S_{13} and S_{31}. The effect may be likened to a kind of Darwinian competition, in which the strong get stronger and the weak eventually succumb.

Previous simulations of the two spin NOE[2,8] have always shown the cross peak intensity to be less than or equal to the diagonal peak intensity. In the present case, it is evident that in three-spin and probably in higher order systems, it is possible for the cross peak to be larger than the diagonal peak.

V. Discussion and Conclusions

The analysis presented here offers a simple and verifiable approach to rate-measurement problems, by back transforming the NOESY mixing coefficients. In particular, the SKEWSY experiment is well suited to studies of the nuclear Overhauser effect in ^{1}H NMR spectroscopy, owing to the information obtainable from cross peaks and the absence of zero-quantum coherences.

In view of the general nature of the $\hat{\mathbf{a}} \rightarrow \hat{\mathbf{R}}$ transformation, the question arises as to why a combined SKEWSY/NOESY experiment is preferred over either a single NOESY or a single SKEWSY experiment. In the former case a NOESY spectrum might be expected to give a direct map of the $\hat{\mathbf{a}}$ matrix, in which n diagonal peaks correspond to n diagonal coefficients and m cross peaks to m off-diagonal terms. Two problems arise in this instance. The first stems from the need to calibrate the spectrum by measuring the equilibrium magnetization M_0. Failure to do so leads to a false $\hat{\mathbf{R}}$ matrix, in which each diagonal element must corrected by the additive constant $(-1/\tau_m)$ ln C, C being the calibration constant. If ratios are used to eliminate the dependence on M_0, as in eq 20, then the NOESY spectrum can yield only $n + m - 1$ equations.

This result obviates the second problem associated with NOESY experiments. The $n + m - 1$ equations can only be formed if all of the diagonal peaks are resolved, a situation rarely encountered in practice. In the worst case, where no diagonal peaks can be distinguished, only $m - 1$ equations are obtained.

By contrast, the SKEWSY experiment projects diagonal peak information onto the cross peaks, and $m + n - 2$ equations can be obtained from cross peak/cross peak ratios. Thus, if two diagonal peaks are resolved, a single SKEWSY spectrum will contain sufficient information for a tractable solution. In this context the combination of a SKEWSY and NOESY experiment diminishes the required number of resolvable diagonal peaks, from two to one, or, in the case of the iterative solution (eq 21), to none.

Like all matrix solutions, a two-dimensional approach to the measurement of n-site exchange will be prone to error propagation. For this reason, it may prove useful to conduct several SKEWSY experiments and subject the solution to a least-squares analysis. In such circumstances the SKEWSY mixing time τ_s could be changed while the NOESY mixing time is kept constant. All the consequent SKEWSY peaks could then be used with the same reference NOESY spectrum. A natural extension of this approach would be to apply an accordion-type increment[11,12] to τ_s and obtain the time dependence of the cross peaks by deconvolution of selected cross sections in the ω_1 domain.

The two-dimensional measurement of cross relaxation promises considerable time savings compared to one-dimensional methods. In the experimental scheme presented here two principal obstacles, J-cross peaks and the overlap of diagonal peaks, have been removed. If, in addition, the back-transform method can provide accurate relaxation data, a more efficient approach to the study of molecular structure and dynamics will be possible.

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Functional Capsule Membranes. 8.[1] Signal-Receptive Permeability Control of NaCl from a Large Nylon Capsule Coated with Phospholipid Bilayers

Yoshio Okahata* and Han-Jin Lim

Contribution from the Department of Polymer Science, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan. *Received December 27, 1983*

Abstract: Nylon ultrathin capsules coated with bilayers of synthetic phospholipids (1,3-dipalmitoylglycero-2-phosphocholine and/or 1,3-dipalmitoylglycero-2-phosphoethanolamine) were prepared. Coating phospholipids were proved to exist as multilamellar bilayers from studies of X-ray diffraction and electron microscopy. The permeation of NaCl trapped in the inner aqueous phase was reduced by factors of 10–500 relative to that of the uncoated capsule and drastically decreased above the phase transition temperature (T_c) of coating phospholipid bilayers. When the capsule was coated with phospholipid bilayers containing the phosphatidylethanolamine moiety, the permeation of NaCl was *reversibly* controlled by the interaction and then the removal of divalent cations; the NaCl permeation was increased by treating with aqueous Ca^{2+} from outside and reverted by washing capsules with aqueous EDTA. The permeation mechanism of NaCl was also discussed from the activation energy data of Arrhenius plots.

