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Pressure-induced phase transitions of lipid bilayers observed by fluorescent probes Prodan and Laurdan

Masataka Kusube, Nobutake Tamai, Hitoshi Matsuki, Shoji Kaneshina*

Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minamijosanjima, Tokushima 770-8506, Japan

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Abstract

The fluorescence spectra of 6-propionyl-2-(dimethylamino)naphthalene (Prodan) and 6-dodecanoyl-2-(dimethylamino)naphthalene (Laurdan) in bilayer membranes of 1,2-distearoylphosphatidylcholine (DSPC) were observed as a function of pressure at constant temperature. The emission spectra of Prodan and Laurdan varied with the pressure-induced states of bilayer membranes. The maximum emission wavelength (λ_{max}) of Prodan characteristic of the liquid crystalline (L_α), lamellar gel (L'_β) and pressure-induced interdigitated gel ($L_\beta I$) phases of the DSPC bilayer was 480, 440 and 500 nm, respectively. On the other hand, the λ_{max} of Laurdan characteristic of the L_α and L'_β phases was 480 and 440 nm in a similar manner as Prodan probe. However, no change in the λ_{max} was observed in spite of the occurrence of the interdigitation of bilayer. Since the λ_{max} reflects the solvent property around the probe molecules, we could speculate about the location of fluorescent probe in the bilayer membranes. In the L_α phase the same chromophore group of Prodan and Laurdan probes distributes around phosphate group of lipid (i.e., polar region). The transformation of bilayer into the L'_β phase causes the Prodan and Laurdan molecules to move into the glycerol backbone (i.e., less polar) region. In the ripple gel (P'_β) phase, the emission spectrum of Prodan shows a broad peak at about 480 nm and a shoulder around 440 nm, which means that the Prodan molecules are widespread over the wide range from the glycerol backbone to the hydrophilic part of bilayer. The $P'_\beta/L_\beta I$ phase transition causes the Prodan molecule to squeeze out from the glycerol backbone region and to move the hydrophilic region near the bilayer surface. Contrarily, the Laurdan molecule was not squeezed out from the glycerol backbone region because the long acyl chain of Laurdan serves as an anchor in the hydrophobic core of bilayer. The ratio of fluorescence intensity of Prodan at 480 nm to that at 440 nm, F_{480}/F_{440}, is available to observation of bilayer phase transitions. The plot of F_{480}/F_{440} versus pressure seems to be useful for the recognition of bilayer phase transition, especially the bilayer interdigitation.

Keywords: Fluorescence; Laurdan; Lipid bilayer; Phase transition; Pressure; Prodan

1. Introduction

High-pressure studies on lipid bilayer membranes have been started at first in the interest of a more complete understanding of pressure–anesthetic antagonism [1,2], and extended to pressure adaptation of deep-sea organisms [3] and sterilization by high pressure in food processing [4–9]. Such studies have concentrated on a few members of phosphatidylcholines containing two identical linear saturated fatty acyl chains and the thermotropic phase behavior of some phosphatidylcholines (especially dipalmitoylphosphatidylcholine, DPPC) has been relatively well understood. The succeeding high-pressure studies on the DPPC bilayer membrane have been performed with various physical techniques including electron spin resonance [10], dilatometry [11,12], calorimetry [13,14], X-ray diffraction [15], dynamic light scattering [16], Raman spectroscopy [17,18], FT-IR [19], neutron diffraction [20,21], light transmittance [22,23] and NMR [24–26]. These measurements have revealed that the temperature of main transition from the gel phase to the liquid crystalline phase is elevated linearly by applying pressure. Our previous studies have demonstrated the temperature–pressure phase diagrams of bilayer membranes of phospholipids with various acyl chain lengths

* Corresponding author. Tel.: +81 88 656 7513; fax: +81 88 655 3162.
E-mail address: kanesina@bio.tokushima-u.ac.jp (S. Kaneshina).

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[27–30], unsaturated acyl chains [31,32] and different polar head groups [33,34].

