Static and Kinetic Investigations of the Structural Ordering of Phospholipid Membranes Doped with Azobenzene Derivative

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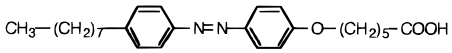
An azobenzene containing a fatty acid derivative, 4-octyl-4′-(5-carboxypentamethyleneoxy)azobenzene (8A5), was incorporated into large unilamellar vesicles (LUVs) of L-α-dimyristoylphosphatidylcholine (DMPC), and its effects before and after photoisomerization (in trans and cis forms, respectively) on fast osmotic processes were observed by stopped-flow light scattering measurements. Differential scanning calorimetry (DSC) measurements revealed 8A5, in particular the cis form, to strongly perturb the membrane structure. More specifically, fast osmotic shrinkage of the LUVs was detected by the changes in the light scattering at 500 nm following the mixing of DMPC LUVs and an aqueous solution of betaine at high flow velocity. A rapid and novel type of the scattering signal was observed in the vicinity of the phase-transition temperature, suggesting the thermodynamic lateral reordering in the membrane due to the imposed osmotic pressure difference.

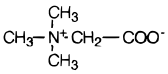
Introduction

A large number of different lines of evidence indicate that lateral ordering of lipid molecules plays an important role in functions of biomembranes.[1−3] Lipid bilayer is a fundamental structure of biomembranes, and it helps to maintain an osmotic gradient between cells; however, the permeability of small cations as well as water across the membrane is still large. Papahadjopoulos et al.[4] had reported that lipid bilayers become leaky to small cations in the main (e.g., gel to fluid) phase-transition region. It was suggested[4] that this permeability anomaly is related to lateral fluctuation in the lipid membranes, thus resulting in the formation of especially leaky interfacial regions between those gel and fluid domains that are formed in the transition region.

Artificial bilayer lipid membranes containing photochromic compounds are attractive because the molecular interaction between the dopants and the lipid molecules can be regulated by the photochemical reactions of the dopants.[5,6] The photochromic reaction causes a change in the shape of the chromophore of the molecules that leads to a reversible change in the membrane properties. Previously, Tanaka et al.[7,8] had incorporated an amphiphilic azobenzene derivative, 4-octyl-4′-(5-carboxypentamethyleneoxy) azobenzene (8A5), into the single unilamellar vesicles (SUVs) of L-α-dimyristoylphosphatidylcholine (DMPC). 8A5 molecules undergo trans-to-cis photoisomerization under UV irradiation, resulting in the increase of the K^+ ion permeability of the DMPC membrane. In addition, an anomalous ion permeability enhancement was observed in the vicinity of the main chain melting phase-transition temperature of the membrane. It was concluded that 8A5 molecules were located around the phase separation boundaries and underwent trans-to-cis photoisomerization, resulting in a specific conformational disordering of lipid bilayers.



8A5



betaine

In this study, we incorporated 8A5 into large unilamellar vesicles (LUVs) of DMPC with diverse dopant concentrations. Fast osmotic shrinkage was induced by the concentration difference of betaine between the inside and outside of liposomes. Compared with SUVs, LUVs are suited for the study of the osmotic swelling/shrinkage of the vesicles because they take fairly large, flexible, and defectless structures. Betaine is a natural, nontoxic compound, and it is impermeable through the lipid membrane because of its large hydration radius. One of the important functions of betaine in animal and plant metabolism is its ability to increase the osmotic strength of cells under salinity or drought stress, thereby preventing water loss.[9]

Light scattering methods have been used to determine sizes and shapes of cells, subcellular particles, and phospholipids dispersed in water.[10,11] Such an optical method is easily used, and the sensitivity is high enough to follow a rapid volume change of liposomes without any perturbation of the system itself. In this study, shrinkage of LUVs was detected by means of high time resolution (milliseconds) stopped-flow light scattering measurements at different temperatures. The effects before and after photoisomerization of 8A5, in trans and cis forms, respectively, on structural ordering and the water

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permeability of the membrane were carefully examined from the combination of static (differential scanning calorimetry and surface pressure measurements) and kinetic investigations (stopped-flow light scattering measurements).

