pH-Sensitive Capsule Membranes. Reversible Permeability Control from the Dissociative Bilayer-Coated Capsule Membrane by an Ambient pH Change[1,2]

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Abstract: Nylon ultrathin capsules coated with synthetic bilayers having dissociative head groups ($2C_{12}PO_4^-$, $2C_{12}$-cys-$2COO^-$, $2C_{12}$-scu-COO^-, and $2C_{16}$-gly-NH_3^+) were prepared. Permeations through the membrane of a fluorescent probe trapped in the inner aqueous phase could be reduced by factors of 50–100 relative to that of the uncoated capsule and reversibly controlled by pH changes of the outer medium: in the case of the capsule coated with bilayers containing phosphate ($2C_{12}PO_4^-$) and carboxylate head groups ($2C_{12}$-cys-$2COO^-$ and $2C_{12}$-suc-COO^-), the permeation was redused in the neutral medium, increased in the acidic medium, and reverted to the original slow rate in the neutral medium, depending on the dissociation of head groups of coating bilayers. When the capsule coated with bilayers containing an ammonium head group ($2C_{16}$-gly-NH_3^+) was employed, the reverse occurred: the permeability was increased and decreased in the basic and neutral medium respectively. This pH-sensitive permeation occurred only above the phase transition temperature (T_c) of coating bilayers but not below their T_c. In the case of the capsule coated with the neutralized amphiphiles ($2C_{12}PO_4H$, $2C_{12}$-cys-2COOH, $2C_{12}$-suc-COOH, and $2C_{16}$-gly-NH_2) which cannot form bilayer structures, the permeability was hardly reduced relative to that of the uncoated capsule, as expected. In the neutral medium, however, the coatings could form bilayer structures and provide a high barrier to the permeation, only in the fluid state above their melting point.

Development of relatively simple synthetic membrane analogues has been prompted by the need to study the properties of biological membranes. Liposomes[3-5] and synthetic bilayer vesicles[6-9] have been widely used as closed membrane analogues. These lipid–bilayer vesicles, however, have several disadvantages when trapping and kinetically studying the permeability: very small inner aqueous phase, size distribution, and relatively breakable bilayer wall. Some of these weak points are being improved by polymerization of the bilayer wall.[10-15] As another type of closed membrane analogues, nylon capsule membranes entrapping enzymes in the inner aqueous phase have been studied.[16-18] Capsule membranes, however, are semipermeable and therefore have a disadvantage in that they cannot trap small substances in the inner aqueous phase.

To overcome the above problems from both types of closed membrane analogues, we recently prepared novel functional nylon capsules whose porous membranes were coated with phospholipids or synthetic bilayer-forming amphiphiles.[19-25] The capsule is formed by physically strong, ultrathin nylon membranes, and the coating shows characteristics of bilayer vesicles. Permeation through the membrane of water-soluble substances such as NaCl trapped in the inner aqueous phase was reversibly controlled by stimuli from outside such as temperature,[1,19-25] photoirradiation,[20] ultrasonic power,[22] electric field,[25] and interaction with divalent cations.[21] Their signal-receptive permeability control is explained by changes in the physical state of coating bilayers which act as "*an ion gate*". Aqueous synthetic bilayer vesicles as well as liposomes could not achieve such a reversible permeability control because of their easily breakable bilayer walls.

In this paper, we describe how an ambient pH can also act as a signal which causes the reversible permeation control from nylon capsules coated with synthetic bilayers having a dissociative, hydrophilic head group such as carboxylate ($2C_{12}$-suc-COO^-, $2C_{12}$-cys-$2COO^-$), phosphate ($2C_{12}PO_4^-$), or primary ammonium groups ($2C_{16}$-gly-NH_3^+). Anionic (**1**), cationic (**2**), and zwitterionic (**3**) fluorescent probes were employed as a permeant entrapped in the inner aqueous phase. A schematic representation of the capsule is shown in Figure 1. Yatvin et al.[26] reported that pH-sensitive liposomes would be useful for clinical implications; they would release encapsulated drug when passing around tumor cells that have a considerably lower pH than normal tissues.

Experimental Section

Preparation of Amphiphiles. The structures of the neutral and salt forms of amphiphiles were confirmed by thin-layer chromatography with a flame-ionization detector (Iatron Laboratories, Japan, Model TF-10), IR and NMR spectroscopy, and elemental analysis. The melting point

