Phospholipid Inverted Micellar Aggregate as an Ionophore in Liquid Membrane System

Kozue KAIBARA,* Keiko ITO, and Kenji MIYAKAWA†
Department of Chemistry, Faculty of Science, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812
†Department of Applied Physics, Faculty of Science, Fukuoka University, Nanakuma, Jonan-ku, Fukuoka 814
(Received July 11, 1988)

Synopsis. Electrochemical, thermochemical, and light scattering investigations were performed on the 1-octanol liquid membrane systems containing dipalmitoyl-DL-phosphatidylcholine (DPPC) as an ionophore. It was suggested that DPPC molecules form inverted micellar aggregate in water saturated 1-octanol and that the membrane processes can be regulated by an endothermic phase transition of the DPPC multimolecular cation carrier.

Phospholipids, as an essential constituent of the biological systems, are important to assemble a bimolecular layer structure of the biomembrane. Bimolecular membrane structure of lipids observed in the artificial planar membrane or liposome has long been a useful model to investigate the biological membrane-related processes. On the other hand, it has been demonstrated that phospholipids dissolved in organic solvent with trace water content form inverted micellar aggregates.[1-5] There is interest in inverted micelles in these days as a mediator of the specific chemical reactions relating to a water pool trapped inside the micelle phase. In the present study, the ionophoretic capabilities of the inverted micellar aggregates of phospholipid in liquid membrane systems were investigated.

Experimental

Liquid membranes were prepared by dissolving dipalmitoyl-DL-phosphatidylcholine (DPPC), purchased from Sigma Chemical Co. and used without further purification, into 1-octanol saturated with water or 10^{-2} mol dm^{-3} aqueous NaCl solution by applying the ultrasonic irradiation above the main transition temperature of DPPC-water system. Three types of experiments, electrochemical, thermochemical, and photon correlation spectroscopic measurements, were carried out: (A) Transmembrane potentials and conductances were measured with varying temperature, 0—50 °C, in an immobilized liquid membrane-aqueous NaCl system to estimate the ion selectivity parameters. The immobilized liquid membrane systems consisted of solution phase I, where NaCl concentration was varied from 10^{-1} to 10^{-3} mol dm^{-3}, and phase II with a fixed NaCl concentration of 10^{-2} mol dm^{-3} separated a poly(tetrafluoroethylene) membrane filter (Fluoropore FP100, pore size: 1.00 μm, Sumitomo Electric Co.) impregnated with a 1-octanol solution of 10^{-2} mol dm^{-3} DPPC saturated with 10^{-2} mol dm^{-3} NaCl solution. (B) A differential scanning calorimeter (SSC/560U, Dainiseikosha Co.) was employed to observe thermal behavior of DPPC molecules using a 50 μl liquid membrane sample containing 1 mg DPPC sealed in a silver pan with a main scanning rate at 1 °C min^{-1}. (C) Laser light scattering equipments and measuring conditions were the same as used in the previous experiments.[6] The liquid membrane samples were illuminated by an argon laser operated in the TEM_{00} mode at 514.5 nm, at a power 100 mW. Providing that autocorrelation function, $C(t)$, in the heterodyne regime has a single exponential decay mode, the relaxation time, τ, was determined from a nonlinear least-squares fit to the relation as:

$$C(t)=A+B\exp(-t/\tau) \quad (1)$$

where A is the background part of $C(t)$ and B is a constant.

Results and Discussion

Figure 1 summarizes the experimental data and the estimated parameters as a function of temperature. The observed membrane potential data indicated that DPPC in the 1-octanol liquid membrane phase acts as a cation carrier with a transport number to Na^+, 0.82—0.92. The transport number to Na^+ increased with raising temperature and decreasing NaCl concentration in phase I. Systematic investigations by Green et al.[7-9] demonstrated that phospholipids function as an ionophore and, in some cases, ionophoretic capabilities are comparable to those of authentic antibiotic ionophores. Highly selective and specific biomembrane transport processes are considered nowadays to be determined by proteinic materials embedded in the phospholipid bilayers such as ionophoretic substances and transport enzymes. However, phospholipids still play some roles in the broad spectrum of biomembrane transport capabilities. In the context of a possible physiological significance of the ionophoretic activities of phospholipids, involvements to the energy coupling process were suggested.[7]