Materials and Methods

Materials. DMPC was obtained from Sigma and 8A5 from Dojindo (Japan). Betaine and *n*-decane were from Finnsugar Bioproducts (Finland) and Merck (Germany), respectively. All of these compounds were used without further purification. Water used throughout this work was freshly deionized in a Milli RO/Milli Q (Millipore) filtering system.

Preparation of Liposomes. After mixing of desired lipid compositions in chloroform, the solvent was removed under a stream of nitrogen. The molar concentration ratios of 8A5 to DMPC, [8A5]/[DMPC], were varied in the range 0−0.10. The dry residues were maintained under reduced pressure for at least 2 h and hydrated in H_2O at 313 K to yield multilamellar vesicles (MLVs) with lipid concentration of 3.0 mM in total. To obtain LUVs (approximately 100 nm in diameter), the lipid dispersions were extruded with a LiposoFast small-volume homogenizer (Avestin, Canada), subjecting the lipid dispersions to 19 passes through 2 polycarbonate filters (0.1 μm pore size; Millipore) as described.[12]

An LH-450 xenon arc lamp (SLM Instruments) was used as the light source for photoisomerization. Ultraviolet (UV) light ($\lambda = 365$ nm) and visible light ($\lambda = 450$ nm) were selected by the combinations of a water thermal filter and glass band-pass filters, XB07 for UV light and XB08 for visible light (Melles Griot AB, Sweden), respectively. The conversion of *trans*-8A5 to *cis*-8A5 was monitored spectrophotometrically from the absorbance of the liposome dispersion at $\lambda = 350$ nm in the manner previously reported.[7,8]

Differential Scanning Calorimetry (DSC). Differential heat capacity scans of the dispersions of the LUVs in water were recorded with a Microcal VP-DSC Micro Calorimeter (Microcal Inc.) at a lipid concentration of 1.0 mM in total (heating rate of 0.5 K min^{-1}). The data were analyzed using the routines of the Origin software (Microcal Inc.).

Surface Pressure Measurements of Monolayers at the Air/Water Interface. Surface pressure measurements of the DMPC monolayer and the DMPC monolayers doped with 8A5 were carried out using a Teflon trough (150 mm × 392 mm). A quantity of 44.25 nmol of lipid dissolved in *n*-hexane (30 μL) was applied onto the water subphase, whereafter surface pressure was monitored with a Wilhelmy plate in the course of compression at a rate of 5.6 Å^2 molecule^{-1} min^{-1}. The data were sent to a 486 PC and analyzed by the Origin software (Microcal).

Stopped-Flow Light Scattering Measurements. Stopped-flow light scattering measurements were performed on an OLIS-RSM 1000 stopped-flow light scattering apparatus (OLIS). The irradiation wavelength of 500 nm was selected by a band-pass filter. Slits of 0.6 mm were used for both irradiation light and scattered light. An amount of 200 μL of 300 μM liposome dispersion and 200 μL of 200 mM aqueous solution of betaine were injected into the cuvette at higher flow velocity. The 90° scattered light was collected on the photomultiplier 1000 times for each measurement. Time constants used for the fast mode and slow mode measurements were 1 and 16 ms scan^{-1}, respectively. The collected data were analyzed by using the software provided by the manufacturer.

TABLE 1: Comparison of the Main Phase-Transition Temperatures and Enthalpies for Large Unilamellar Vesicles (LUVs) and Multilamellar Vesicles (MLVs) with the Indicated Compositions

	T_m/K		ΔH_m/kJ mol^{-1}	
samples	LUVs	MLVs	LUVs	MLVs
DMPC	297.2	296.9	20.6	25.2
[*trans*-8A5]/[DMPC] = 0.02	296.9	296.4	19.2	22.1
[*cis*-8A5]/[DMPC] = 0.02	296.8	296.2	18.6	19.9
[*trans*-8A5]/[DMPC] = 0.05	296.1	296.1	18.7	17.1
[*cis*-8A5]/[DMPC] = 0.05	296.0	295.2	18.7[a]	14.8
[*trans*-8A5]/[DMPC] = 0.10	295.5	295.5	13.9	9.83
[*cis*-8A5]/[DMPC] = 0.10	294.5	294.4	13.2	8.59

[a] This value is likely to be erroneous.