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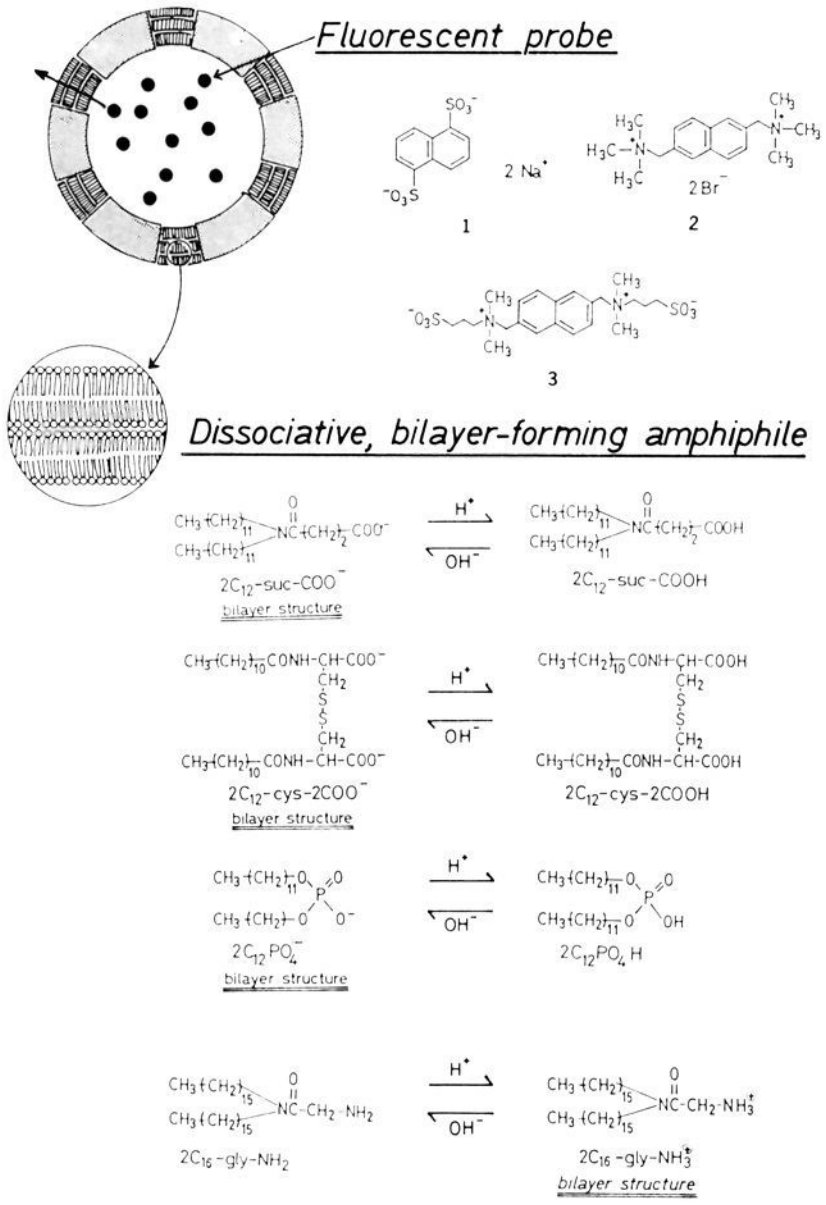

Figure 1. The pH-sensitive capsule membrane coated with dissociative, bilayer-forming amphiphiles.

was measured with a polarizing microscope, and the liquid crystalline region is denoted by the arrow.

Didodecylphosphoric acid ($2C_{12}PO_4H$) was prepared according to the previous paper[27] from $POCl_3$ and dodecylalcohol: colorless needles from *n*-hexane, mp 56–58 °C. The sodium salt ($2C_{12}PO_4^-$) had mp 243→250 °C.

Didodecanolylcystine ($2C_{12}$-cys-2COOH) was obtained from the reaction of cystine (16.0 g, 67 mmol) with dodecanoyl chloride (35.0 g, 0.16 mol) in alkalic aqueous solution (pH 10–11) at 40 °C for 3 h: white needles from ethyl acetate, yield 7.8 g (19%); mp 98–100 °C. Anal. Calcd for $C_{30}H_{56}N_2O_6S_2$: C, 59.6; H, 9.27; N, 4.64. Found C, 54.11; H, 9.54; N, 4.21. The sodium salt ($2C_{12}$-cys-$2COO^-$) had mp 180→190 °C.

N,N-Didodecylsuccinamic acid ($2C_{12}$-suc-COOH) was prepared from the reaction of *N,N*-didodecylamine (16.4 g, 47 mmol) with succinic anhydride (5.5 g, 55 mmol) in tetrahydrofuran at room temperature for 18 h: white needles from *n*-hexane, mp 39 °C, yield 7.2 g (34%). Anal. Calcd for $C_{28}H_{55}NO_3$: C, 74.2; H, 12.1; N 3.1. Found: C, 74.2; H, 12.1; N, 2.9. The sodium salt ($2C_{12}$-suc-COO^-), white granules, had mp 65 °C.

N,N-Dihexadecylglycineamide ($2C_{16}$-gly-NH_2) was obtained from the reaction of *N,N*-dihexadecylchloroacetoamide[1] (4.0 g, 7 mmol) with excess ammonia in ethanol at 50 °C for 24 h: white granules, mp 33 °C. Anal. Calcd for $C_{34}H_{70}N_2O$: C, 78.2; H, 13.4; N, 5.36. Found: C, 78.0; H, 13.2; N, 5.40. The hydrogen chloride salt ($2C_{16}$-gly-NH_3^+), pale yellow granules from *n*-hexane, had mp 51→76 °C.

Preparation of Fluorescent Probes. Reagent grade disodium naphthalene-1,5-disulfonate (**1**) was used for an anionic fluorescent probe without further purification.

A cationic fluorescent probe **2** (2,6-bis(trimethylammoniomethyl)-naphtalene dibromide) was prepared from 2,6-bis(bromomethyl)-naphthalene[28] (1.0 g, 3.2 mmol, mp 185 °C) and trimethylamine in dimethylformamide at 60 °C for 12 h: white granules from acetone–water, yield 1.0 g (72%); mp > 300 °C; NMR δ 3.0 (s, 18 H), 4.6 (s, 4 H), 8.0 (m, 4 H). Anal. Calcd for $C_{18}H_{28}N_2Br_2$: C, 50.0; H, 6.48; N, 6.48. Found: C, 50.0; H, 6.27; N, 6.42.

A zwitterionic probe **3** (2,6-bis(sulfopropyl-*N,N*-dimethylammoniomethyl)naphthalene) was prepared as follows: 2,6-bis(*N,N*-dimethylaminomethyl)naphthalene (mp 97–99 °C, 4.4 g, 19 mmol), obtained from 2,6-bis(bromomethyl)naphthalene[28] and dimethylamine, was refluxed with propanesultone (5.4 g, 44 mmol) in ethanol for 24 h. The solvent was evaporated and the residue was recrystallized twice from ethanol–water: white granules, yield 5.7 g (64%); mp 286 °C dec; NMR δ (D_2O) 2.5 (m, 4 H), 3.2 (s, 12 H), 3.4 (t, 4 H), 3.6 (t, 4 H), 5.2 (s, 4 H) 7.9 (m, 6 H). Anal. Calcd for $C_{22}H_{34}N_2O_6S_2$: C, 54.3; H, 7.00; N, 5.76. Found: C, 54.0; H, 6.88; N, 5.76.