A fluorescence technique as well as other physical techniques has been used for high-pressure study on lipid bilayers and cellular membranes. In the previous studies, diphenylhexatriene [35,36], perylene [37], pyrene [38], dipyrenylpropane [39] and 12-(9-anthroyloxy) stearate [40] were used to show that pressure increases either the molecular order of the phospholipids or the membrane viscosity. Although much information has been derived from these studies, very little is known about the pressure dependence of probe location in bilayers. A lipid bilayer possesses a polar environment at water–lipid interface and a less polar environment in hydrocarbon core. A polarity-sensitive fluorescent probe may prove useful in elucidating the pressure dependence of probe location in bilayer membranes. For this purpose, a series of fluorescent probes placed at different depths of the bilayer interface has been used. The change of water content depending on the depths of bilayer may give rise to a different polarity of the solvent microenvironment. Since the lipid hydration is inherent in the stability of the lipid membrane in practical processes such as dehydration, lyophilization and freezing in which the lipid bilayer transforms to the gel phase, it is interesting to examine the behavior of bilayer interface by using the polarity-sensitive fluorescent probe.

In the present study, by using two kinds of fluorescent probes, 6-propionyl-2-(dimethylamino)naphthalene (Prodan) and 6-dodecanoyl-2-(dimethylamino)-naphthalene (Laurdan), we demonstrate how pressure affects the polarity and packing properties of the interfacial region included between the hydrocarbon core and the external phosphate groups in distearoylphosphatidylcholine (DSPC) bilayer membrane. Temperature–pressure phase diagram of DSPC bilayer membrane has already been described by our previous study [30].

2. Materials and methods

2.1. Materials

Synthetic DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, was obtained from Sigma-Aldrich Co. (St. Louis, MO) and used without further purification. The fluorescent probes of Prodan and Laurdan were obtained from Molecular Probes Inc. (Eugene, OR). Water was distilled twice from dilute alkaline permanganate solution. The multilamellar vesicles of DSPC containing Prodan and Laurdan were prepared by the following Bangham's method [41]. The chloroform stock solution of DSPC was mixed with the ethanol solution of a probe, and then the mixed solution was dried in vacuum to remove all residual solvents till it became a dry film. Distilled water was added to the dry film, and the suspension was stirred by a vortex mixer and sonicated at a temperature above the main-transition temperature of DSPC bilayer membrane. In all experiments, the total concentration of the lipid was 1.0 mmol kg^{-1} and the molar ratio of fluorescent probe to the lipid was 1 : 500.

2.2. Fluorescence measurements under high pressure

The fluorescence measurements under high pressure were carried out with Hitachi Fluorescence Spectrophotometer Model F-2500. A high-pressure cell assembly with quarts windows was supplied by Teramecs Co. (Kyoto). Pressure was generated by a hand-operated KP-3B hydraulic pump (Hikari High Pressure Instruments, Hiroshima) and measured within an accuracy of ±0.2 MPa by a Heise gauge. Temperature of the high-pressure cell was controlled within an accuracy of ±0.1 °C by circulating water from a water bath through the jacket enclosing the pressure cell. The sample solutions were compressed slowly and stepwise, that is, pressure was increased by approximately 10 MPa in each step at a desired temperature. The excitation wavelength was 361 nm and the emission spectra were obtained in the range of wavelengths from 390 to 550 nm.

3. Results and discussion

3.1. Fluorescent properties of probes

The maximum emission wavelengths (λ_{max}) of Prodan and Laurdan in various solvents are respectively shown in Fig. 1 as a function of solvent dielectric constant. The values of λ_{max} of Prodan and Laurdan in various solvents were in good agreement with each other. The λ_{max} of both probes in

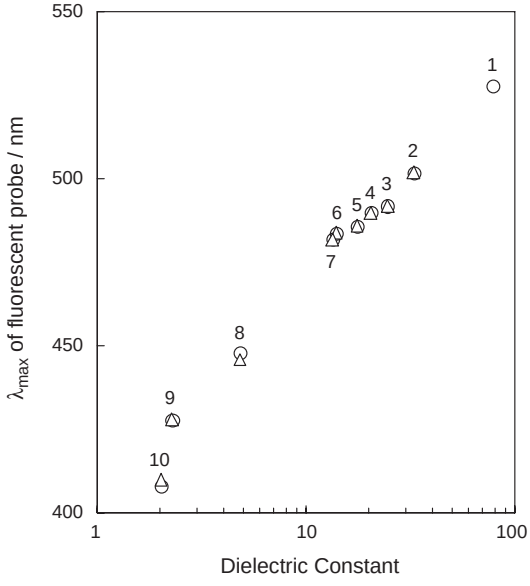

Fig. 1. Wavelength of fluorescence emission maximum for various solvents as a function of solvent dielectric constant at 25 °C and 0.1 MPa: (1) water, (2) methanol, (3) ethanol, (4) 1-propanol, (5) 1-butanol, (6) 1-pentanol, (7) 1-hexanol, (8) chloroform, (9) benzene and (10) cyclohexane. Circle and triangle indicate the fluorescence of Prodan and Laurdan, respectively.