Recently we prepared newly functional nylon capsules whose porous membranes were coated with synthetic amphiphile bilayers.[1-7] The capsule has some advantages of both nylon capsules and bilayer vesicles: the large inner aqueous phase, the physically strong wall, and the characteristics of bilayer properties.

We describe in this paper the nylon capsule membrane coated with biologically interesting phospholipid bilayers of 1,3-di-

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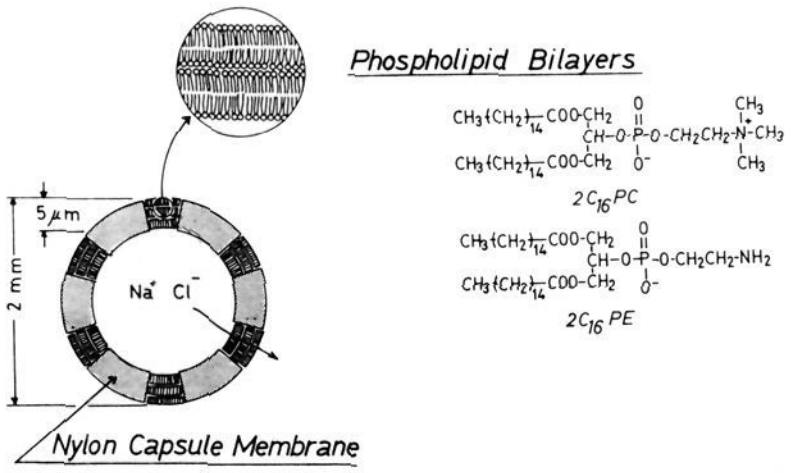

Figure 1. Schematic illustration of the large nylon capsule membrane coated with phospholipid bilayers.

palmitoylglycero-2-phosphocholine ($2C_{16}PC$) and/or 1,3-dipalmitoylglycero-2-phosphoethanolamine ($2C_{16}PE$). A schematic illustration of the capsule is shown in Figure 1. The permeability of water-soluble substances trapped in the inner phase was reversibly regulated not only by the phase transition temperature of bilayer coats but also by the interaction of Ca^{2+}/EDTA from outside to $2C_{16}PE$-containing bilayers. This means phospholipid–bilayer coats act as a kind of valve which opens or shuts by physical state changes of bilayers, responding to stimuli from outside such as temperature and divalent cation interaction. Liposomal membranes cannot achieve such a reversibly signal-receptive permeability control because of their easily breakable bilayer walls.

Synthetic 2-phosphatidyl lipids were selected as a covering bilayer for the chemical stability compared with naturally occurring 3-phosphatidyl lipids which have the labile fatty acid ester of the secondary hydroxy of glycerol.[8] Nylon capsules coated with natural egg lecithin have been reported by Chang and coworkers in the connection with their studies on the artificial cell.[9,10]

Experimental Section

Preparation of Lipids. 1,3-Dipalmitoylglycero-2-phosphocholine ($2C_{16}PC$) and 2-phosphoethanolamine ($2C_{16}PE$) were prepared according to the literature.[11-14] 1,3-Dipalmitoylglyceride was prepared according to the method of Mank et al.:[12] mp 73–74 °C, R_f 0.78 (toluene:ether 4:1). The 1,3-disubstituted structure was confirmed by ^{13}C NMR spectra.

A chloroform solution (40 mL) of 2-chloro-2-oxo-1,3,2-dioxaphospholane[15] (7.6 g, 53 mmol) was added dropwise to the chloroform solution (200 mL) of 1,3-dipalmitoylglyceride (15 g, 26 mmol) and triethylamine (2.7 g, 27 mmol) at 5–10 °C for 30 min, and the stirring was continued for 10 h at 50 °C. After the reaction, the mixture was washed with excess water, dried over Na_2SO_4, and evaporated in vacuo. The white residue was recrystallized from *n*-hexane 3 times: white granules, mp 43–45 °C, yield 13.4 g (75%).

$2C_{16}PC$ was obtained from the reaction of 1,3-dipalmitoylglycero-2-dioxaphospholane (13.4 g, 20 mmol) with acetonitrile solution (50 mL) of excess trimethylamine at 60 °C for 3 days in a pressure bottle: white granules from tetrahydrofuran, mp 70 → 240 °C (a thermotropic liquid crystalline behavior was observed in this temperature range) (lit.[16] mp 213–214 °C), yield 13.0 g (85%). Anal. Calcd for $C_{40}H_{80}O_8NP{\cdot}2H_2O$: C, 62.4; H, 11.0; n, 1.8. Found: C, 62.7; H, 11.0; N 1.8.