Preparation of Capsules. Large nylon-2,12 capsules were obtained from ethylenediamine and 1,10-bis(chlorocarbonyl)decane by interfacial polymerization with use of a drop technique in the presence of a small amount of a cross-linking agent (trimesoyl chloride) according to previous methods.[1,19-25] Nylon capsules with an ultrathin membrane thickness of 5.0 ± 0.3 μm and a large diameter of 2.0 ± 0.1 mm were obtained. The nylon capsule was found to have a small amount of the residual COOH and NH_2 end groups (ca. 1×10^{-4} equiv/g) by acid–base titrations.[29] In order to avoid the effect of ionization of these groups on pH change experiments, the residual end groups were changed to $COOCH_3$ and $NHCOCH_3$ groups by methylation with CH_3OH/H_2SO_4 and amidation with acetic anhydride, respectively.[29] Contents of residual end groups were confirmed to be less than 1×10^{-5} equiv/g by titrations. The capsules having blocked end groups were dialyzed against an aqueous solution of 0.01 M phosphate buffer (pH 7) containing an appropriate fluorescent probe (1×10^{-3} M) to give capsules trapped by a probe.

Amphiphile-coated capsules were prepared as follows:[1,19-21] 20 pieces of capsule-entrapped fluorescent probe were transferred to a dodecane solution (3 mL) of dialkyl amphiphile (50 mg) and maintained at 60 °C for 5 min. After being cooled, amphiphile-coated capsules were picked up, rolled on filter paper to remove excess dodecane solution, and kept in aqueous solution containing 1×10^{-3} M fluorescent probe. The amphiphile content on the capsule was 20 ± 3 μg per capsule, and dodecane was confirmed not to be involved as impurities in the coating amphiphiles by elemental analysis.[21]

Measurements. X-ray measurements with Ni-filtered Cu Kα radiation were carried out by using a flat-plate camera. The crushed, amphiphile-coated capsule membranes were piled up and cut into fine strips with dimensions of about 2 × 0.3 × 0.3 mm. The specimens were mounted on the sample holder with the intersection of the capsule membranes perpendicular to the incident beam.

The multilamellar structure of the coating bilayers was observed by a Hitachi H-500 electron microscope (TEM). Capsules were coated with amphiphiles in 2% aqueous solution (pH 3) of uranyl acetate, after which the negatively stained sample was cut to ultrathin pieces and applied to carbon-coated grids.

Differential scanning calorimetry (DSC) of the coating amphiphile was carried out with a Perkin Elmer DSC-2 instrument. Five crushed capsules were sealed with 10μL of water in a sample pan and heated from 5 to 90 °C at a rate of 10 °C/min.

Permeability of capsules toward the fluorescent probe trapped in the inner aqueous phase was measured by detecting increases in the relative fluorescence intensity at 340 nm (excitation at 280 nm for **1**, and at 290 nm for **2** and **3**) of the outer water phase, after dropping one capsule into 3 mL of deionized water in a quartz cell. pH values of the outer medium were changed by adding aliquots of 1 M HCl or NaOH aqueous solution.

Results and Discussion

Characterization of Capsules. It has been confirmed[1,19-21] the original nylon capsules have the porous membrane structure (pore size 0.1–0.3 μm) and coating amphiphiles cover entirely these pores as multilamellar bilayers in the case of the capsule coated with phospholipid or other synthetic bilayer-forming amphiphiles. In order to clarify whether the coating amphiphiles having dissociative head groups can also exist as bilayer structures on the capsule membrane or not, the amphiphile-coated capsule was characterized by X-ray analysis, transmission electron microscopy (TEM), and differential scanning calorimetry (DSC).

The X-ray diffraction pattern of the anionic $2C_{12}$-suc-COO^--coated capsule membrane is shown in Figure 2a as a typical example. The series of strong reflection arcs with 32-Å spacing was observed and consistent with the bimolecular length of $2C_{12}$-suc-COO^- amphiphiles. The similar diffraction pattern was

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Table I. pH-Sensitive Permeation Rates across Capsule Membranes Coated with Dissociative Bilayers, Depending on Temperature

capsule membrane	probe	temp, °C	P, 10^6 cm s^{-1} pH 2	pH 7	pH 11	$P_{pH\ 2}/P_{pH\ 7}$	$P_{pH\ 11}/P_{pH\ 7}$
uncoated	anionic **1**	25	65	66	70	*c*	*c*
		60	210	210	215	*c*	*c*
	cationic **2**	25	55	60	58	*c*	*c*
		60	195	200	195	*c*	*c*
	zwitterionic **3**	25	15	18	15	*c*	*c*
		60	35	38	36	*c*	*c*
bilayer coating with:[a]							
$2C_{12}$-suc-COO^-	*anionic* **1**	25	*1.0*	*0.98*	*1.2*	*c*	*c*
(pK_a = 5.5, T_c = 29 °C)		60	60	6.0	6.5	10	*c*
$2C_{12}$-cys-$2COO^-$	anionic **1**	25	0.44	0.43	0.45	*c*	*c*
(pK_a = 3.5, T_c = 42 °C)		60	70	4.5	4.8	16	*c*
$2C_{12}PO_4^-$	anionic **1**	25	0.13	0.12	0.15	*c*	*c*
(pK_a = 3.2, T_c = 45 °C)		60	69	6.8	7.0	10	*c*
	NaCl[b]	25	0.54	0.55	0.56	*c*	*c*
		60	70	6.8	7.0	10	*c*
$2C_{16}$-gly-NH_3^+	cationic **2**	25	0.44	0.42	0.38	*c*	*c*
(pK_a = 9.2, T_c = 51 °C)		60	4.0	3.8	27	*c*	7.1
	zwitterionic **3**	25	0.95	0.93	0.98	*c*	*c*
		60	4.2	4.0	9.2	*c*	2.3

[a] pK_a values and T_c of coating bilayers were obtained from pH–rate profiles of permeations and DSC measurements, respectively. [b] Permeation rates of NaCl were obtained by detecting increases in the electrical conductance in the outer water phase. [c] Ratios of both $P_{pH\ 2}/P_{pH\ 7}$ and $P_{pH\ 11}/P_{pH\ 7}$ were nearly unity (in the range of 0.8–1.2).