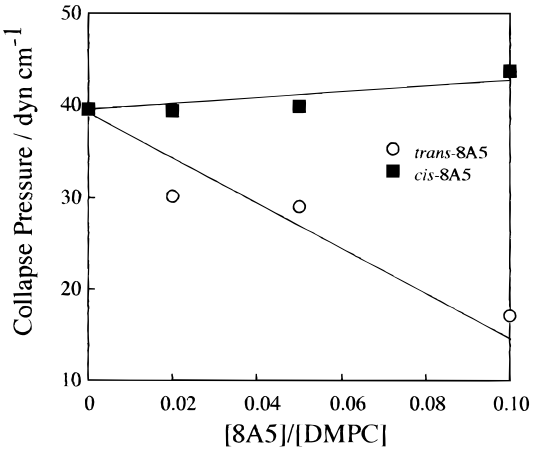

Figure 1. Collapse pressures of the pure DMPC monolayer and the DMPC monolayers doped with *trans*-8A5 and *cis*-8A5 molecules. The molar concentration ratio of 8A5 to DMPC, [8A5]/[DMPC], was varied in the range 0−0.10.

Results

DSC Measurements. Changes in the DSC of the DMPC LUVs and the LUVs doped with various concentrations of 8A5 are compiled in Table 1. These data are the average of the two measurements for each sample, and the peak temperatures are reproducible to better than ±0.05 K. The phase-transition temperature, T_m, of the pure DMPC LUVs was 297.2 K, and the phase-transition enthalpy, ΔH_m, was 20.6 kJ mol^{-1}. Presence of *trans*-8A5 in the DMPC LUVs caused T_m to decrease and broadened the main transition peak. For example, when [*trans*-8A5]/[DMPC] = 0.10, T_m was 295.5 K and ΔH_m amounted to 13.9 kJ mol^{-1}. Notably, perturbation of the structural ordering of the LUVs with *cis*-8A5 seems to become augmented. When [*cis*-8A5]/[DMPC] = 0.10, T_m and ΔH_m were reduced to 294.5 K and 13.2 kJ mol^{-1}, respectively. Both isomers abolished the pretransition peaks around 287 K. For the reference, the data of DMPC MLVs were also shown in Table 1.

Surface Pressure Measurements of Monolayers at the Air/Water Interface. The limiting areas of DMPC, *trans*-8A5, and *cis*-8A5 roughly extrapolated from the linear part of the condensed region to zero pressure were approximately 60, 20, and 160 Å^2 molecule^{-1}, respectively. Collapse pressures of the DMPC monolayer and DMPC monolayers doped with 8A5 were obtained from the $\pi-A$ isotherms, and they are summarized in Figure 1. The collapse pressure of the DMPC monolayer was 40 dyn cm^{-1}. When *cis*-8A5 molecules were incorporated into the monolayer, only a slight increase in the collapse pressure was evident. At [*cis*-8A5]/[DMPC] = 0.10, the monolayer became unstable at 44 dyn cm^{-1}. However, the trans isomer

