

# Bilayer phase transitions of *N*-methylated dioleoylphosphatidylethanolamines under high pressure

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Received 21 November 2005; received in revised form 12 March 2006; accepted 15 March 2006

Available online 4 April 2006

## Abstract

The bilayer phase transitions of four kinds of unsaturated phospholipids with different-sized polar head groups, dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidyl-*N*-methylethanolamine (DOMePE), dioleoylphosphatidyl-*N,N*-dimethylethanolamine (DOME<sub>2</sub>PE) and dioleoylphosphatidylcholine (DOPC), were observed by means of differential scanning calorimetry (DSC) and high-pressure light-transmittance. DSC thermogram and light-transmittance curve for each phospholipid vesicle solution exhibited only one phase transition under ambient pressure, respectively. The light-transmittance of DOPC solution at pressure higher than 234 MPa abruptly increased stepwise at two temperatures, which corresponds to the appearance of stable subgel and lamellar gel phases under high pressure in addition to the liquid crystal phase. The constructed temperature (*T*)–pressure (*p*) phase diagrams were compared among these phospholipids. The phase-transition temperatures of the phospholipids decreased stepwise by *N*-methylation of the head group. The slopes of the *T*–*p* phase boundary ( $dT/dp$ ) of DOPE, DOMePE and DOME<sub>2</sub>PE bilayers (0.127–0.145 K MPa<sup>-1</sup>) were found to be close to that of the transition from the lamellar crystal (or subgel; L<sub>c</sub>) phase to the liquid crystal (L<sub>α</sub>) phase for DOPC bilayer (0.131 K MPa<sup>-1</sup>). On the other hand, the  $dT/dp$  value of the main transition from the lamellar gel (L<sub>β</sub>) phase to the L<sub>α</sub> phase for DOPC bilayer (0.233 K MPa<sup>-1</sup>) was significantly different from that of the L<sub>c</sub>/L<sub>α</sub> transition, hence both curves intersected with each other at 234 MPa. The thermodynamic quantities associated with the phase transition of DOPE, DOMePE and DOME<sub>2</sub>PE bilayers had also similar values to those for the L<sub>c</sub>/L<sub>α</sub> transition of DOPC bilayer. Taking into account of the values of transition temperature,  $dT/dp$  and thermodynamic quantities compared with the corresponding results of saturated phospholipids, we identified the phase transitions observed in the DOPE, DOMePE and DOME<sub>2</sub>PE bilayers as the transition from the L<sub>c</sub> phase to the L<sub>α</sub> phase although they have been regarded as the main transition in the previous studies. The L<sub>β</sub> phase is probably unstable for DOPE, DOMePE and DOME<sub>2</sub>PE bilayers at all pressures, it exists as a metastable phase at pressures below 234 MPa while as a stable phase at pressures above 234 MPa in DOPC bilayer. The difference in phase stability among the phospholipid bilayers is originated from that in hydration structure of the polar head groups.

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**Keywords:** Bilayer membrane; *N*-Methylation; Phase transition; Pressure; Unsaturated phospholipid; Vesicle

## 1. Introduction

Biological membranes of organisms living at lower temperatures or high pressures contain a great number of phospholipids with unsaturated fatty acids as hydrophobic acyl chains. Since the melting points of unsaturated

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fatty acids are much lower than those of saturated fatty acids (Quinn et al., 1989), unsaturated phospholipids are believed to play an important role for controlling membrane fluidity (Ichimori et al., 1999; Kaneshina et al., 1998, 2003; Matsuki et al., 2003; Mentré and Hoa, 2001; Siminovitch et al., 1987). The introduction of *cis* double bond into acyl chains of phospholipids actually causes the decrease in the temperature of main transition from the gel phase to the liquid crystal phase down to less than below 0 °C at ambient pressure (Barton and Gunstone, 1975; Davis and Keough, 1983; Lewis et al., 1988). Hence, it is usually difficult to detect phase transitions of unsaturated phospholipids by conventional techniques due to the freezing of solvent water. Differential scanning calorimetric (DSC) measurements on liposomes of unsaturated phospholipids at lower temperatures have been often performed in solution containing ethylene glycol as an antifreeze in order to avoid the water freezing (Newman et al., 1975). We have shown that phase transitions undergone at a low temperature below 0 °C can be easily observed by applying pressure without additives because water does not freeze at 0 °C under high pressure and the transition temperatures increase up to the observable temperature range (Kaneshina et al., 1998). The pressure study of phospholipids bilayers is a highly effective means of investigating the phase behavior of unsaturated phospholipids.