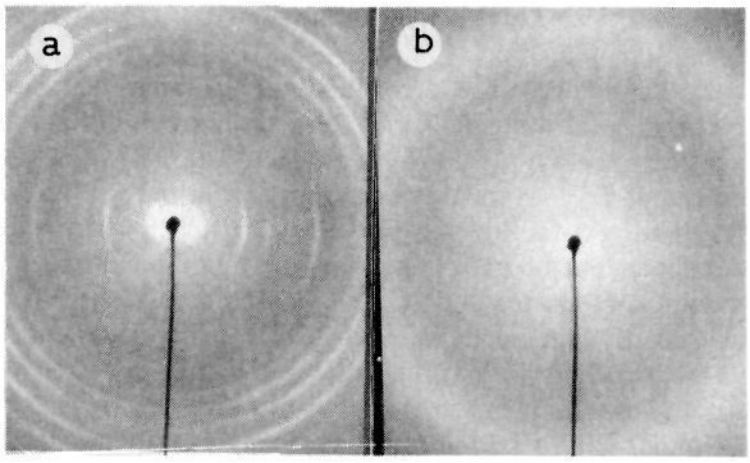

Figure 2. X-ray diffraction patterns of the capsule coated with (a) anionic $2C_{12}$-suc-COO^- and (b) neutralized $2C_{12}$-suc-COOH amphiphiles.

also observed in the capsule membrane coated with other anionic $2C_{12}PO_4^-$, $2C_{12}$-cys-$2COO^-$, and cationic $2C_{16}$-gly-NH_3^+ amphiphiles by X-ray analysis, as previously reported in the case of other bilayer-coated capsules.[1,19,20]

In the transmission electron microscopy (TEM), an enlargement of a favorable area of the ultrathin section of $2C_{12}$-suc-COO^--coated capsule membrane showed the distinct multilamellar structure whose mean thickness is estimated to be ca. 35 Å, as previously reported in the case of phospholipid-coated capsules.[21] Similar TEM observations were obtained in the case of capsules coated with other amphiphiles ($2C_{12}PO_4^-$, $2C_{12}$-cys-$2COO^-$, and $2C_{12}$-gly-NH_3^+). These findings clearly prove that the ionized amphiphile coating exists as multilamellar bilayers, which pile growing up parallel to the capsule membrane, as illustrated in Figure 1. The coating bilayers of ionized amphiphiles were also proved to have the phase transition temperature (T_c) from gel to liquid crystalline state as an endothermic peak by DSC measurements: T_c = 29 °C for $2C_{12}$-suc-COO^-, T_c = 42 °C for $2C_{12}$-cys-$2COO^-$, T_c = 45 °C for $2C_{12}PO_4^-$, and T_c = 51 °C for $2C_{16}$-gly-NH_3^+.

On the contrary, the capsule membrane coated with the neutralized amphiphiles of $2C_{12}$-suc-COOH, $2C_{12}$-cys-2COOH, $2C_{12}PO_4H$, and $2C_{16}$-gly-NH_2 did not show the clear X-ray diffraction patterns and the lamellar structure by TEM observations. The X-ray diffraction pattern of the $2C_{12}$-suc-COOH-coated capsule was shown in Figure 2b as a typical example. In DSC measurements these capsules showed an endothermic peak only at the position of a melting point of corresponding amphiphiles: $2C_{12}$-suc-COOH at 39 °C; $2C_{12}$-cys-2COOH at 98 °C; $2C_{16}$-gly-NH_2 at 33 °C. These results suggest that the neutral

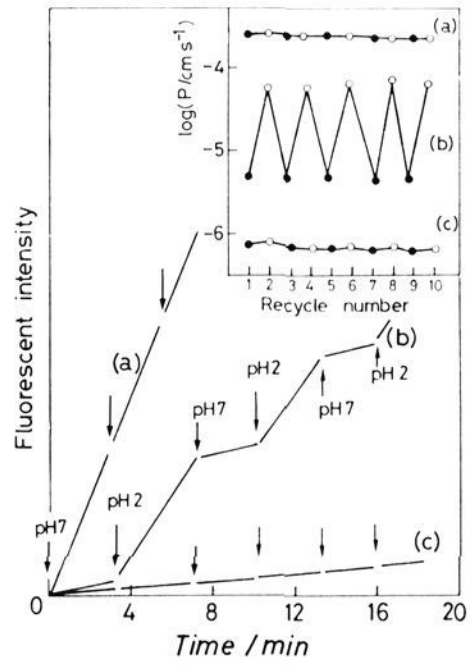

Figure 3. pH-sensitive permeations of the anionic fluorescent probe **1** from capsule membranes by pH changes between 2 and 7 of the outer medium. The inserted figure shows the reversibility of pH-sensitive permeations (open circles, pH 2; closed circles, pH 7). (a) The uncoated capsule at 60 °C, (b) the $2C_{12}$-suc-COO^--coated capsule at 60 °C, (c) the $2C_{12}$-suc-COO^--coated capsule at 25 °C.

form of amphiphiles cannot form bilayer structures on the capsule membrane, in contrast to ionized amphiphiles.

pH-Sensitive Permeation. Permeability of capsules toward a fluorescent probe trapped in the inner aqueous phase was followed by detecting increases in the relative fluorescent intensity of the outer water phase at various pH values. Permeation rates were calculated from the following equation[1,19]

$$P = \frac{1}{6}\frac{kd}{\Delta C} \tag{1}$$

where k and d are the increase in the fluorescence intensity with time (the slope of Figure 3) and the capsule diameter, respectively. ΔC denotes a change of the fluorescence intensity after crushing a capsule, which means the concentration of the probe trapped in the inner aqueous phase. Permeation rates obtained from eq 1 at pH 2, 7, and 11 of the outer medium were summarized in Table I.