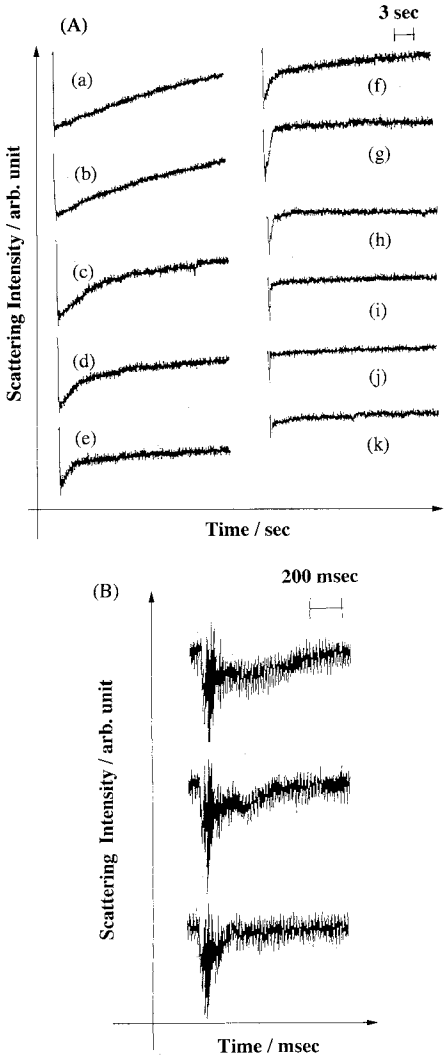

Figure 2. (A) Stopped-flow light scattering signals of the pure DMPC LUVs. An amount of 200 μL of 300 μM liposome dispersion and 200 μL of 200 mM aqueous solution of betaine were injected into the cuvette at the time indicated by an arrow. Time constant used throughout this measurements was 16 ms scan^{-1}. Temperatures were (a) 292.5, (b) 293.4, (c) 294.3, (d) 295.4, (e) 296.1, (f) 296.6, (g) 297.0, (h) 297.5, (i) 298.4, (j) 299.5, and (k) 300.4 K. (B) Stopped-flow light scattering signals of the pure DMPC LUVs around the phase-transition temperature (T_m = 296.9 K). Time constant used here was 1 ms scan^{-1}. The samples were the same as in panel A. Temperatures were (top) 297.0, (middle) 297.5, and (bottom) 298.4 K.

of 8A5 caused the collapse of the monolayer to occur at a much lower surface pressure. For example, when [*trans*-8A5]/[DMPC] was 0.10, the collapse was observed at 17 dyn cm^{-1}.

Stopped-Flow Light Scattering Measurements. The light scattering signals of pure DMPC LUVs are shown in Figure 2A. On the injection of LUVs dispersion together with the aqueous solution of betaine, the scattering intensity rose because of the shrinkage induced by the osmotic gradient between the inside and the outside of the lipid bilayer. From T = 292.5 to 296.6 K, where the bilayer is in the gel phase, the scattering intensity increased rather slowly as a function of time. The rate of shrinkage became faster with the increase of the temperature. In this temperature range, the kinetics of shrinkage seemed to obey the slow first-order kinetics. Intriguingly, however, when the temperature was increased to T = 297.0 K, i.e., close to the phase-transition temperature (T_m = 296.9 K), a different type of signal appeared. The light scattering signal did not obey first-order kinetics, especially in its earlier stage.

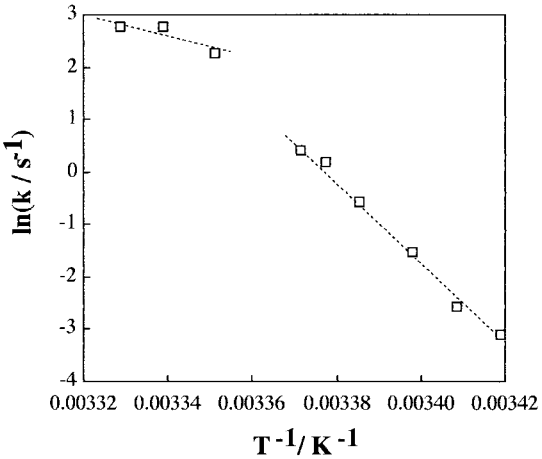

Figure 3. Arrhenius plot of ln k against 1/T for the light scattering increase due to the osmotic shrinkage of the pure DMPC LUVs. The rate constant of the scattering increase, k, was calculated by a first-order approximation.

To see the fast process around here, measurements at higher time resolution (1 ms scan^{-1}) were also carried out (Figure 2B). At T = 297.0 K (top) and 297.5 K (middle), a short time lag was observed within the first approximately 200 ms. After this time lag, the light scattering was increased to its terminal state. Such signals were reproducible throughout this series of measurements, and they were observed only in this temperature range; then it disappeared at T = 298.4 K (bottom). Above this temperature, all the signals also obeyed the faster first-order kinetics. The rate constant of the scattering increase, k, was calculated by the first-order approximation. For example, at T = 292.5 K, when the membrane is in the gel phase, k = 0.045 s^{-1}. On the other hand, for the fluid bilayer, e.g., at T = 299.5 K, k was approximately 16 s^{-1}. The Arrhenius plot of ln k against 1/T for this process is shown in Figure 3. The apparent activation energy, E_a, of the osmotic shrinkage in the gel phase was evaluated from the slope; E_a = 653 kJ mol^{-1}. E_a in the fluid phase seemed to be smaller than that in gel phase, although the exact value was not to be determined because of the experimental errors. Despite the different mechanism in the two different phases, it is noticeable that the osmotic shrinkage processes in both the gel phase and the fluid phase obey first-order kinetics.