water was found to be 528 nm while it was 428 nm in benzene. A good correlation was confirmed between the solvent polarity and the λ_{max} of Prodan and Laurdan because of their same chromophore group. A higher value of λ_{max} is attributed to probes in polar or hydrophilic environment while a lower value of λ_{max} is attributed to probes in less polar or hydrophobic environment. The emission maximum of probe-containing vesicles varied with states of the bilayer membranes. The variation of λ_{max} of probes in the bilayers associated with the pressure-induced phase transition probably responsible for a displacement of probe molecules in the bilayer membranes. A change of λ_{max} into the higher value (i.e., the red shift) corresponds to the movement of probes into polar or hydrophilic environment of the bilayers while change of λ_{max} into the lower value (i.e., the blue shift) corresponds to that of probes in less polar or hydrophobic environment of the bilayers. Therefore, it is possible to speculate the location of probes in bilayer membranes from the emission maxima.

3.2. Prodan fluorescence in DSPC bilayer

Fluorescence spectra of Prodan in the DSPC bilayer were observed as a function of pressure at 63.0 and 49.0 °C, which are shown in Figs. 2(A) and (B), respectively. The pressurizing process is shown in the phase diagram (Fig. 2(C)) [30] as broken lines. The DSPC bilayer undergoes two phase transitions with increasing pressure at 63.0 °C: one is the main transition from the liquid crystalline (L_α) phase to the ripple gel (P'_β) phase and the other is the transition from the P'_β phase to the interdigitated gel ($L_\beta I$) phase. The bilayer state corresponds to the L_α phase at low pressure below 30 MPa. The emission spectrum has a maximum at about 480 nm in the pressure range. At pressures between 40 and 80 MPa, namely, in the P'_β phase, the emission spectrum transforms the shape into a peak at about 480 nm and a shoulder around 440 nm. A broad peak of emission spectrum is observed at about 500 nm at higher pressures than 80 MPa at which the $L_\beta I$ phase is induced.

The emission spectra of Prodan at 49.0 °C are shown in Fig. 2(B). The DSPC bilayer undergoes the transition from the lamellar gel (L'_β) phase to the $L_\beta I$ phase with increasing pressure at the temperature. The emission spectrum shows a peak at about 440 nm and a shoulder around 480 nm in the L'_β phase of DSPC bilayer at lower pressures than 80 MPa. When the $L_\beta I$ phase appeared at higher pressures than 80 MPa, the emission spectrum became a broad peak at about 500 nm like the spectrum observed at 63.0 °C.

Since the emission spectrum of Prodan varies its shape depending on the bilayer phase states, the Prodan fluorescence method is useful for the discrimination between bilayer phases and for the determination of Prodan location in the bilayer.

3.3. Laurdan fluorescence in DSPC bilayer

Fluorescence spectra of Laurdan in the DSPC bilayer were also observed as a function of pressure at 62.0 and 50.0 °C and are shown in Figs. 3(A) and (B), respectively. The emission spectrum of Laurdan has a maximum at about 480 nm in the L_α phase of bilayer at low pressures below 30 MPa at 62°C. We found that there is a strong resemblance between emission spectra of Prodan and Laurdan in the L_α phase of DSPC bilayer. This fact means that the chromophore group for both probes are in the same location and/or a polar environment in the L_α phase. On the other hand, the