Figure 3 shows typical time courses of permeations of an anionic fluorescent probe, **1**, from capsule membranes coated with anionic $2C_{12}$-suc-COO^- bilayers. In the case of the uncoated, semipermeable capsule membrane, the permeation of fluorescent probes

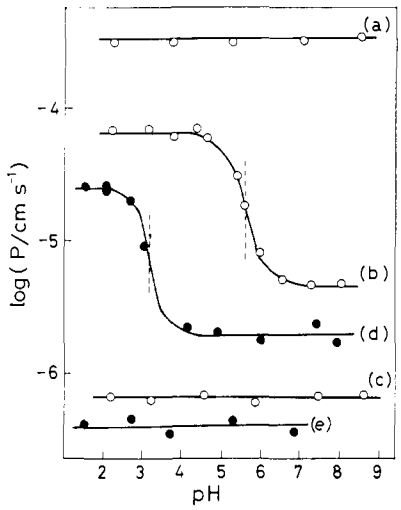

Figure 4. pH dependence of the permeation rate of the anionic probe **1** from anionic bilayer-coated capsules. (a) The uncoated capsule at 60 °C, (b) the $2C_{12}$-suc-COO^--coated capsule at 60 °C, (c) the $2C_{12}$-suc-COO^--coated capsule at 25 °C, (d) the $2C_{12}PO_4^-$-coated capsule at 60 °C, (e) the $2C_{12}PO_4^-$-coated capsule at 25 °C.

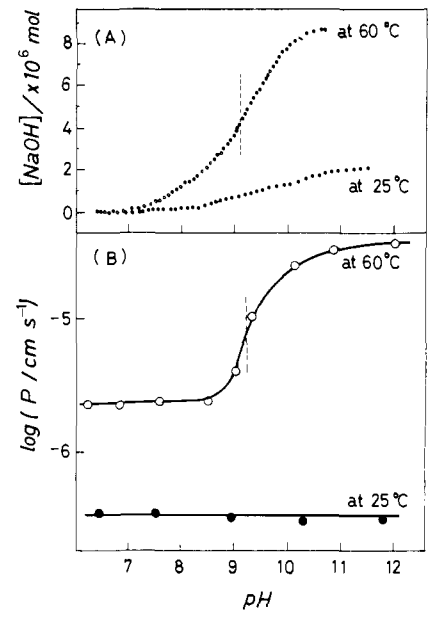

Figure 5. (A) Base titration curves of $2C_{16}$-gly-NH_3^+ bilayers on the capsule membrane and (B) pH dependences of the permeation of the probe **2** from $2C_{16}$-gly-NH_3^+-coated capsules.

was very fast and not affected by a change in pH from 2 to 7. When the capsule coated with anionic $2C_{12}$-suc-COO^- bilayers was employed, the permeation of the anionic probe **1** was remarkably reduced at an ambient pH of 7, suggesting that anionic $2C_{12}$-suc-COO^- bilayer coats provide a high barrier to the permeation, as well as other bilayer-coated capsules.[1,19-21] Upon changing the pH of the outer medium from 7 to 2 at 60 °C, the permeability of the coated capsule was immediately enhanced by a factor of 10 and reduced again nearly to the original slow rate by returning the ambient pH to 7. In the case of the capsule coated with cationic $2C_{16}$-gly-NH_3^+ bilayers, the similar pH-sensitive permeation was observed at 60 °C in the basic medium; the permeations of a cationic probe **2** were smaller at pH 7 and increased drastically at pH 11 (see Table I). As shown in the insert of Figure 3, this permeability regulation by ambient pH changes at 60 °C could be repeated over and over again without damaging coating bilayers of $2C_{12}$-suc-COO^- or $2C_{16}$-gly-MH_3^+, until most of the probe had been released. The probe permeation from the anionic and cationic bilayer-coated capsule at 25 °C, however, was very slow and not affected by pH changes of the outer medium (see curve c of Figure 3).

As shown in Table I, the permeation of all probes (**1–3**) from the uncoated capsule was not affected by pH changes from 2 to 11 independent of hydrophilic head groups. When the capsule coated with other anionic bilayers such as $2C_{12}PO_4^-$ and $2C_{12}$-cys-$2COO^-$ was employed, the permeation of the anionic probe **1** was reduced at the ambient pH of 7 and 11 and increased in the range of 10–16 at pH 2 only at 60 °C but not at 25 °C. This pH-sensitive permeation at 60 °C was also observed on the permeation of a zwitterionic probe **3** and NaCl in the case of the capsule coated with cationic $2C_{16}$-gly-NH_3^+ and anionic $2C_{12}PO_4^-$ bilayers, respectively. However, in the combination of an anionic probe and cationic bilayer coatings (e.g., probe **1** and $2C_{16}$-gly-NH_3^+ coatings) and in the reverse combination (e.g., cationic probe **2** and $2C_{12}$-suc-COO^- coatings), probes were not entrapped in the inner aqueous phase, and the strange permeation behavior was observed because of the formation of hydrophobic ion pairs between opposite charged probes and amphiphiles.