The light scattering signals of DMPC LUVs doped with *trans*-8A5 are exhibited in Figure 4A. The molar concentration ratio of 8A5 to DMPC was 0.05. When 8A5 molecules were doped into DMPC LUVs, a fairly long delay was observed in the range $T < T_m$. For example, it lasts almost 3 s at T = 291.5 K. Then the signal was increased to its terminal value. On the other hand, concerning the fast process (Figure 4B), the scattering increase also obeyed the fast first-order kinetics. similar to DMPC LUVs when $T > T_m$. The signals with the short time lag were also observed around T_m (T_m = 296.1 K), whose shapes were very similar to that of DMPC LUVs. Figure 5A shows the light scattering signals of the LUVs with *cis*-8A5. In the range $T < T_m$, the signal did not obey first-order kinetics either, but the delays were rather short and fast compared with those observed in LUVs with *trans*-8A5. Around T_m (T_m = 295.2 K), the signal shapes were almost the same as those observed in Figures 2B and 4B, and the signal increase also obeyed the fast first-order kinetics in the range $T > T_m$ (Figure 5B).

Discussion

From the calorimetric studies, it has been demonstrated that 8A5 molecules act as strong substitutional impurities to the

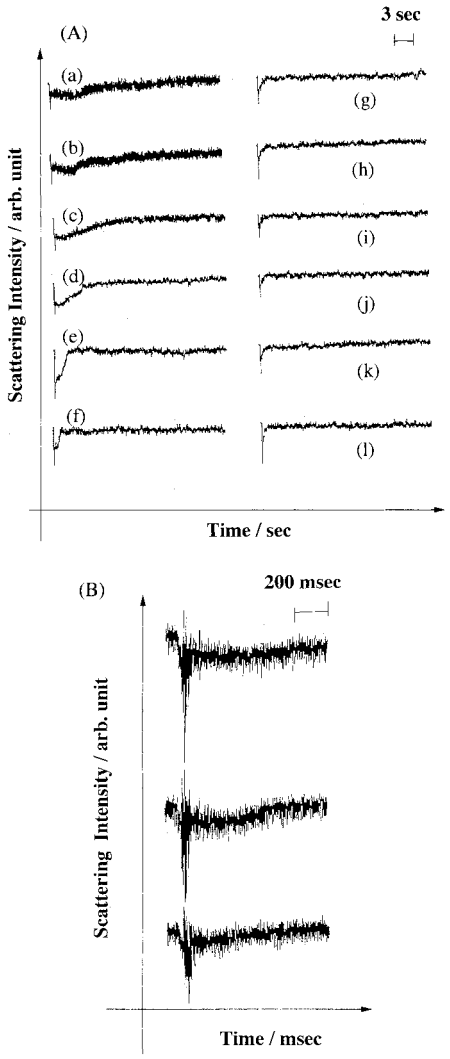

Figure 4. (A) Stopped-flow light scattering signals of the DMPC LUVs doped with *trans*-8A5. The concentrations of total lipid and betaine were 300 μM and 200 mM, respectively. [8A5]/[DMPC] = 0.05. Time constant was 16 ms scan^{-1}. Temperatures were (a) 291.5, (b) 292.4, (c) 293.4, (d) 294.3, (e) 295.4, (f) 296.0, (g) 296.6, (h) 297.1, (i) 297.5, (j) 298.5, (k) 299.5, and (l) 300.3 K. (B) Stopped-flow light scattering signals of the DMPC LUVs with *trans*-8A5 around the phase-transition temperature (T_m = 296.1 K). Time constant was 1 ms scan^{-1}. The samples were the same as in panel A. Temperatures were (top) 295.4, (middle) 296.0, and (bottom) 296.6 K.

