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# Chain asymmetry alters thermotropic and barotropic properties of phospholipid bilayer membranes

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ABSTRACT

The alignment of the *sn*-1 and *sn*-2 acyl chains at the terminal methyl ends generally produces significant influence on the thermodynamic properties of the bilayer phase transitions. We investigated the bilayer phase behavior of asymmetric phospholipids, myristoylpalmitoylphosphatidylcholine and palmitoylmyristoylphosphatidylcholine, by high-pressure light-transmittance and Prodan-fluorescence techniques and differential scanning calorimetry. Constructed temperature–pressure phase diagrams revealed that no stable $L'_{\beta}$ phase can exist in the whole pressure range because of the formation of the most stable $L_c$ phase. Nevertheless, the pretransition, the detection of which is severely hampered by the exceptionally prompt formation of the $L_c$ phase, was successfully observed. Moreover, the effect of the total and difference of the *sn*-1 and *sn*-2 acyl chain lengths on minimal interdigitation pressure (MIP) was summarized in a MIP vs. chain-length inequivalence parameter plot, where the effect was proved to be classified in three zones depending on the alignment of both terminal methyl ends.



## 1. Introduction

Phospholipids found in most biological membranes have two different acyl chains attached on glycerol backbone in the molecules. Generally, the acyl chain in the *sn*-1 position is saturated, whereas that in the *sn*-2 position is usually unsaturated and has different numbers of carbon atoms from that in the *sn*-1 position. Such phospholipids are called asymmetric phospholipids (Huang and Mason, 1986). Some investigations on bilayer membranes of asymmetric phospholipids under ambient pressure have been performed by conventional differential scanning calorimetry (DSC) (Huang and Mason, 1986; Stümpel et al., 1983; Serrallach et al., 1984; Mattai et al., 1987), high-sensitivity DSC (Chen and Sturtevant, 1981; Lewis et al., 1994a), X-ray diffraction (Serrallach et al., 1984; Mattai et al., 1987; Lewis et al., 1994a), Raman and IR spectroscopy (Lewis et al., 1994a,b) and NMR (Lewis et al., 1984, 1994b). A consistent feature from all studies for asymmetric phosphatidylcholine (PC) bilayers is that the contributions of the two acyl chains to thermodynamic properties associated with bilayer phase transitions are not equivalent. In the case of the asymmetric PC with two saturated acyl chains, the bilayer of a lipid having a longer chain in the *sn*-2 position always exhibits significantly higher temperature and larger enthalpy change of the main transition than those for the isomer with the chain-positions reversed (Huang and Mason, 1986). The phenomena can be rationalized by the conformational difference between the *sn*-1 and *sn*-2 acyl chains of PCs. The terminal methyl groups of the *sn*-1 and *sn*-2 acyl chains are out of register by 1.5 carbon–carbon lengths along the long molecular axis because the acyl chain in the *sn*-2 position begins roughly parallel to the bilayer surface before bending at C-2 to orient the hydrocarbon chain perpendicular to the bilayer surface (Bultman et al., 1991). Saturated asymmetric PCs with a longer chain at *sn*-2 position reduce the intrinsic chain-length mismatch between the acyl chains in the *sn*-1 and *sn*-2 positions, while the mismatch is accentuated in the position-reversed isomer. Thus, the enhanced cohesive interaction between the two chains stabilizes the former bilayer and causes higher transition temperatures and larger enthalpy changes compared with the latter bilayer. In addition, it has been reported that the reduction of the effective chain-length mismatch between the *sn*-1 and *sn*-2 acyl chains promotes the formation of the subgel, namely lamellar crystal ($L_c$) phase and the interdigitated gel ($L_{\beta}I$) phase for bilayer membranes of asymmetric saturated PCs (Huang and Mason, 1986; Huang, 1990).

Our previous study (Goto et al., 2008) on the bilayer membranes of asymmetric PCs, palmitoylstearoyl-PC (PSPC) and stearoylpalmitoyl-PC (SPPC), has also shown that the contributions of two acyl chains to the thermodynamic properties associated with their bilayer phase transitions are not equivalent under high

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pressure as well as at ambient pressure. The *sn*-2 acyl chain length, and not *sn*-1 acyl chain length, governed primarily the bilayer properties such as transition temperature, enthalpy change and minimal pressure at which the interdigitation occurs. Further, when acyl chains are tilting in the gel phase, the bilayer of diheptadecanoyl-PC (C17PC), which is a symmetric PC with the same total chain length in the two acyl chains as PSPC and SPPC, showed the most stable acyl chain packing in the gel phase.