On the other hand, the difference in polar head groups among phospholipids greatly influences membrane properties, especially, the packing of bilayer (Berde et al., 1980; Cevc et al., 1986; Gruner et al., 1988; Ho et al., 1995). Recently, we have revealed (Kusube et al., 2005) that the difference in polar head group size of saturated phospholipids also provides significant influence on the barotropic phase transitions of bilayer membranes. It is now interesting to see how the bilayer phase transitions of unsaturated phospholipids under high pressure vary with the size of polar head group. There have been no reports on high-pressure studies of unsaturated phospholipids with different-sized head groups.

In the present study, we report the pressure effect on bilayer phase transitions of four kinds of unsaturated phospholipids, dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidyl-*N*-methylethanolamine (DOMePE), dioleoylphosphatidyl-*N,N*-dimethylethanolamine (DOME<sub>2</sub>PE) and dioleoylphosphatidylcholine (DOPC). The bilayer phase transitions of *N*-methylated DOPEs under high pressure are discussed from the constructed temperature–pressure phase diagrams and the comparison with corresponding results of saturated phospholipids (Kusube et al., 2005).

## 2. Materials and methods

### 2.1. Sample preparations

1,2-Dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) were purchased from Sigma Chemical Co. (St. Louis, MO). The intermediate *N*-methylated DOPEs; 1,2-dioleoyl-*sn*-glycero-3-phosphatidyl-*N*-methylethanolamine (DOMePE) and 1,2-dioleoyl-*sn*-glycero-3-phosphatidyl-*N,N*-dimethylethanolamine (DOME<sub>2</sub>PE) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). These phospholipids were used without further purification. The phospholipid vesicle was prepared by sonication using a Branson model 185-sonifier at several temperatures above the main transition of each phospholipid in order to prepare the multilamellar vesicle suitable for the phase transition measurements by the optical method (Kaneshina et al., 1998; Maruyama et al., 1996). The sample solutions of phospholipids were prepared at 1.0 or 5.0 mmol kg<sup>-1</sup> in water which is distilled twice after a de-ionized treatment. The solution was kept in freeze storage at -20 °C in a freezer and sonicated for a short time after the melting of frozen sample in ice water. DOPC was also dispersed in 50% ethylene glycol aqueous solution to induce the lamellar crystal phase and the pretreatment prior to measurements was carried out as mentioned above.

### 2.2. Differential scanning calorimetric (DSC) measurements

DSC measurements were performed by using a SSC 5200-DSC 120 calorimeter (SII Nanotechnology Co. Ltd., Chiba). Vesicle solutions prepared at 5.0 mmol kg<sup>-1</sup> were sealed up to the amount of 60 μl in a DSC silver cell. Water or 50% ethylene glycol aqueous solution was used as the reference solution. The measurements were carried out at the heating rate of 0.2 or 0.5 K min<sup>-1</sup>. The enthalpy changes of phase transitions were determined as an average value from peak areas of the transitions in several DSC measurements.

### 2.3. Light-transmittance measurements

Phase transitions of bilayer membranes under ambient and high pressure were observed by two kinds of optical measurements, isobaric thermotropic and isothermal barotropic determinations, which were described previously (Maruyama et al., 1996). The pressures were monitored within an accuracy of 0.2 MPa by using a

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