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Thermotropic and barotropic phase transitions of *N*-methylated dipalmitoylphosphatidylethanolamine bilayers

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Abstract

In order to understand the effect of polar head group modification on the thermotropic and barotropic phase behavior of phospholipid bilayer membranes, the phase transitions of dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidyl-*N*-methylethanolamine (DPMePE), dipalmitoylphosphatidyl-*N*,*N*-dimethylethanolamine ($DPMe_2PE$) and dipalmitoylphosphatidylcholine (DPPC) bilayer membranes were observed by differential scanning calorimetry and high-pressure optical methods. The temperatures of the so-called main transition from the gel (L_β) or ripple gel (P'_β) phase to the liquid crystalline (L_α) phase were almost linearly elevated by applying pressure. The slope of the temperature–pressure boundary, dT/dp, was in the range of 0.220–0.264 K MPa^{-1} depending on the number of methyl groups in the head group of lipids. The main-transition temperatures of *N*-methylated DPPEs decreased with increasing size of head group by stepwise *N*-methylation. On the other hand, there was no significant difference in thermodynamic quantities of the main transition between the phospholipids. With respect to the transition from the subgel (L_c) phase to the lamellar gel (L_β or L'_β) phase, the transition temperatures were also elevated by applying pressure. In the case of DPPE bilayer the L_c/L_β transition appeared at a pressure higher than 21.8 MPa. At a pressure below 21.8 MPa the L_c/L_α transition was observed at a temperature higher than the main-transition temperature. The main (L_β/L_α) transition can be recognized as the transformation between metastable phases in the range from ambient pressure to 21.8 MPa. Polymorphism in the gel phase is characteristic of DPPC bilayer membrane unlike other lipid bilayers used in this study: the L'_β, P'_β and pressure-induced interdigitated gel ($L_\beta I$) phases were observed only in the DPPC bilayer. Regarding the bilayers of DPPE, DPMePE and $DPMe_2PE$, the interdigitation of acyl chain did not appear even at pressures as high as 200 MPa.

Keywords: Bilayer membrane; *N*-methylated phosphatidylethanolamine; Phase transition; Phospholipid; Pressure; Vesicle

1. Introduction

The effect of pressure on lipid bilayer membranes and cellular membranes is of particular interest to studies of pressure–anesthetic antagonism [1], pressure adaptation in deep sea organisms [2], and high-pressure sterilization in food processing [3–6]. Lipid bilayer membranes composed of phosphatidylcholines (PCs) containing two identical linear saturated fatty acyl chains have been most thoroughly studied under high pressure [7–10]. PCs in vivo can be derived from sequential methylation of the amino group of phosphatidylethanolamines (PEs) by methyltransferases. Therefore, there exist two intermediates which contain one and two methyl groups in the ethanolamine moiety, respectively. Since the state of biological membranes is regulated not only through changes in the nature of the lipid acyl chains but also through changes in the head group, it is plausible that the partially methylated PE may play a role in the regulation of membrane state [11,12]. So far, bilayer membranes of *N*-methylated dipalmitoylphosphatidylethanolamines (DPPEs) have been studied with regard to the thermotropic phase behavior [13–15], sodium and glucose permeabilities [16], membrane fluidity [14,17], volume changes associated with the gel to liquid crystalline phase transition [18] and miscible behavior of *N*-methylated DPPE

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mixtures in the bilayers [15]. However, the effect of pressure on bilayer phase behavior of *N*-methylated DPPEs has not yet been confirmed. With respect to dipalmitoylphosphatidylcholine (DPPC), pressure studies on the bilayer phase transition have been reported by various physical techniques including ESR [19], dilatometry [20,21], calorimetry [22,23], X-ray diffraction [24], dynamic light scattering [25], Raman spectroscopy [26,27], adiabatic compression [28], fluorescence [29,30], FT-IR [31], neutron diffraction [32,33], light transmittance [34,35], and NMR [36–38]. These measurements have revealed the phase behavior of DPPC bilayer membranes. A new pressure-induced gel phase, i.e., the interdigitated gel phase, as well as the liquid crystal, ripple gel and lamellar gel phases has been observed under high pressure [7–10,32,33,35].