In the present study, we selected two different kinds of asymmetric PCs, myristoylpalmitoyl-PC (MPPC) and palmitoylmyristoyl-PC (PMPC), and investigated the thermotropic and barotropic phase behavior of their bilayers in order to expand the research on the bilayer phase behavior of asymmetric saturated PCs in a systematic way. MPPC and PMPC have shorter acyl chains than PSPC and SPPC, but as far as the effective chain-length mismatch between the *sn*-1 and *sn*-2 acyl chains is concerned, the former pair of the asymmetric PCs is the same as the latter pair. That is, the *sn*-1 and *sn*-2 acyl chains are out of register at the terminal methyl ends by 0.5 carbon–carbon length along the long molecular axis for MPPC and PSPC whereas by 3.5 carbon–carbon lengths for PMPC and SPPC (Serrallach et al., 1984). There are some studies dealing with the MPPC and PMPC bilayers (Chen and Sturtevant, 1981; Stümpel et al., 1983; Serrallach et al., 1984; Lin et al., 1991; Tristram-Nagle et al., 1999), which have already revealed fundamental but significant properties and structure of their bilayer, such as bilayer thickness and bilayer phase transitions at ambient pressure. Especially, the X-ray study (Serrallach et al., 1984) has firmly established the phase behavior at ambient pressure for those bilayers and also clearly revealed that the ripple gel ($P'_\beta$) and also the lamellar gel ($L'_\beta$) phases can be formed below the $L_c/P'_\beta$ transition temperature as a kind of supercooled state. However, no sufficiently clear DSC thermogram that well corresponds to the structural changes including the transitions between metastable phases has been obtained so far to the best of our knowledge. It should be noted that though the terms of "metastable phase" are not used in their report (Serrallach et al., 1984), we use the term "metastable phase" in this paper to designate phases formed at a certain temperature and pressure that are distinct from the most stable phase formed under the same condition. In addition, the bilayer phase behavior under high pressure has not yet been clarified and thus the information on the pressure-induced interdigitation of their bilayers is not accessible to us. Our aim of this paper is to clarify the bilayer phase behavior of MPPC and PMPC under high pressure and thereby to explain the stability of the gel phase associated with that of the $L_c$ phase. For this purpose, we constructed the temperature ($T$)–pressure ($p$) phase diagrams for the MPPC and the PMPC bilayers on the basis of the experimental data from high-sensitivity DSC and the high-pressure light-transmittance measurements, and the high-pressure fluorescence technique using 6-propionyl-2-(dimethylamino)naphthalene (Prodan) as a probe. We discuss the phase diagrams and the thermodynamic data, such as transition temperature and transition enthalpy and volume changes, by comparing them with those for bilayer membranes of symmetric PCs, dimyristoyl-PC (C14PC) and dipentadecanoyl-PC (C15PC).

## 2. Experimental

### 2.1. Materials

Asymmetric phospholipids, 1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine and 1-palmitoyl-2-myristoyl-*sn*-glycero-3-phosphocholine, were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and symmetric phospholipids, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine, were purchased from Sigma Chemical Co. (St. Louis, MO). They were used as received. A fluorescence probe, 6-propionyl-2-(dimethylamino)naphthalene, was obtained from Molecular Probes, Inc. (Eugene, OR). Water was distilled twice from a dilute alkaline permanganate solution. We employed two methods of vesicle preparation. One is a sonication method, which was used for DSC and light-transmittance measurements. Phospholipid multilamellar vesicles were prepared by suspending each phospholipid in water at 1.0 mmol $kg^{-1}$. The suspensions were sonicated for a few minutes by using a sonifier (Branson Model 3510J-DTH) and a cup horn at a temperature several degrees above the main-transition temperature for each lipid. These dispersions were annealed by repeating thermal cycling at least seven times between frozen storage at −30 °C (for one day) and cold storage at 5 °C (for one day) to induce the $L_c$ phase, and they were subsequently refrigerated. The other is the Bangham's method (Bangham et al., 1967) and used for fluorescence measurements. A chloroform stock solution of the lipid was mixed with an ethanol solution of Prodan. The mixed solution was dried in vacuum to remove all residual solvents and finally to get a dry film. Water was added to the dry film, and the lipid samples were hydrated by a treatment of vortex. The suspensions were sonicated at a temperature above the main-transition temperature for each lipid for a short time (ca. 3 min) in order to prepare the multilamellar vesicle suitable for the fluorescence measurements. The total concentration of a lipid was 1.0 mmol $kg^{-1}$ and the molar ratio of Prodan to the lipid was 1:500. The sample solutions were protected from light until measurements.

### 2.2. Differential scanning calorimetry

The phase transitions of multilamellar vesicles for all phospholipids under ambient pressure were observed by a MicroCal MCS high-sensitivity differential scanning calorimeter (Northampton, MA). The heating rate was 0.75 °C $min^{-1}$. The enthalpy changes of phase transitions were determined from the endothermic peak areas as average values over several DSC measurements by use of software Origin-DSC Data Analysis 2.9 (OriginLab Corp., Northampton, MA).

### 2.3. Light-transmittance measurements

The light-transmittance measurements at ambient and high pressures were carried out with a U-3010 spectrophotometer (Hitachi Co., Tokyo, Japan) equipped with a Model PCI-400 high-pressure cell assembly with two sapphire windows (Teramecs Co., Kyoto, Japan). The wavelength of the incident beam is 560 nm. The temperature of high-pressure cell was controlled within ± 0.1 °C by circulating water from a water bath through the jacket enclosing the measurement cell. Pressure was generated by a hand-operated KP-3B hydraulic pump (Hikari High Pressure Instruments, Hiroshima, Japan) and monitored within an accuracy of 0.2 MPa by using a Heise gauge (Heise Co., Newtown, CT). The first heating scan was performed at heating rate of 0.33 °C $min^{-1}$ from temperature below 20 °C and subsequently the second scan was started on the same condition immediately after the sample solution was cooled down below 20 °C. The transmittance vs. temperature profile obtained was differentiated with respect to temperature to determine the transition temperatures. An abrupt change of the transmittance in the original profile changes into a peak by differentiating the curve with respect to temperature and thus the transition temperature was determined as a peak temperature in the derivative curve. The pressure, at which the heating of high-pressure cell started, was recorded because the pressure of closed system was slightly elevated by the heating in the measurement. We used also a model 100-60 spectrophotometer (Hitachi Co., Tokyo Japan) in