$2C_{16}PE$ was obtained from the reaction of 1,3-dipalmitoylglycero-2-dioxaphospholane with excess NH_3 in a similar way: white granules from tetrahydrofuran, mp 94–96 °C, yield 12 g (75%). Anal. Calcd for $C_{37}H_{78}O_8NP{\cdot}2H_2O$: C, 62.5; H, 11.4; N, 2.0. Found: C, 62.5; H,, 11.5; N, 2.2.

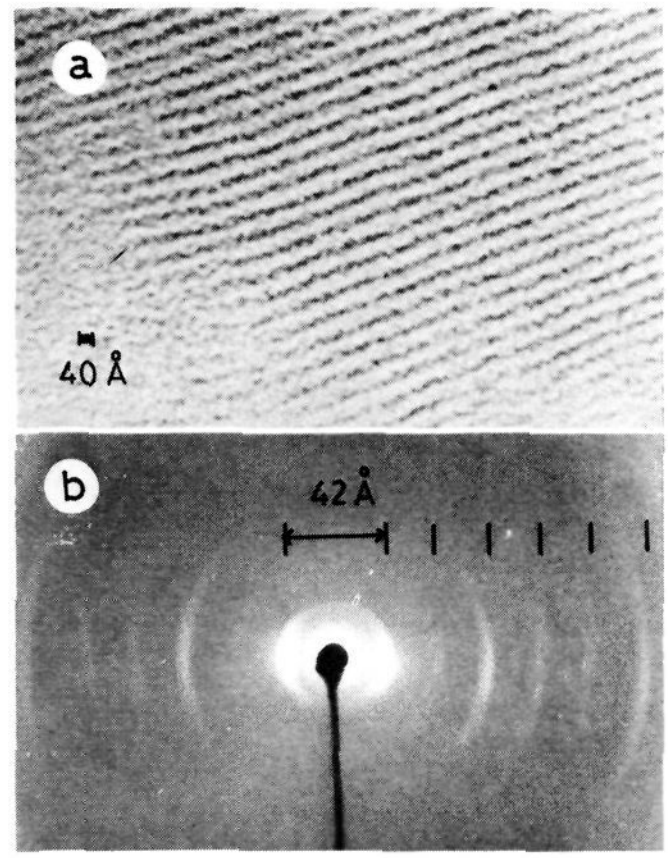

Figure 2. Transmission electron micrograph (a) and a X-ray diffraction pattern (b) of $2C_{16}PC$-coated capsule membranes.

$2C_{16}PC$ and $2C_{16}PE$ were confirmed to migrate as a single peak containing no other isomers and impurities on the thin-layer chromatography with a flame-ionization detector (IATRON Laboratories, Japan, Model TF-10) in a chloroform/methanol/water solvent system (65:25:4).

Preparation of Capsules. Large, semipermeable nylon capsules were prepared from ethylenediamine and 1,10-bis(chlorocarbonyl)decane by interfacial polymerization according to previous methods.[1-7] Nylon capsules which have an ultrathin membrane thickness (5.0 ± 0.1 μm) and a large diameter (2.0 ± 0.1 mm) were obtained. After removal of the outer organic solvent, the capsules were dialyzed against 0.2 M aqueous NaCl solution for 2–3 days.

Phospholipid-coated capsules were prepared as follows. The NaCl trapped capsules (20 pieces) were transferred to a dodecane solution (3 mL) of phospholipids (50 mg) at 60 °C and maintained for 5 min. After cooling slowly, lipid-coated capsules were transferred to aqueous 0.2 M NaCl in order to remove excess dodecane solution. The phospholipid content on the capsule was 5.0 ± 0.5 μg per capsule.

Experimental Procedures. The structure of coating lipids on the capsule membrane was observed by a Hitachi H-500 transmission electron microscope (TEM). Nylon capsules which had been kept in aqueous 2% uranyl acetate (negatively staining agents) were coated with $2C_{16}PC$ lipids in a dodecane solution. The negatively stained, lipid-coated capsule was cut to an ultrathin section and applied to TEM observations.