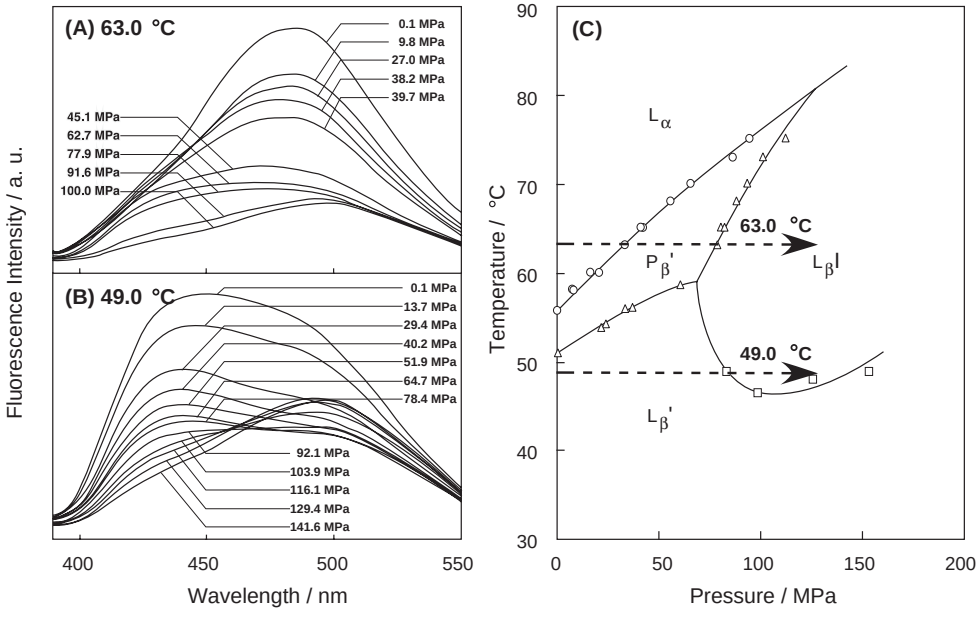

Fig. 2. Fluorescence spectra of Prodan in DSPC bilayer membrane at (A) 63.0 °C and (B) 49.0 °C. (C) Temperature–pressure phase diagram of DSPC bilayer, which is from Ichimori et al. (1998) [30]. Broken lines in the phase diagram mean pressurizing process.

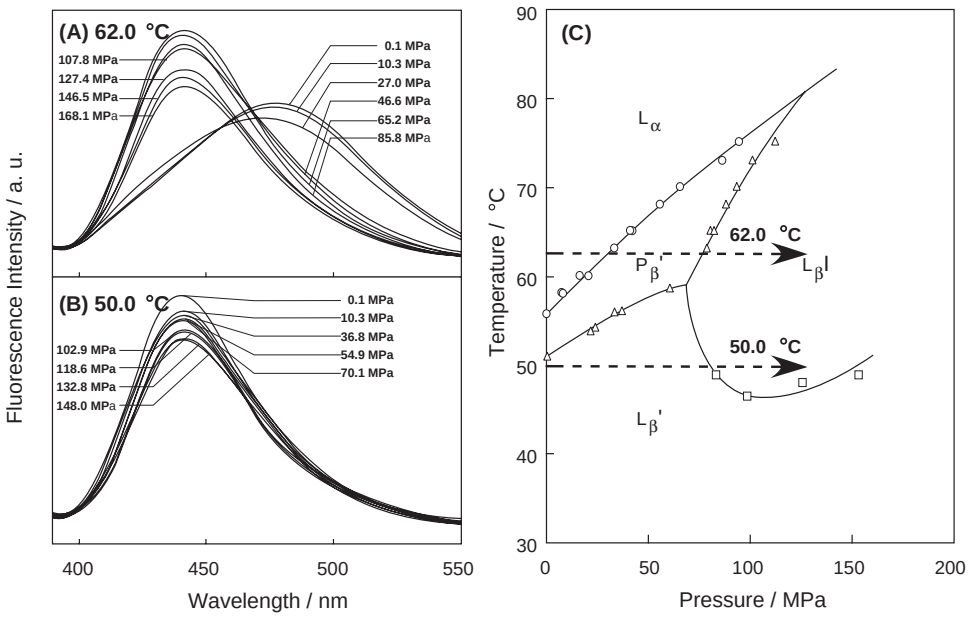

Fig. 3. Fluorescence spectra of Laurdan in DSPC bilayer membrane at (A) 62.0 °C and (B) 50.0 °C. (C) Temperature–pressure phase diagram of DSPC bilayer, which is from Ichimori et al. (1998) [30]. Broken lines in the phase diagram mean pressurizing process.

emission spectrum shows a sharp peak at about 440 nm at higher pressures than 40MPa. Although the DSPC bilayer undergoes the phase transition from the P'_β phase to the $L_\beta I$ phase at about 80 MPa at 62°C, no change of the emission maximum of Laurdan was observed. Moreover, the emission spectrum of Laurdan at 50.0 °C shows only a sharp peak at about 440 nm in the whole ranges of pressure up to 150 MPa as shown in Fig. 3(B). We also could not observe any change in the emission maximum in spite of the induction of phase transition from the L'_β phase to the $L_\beta I$ phase. The emission spectra in the L'_β and $L_\beta I$ phase are no longer distinguishable by the Laurdan fluorescence. Therefore, the Laurdan fluorescence method is available to the gel to liquid crystal phase transition only.