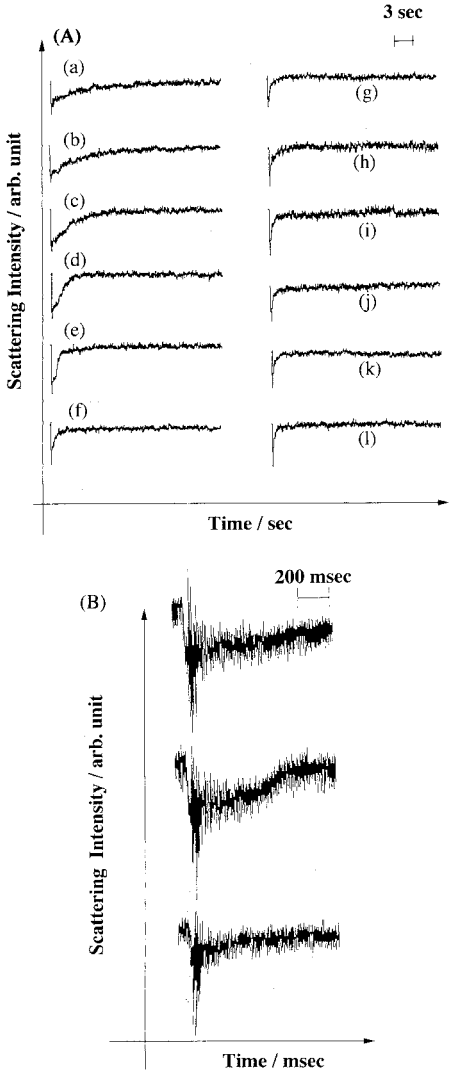

Figure 5. (A) Stopped-flow light scattering signals of the DMPC LUVs doped with *cis*-8A5. The concentrations of total lipid and betaine were 300 μM and 200 mM, respectively. [8A5]/[DMPC] = 0.05. Time constant was 16 ms scan^{-1}. Temperatures were (a) 291.8, (b) 292.7, (c) 293.5, (d) 294.4, (e) 295.3, (f) 296.0, (g) 296.5, (h) 297.0, (i) 297.7, (j) 298.5, (k) 299.5, and (l) 300.3 K. (B) Stopped-flow light scattering signals of the DMPC LUVs with *cis*-8A5 around the phase-transition temperature (T_m = 295.2 K). Time constant was 1 ms scan^{-1}. The samples were the same as in panel A. Temperatures were (top) 294.4, (middle) 295.3, and (bottom) 296.0 K.

structural ordering of the DMPC bilayers. Both the phase-transition temperature, T_m, and the phase-transition enthalpy, ΔH_m, were reduced by doping of 8A5 molecules, especially when they take the cis form. These results are considered with the "bulky" structure of *cis*-8A5 in the monolayer. However, the collapse of the DMPC monolayer doped with 8A5 molecules occurred in a different way. Despite the smaller area per one *trans*-8A5 molecule expected from the $\pi-A$ isotherm, the DMPC monolayer doped with *trans*-8A5 collapsed at significantly lower surface pressures, while the collapse pressure of the monolayer with "bulky" *cis*-8A5 was slightly higher than that of pure DMPC monolayer. It is difficult to define the exact physical meaning of the collapse pressure of the monolayer at the interface; however, we interpret at present that these different properties would reflect the variation in the bending elasticity of the monolayers induced by dopant molecules.

The osmotic behaviors of phospholipid liposomes have been often investigated by a turbidimetric method.[13−17] The relationship between the turbidity and the vesicle volume can be roughly represented as

$$dV/dt = k(d(1/Abs)/dt)$$

where V and Abs mean the vesicle volume and the absorbance at time t.[18] Though there have been several investigations of the osmotic behaviors and permeability of liposomes,[18−20] kinetic studies of the osmotic processes, especially in their earlier stages, remain difficult because it would be competitive with the mixing processes. In this sense, the stopped-flow light scattering method employed here has a promising possibility because the mixing process is almost neglectable. This method enables us to analyze the osmotic processes on a time scale of milliseconds.

