that of NaCl.[39] This indicates that the electrostatic forces between permeants and electrodes are not important for the observed permeation changes. When the relatively large substances such as water-soluble naphthalene probes **1–3** were employed as permeants, a similar rate enhancement was observed. The surface charge of corking bilayers seems not to be important for the electrically induced permeation change, because the similar effect was observed on the capsule corked with either cationic or nonionic lipid bilayers.

Permeation of the small NaCl and the large nonionic probe **3** was studied at temperatures below and above T_c under the electric field (60 V). Permeability was enhanced 3–15 times relative to that without the electric field over the whole temperature range and was accelerated faster at temperatures below T_c than above T_c. Arrhenius plots of permeation of both NaCl and the probe **3** under the electric field gave a simple straight line without inflections (E_a = 5.2–5.7 kcal mol^{-1}), in contrast to the inflected lines shown in Figure 2 without the electric field. E_a values of both permeants under the electric field are small and nearly equal to that in the permeation through the uncorked capsule membrane, in which permeants pass through pores by a diffusion process. This suggests that the transmembrane potential induced by a electric field generates many transient pores in the corking bilayers, and then NaCl or the large probe pass rapidly through these pores with a small activation energy.

Although the permeation of NaCl, glucose, and up to water-soluble naphthalene molecules **1–3** was enhanced under the electric field, the permeation of the much larger pyrene-1,3,6,9-tetrasulfonate **4** was not affected



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at all. Thus, transient pores induced by the electrical breakdown are permeable to the molecular size up to naphthalene molecules but not to the large pyrene molecules. In the pulsation experiments of the planner lipid bilayer membranes, it is reported that the number of transient pores created during a short duration of the electrical breakdown is estimated to be 10^7/cm^2, and the pore radius is calculated to be 4 nm.[58]

Concluding Remarks

Although the nylon capsule membrane is simply semipermeable, the permeation of water-soluble substances through the bilayer-corked capsule membrane can be reversibly controlled. The bilayer corking acts as a valve that opens or shuts, responding to stimuli from outside such as temperature changes, photoirradiations, ambient pH changes, addition of Ca^{2+} ions, and external electric fields. The permeability of the bilayer-corked capsule membrane can also be changed by the intermittent irradiation of ultrasonic power.[59] The bilayer-corked capsule can selectively preserve the high concentration (0.1–0.2 M) of acids or alkalis in the inner aqueous core depending on the charge of head groups of bilayers and can form the high electrochemical gradient of H^+ or OH^- across the membrane,[60,61] which can be used for energies of an active transport. When enzymes are immobilized in the capsule membrane together with bilayers, the activity of enzymes can be regulated by the phase transition of bilayers.[62] The capsule having the surface-grafted polymer can also control the permeability, in which the grafted polymer acts as a permeation valve owing to the conformational change by stimuli from outside.[63–65]

The bilayer-corked capsule membrane is interesting both as a physically strong model of lipid bilayer vesicles and as a functional microcapsule. The capsule membrane having the signal-receptive permeation valve should be useful for a drug-release device and a model synaptic system in which a nerve impulse initiates the rapid release of chemical substances such as acetylchorine.

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