As can be seen in Fig. 1-A, the curve relating the membrane conductance, G_m, with temperature shows a change of gradient at around 18 °C. And, the curve relating the membrane permeabilities to Na^+ and Cl^-, P_{Na} and P_{Cl}, with temperature in Fig. 1-B shows a change of gradient as corresponding with the membrane conductance change as a function of temperature. P_{Na} and P_{Cl}, indicating the membrane permselectivities by referring to the permeating speed of respective ions, can be calculated from the membrane potential and conductance data based on the preceding membrane theory.[10,11] The membrane permeability ratio, P_{Cl}/P_{Na}, decreased slightly with raising temperature.

It is a well-known fact that the conductance of phospholipid bilayer membrane changes drastically at the temperature corresponding to the gel-liquid crystal phase transition temperature observed in phospholipid-water system. Differential scanning calorimetry (DSC) measurements on the DPPC-1-octanol liquid membrane were carried out to examine a phase behavior of DPPC molecule at around 18 °C where the temperature-dependent characteristics of membrane transport altered. Examples of the DSC thermogram of the DPPC-1-octanol liquid membrane fluid are

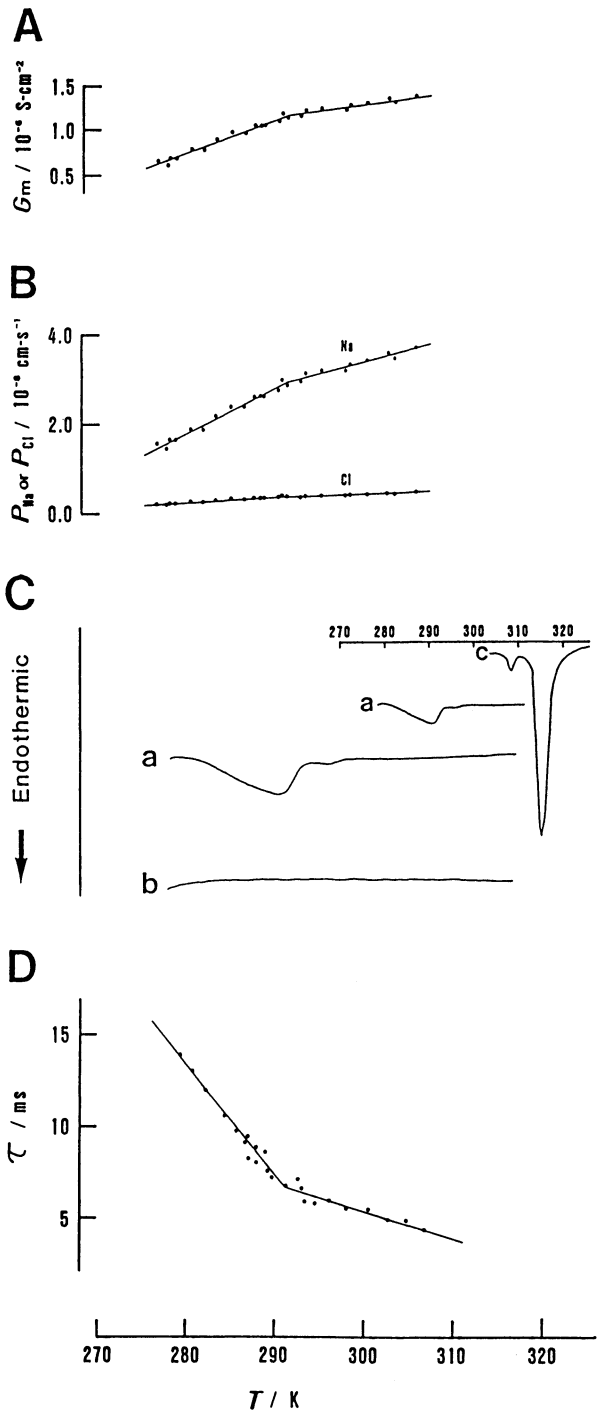

Fig. 1. Membrane conductance [A], membrane permeabilities to Na^+(●) and Cl^-(○) [B], DSC thermogram [C], and relaxation time obtained by the light scattering study [D] as a function of temperature. The membrane conductance and permeability values were obtained for 10^{-2} mol dm^{-3} NaCl(I)-10^{-2} mol dm^{-3} NaCl(II) system. In Fig. 1-C, thermograms a, b, and c were recorded for the systems in which DPPC dissolved in 1-octanol saturated with 10^{-2} mol dm^{-3} NaCl aqueous solution, in pure 1-octanol, and in 10^{-2} mol dm^{-3} NaCl aqueous solution, respectively. The scale of the upperright frame comparing the thermograms a and c is reduced to 1/2 of the original scale for the thermograms a and b.

shown in Fig. 1-C. As illustrated in the thermogram a, an endothermic peak was detected at around 18 °C in 1-octanol solution of DPPC saturated with water or aqueous NaCl solution. None of the detectable thermal changes were observed, on the contrary, in the pure 1-octanol solution of DPPC (thermogram b) and in the system without DPPC. Up to 80—90 °C, additional thermal changes were not observed in these systems. The peak height of the thermogram a is approximately 1/10 of that of the established endothermic changes for the gel-liquid crystal transition of DPPC-water system (compare the thermograms a and c). The thermograms a, b, and c were recorded from the samples containin 20 mg ml^{-1} DPPC dissolved into respective solvents. The present DSC results suggest that the changes in mode of the DPPC-mediated cation transport at critical temperature, 18 °C, are related in some way to endothermic phase transitions of DPPC in the liquid membrane phase.