X-ray measurements with the Ni-filtered Cu Kα radiation were carried out by using a flat-plate camera. $2C_{16}PC$ lipid-coated capsule membranes were crushed, piled up, and cut into a fine strip with diameter about 2 × 0.3 × 0.3 mm. The intersection of the capsule membranes was exposed with the incident beam.

Differential scanning calorimetry (DSC) of the coating phospholipids was carried out with a Daini-Seikosha Model SSC-560 instrument. Five crushed capsules were sealed with 50 μL of water in Ag-sample pans and heated from 5 to 90 °C at a rate of 2 °C min^{-1}.

The permeation of NaCl from the inner aqueous phase was measured by detecting increases in the electrical conductance of the outer aqueous phase after dropping one capsule into 50 mL of the deionized water in a constant-temperature cell.[1-7]

Results

Characterization of Capsules. The scanning electron microscopy had confirmed that original nylon capsules had a porous membrane structure, and coating lipids covered these pores entirely (diameter: 0.1–0.3 μm) in the capsule membrane.[2,3,5,6] It is important whether the coating lipid can form bilayer structures on the capsule membrane or not. Figure 2a is an enlargement of a favorable area of the ultrathin section of the $2C_{16}PC$-coated capsule membrane stained negatively by uranyl acetate and shows the distinct multilamellar structure whose mean thickness is estimated to be ca. 40 Å. The X-ray diffraction pattern of the $2C_{16}PC$-coated

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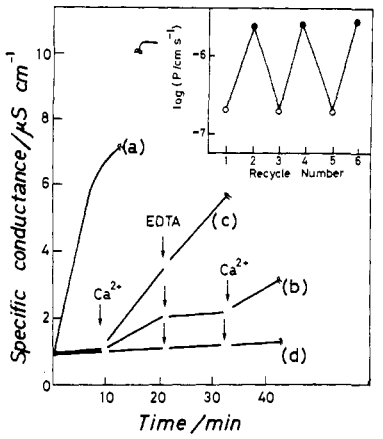

Figure 3. Time courses of NaCl permeation from Nylon capsules at 55 °C. (a) The uncoated capsule, (b) the capsule coated with a mixed bilayer of $2C_{16}PC/2C_{16}PE$ (30:70), (c) the capsule coated solely with $2C_{16}PE$ bilayers, and (d) the capsule coated solely with $2C_{16}PC$ bilayers. The insert shows the repeated permeability control by Ca^{2+} (●) and then EDTA (○) treatments of $2C_{16}PC/2_{16}PE$-coated capsules (curve b).

capsule membrane is shown in Figure 2b, in which the incident beam was exposed perpendicular to the intersection of capsule membranes. The series of strong reflection arcs with 42-Å spacing are very consistent with both the bimolecular length of $2C_{16}PC$ (40–45 Å, estimated from CPK molecular models) and one white-line width (one bimolecular layer) of lamellar structures shown in Figure 2a. These findings strongly indicate that coating phospholipids exist as well-oriented, multilamellar bilayers, which pile growing up parallel to the capsule membrane plane, as illustrated in Figure 1. Similar results were obtained in the case of capsules coated with $2C_{16}PE$ lipids or a mixture of $2C_{16}PC/2C_{16}PE$ (30:70).

The liquid crystalline property is one of the fundamental physicochemical characteristics of phospholipid bilayers. Phospholipid-coated capsules showed a sharp endothermic peak by DSC measurements, which indicates a phase transition of coating phospholipid bilayers from a crystalline to a liquid crystalline state: $2C_{16}PC$ at 49 °C (ΔH = 12 kcal mol^{-1}), $2C_{16}PE$ at 55 °C (ΔH = 15 kcal mol^{-1}), and a mixture of $2C_{16}PC/2C_{16}PE$ (30:70) at 54 °C (ΔH = 11 kcal mol^{-1}). The obtained phase transition temperatures (T_c) of coating phospholipids were 10–15 °C higher than those of aqueous unilamellar (T_c = 34 °C)[17] and multilamellar liposomes (T_c = 37–38 °C).[18] The pretransition endothermic peak was not seen in the temperature range of 5–90 °C. A slightly high T_c of coating lipids may reflect the well-oriented lamellar structure on the capsule membrane.

