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High-pressure study on bilayer phase behavior of oleoylmyristoyl- and myristoyloleoyl-phosphatidylcholines

Kaori Tada [a], Kensuke Saito [a], Masaki Goto [b], Nobutake Tamai [b], Hitoshi Matsuki [b,*], Shoji Kaneshina [b]

[a] Department of Biological Science and Technology, Faculty of Engineering, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan
[b] Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan

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ABSTRACT

We investigated the thermotropic and barotropic bilayer phase behavior of 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC) and 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (OMPC) by means of the differential scanning calorimetry (DSC) and high-pressure light-transmittance technique. Water could be used as a solvent for measurements at high pressures because of the elevation of the transition temperatures above 0 °C by pressurization, whereas aqueous 50 wt.% ethylene glycol solution was used mainly for those at low pressures. Only one phase transition was observed in the DSC thermogram of the MOPC bilayer membrane as an endothermic peak, and also observed at high pressures as an abrupt change of the light-transmittance. The transition was assigned as a main transition between the lamellar gel (L_{β}) and liquid-crystalline (L_{α}) phases on the basis of the values of enthalpy change (ΔH) and slope of the transition temperature with respect to pressure (dT/dP). The DSC thermogram of the OMPC bilayer membrane similarly showed a single endothermic peak but two kinds of phase transitions were observed at different temperatures in the light-transmittance profile at high pressures. The extrapolation of the lower-temperature transition in the high-pressure range to an ambient pressure coincided with the transition observed in the DSC thermogram. This transition was identified as a transition between the lamellar crystal (L_c) and L_{α} (or L_{β}) phases from the ΔH and dT/dP values. The higher-temperature transition, appearing only at high pressures, was identified as the L_{β}/L_{α} transition considering the topological resemblance of its temperature–pressure phase diagram as that of the dioleoylphosphatidylcholine bilayer membrane. The phase diagram of the OMPC bilayer membrane demonstrated that the L_{β} phase cannot exist at pressures below ca. 190 MPa while it can exist stably in a finite temperature range at pressures above the pressure.

1. Introduction

Biological membranes contain many kinds of phospholipids and the biological significance of the diversity of the lipid species is still a matter of great interest. Unsaturated phospholipids, which have mono- or poly-unsaturated acyl chains such as oleoyl-, linoleoyl- and arachidonoyl-chains, are known as major components of biomembranes. The bilayer phase behavior of unsaturated phospholipids has been intensely investigated. Lewis et al. [1] have demonstrated that the transition temperatures of bilayer membranes of the dioleoylphosphatidylcholine (DOPC) and the six kinds of its longer acyl-chain homologues are much lower than those of their saturated *n*-acyl counterparts. They have also revealed that the phase transition of the DOPC bilayer observed at −11.8 °C is a transition between the lamellar crystal (L_c) and liquid-crystalline (L_{α}) phases and not the main transition between the lamellar gel (L_{β}) and L_{α} phases. Our previous studies on the DOPC bilayer membrane [2–4] has shown that the L_{β} phase can exist as a stable phase at high pressures above ca. 300 MPa in aqueous 50 wt.% ethylene glycol solution and above 234 MPa in water. Further, the linear extrapolation of the high-pressure data to the atmospheric pressure has given not only the confirmation that the transition temperature of the L_c/L_{α} phase transition is about −12 °C at the atmospheric pressure, but also that the temperature of a *hypothetical* main transition at the atmospheric pressure is −40.3 °C. This temperature is 95 °C lower than that of the distearoylphosphatidylcholine (DSPC) bilayer membrane (55 °C).

An outstanding feature in naturally occurring unsaturated phospholipids is that they usually have one unsaturated acyl chain in the *sn*-2 position of the glycerol backbone and that almost all the double bonds have the *cis*-configuration [5]. Such phospholipids are generally classified as asymmetric unsaturated phospholipids. Despite the biological importance of why biomembranes show such configurational preference as well as the scientific interest in how the chain unsaturation and the asymmetry of the *sn*-1 and *sn*-2 acyl chains affect the membrane properties, only a few researches have been conducted [3,6–11]. Fundamental data are thought to be necessary in order to clarify the effect of the acyl-chain asymmetry on the membrane properties in a systematic way. Although the effect has been often dealt

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

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with for asymmetric phospholipids with two saturated acyl chains of different length in relation to the bilayer interdigitation [12], it has not yet been understood for those with a saturated and an unsaturated chain.