It is also a common observation that addition of short-chain normal alkanols cause broadening of the thermal peak and lowering of the temperature for the endothermic gel-liquid crystal transition of phospholipid-water system.[12,13] These alterations in the endothermic behavior were suggested to be due to the penetrations of additives into the phospholipid bilayer structure. The present DPPC-1-octanol liquid membrane systems are different definitely in 1-octanol/water ratio from these phospholipid-excess water system with adding alcohols. It is expected, accordingly, in the present DPPC-1-octanol with trace water system (solubility of water in 1-octanol at 25 °C: ca. 3.5 wt%)[14] that the DPPC molecules form inverted micellar aggregates trapping a pool water phase. The formation of micelles in nonaqueous solvent is suggested to be affected by a trace amount of water existing in the solvent.[2,3] In the present system, appearance and peak height of the endothermic change illustrated in the DSC thermogram Fig. 1-C-a were quite sensitive to water content in the 1-octanol liquid membrane phase (unpublished observations).

To confirm the participations of DPPC inverted micellar aggregate in the cation transport across liquid membrane, the micelle formation was detected directly by means of the photon correlation spectroscopy. The mode of temperature dependences of the relaxation time constant obtained from the analyses of laser light scattering measurements on the DPPC-1-octanol membrane fluid samples saturated with water or aqueous NaCl solution is shown in Fig. 1-D. In case of the samples with pure 1-octanol as well as the samples without DPPC, none of the evidence indicating aggregate formations was obtained. The DPPC inverted micellar diameter at 18 °C can be estimated approximately at 0.1 μm, on the assumption of a formation of spherical aggregates. As illustrated in Fig. 1-D, the curve relating the relaxation time constant with temperature shows a distinct change of slope, and, accordingly, indicates an alteration of the temperature dependences of diffusion coefficient of the DPPC aggregate at around 18 °C. Possible mechanisms to decrease the diffusion coefficient of DPPC inverted micellar aggregate in lower temperature

region are increases in micellar diameter, changes in micellar shape, fusion of micellar aggregate, etc. It was reported that the inverted micellar size increases with decreasing solvent dielectric constant and with lowering temperature.[1,15] An increase in micellar diameter due to the fusion of DPPC aggregates is possible in relation to the decrease in water solubility into 1-octanol. Changes in the surface charge conditions of DPPC aggregates are also possible in addition to these mechanisms.