A novel type of light scattering signal with a short time lag near the phase-transition temperature was observed for the first time. In the vicinity of the phase-transition region, it is generally accepted that the gel and fluid phases coexist in lipid bilayers.[21−23] The lipid bilayer fluctuations in this temperature range have

their most prominent macroscopic significance in terms of strong anomalies in thermodynamic response functions, especially apparent singularities in the specific heat[24,25] and the lateral area compressibility.[26] Recently, Jutila and Kinnunen supposed novel features of the main transition of DMPC bilayers by fluorescence spectroscopy.[27] Such observations are indicative that the main transition of lipid bilayers is very close to a critical point (pseudocritical).[25,28−30] In the case of the pure DMPC LUVs (Figure 2B), the time lags were seen in a very narrow temperature range around T_m (they were not to be seen at $T =$ 296.6 and 298.4 K), suggesting the close relationship to this phase-coexisting state. We assume these short time lags to be caused by thermodynamic lateral reordering in the membrane due to the imposed osmotic pressure difference, thus relating to anomalies in other thermodynamic response functions. Signals with very similar shapes were also obtained in the LUVs with 8A5 (Figures 4B and 5B) just around the phase-transition temperatures evaluated by the DSC analyses. However, fairly long delays were observed at lower temperatures, especially when 8A5 took the trans form. As shown in Figure 4A, the time lag lasts almost 3 s at $T = 292.5$ K. It is noticeable that this temperature is obviously lower than the peak temperature obtained by the DSC analysis, where even a slight increase was hardly observed. On the other hand, the shapes of the signals of the LUVs compared with that of *cis*-8A5 (Figure 5A) seem to be rather similar to those of pure DMPC LUVs. If these delays are regarded as the thermodynamic lateral reordering in the membrane as well, it is plausible that they would be dependent on the bending elasticity of the membrane, which was suggested by the collapse phenomena of the monolayers. In the fluid-phase membranes, it was difficult to see the quantitative difference between the water permeability of the pure DMPC LUVs, the LUVs with *trans*-8A5, and the LUVs with *cis*-8A5. The rate constant were almost the same order for both isomers. In this phase, the fast water permeation through the bulk lipid phase was dominant; hence, the effects of *cis*-8A5 on local packing of lipids, e.g., permeability of the membrane, should be hindered.

It had been reported[7,8] that the rate of trans-to-cis photoisomerization of 8A5 molecules in DMPC SUVs took its maximum around T_m. From these results, it was concluded that 8A5 molecules were located around the phase-separation boundaries. On the other hand, either in the gel or in the fluid phase, 8A5 molecules were distributed in the membrane homogeneously. To see the local circumstances around 8A5 molecules, we estimated the isomerization rate in the same manner in this LUVs system. As a result, we could see the same tendencies, indicating that 8A5 molecules also colocalize around the phase-separation boundaries between the gel and fluid phases.

Conclusions

Structural ordering of the DMPC membranes doped with *trans*-8A5 and *cis*-8A5 was investigated by the combination of static and kinetic studies. In the kinetic investigation using stopped-flow light scattering measurements, a novel type of signal with short time lags was first observed in the vicinity of the phase-transition temperatures, which were evaluated by the static DSC analyses. These time lags would be closely related to the structural reordering in the gel and fluid coexisting phase of lipid membranes. In the LUVs with *trans*-8A5, different types of signals with fairly long delays were observed, suggesting the effect of the bending elasticity of the membrane observed in the static study of the monolayers. The combined investigations of static and kinetic analyses demonstrated here would allow a better knowledge of the effect of doping and photoisomerization of the azobenzene derivatives on the structural ordering of the lipid membranes and, consequently, on their function.