In this paper, we report on the thermotropic and barotropic phase behavior of the 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC) and 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (OMPC) bilayer membranes revealed by using differential scanning calorimetry (DSC) and high-pressure light-transmittance technique. The chemical structures of these phospholipids are illustrated in Fig. 1. In the case of symmetric saturated phospholipids, the *sn*-1 acyl chain is virtually longer than the *sn*-2 acyl chain by 1.5 carbon–carbon bond lengths due to the inherent difference of the conformation near the ester linkage on the glycerol backbone between the *sn*-1 and *sn*-2 acyl chains [13]. As seen from the figure, this inherent separation at the terminal methyl ends enhances the chain inequivalence for the OMPC molecule whereas it conversely reduces the inequivalence for the MOPC one. This means that MOPC and OMPC are just a pair of positional isomers but very different from each other in terms of the acyl-chain inequivalence. As far as we know, there is no report on the bilayer phase behavior of the MOPC and OMPC bilayer membranes. The lack of data on these lipids is mainly attributable to the experimental difficulty in detecting the lower phase-transition temperatures of these lipids than the freezing temperature of water. To prevent water from freezing we used aqueous 50 wt.% ethylene glycol solution as an antifreeze solvent. Ethylene glycol solution is generally used for studies on low-temperature transitions of phospholipid bilayer membranes. It is known for bilayer membranes of typical saturated PCs that the bilayer interdigitation is induced in the presence of 20–30 wt. % ethylene glycol [14]. For phospholipid bilayer membranes that are inherently difficult to transform into the interdigitated structure, such as unsaturated PC bilayers, however, its presence is believed to produce no drastic effect on the bilayer phase behavior itself though it surely tends to shift the transition temperature to slightly higher values and to bring about larger quantity of enthalpy change for the bilayer transition relating to the L_c phase [1,2].

The application of pressure produces several advantages. First, it allows us to determine the pressure dependence of the transition temperature (i.e., dT/dP). It is one of important thermodynamic properties because its value is closely related to the kind of the bilayer phase transition, such as the main transition and subtransition, as expected from the Clapeyron equation (dT/dP=$T\Delta V/\Delta H$). Second, the pressurization causes the elevation of the transition temperature, and the transition temperature below 0 °C at an ambient pressure can be shifted toward higher-temperature region above 0 °C at high pressures. This elevation allows us to use water as a solvent in the high-pressure region and to compare the results for the phospholipid bilayer systems in water and in ethylene glycol. This paper discusses the phase behavior by constructing the temperature–pressure phase diagrams and the thermodynamic properties of the phase transitions for the MOPC and OMPC bilayer membranes.

2. Experimental

2.1. Materials and sample preparation

Asymmetric unsaturated phospholipids, 1-myristoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (MOPC) and 1-oleoyl-2-myristoyl-*sn*-glycero-3-phosphocholine (OMPC), were purchased from Avanti Polar Lipids Inc. (Alabaster, AL) and used without further purification. Ethylene glycol (purity>99.5%) was purchased from Kanto Chemical Co., Inc. (Tokyo). A translucent phospholipid suspension was obtained by sonicating the mixture of an appropriate amount of the phospholipid sample and water purified by double distillation or aqueous 50 wt.% ethylene glycol solution for about 10 min. The lipid concentrations were adjusted to 10.0 mmol kg^{-1} (7.26×10^{-1} wt.%) in 50 wt.% ethylene glycol solution for the DSC measurements, and to 1.0 mmol kg^{-1} (7.26×10^{-2} wt.%) in water and 2.0 mmol kg^{-1} (1.45×10^{-1} wt.%) in 50 wt. % ethylene glycol solution for the light-transmittance measurements, respectively. The dispersion in ethylene glycol solution was usually more transparent than that in water at the same lipid concentration. In order to obtain the sufficient turbidity for the light-transmittance measurements, the lipid concentration in ethylene glycol solution was adjusted to be two times higher than that in water. All the sample solutions were prepared without thermal pretreatments, such as cold storage for a long time.

2.2. DSC and light-transmittance measurements

DSC measurements were carried out using an SSC 5200-DSC 120 calorimeter (SII Nanotechnology Co. Ltd., Chiba) with an externally attached equipment for cooling sample and reference solutions below 0 °C. A 60-μL aliquot of sample solution and an identical volume of solvent (reference solution) were separately poured into DSC silver cells, and the silver cells were sealed with silver lids. The sample and reference cells were kept standing at ca. −30 °C in the furnace chamber for 10 min or more before every scan for the equilibration. The heating rate was 0.30 K min^{-1}. The enthalpy changes of the bilayer phase transitions were determined from areas of endothermic peaks in the DSC thermograms and averaged over at least 5 scans.

Light-transmittance measurements were performed using a U-3010 spectrophotometer (Hitachi High-Technologies Corp., Tokyo) equipped with a high-pressure cell assembly PCI-400 (Teramecs, Kyoto). Pressure applied to the sample solution was generated by a

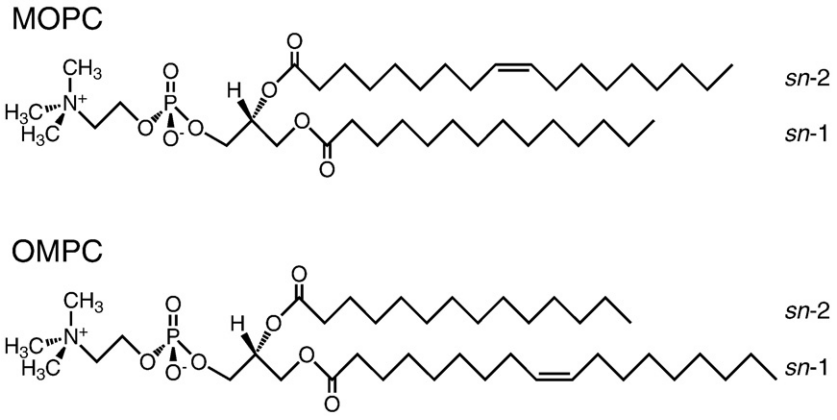

Fig. 1. Chemical structures of MOPC and OMPC molecules.