Since the lipids were deposited on the capsule membrane from the interface of water and dodecane, it should be confirmed whether the hydrocarbon solvent is involved as impurities in the coating bilayer or not. The covering $2C_{16}PC$ lipids were extracted with chloroform from 100 pieces of capsules, and the extract was analyzed. The ratio between C, N, and P of the extract was very consistent with that of the $2C_{16}PC$ molecule within experimental errors. This clearly indicates the covering lipid deposited from the dodecane solution does not include hydrocarbons as impurities and agrees with the results of DSC and X-ray measurements.

Permeation of NaCl. The permeation of NaCl from the inner phase was followed by detecting increases in electrical conductance in the outer water phase.[1-7] Figure 3 shows typical time courses of the NaCl release to the outer water phase at 55 °C. When the uncoated capsule membrane was employed, NaCl was completely released within 20 min (curve a). A marked decrease in NaCl efflux was observed at 55 °C when the capsule membrane was coated with phospholipid bilayers (initial parts of curves b, c, and d). At the lower temperature, the permeability of the lipid-coated capsule was not drastically reduced compared with that of the uncoated one (see the effect of temperature).

Table I. Permeability Constants of NaCl across Capsule Membranes at 55 °C

capsules	P, ×10^6 cm s^{-1} treated with Ca^{2+}		then, treated with EDTA
uncoated	120	123	125
coated with $2C_{16}PC$ sole bilayers	0.32	0.35	0.31
coated with mixed bilayers of $2C_{16}PC/2C_{16}PE$			
(30:70)	0.25	2.5	0.27
(75:25)	0.20	1.2	0.23
coated with $2C_{16}PE$ sole bilayers	0.12	11	10

Permeability constants P (cm s^{-1}) can be calculated from eq 1,[3,5] where k and d are the slope of Figure 3 and the capsule

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

diameter, respectively. ΔC denotes a change of the electric conductance after crushing a capsule, which means the concentration of NaCl trapped in the inner phase. From ΔC values, the concentration of the trapped NaCl was estimated to be 0.19 ± 0.01 M, which was nearly equal to that of the dialysis solution (0.2 M).

Permeability constants at 55 °C obtained from Figure 3 and eq 1 were summarized in Table I.

Effect of Ca^{2+} Ion Interaction. Divalent cations are well-known to cause the fusion and the release of encapsulated drugs of negatively charged liposomes from acidic phospholipids.[19,20] Thus, it is interesting to examine the effect of divalent cations on the permeability of capsules coated with phospholipid bilayers containing the $2C_{16}PE$ moiety.

Treatment of capsules with aqueous Ca^{2+} ions or EDTA (ethylenediaminetetraacetic acids disodium salts) was carried out as follows: a capsule was picked from a cell, immersed in 0.1 M phosphate buffer (pH 7) containing 0.05 M $CaCl_2$ or EDTA at 55 °C for 2 min, and returned to the cell. Although the ethanolamine group in $2C_{16}PC$ bilayers is partially protonated in aqueous pH 7, the presence of excess Ca^{2+} might be enough to interact with PE groups by shifting the apparant pK_a of the ethanolamine group (pK_a = ca. 8).

When a capsule coated with phospholipid bilayers containing a large amount of the phosphatidylethanolamine moiety ($2C_{16}PC/2C_{16}PE$ = 30:70) was employed, the permeability of the trapped NaCl was enhanced 12 times by treating it with aqueous Ca^{2+} at 55 °C. The permeability could be reduced to the original rate by removing Ca^{2+} ion from the capsule with the EDTA treatment (Figure 3, curve b, and Table I). As shown in the inserted figure of Figure 3, this reversible permeability control could be repeated several times without damaging both coating lipids and capsule membranes. In the case of the capsule coated with a small amount of $2C_{16}PE$ containing bilayers ($2C_{16}PC/2C_{16}PE$ = 75:25), the effect of Ca^{2+} ion on the permeability was relatively small (6 times the rate enhancement) and the treatment with EDTA properly reduced the NaCl release to the original rate (Table I). When the capsule was treated with the more concentrated aqueous Ca^{2+} (0.3–0.5 M) or at the higher temperature (above 65 °C), the enhanced permeability was not reduced completely to the original rate by treatment with EDTA, probably because of the damage to coating bilayers.

When the capsule coated solely with $2C_{16}PE$ lipid bilayers was treated with aqueous $CuCl_2$ above their T_c, the ca. 100 times permeability enhancement was observed. The permeability, however, was not reduced again in the least by washing with EDTA (Figure 3, curve c, and Table I).