pH–Rates Profiles and Titrations. Since the dissociation of head groups of coating bilayers seemed to play an important role for the observed pH-sensitive permeations, pH–rate profiles of the probe permeation were studied at both 60 °C and 25 °C and are shown in Figure 4. The permeability of $2C_{12}$-suc-COO^--coated and $2C_{12}PO_4^-$-coated capsules was greatly changed near pH 5.5 and 3.2, respectively, at 60 °C, suggesting that the neutralization of anionic head groups ($-COO^-$ and $-PO_4^-$) of the coating bilayers directly affects the observed pH-sensitive permeation. Thus, $2C_{12}$-suc-COO^- coatings provide a high barrier to the probe permeation in the anionic bilayer form above pH 6 but not in the neutral form below pH 5. In the case of the $2C_{12}PO_4^-$-coated capsule, anionic bilayers provide a high barrier to the permeation above pH 4. At the low temperature (25 °C) the permeability was independent of pH changes of the outer medium in both $2C_{12}$-suc-COO^--coated and $2C_{12}PO_4^-$-coated capsules. When the capsule coated with anionic $2C_2$-cys-$2COO^-$ bilayers was employed at 60 °C, the pH–rate profile of the permeation inflected at pH 3.5 (not shown in the figure), suggesting that $2C_{12}$-cys-$2COO^-$ amphiphiles can form anionic bilayer coatings above their first pK_a of two carboxyl head groups. The apparent pK_a values of coating bilayers obtained from the inflection point of pH–rate profiles are summarized in Table I.

Figure 5 shows titration curves of cationic $2C_{16}$-gly-NH_3^+-coated capsule membranes, together with the pH dependence of the permeation of a cationic probe **2**. The titration of amphiphiles on the capsule membrane was performed as follows: the aqueous suspension of 300 pieces of crushed capsules coated with $2C_{16}$-gly-NH_3^+ amphiphiles (amphiphile contents 5.1 ± 0.3 mg (17 ± 1 μg per capsule), ca. 9.0×10^{-6} mol) was titrated from pH 6 to 11 by adding 0.1 N NaOH at both 60 °C and 25 °C. At 60 °C, coating amphiphiles on the capsule were practically titrated (8.9×10^{-6} mol) within experimental errors. The inflection point of the titration curve appeared at pH 9.2 and was consistent with that of the pH–rate profile of the permeation at 60 °C. This indicates that a pK_a value of the primary amino group of $2C_{16}$-gly-NH_3^+ amphiphiles is estimated to be 9.2, and the amphiphiles exist in the cationic bilayer form (providing a high barrier to the permeation) below pH 9 and neutralize easily to the $2C_{16}$-gly-NH_2 form (providing a low barrier) above pH 10 at 60 °C. On the other hand, at 25 °C, only 20% of the coating amphiphile on the capsule membrane was titrated, and the permeability was small and hardly affected by pH changes from 7 to 12. This suggests that the coating $2C_{16}$-gly-NH_3^+ amphiphiles still exist in a cationic form and provide a high barrier to the permeation even in the basic medium (above pH 10) at the low temperature. Similar titration behaviors at both 60 °C and 25 °C were observed in the case of capsules coated with anionic bilayers ($2C_{12}$-cys-$2COO^-$, $2C_{12}$-suc-COO^-, and $2C_{12}PO_4^-$).

Effect of Temperature: Arrhenius Plot. In contrast to the results at 60 °C, only a minimum effect was observed at 25 °C on the permeation and titration results of all the bilayer-coated capsules (Figures 3–5). It is well-known that the bilayer property varies largely near a phase transition temperature (T_c) of bilayers in the

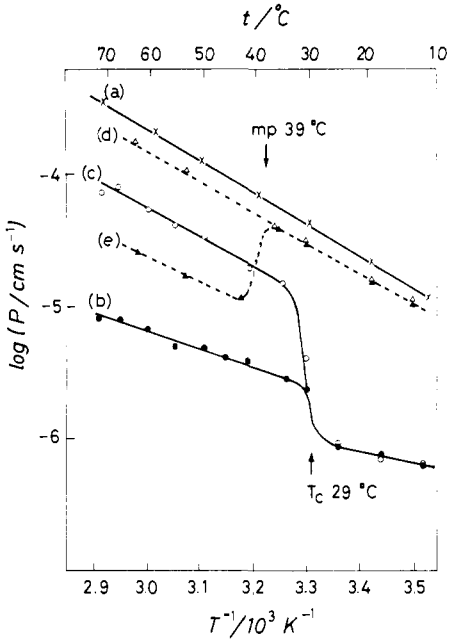

Figure 6. Arrhenius plots of the permeation of the anionic probe **1** from the uncoated capsule (a), capsules coated with anionic $2C_{12}$-suc-COO^- bilayers (solid lines: (b) pH 7, (c) pH 2), and capsules coated with neutralized $2C_{12}$-suc-COOH amphiphiles (dotted lines: (d) pH 2, (e) pH 7). Arrows show T_c of anionic $2C_{12}$-suc-COO^- bilayers and melting point of neutralized $2C_{12}$-suc-COOH amphiphiles, respectively.