3.4. Location of probe molecules in DSPC bilayer

Location of probe molecules in lipid bilayers has been discussed on the basis of the fluorescent behavior by several researchers. Chong [42] observed that the emission spectrum of Prodan in dimyristoylphosphatidylcholine bilayer membrane exhibits two local maxima: one centers at around 435 nm and the other at about 510 nm. Pressure induces the relocation of Prodan molecules from the "polar" disposition (the 510 nm peak) to the "less polar" disposition (the 435 nm peak); Prodan favors a more hydrophobic environment under high pressure. Zeng and Chong [43] also revealed that the emission spectrum of the Prodan in DPPC bilayer membrane changes dramatically with the ethanol-induced phase transition from the noninterdigitated gel state to the fully interdigitated gel state. Spectral changes are attributed to the probe relocation from a less polar environment to a more polar environment due to the bilayer interdigitation. Bernik et al. [44] examined the fluorescence properties of probes inserted at different levels of bilayer interface and suggested that Prodan locates in the region between the ester carbonyls at glycerol backbone and the phosphate group. Regarding a polar environment in the lipid bilayers, Okamura and Nakahara [45] demonstrated that hydrated lipid bilayers could be divided into three zones. Zone I is the hydrophilic part of bilayer, which includes the polar head groups consisting of choline and phosphate groups. Zone II is the interfacial region between the hydrophilic and the hydrophobic parts of the lipid. Glycerol backbone and ester carbonyl group belong to this region. Zone III is the hydrocarbon chain region and composed by the bilayer core. They described how the dielectric constant ε or the water density ρ (H_2O) distributes along the lipid molecule in the bilayer. A significant decrease in ε or ρ (H_2O) with a large gradient is characteristic of the zone II.

We consider the location of the probe molecules in different phases of the DSPC bilayer on the basis of the present results and above discussion. The 440 nm peak of fluorescence spectra means the existence of probe molecules in less polar region of the lipid bilayer around glycerol backbone, the 480 nm peak does that in polar region of lipid bilayer around phosphate, and the 500 nm peak does that in hydrophilic region around lipid head group. This polar gradient in the bilayer is responsible to the amphiphilic structure of phospholipids. We suggested from the dramatic shifts of λ_{max} that the positioning of chromophore along the phospholipid molecule in lipid bilayer could be determined. The values of λ_{max} characteristic of the L'_β, L_α and $L_\beta I$ phase were 440, 480 and 500 nm, respectively. The location of the Prodan and Laurdan molecules in lipid bilayers on the basis of the above discussion is schematically depicted in Fig. 4.