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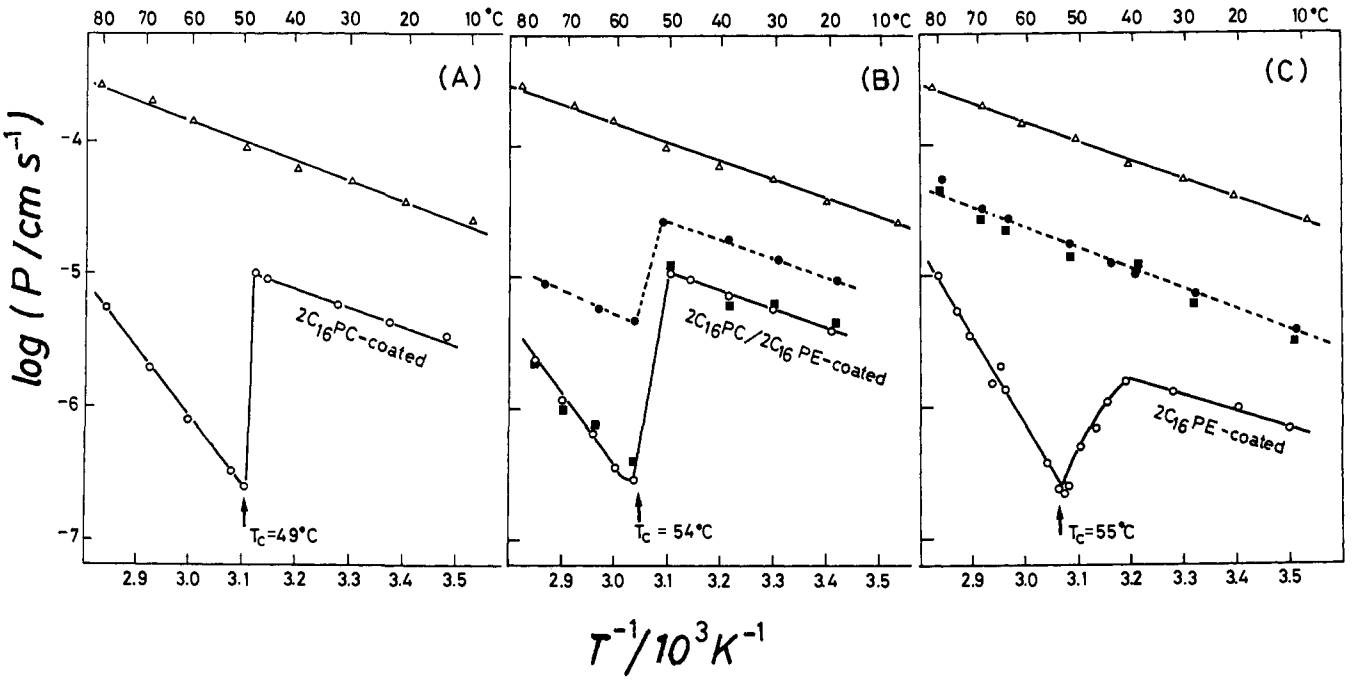

Figure 4. Arrhenius plots of NaCl permeation across capsule membranes coated with $2C_{16}PC$ sole bilayers (A, —○—), coated with a mixed bilayer of $2C_{16}PC/2C_{16}PE$ (30:70) (B, —○—), and coated with $2C_{16}PE$ sole bilayers (C, —○—). Arrhenius plots of the uncoated capsule (—△—) are also shown. Dotted lines (---●---) show Arrhenius plots of Ca^{2+}-coordinated capsules. Closed squares show the permeability after treating the Ca^{2+}-coordinated capsule with aqueous EDTA. Arrows show phase transition temperatures of respective coating lipid bilayers obtained from DSC measurements.

Other divalent cations such as Ba^{2+}, Cu^{2+}, and Mg^{2+} were also effective on the permeability enhancement of all the capsules coated with $2C_{16}PE$ containing bilayers. The treatment with alkali metal ions (Na^+ and K^+) did not change the permeability at all.

The permeability of the zwitterionic $2C_{16}PC$-coated capsule, whose coating lipids do not have an acidic phosphate group, was not changed by treatment with Ca^{2+}/EDTA (Figure 3 curve d, and Table I).