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References and Notes

(1) Sackmann, E. In *Biological Membranes*; Chapman, D., Eds.; Academic Press: London, 1980; p 105.
(2) Kinnunen, P. K. J. *Chem. Phys. Lipids* **1991**, *57*, 375.
(3) Knoll, W.; Schmidt, G.; Rötzer, H.; Henkel, T.; Pfeiffer, W.; Sackmann, E.; Mittler-Nehr, S.; Spinke, I. *Chem. Phys. Lipids* **1991**, *57*, 363.
(4) Papahadjopulos, D.; Jacobsen, K.; Nir, S.; Isac, T. *Biochim. Biophys. Acta* **1973**, *311*, 330.
(5) Polymeropoulos, E. E.; Möbius, D. *Ber. Bunsen-Ges. Phys. Chem.* **1979**, *83*, 1215.
(6) Ahuja, R. C.; Maack, J.; Tachibana, H. *J. Phys. Chem.* **1995**, *99*, 9221.
(7) Tanaka, M.; Sato, T.; Yonezawa, Y. *Langmuir* **1995**, *11*, 2834.
(8) Tanaka, M.; Sato, T.; Yonezawa, Y. *Thin Solid Films* **1996**, *284−285*, 829.
(9) McCue, K.; Hanson, A. *Trends Biotechnol.* **1990**, *8*, 358.
(10) Atwood, D.; Saunders, L. *Biochim. Biophys. Acta* **1965**, *98*, 344.
(11) Chong, C. S.; Colbow, K. *Biochim. Biophys. Acta* **1976**, *436*, 260.
(12) MacDonald, R. C.; MacDonald, R. I.; Menco, B. M.; Takeshita, K.; Subbarao, N. K.; Hu, L. R. *Biomchim. Biophys. Acta* **1991**, *1061*, 297.
(13) (a) Tedeschi, H.; Harris, D. L. *Arch. Biochem. Biophys.* **1955**, *58*, 52. (b) Tedeschi, H.; Harris, D. L. *Biochim. Biophys. Acta* **1958**, *28*, 392.
(14) (a) Sidel, V. W.; Solomon, A. K. *J. Gen. Physiol.* **1957**, *41*, 243. (b) Sha'afi, R. I.; Rich, G. T.; Sidel, V. W.; Bossert, W.; Solomon, A. K. *J. Gen. Physiol.* **1967**, *50*, 1377.
(15) Bangham, A. D.; de Gier, J.; Greville, G. D. *Chem. Phys. Lipids* **1967**, *1*, 225.
(16) Kamino, K.; Inouye, A. *Biochim. Biophys. Acta* **1969**, *183*, 36.
(17) Bittman, R.; Blau, L. *Biochemistry* **1972**, *11*, 4831.
(18) de Gier, J. *Chem. Phys. Lipids* **1993**, *64*, 187.
(19) Carruthers, A.; Melchior, D. L. *Biochemistry* **1983**, *22*, 5797.
(20) White, G.; Pencer, J.; Nickel, B. G.; Wood, J. M.; Hallet, F. R. *Biophys. J.* **1996**, *71*, 2701.
(21) Marsh, D.; Watts, A.; Knowles, P. F. *Biochim. Biophys. Acta* **1977**, *465*, 500.
(22) Freire, E.; Biltonen, R. L. *Biochim. Biophys. Acta* **1978**, *514*, 54.
(23) Mouritsen, O. G.; Jørgensen, K.; Hønger, T. In *Permeability and Stability of Lipid Bilayers*; Dissalvo, E. A., Simon, S. A., Eds.; CRC Press: Boca Raton, FL, 1995; p 137.
(24) Albon, N.; Sturtevant, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2258.
(25) Biltonen, R. L. *J. Chem. Thermodyn.* **1990**, *22*, 1.
(26) Evans, E.; Kwok, R. *Biochemistry* **1982**, *21*, 4874.
(27) Jutila, A.; Kinnunen, P. K. J. *J. Phys. Chem. B* **1997**, *101*, 7635.
(28) Mouritsen, O. G.; Zuckermann, M. J. *Eur. Biophys. J.* **1985**, *12*, 75.
(29) Zhang, Z.; Laradji, M.; Guo, H.; Mouritsen, O. G.; Zuckermann, M. J. *Phys. Rev. A* **1992**, *45*, 7560.
(30) Zhang, Z.; Zuckermann, M. J.; Mouritsen, O. G. *Phys. Rev. A* **1992**, *46*, 6707.