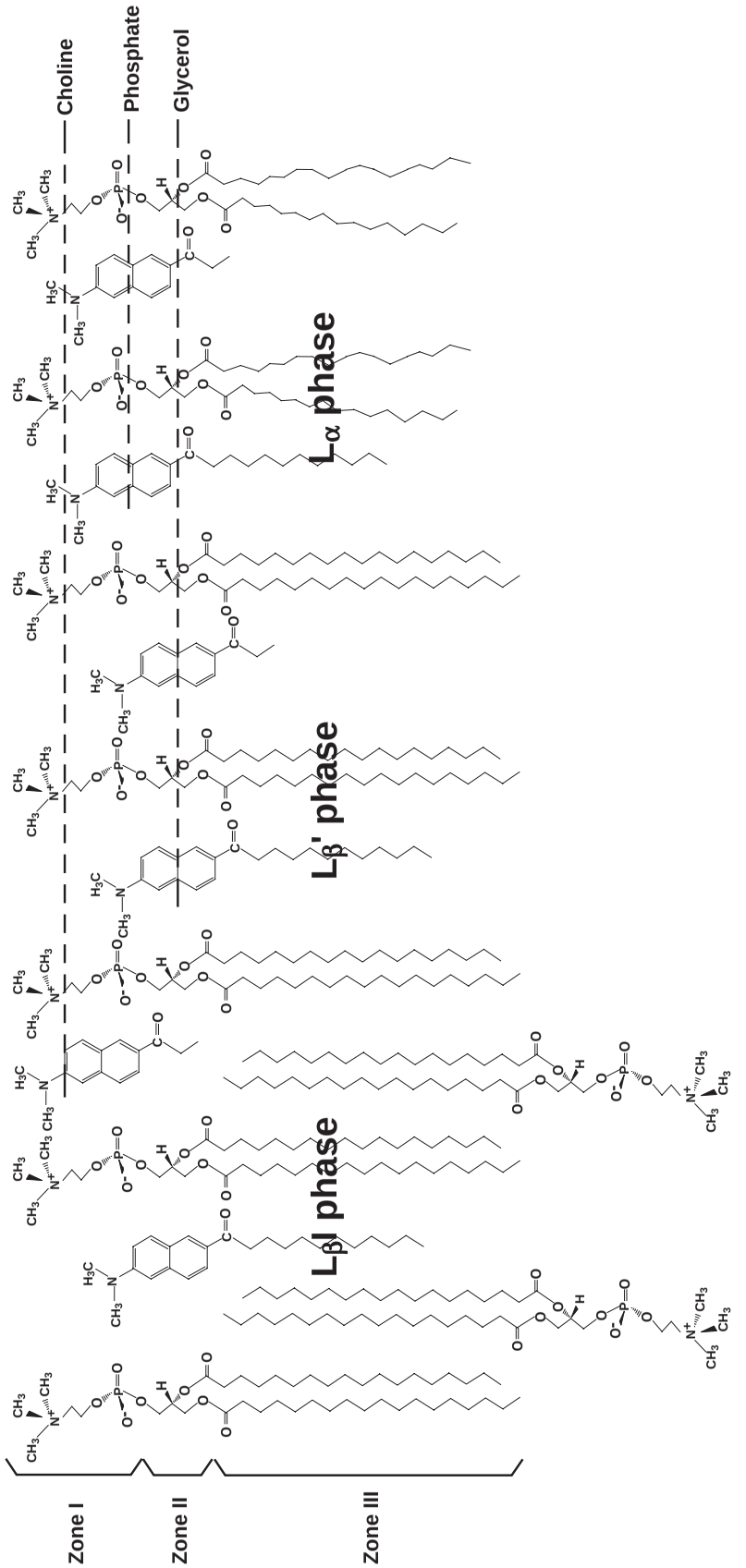

Fig. 4. Schematic drawing for the location of fluorescent probes in different phases of DSPC bilayer.

The same chromophore group of Prodan and Laurdan molecules distributes around phosphate group of lipid (zone I) in the L_α phase of DSPC bilayer. The L_α/P'_β phase transition causes the Prodan and Laurdan molecules to move into the glycerol backbone (less polar) region (zone II). In the P'_β phase of DSPC bilayer, the Prodan molecules are widespread in the region covering zones I and II, while the most Laurdan molecules are localized into zone II. As the pressure increases succeedingly, the pressure-induced interdigitation causes the Prodan molecule to squeeze out from the glycerol backbone region (zone II) and to move up to the hydrophilic region near the bilayer surface (zone I). However, the Laurdan molecule was not squeezed out from the glycerol backbone region because the long acyl chain of the Laurdan molecule serves as an anchor in the hydrophobic core of bilayer.

As is shown in Fig. 2, the emission spectra of Prodan in the L'_β and P'_β phases are slightly different: the emission spectrum shows a broad peak at about 440 nm and a shoulder around 480 nm in the L'_β phase, while a peak at about 480 nm and a shoulder around 440 nm are observed in the P'_β phase. The Prodan molecules in the L'_β phase as well as those in the P'_β phase are widely distributed between zones I and II. However, the distribution of Prodan into zone II is dominant in the L'_β phase. Since the emission maximum of Laurdan was no longer distinguishable in the L'_β, P'_β and $L_\beta I$ phases and observed at 440 nm as seen in Fig. 3, the Laurdan molecule in these phases of DSPC bilayer seems to be almost localized at zone II.