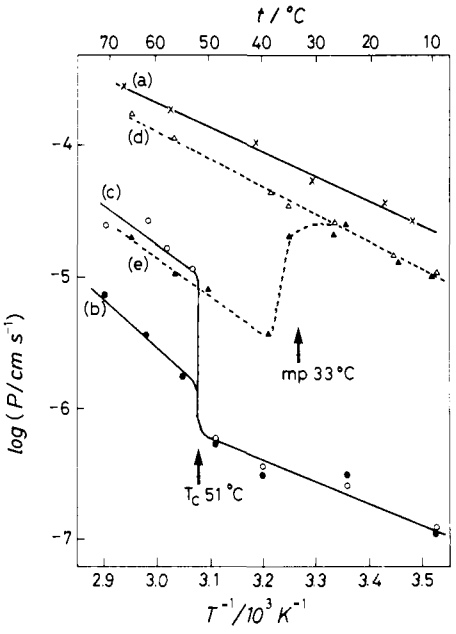

Figure 7. Arrhenius plots of the permeation of the cationic probe **2** from the uncoated capsule (a), capsules coated with cationic $2C_{16}$-gly-NH_3^+ bilayers (solid lines: (b) pH 7, (c) pH 11), and capsules coated with neutralized $2C_{16}$-gly-NH_2 amphiphiles (dotted lines: (d) pH 11, (e) pH 7). Arrows show T_c of cationic $2C_{16}$-gly-NH_3^+ bilayers and melting point of neutralized $2C_{16}$-gly-NH_2 amphiphiles, respectively.

case of other bilayer-coated capsule membranes,[1,19-21] as well as aqueous suspensions of liposomes and synthetic bilayer vesicles. In order to clarify the effect of temperature on the permeability, permeation rates were obtained in a range of 10–70 °C at different pH. Arrhenius plots of the anionic $2C_{12}$-suc-COO^--coated capsule obtained at both pH 2 and 7 and of the cationic $2C_{16}$-gly-NH_3^+-coated capsule at both pH 7 and 11 were shown in Figures 6 and 7, respectively, together with that of the uncoated capsule.

In the case of the uncoated capsule, the plot of log P vs. T^{-1} gave a simple straight line. On the contrary, Arrhenius plots of the $2C_{12}$-suc-COO^- bilayer-coated capsule (solid lines in Figure

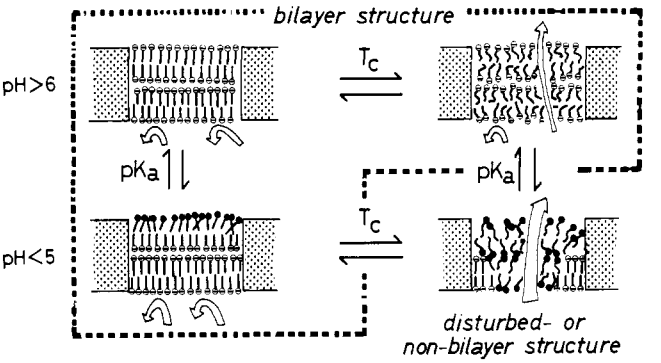

Figure 8. A schematic illustration of the supposed mechanism of pH-sensitive permeation across the $2C_{12}$-suc-COO^--coated capsule, depending on the phase transition of bilayers. Symbols of —⊖ and —● show the anionic and the neutralized amphiphiles, respectively.

6) gave an inflection near 29 °C at both pH 2 and 7, consistent with a phse transition temperature (T_c) from gel to liquid crystalline state of coating bilayers obtained from DSC measurements (shown as an arrow in the figure). The cationic $2C_{16}$-gly-NH_3^+ bilayer-coated capsule also gave inflected Arrhenius plots near T_c = 51 °C at both pH 7 and 11 (solid lines in Figure 7). In both cases, the effect of the ambient pH on the permeability was not observed at the temperature below T_c of coating bilayers, but clearly at the temperature above their T_c. When capsules coated with other anionic $2C_{12}$-cys-$2COO^-$ and $2C_{12}PO_4^-$ bilayers were employed, Arrhenius plots gave an inflection near respective T_c of coating bilayers and the permeability at pH 2 was largely increased above T_c, but not affected by pH changes below T_c.

The observed results (Figure 3–5 and Table I) that pH-sensitive permeations occurred only at 60 °C but not at 25 °C may be concluded due to the phase transition of coating bilayers. A supposed mechanism of pH-sensitive permeations is shown in Figure 8, as an example in the case of the $2C_{12}$-suc-COO^- bilayer-coated capsule. When the coating amphiphiles exist as the anionic bilayer form in the neutral and basic medium (e.g., pH >6), which provides a high barrier to the permeation, the permeation of fluorescent probes is slow, and the small inflection near T_c of Arrhenius plots (curve b of Figure 6) is the result of the phase transition from the rigid gel to the fluid liquid crystal of the bilayers. The permeation enhancement above T_c has been frequently observed in other bilayer-coated capsules.[1,19-21] In the case of the acidic medium (pH <5), the fluid anionic bilayers above T_c are immediately neutralized and the permeability is drastically enhanced probably because of the disturbed or non-bilayer structure of the neutralized amphiphiles (curve c Figure 6). In the rigid gel state below T_c, only the surface of multiple bilayer coats may be neutralized and inner, most of the bilayer coats still exist in an anionic form (providing a high barrier to the permeation) even at the ambient pH <5, which can be expected from the titration data of Figure 5. Thus, the permeability was affected by ambient pH only in the fluid state above T_c (two illustrations on the right-hand side of Figure 8), but not below T_c (two illustrations on the left-hand side of Figure 8). A similar explanation can be attributed to the cationic $2C_{16}$-gly-NH_3^+-coated capsule membrane: the permeability was drastically increased only in the fluid state of bilayers when the ambient pH was above 10, but it hardly increased in the rigid gel state even in the basic medium.

In our experiment, the anionic or cationic fluorescent probe was mainly used as a permeant from the capsule coated with anionic or cationic bilayers, respectively. It should be considered whether the permeability enhancement at the neutralized coatings is due to the disappearance of the electrostatic repulsion between probes and ionic bilayers or not. Since permeations of zwitterionic fluorescent probe and NaCl were also controlled by the ambient pH changes in the fluid state of coating bilayers though the effect was relatively small (Table I), the permeability enhancement at the neutralized coatings is concluded mainly due to the disturbed, nonbilayer structures.