The present results, especially the DSC and light scattering investigations demonstrating the importance of trace water content in the present liquid membrane system, suggest that the DPPC molecules form inverted micellar aggregates and function as a multimolecular cation carrier across the liquid membrane phase. Cations are thought to be transported by means of the trapping into a pool water phase as well as of the binding to the phosphate charges on the inverted micellar surface. The multimolecular ion carrier is expected to serve more possibilities to regulate membrane transport processes than the usual unimolecular ion carrier as shown in the present results, in which the effects of temperature on membrane permeability to Na^+ are demonstrated based on the endothermic phase transition of the DPPC inverted micellar aggregates. A detailed description of the phase behavior of the DPPC-1-octanol-trace water system requires further careful experiments, since the contents of trace amount of water seem to play a critical role for the phase behavior and the contributions to the liquid membrane transport. These experiments are also required for more direct evidence indicating the existence of the DPPC inverted micellar aggregates in the liquid membrane phase. The phase behavior studies of phospholipid-water system with and without addition of organic substances have been carried out extensively. For better understanding of the biological importance of phospholipids, however, systematic investigations on the phase behavior of phospholipid-organic solvent with trace water system are required. In the context of an existence of trace amount of water within membrane phase, the liquid membrane systems containing inverted micellar aggregates as an ion carrier provide an interesting model system to analyze the ion transport mechanisms of highly hydrophobic fluorinated ion-exchange membrane,[16,17] of which technological importance in membrane utilizing process is recognized in these days.

The authors wish to thank Prof. Shoji Kaneshina of Tokushima University for facilities for the DSC measurements. The authors are grateful to the Ministry of Education, Science and Culture of Japan for a Grant-in-Aid for Scientific Research on Priority Areas and one of the authors (K.K.) is also grateful to the Kurata Foundation for the research grant support.

References

1) P. H. Elworthy and D. S. McIntosh, *J. Pharm. Pharmacol.*, **13**, 663 (1961).
2) R. Kanamoto, Y. Wada, G. Miyajima, and M. Kito, *J. Am. Oil Chem. Soc.*, **58**, 1050 (1981).
3) M. Hayashi, K. Fukushima, and A. Kitamura, *Chem. Phys. Lipids*, **33**, 1 (1983).
4) V. V. Kumar, C. Kumar, and P. Raghunathan, *J. Colloid Interface Sci.*, **99**, 315 (1984).
5) M. Hayashi and Y. Maeda, *J. Jpn. Oil Chem. Soc.*, **34**, 758 (1985).
6) K. Miyakawa, T. Hirakawa, and S. Yamashita, *J. Phys. Soc. Jpn.*, **54**, 445 (1985).
7) D. E. Green, *Ann. N. Y. Acad. Sci.*, **264**, 61 (1975).
8) C. A. Tyson, H. Vande Zande, and D. E. Green, *J. Biol. Chem.*, **251**, 1326 (1976).
9) D. E. Green, M. Fry, and G. A. Blondin, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 257 (1980).
10) K. Kaibara, H. Sonoda, Y. Nagata, and H. Kimizuka, *Bull. Chem. Soc. Jpn.*, **56**, 1346 (1983).
11) K. Kaibara, Y. Nagata. T. Kimotsuki, and H. Kimizuka, *J. Membr. Sci.*, **29**, 37 (1986).
12) A. W. Eliasz, D. Chapman, and D. F. Ewing, *Biochim. Biophys. Acta*, **448**, 220 (1976).
13) E.S. Rowe, *Mol. Pharmacol.*, **22**, 130 (1982).
14) J. M. Sørensen and W. Arlt, "Liquid-Liquid Equilibrium Data Collection, Binary Systems," Deutschen Gesellschaft für Chemisches Apparatewesen, Frankfurt (1979).
15) N. Muller, "Micellization, Solubilization and Microemulsions," ed by K. L. Mittal, Plenum Press, New York (1977), Vol. 1, p. 229.
16) T. G. Gierke and W. H. Hsu, "Perfluorinated Ionomer Membranes," American Chemical Society, Washington, DC (1982), p. 283.
17) R. Wódzky, A. Narebska, and W. K. Nioch, *J. Appl. Polym. Sci.*, **30**, 769 (1985).