3.5. Observation of phase transition with fluorescence intensity

From Figs. 2 and 3, the emission spectra of Prodan in the DSPC bilayer during the phase transition may contain two peaks of similar intensities, making the selection of the emission maximum difficult. The ratio of fluorescence intensity at 480 nm to that at 440 nm, F_{480}/F_{440}, is available to determination of bilayer phase transition. The values of F_{480}/F_{440} were plotted against pressure in Fig. 5. In the figure are also shown the phase transition pressures by arrows, which were taken from the temperature–pressure phase diagram [30]. The values of F_{480}/F_{440} exhibit a sharp decrease and a subsequent plateau with increasing pressure, which corresponds to the phase transition from the L_α to the P'_β phase. As the pressure increases subsequently, the values of F_{480}/F_{440} show an abrupt increase at a certain pressure, which corresponds to the transition from the P'_β to the pressure-induced $L_\beta I$ phase, namely interdigitation of bilayer. Although the emission maximum characteristic of the $L_\beta I$ phase is found at 500 nm, the plot of F_{480}/F_{440} versus pressure for Prodan can be utilized for recognition of bilayer interdigitation because the 500 nm peak is broad and close to the 480 nm peak. On the other hand Laurdan could not be utilized for recognition of the interdigitation because no change of F_{480}/F_{440} associated with the bilayer interdigitation was observed.

In the previous studies, the pressure-induced interdigitation has been detected from the bilayer thickness determined by the X-ray diffraction [46,47] and the neutron diffraction methods [20,21]. The present results using Prodan fluorescence propose an easy and convenient recognition method for the pressure-induced interdigitation of lipid bilayers instead of the previous elaborate time-consuming methods.

4. Conclusions

Fluorescence spectra of Prodan in the DSPC bilayer membranes show the maximum emission wavelength

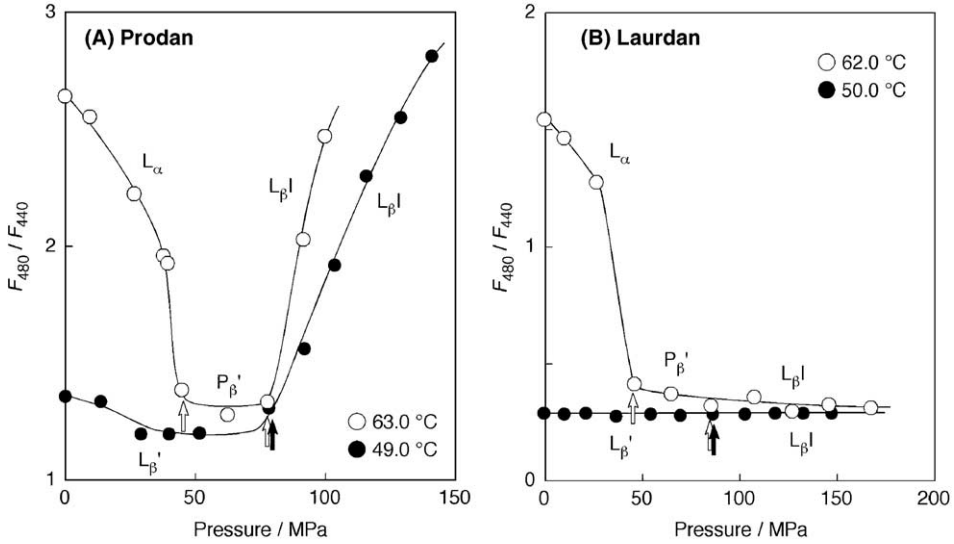

Fig. 5. Ratio of fluorescence intensity at 480 nm to that at 440 nm, F_{480}/F_{430} versus pressure. Fluorescence intensity ratio of (A) Prodan and (B) Laurdan. The phase–transition pressures, which were taken from the temperature–pressure phase diagram, are shown by arrows.

characteristic of the states of bilayers; (1) a 480 nm peak in the L_α phase, (2) a 480 nm peak and a 440 nm shoulder in the P'_β phase, (3) a 440 nm peak and a 480 nm shoulder in the L'_β phase and (4) a 500 nm broad peak in the $L_\beta I$ phase. On the other hand, fluorescence spectra of Laurdan in the DSPC bilayers show a 480 nm peak in the L_α phase and a 440 nm peak in the P'_β, L'_β and $L_\beta I$ phases. The positioning of probe molecules along the lipid molecule in the bilayer was determinable from the dramatic shift of maximum emission wavelength. The plot of F_{480}/F_{440} versus pressure for Prodan was useful for recognition of the bilayer interdigitation. On the other hand Laurdan could not be utilized for recognition of the interdigitation, although the fluorescence spectra of Laurdan were useful for discrimination between gel and liquid crystal phases.

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