The present study demonstrates the pressure effect on the phase behavior of bilayer membranes of DPPE, dipalmitoylphosphatidyl-*N*-methylethanolamine (DPMePE), dipalmitoylphosphatidyl-*N*,*N*-dimethylethanolamine ($DPMe_2PE$) and DPPC, and reveals the effect of polar head group modification on the barotropic phase behavior of lipid bilayer membranes.

2. Experimental

2.1. Materials

Highly pure phospholipids, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (DPPE) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC, i.e., $DPMe_3PE$), were obtained from Sigma Chemical Co. (St. Louis, MO). Other lipids, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyl-*N*-methylethanolamine (DPMePE) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidyl-*N,N*-dimethylethanolamine ($DPMe_2PE$), were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). These phospholipids were used without further purification. Water was distilled twice from dilute alkaline permanganate solution. The phospholipid multilamellar vesicles were prepared by suspending each phospholipid in water at 0.5–1.0 mmol kg^{-1}, using a Branson model 185 sonifier at a temperature several degrees above the main transition for a short time (approximately 3 min) in order to prepare the multilamellar vesicle suitable for the optical measurements of phase transition. The average size of vesicles was found to be 200–300 nm, which was determined by a light scattering method.

2.2. Differential scanning calorimetry

Calorimetric scans were performed with a MicroCal MCS (Northampton, MA) highly sensitive differential scanning calorimeter at a heating rate of 0.75 K min^{-1}. The enthalpy changes of phase transitions were determined as an average value for several DSC measurements.

2.3. Phase transition measurements under high pressure

In order to transform completely into the subgel phase of lipid bilayer, vesicle suspensions were kept in a refrigerator (at about 5 °C) for 2 or 3 days and then transferred to a freezer (at about −20 °C). The thermo-cycle was repeated five times or more. The sample was periodically shaken by vortex during the storage to prevent from precipitating. Phase transitions under high pressure were observed by two kinds of optical methods. One is the observation of isothermal barotropic phase transition and the other is the isobaric thermotropic phase transition. A high-pressure cell assembly with sapphire windows, which was made of SUS 630 stainless steel supplied by Hikari High Pressure Instruments (Hiroshima, Japan), was connected to a spectrophotometer through an optical fiber. The light transmittance of the vesicle suspension was determined at a suitable interval of pressure (or temperature) by a Photal model IMUC 7000 spectrophotometer equipped with a photodiode array of 512 ch. (Otsuka Electronics, Osaka).

Pressures were generated by a hand-operated KP-3B hydraulic pump (Hikari High Pressure Instruments) and measured within an accuracy of 0.2 MPa by a Heise gauge. The temperature of the high-pressure cell was controlled by circulating water from a water bath through the jacket enclosing the pressure cell. In the isobaric thermotropic phase transition measurements, the abrupt change in transmittance accompanying the phase transition was followed at 560 nm. The heating rate at a given pressure was 0.33 K min^{-1}. In the isothermal barotropic phase transition, vesicle suspension was compressed slowly and stepwise, i.e., the pressure was increased by approximately 5 MPa in each step in the vicinity of the phase transition, and allowed to stand for 15 min. in each step.

3. Results and discussion

3.1. Phase transitions of DPPE bilayer membrane

The heating DSC thermograms of DPPE bilayer membrane showed two kinds of endothermic transitions (curve 1 in Fig. 1). Higher-temperature transition obtained by the first scan can be assigned as the transition from the subgel or lamellar crystal (L_c) phase to the liquid crystalline (L_α) phase. On the other hand, lower-temperature transition observed by the second scan can be assigned as the main transition from the gel (L_β) phase to the L_α phase. In the figure are also included the results of *N*-methylated DPPE bilayers. The DSC thermograms of DPMePE and $DPMe_2PE$ bilayer membranes showed two endothermic transitions (curves 2 and 3 in Fig. 1). Higher-temperature transition can be assigned as the main transition from the L_β phase to the L_α phase, which was in good agreement with previous observation [11–14]. Lower-temperature transition was observed newly after cold storage and was not observed