Effect of Temperature: Arrhenius Plots. It has been reported that the permeability of liposomes from pure lipids toward water-soluble substances changes near their phase transition temperatures.[21-25] In general, the permeability of liposomal membranes increases in the fluid state above their T_c.[21,22,26] Permeability constants (P) of bilayer-coated capsule membranes were obtained at various temperatures (10–80 °C) to study the effect of the phase transition on the NaCl permeation. Arrhenius plots of capsules coated with $2C_{16}PC$ bilayers, a mixed bilayer of $2C_{16}PC/2C_{16}PE$ (30:70), and $2C_{16}PE$ bilayers are shown in Figure 4 (A, B, and C, 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 straight line. In contrast, Arrhenius plots gave abrupt inflections in the temperature range of 45–55 °C in all the phospholipid-coated capsules. These inflections correspond well to T_c of the respective bilayers obtained from DSC measurements (shown by arrows in Figure 4). The permeability of all the lipid-coated capsules was unexpectedly reduced at the temperature above their respective T_c relative to below T_c. The permeability decrease above T_c is quite opposite to that of liposomal membranes, in which the permeability increases above their T_c.[21,22,26]

Arrhenius plots of Ca^{2+}-coordinated capsules coated with $2C_{16}PE$-containing bilayers are shown as dotted lines in Figure 4B,C. When the capsule coated with a mixed bilayer of $2C_{16}PC/2C_{16}PE$ (30:70) was treated with Ca^{2+} ions, the permeability of NaCl increased by a factor of 3–12 relative to the Ca^{2+}-untreated capsule over the whole temperature range. The Arrhenius plot of Ca^{2+}-coordinated capsules still showed a small inflection near T_c (Figure 4B, --- ● --). The permeability of the Ca^{2+}-treated capsule was reverted to the original rate after washing with aqueous EDTA over the whole temperature range (—■—). On the other hand, in the case of the capsule coated solely with $2C_{16}PE$ bilayers, the Ca^{2+}-treated capsule gave the straight Arrhenius plot with the large permeability enhancement (10–100 times relative to that of the Ca^{2+}-untreated capsule). The permeability, however, was not reverted at all by washing with the EDTA solution (Figure 4C, ----■---).

Activation energies (E_a) were calculated from Arrhenius slopes above and below T_c and summarized in Table II.

Table II. Activation Energy Data on NaCl Permeation across Capsule Membranes

capsules	E_a, kcal mol^{-1} above T_c	E_a, kcal mol^{-1} below T_c
uncoated	5.5	
coated with $2C_{16}PC$ sole bilayers	23	6.0
coated with mixed bilayers of $2C_{16}PC/2C_{16}PE$ (30:70)	25	5.8
Ca^{2+}-coordinated capsule	6.5	5.9
coated with $2C_{16}PE$ sole bilayers	27	5.5
Ca^{2+}-coordinated capsule	6.0	

[a] Obtained from slopes of Arrhenius plots of Figure 4.

Discussion

Effect of Phase Transition on Permeability. The NaCl permeation across the phospholipid-coated capsule membrane was reduced 10–500 times relative to that of the uncoated capsule membrane and drastically decreased at the temperature above T_c, compared to below T_c, of coating bilayers. This permeability change near T_c is quite opposite to that of liposomal membranes, whose permeability enhances above their T_c. That is, lipid bilayers on the capsule provide a higher barrier to the permeation in the fluid liquid crystalline state above T_c than in the rigid gel state below T_c.

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This anomalous permeation behavior below and above T_c of the lipid-coated capsule may be explained as follows from activation energy data in Table II. When a hydrated electrolyte such as NaCl permeates through the hydrophobic matrix, the large E_a value will result. E_a values of the permeation of Na^+ or Cl^- through egg lecithin vesicles (in the liquid crystalline state) have been reported[27-29] to be 15–30 kcal mol^{-1}. In the case of bilayer-coated capsules, E_a values above T_c are always larger than those below T_c (Table II). At the temperature above T_c, NaCl may permeate through the fluid, though hydrophobic bilayer matrix of phospholipids with the relatively high activation energy (23–27 kcal mol^{-1}).