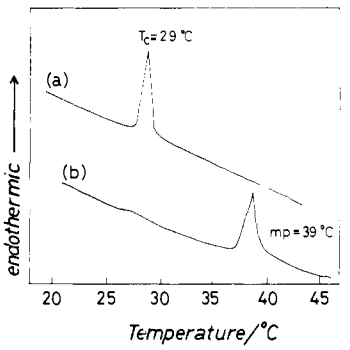

Figure 9. DSC curves of (a) the $2C_{12}$-suc-COO^--coated capsule membrane and (b) the $2C_{12}$-suc-COO^--coated capsule membrane after being soaked in pH 2 solution at 60 °C (above T_c) for 2 min.

DSC and X-ray Measurements. The change in the physical state of coating bilayers by the ambient pH could be directly confirmed by DSC measurements and X-ray analysis. As shown in Figure 9, when the capsule coated with anionic $2C_{12}$-suc-COO^- bilayers (T_c = 29 °C) was immersed in the acidic solution of pH 2 at 60 °C (above T_c), an endothermic peak appeared at 39 °C consistent with the melting point of the neutralized $2C_{12}$-suc-COOH amphiphiles, instead of at 29 °C (T_c of bilayers). When the capsule was immersed in the acidic solution at 25 °C below T_c, the endothermic peak still appeared at 29 °C. Similar DSC results depending on the treating temperature were observed in the case of the capsule coated with cationic $2C_{16}$-gly-NH_3^+ bilayers (T_c = 51 °C): the endothermic peak moved to 33 °C (melting point of $2C_{16}$-gly-NH_2 amphiphile) when the capsule was immersed in pH 12 aqueous solution above T_c, but not below T_c.

Although the $2C_{12}$-suc-COO^--coated capsule showed the distinct X-ray diffraction patterns of bilayer spacings (Figure 2a), the capsule which was immersed in pH 2 solution at 60 °C above T_c did not show any diffraction patterns, as well as that of the capsule coated with neutralized $2C_{16}$-gly-NH_2 amphiphiles (Figure 2b). When the capsule was treated with a solution of pH 2 below T_c, X-ray diffraction patterns did not disappear. Both DSC and X-ray results clearly prove that the coating bilayers were affected by the ambient pH only in the fluid state above their T_c, but not below T_c.

Capsules Coated with Neutralized Amphiphiles. Arrhenius plots of the capsule coated with the neutralized amphilphiles ($2C_{12}$-suc-COOH and $2C_{16}$-gly-NH_2) were also shown by dotted lines in Figure 6 and 7, respectively. The neutralized $2C_{12}$-suc-COOH and $2C_{16}$-gly-NH_2 amphiphiles hardly reduced the permeability relative to that of the uncoated capsule over the whole temperature range in the neutral form at pH 2 and 11, respectively, as expected from X-ray analysis (Figure 2b) and TEM observations. However, when the $2C_{12}$-suc-COOH-coated capsule was employed at pH 7 (pK_a of COOH groups 5.5), the permeability was greatly reduced above 39 °C, consistent with the melting point of the neutralized amphiphile (curve e of Figure 6). In the case of $2C_{16}$-gly-NH_2-coated capsule, the permeabilty at pH 7 where NH_2 groups can be protonated (pK_a 9.2) was also reduced above its melting point (curve e of Figure 7).

When the capsule coated with the $2C_{12}$-suc-COOH amphiphiles (mp 39 °C) was treated with the basic medium (pH 9) at 60 °C (above mp), the DSC peak moved to 29 °C, suggesting the formation of anionic bilayer structures. In the case of $2C_{16}$-gly-NH_2-coated capsules, a similar DSC result was observed: the DSC peak at 33 °C (melting point) moved to 51 °C (T_c of ionic bilayers) when the capsules were soaked in the pH 2 solution at 60 °C. Thus, only in the fluid state above melting point did the neutral amphiphile seem to ionize and form bilayer structures (providing a high barrier to the permeation). It is interesting that coating amphiphiles can be affected by ambient pH only in the fluid state: the anionic and cationic bilayers are destroyed above their T_c in the acidic and basic medium, respectively, and the neutral coatings are allowed to form bilayers in the basic or acidic medium above their melting points.

Summary

Although nylon capsule membranes are simply semipermeable, the capsule coated with dissociative synthetic bilayers can reversibly regulate the permeation by pH changes of the outer medium, depending on the phase transition temperature of coating bilayers. This is the first example of reversibly pH-sensitive permeation control across the capsule membrane, depending on temperature. It is reported that drug can be specifically released by reflecting an ambient pH from lipsomes in which pH-sensitive molecules are incorporated.[26] It is difficult, however, to realize the reversible permeation control in liposomal membranes, because they are easily damaged or fused to each other by changes of an ambient pH.[30] On the contrary, the bilayer-coated capsule membranes are not damaged by continuous pH changes, because lipid bilayers are supported by the physically strong capsule wall. These pH-sensitive, bilayer-coated capsule membranes should be useful for biological and industrial uses.

Registry No. 1, 1655-45-4; **2**, 91606-37-0; **3**, 93280-02-5; Na_2-($2C_{12}$-cys-$2COO^-$), 93380-91-7; Na($2C_{12}$-suc-COO^-), 93280-00-3; Na-($2C_{12}PO_4^-$), 17026-45-8; ($2C_{16}$-gly-NH_2)·HCl, 93280-01-4; nylon-2,12 (SRU), 41724-60-1; nylon-2,12 (copolymer), 41510-72-9; 2,6-bis(*N*,*N*-dimethylaminomethyl)naphthalene, 72461-76-8; 2,6-bis(bromomethyl)-naphthalene, 4542-77-2.