When the bilayer is in the gel state below T_c, the permeation through the rigid hydrophobic matrix becomes difficult and NaCl may permeate only through defectives in multilamellar bilayer coats, instead. Phospholipid bilayers, which were deposited in porous capsule membranes from the interface of water and dodecane, seem to easily produce defectives between lamellae in the crystalline state, and the permeation of NaCl below T_c is relatively fast. E_a values below T_c (5.5–6.0 kcal mol^{-1}), then, become similar to that of the uncoated capsule (E_a = 5.5 kcal mol^{-1}), in which the NaCl permeation mainly proceeds by the diffusion process (E_a = 3–6 kcal mol^{-1}).[30-32] Since these defective pores may disappear in the fluid liquid crystalline state, the NaCl permeation is decreased above T_c (see Figure 4).

Ca^{2+}-Induced Permeability Control. The permeability of capsule membranes coated with the mixed bilayer of $2C_{16}PC/2C_{16}PE$ (30:70) was reversibly controlled by treating with divalent cations (Ca^{2+}) and then EDTA, alternately (Figure 3, curve b, and the insert). The Ca^{2+}-treated capsule had only a smaller and broader endothermic peak near 55 °C (ΔH = 2.3 kcal mol^{-1}) than that of the original bilayer-coated capsule (ΔH = 11 kcal/mol) by DSC measurements. After the Ca^{2+}-coordinated capsule was washed with aqueous EDTA, DSC measurements showed the ca. 90% recovery of the sharp endothermic peak at 53 °C (ΔH = 9.5 kcal mol^{-1}). It was also confirmed that the Ca^{2+}-treated capsule did show only the weak X-ray diffraction patterns of bilayer spacings relative to those of original bilayer coats. These findings indicate that the fast release of NaCl by treatment with Ca^{2+} is due to the partially disturbed bilayer structure (or the non-bilayer structure) by chelating the phosphoethanolamine group of mixed bilayers with divalent cations, and the revertion of the permeability by washing with EDTA is explained by the regeneration of bilayers due to the removal of Ca^{2+} ions.

In the case of the capsule coated solely with phosphoethanolamine ($2C_{16}PE$) bilayers, the endothermic peak at 55 °C (ΔH = 15 kcal mol^{-1}) almost disappeared by treating with Ca^{2+} and was not regenerated by washing with EDTA. This is consistent with the irreversible permeation behavior in Figure 3 (curve c) and Figure 4C. Thus, divalent cations can interact strongly with phosphoethanolamine groups of $2C_{16}PE$ sole bilayers. Therefore, the NaCl permeation increased drastically and was not reverted at all by washing with aqueous EDTA.

Activation energies (E_a = 5.9–6.5 kcal mol^{-1}) of Ca^{2+}-coordinated capsules coated with $2C_{16}PE$-containing bilayers are almost equal to that of the uncoated capsule (E_a = 5.5 kcal mol^{-1}). The enhanced permeation of NaCl from the Ca^{2+}-treated capsule is explained by the fact that NaCl permeates through defective pores of Ca^{2+}-coordinated, disturbed bilayers.

Conclusions

The permeability of water-soluble substances such as NaCl from the large nylon capsule coated with phospholipid bilayers can be reversibly controlled by using the phase transition and/or the divalent cation coordination from outside. It is well-known that the permeability of liposomal membranes can be increased above their phase transition temperatures and drastically enhanced by the Ca^{2+} coordination to negatively charged lipids. It is difficult, however, to realize the reversible permeation control in liposomal membranes because they are easily damaged and fused to each other by the addition of divalent cations. On the other hand, the lipid-coated capsule membrane is not damaged by the repeated divalent cation treatments because lipid bilayers are supported by the physically strong capsule wall.

The coating phospholipid bilayers on the capsule act as a kind of a gate valve which responds to stimuli from outside such as the temperature and the ion interaction. When other synthetic amphiphile bilayers are chosen, we can prepare other signal-receptive capsule membranes which respond to temperature,[2,3] photoirradiation,[1,4] and ultrasonic power.[7] These signal-receptive, lipid-coated capsules should be interesting for a model synaptic system in which a nerve impulse initiates the rapid release of a chemical intermediary such as acetylcholine.

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Registry No. $2C_{16}PC$, 59540-22-6; $2C_{16}PE$, 67303-93-9; NaCl, 7647-14-5; NaCl, 7647-14-5; Ca, 7440-70-2; 2-chloro-2-oxo-1,3,2-dioxaphospholane, 6609-64-9; 1,3-dipalmitoylglyceride, 502-52-3; 1,3-dipalmitoylglycero-2-dioxaphospholane, 59540-21-5; trimethylamine, 75-50-3; ethylenediamine–1,10-bis(chlorocarbonyl) = decane copolymer, 41510-72-9.

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