



Thermotropic and barotropic phase transitions on diacylphosphatidylethanolamine bilayer membranes

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ABSTRACT

The bilayer phase transitions of four diacylphosphatidylethanolamines (PEs) with matched saturated acyl chains ($C_n = 12, 14, 16$ and 18) and two PEs with matched unsaturated acyl chains containing a different kind of double bonds were observed by differential scanning calorimetry under atmospheric pressure and light-transmittance measurements under high pressure. The temperature–pressure phase diagrams for these PE bilayer membranes were constructed from the obtained phase-transition data. The saturated PE bilayer membranes underwent two different phase transitions related to the liquid crystalline (L_α) phase, the transition from the hydrated crystalline (L_c) phase and the chain melting (gel (L_β) to L_α) transition, depending on the thermal history. Pressure altered the gel-phase stability of the bilayer membranes of PEs with longer chains at a low pressure. Comparing the thermodynamic quantities of the saturated PE bilayer membranes with those of diacylphosphatidylcholine (PC) bilayer membranes, the PE bilayer membranes showed higher phase-transition temperatures and formed more stable L_c phase, which originates from the strong interaction between polar head groups of PE molecules. On the other hand, the unsaturated PE bilayer membranes underwent the transition from the L_α phase to the inverted hexagonal (H_{II}) phase at a high temperature and this transition showed a small transition enthalpy but high pressure-responsivity. It turned out that the kind of double bonds markedly affects both bilayer–bilayer and bilayer–nonbilayer transitions and the L_α/H_{II} transition is a volume driven transition for the reconstruction of molecular packing. Further, the phase-transition behavior was explained by chemical potential curves of bilayer phases.

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1. Introduction

Glycerophospholipids are main constituent lipids in biological membranes. There are so many glycerophospholipids with different hydrophobic acyl chains and a different hydrophilic polar head group. Phosphatidylcholines (PCs) are widely distributed in biological membranes and the bilayer aggregates such as vesicles and liposomes formed by PC molecules in the aqueous solution are frequently used as model biomembranes. PC bilayer membranes take several membrane states like gel and liquid crystalline phases depending on the orientation or packing of the PC molecules in the bilayer membrane. They vary between membrane states by structural changes called phase transitions. Reports on temperature-induced structural changes, thermotropic phase transitions, of PC bilayer membranes have been made in large numbers [1–3], whereas those on pressure-induced changes, barotropic phase transition, have been made in very limited numbers [4–14]. We have focused our attention on the pressure effect of lipid bilayer

membranes and investigated the bilayer phase transitions of various kinds of PCs with different molecular structures such as chain length, chain unsaturation, chain asymmetry and linkage to a glycerol backbone [15–22]. Thereby, the phase transitions of these PC bilayer membranes depending on temperature and pressure have been elucidated thermodynamically.

In addition to PCs, there are other principal glycerophospholipid species, phosphatidylethanolamines (PEs). PE universally exists in biological membranes from prokaryotes like *Escherichia coli* to eukaryotes like mammals although PC does not in those of prokaryotes. PE has a small-sized polar head group, ethanolamine ($-\text{CH}_2-\text{CH}_2-\text{N}^+\text{H}_3$) group, in contrast with PC with a large-sized head group, choline ($-\text{CH}_2-\text{CH}_2-\text{N}^+(\text{CH}_3)_3$) group. It has been reported that bilayer membranes formed by PEs with saturated fatty acids as acyl chains, which we call them saturated PEs here, exhibit gel and liquid crystalline phases similar to those formed by PCs with the same chains, but the membrane states are considerably different from each other [23–34]. On the other hand, PEs with unsaturated fatty acids as acyl chains, which we call them unsaturated PEs here, form one of nonbilayer phases called an inverted hexagonal phase at higher temperatures beyond the temperature range of the liquid crystalline phase [35–46], the structure of which cannot be formed

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in PC bilayer membranes. It is considered that the inverted hexagonal structure is partially and transiently formed in various cell or organelle membranes and plays an important role as local and transitional intermediates in cellular process related to morphological changes of membranes such as membrane fusion and fission, membrane transport as exo- and endocytosis [45,47–49].

There are many reports on thermotropic phase transitions of PE bilayer membranes though, barotropic phase-transition data are quite lacking and only a few data concerning the pressure effect on the bilayer phase transition of certain PEs have been reported by some groups including us [4,46,50–52]. Hence, the systematic study of the pressure effect on PE bilayer membranes remains to be made. In the present study, for the purpose of examining the phase transitions for the PE bilayer membranes under high pressure, we selected four diacylphosphatidylethanolamines (PEs) with matched saturated acyl chains, dilauroylphosphatidylethanolamine (12:0-PE), dimyristoylphosphatidylethanolamine (14:0-PE), dipalmitoylphosphatidylethanolamine (16:0-PE) and distearoylphosphatidylethanolamine (18:0-PE), and two PEs with matched unsaturated acyl chains containing a different kind of double bonds, dioleoylphosphatidylethanolamine (18:1(*cis*)-PE) and dielaidoylphosphatidylethanolamine (18:1(*trans*)-PE). The phase transitions of these PE bilayer membranes were observed as a function of temperature and pressure by differential scanning calorimetry (DSC) and high-pressure light transmittance measurement. By using the phase-transition data obtained, the thermotropic and barotropic phase transitions of the PE bilayer membranes are characterized from the temperature-pressure phase diagrams and the thermodynamic quantities of phase transitions. Furthermore, comparing the above results with the corresponding results of PCs with the same acyl chains as these PEs [16,17,22], the similarity and difference in phase transitions between the PE and PC bilayer membranes are also discussed.

2. Experimental

2.1. Materials and sample preparation

Four synthetic phosphatidylethanolamines with different linear saturated acyl chains, 12:0-PE (1,2-didodecanoyl-*sn*-glycero-3-phosphoethanolamine), 14:0-PE (1,2-ditetradecanoyl-*sn*-glycero-3-phosphoethanolamine), 16:0-PE (1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine) and 18:0-PE (1,2-dioctadecanoyl-*sn*-glycero-3-phosphoethanolamine), and two PEs with different kinds of unsaturated acyl chains, 18:1(*cis*)-PE (1,2-di[*cis*-9-octadecenoyl]-*sn*-glycero-3-phosphoethanolamine) and 18:1(*trans*)-PE (1,2-di[*trans*-9-octadecenoyl]-*sn*-glycero-3-phosphoethanolamine), were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). They were directly used without further purification. Ligroin (petroleum fraction consisting mostly of C₇ and C₈ hydrocarbons: Kanto Chemical Co., Inc., Japan) was used as a pressure-medium. Water was distilled twice after a deionization, where the second step was done from dilute alkaline permanganate solution.

The multilamellar vesicle dispersions were prepared by suspending each PE in water using a vortex mixer at a concentration of 1.0 mmol kg⁻¹ for PEs with saturated acyl chains and at a concentration of 5.0 mmol kg⁻¹ for PEs with unsaturated acyl chains. They were sonicated for a few minutes by using a Branson model 185 sonifier at a temperature several degrees above the L_β/L_α-transition temperature of each lipid. Then, the dispersions were allowed to stand at about -20 °C for at least 24 h to form the hydrated crystalline phase. For the 16:0-PE and 18:0-PE bilayer membranes, a special pretreatment called thermal annealing was made because it is difficult to induce the hydrated crystalline phase in bilayer membranes of phospholipids with long saturated acyl chains [22,53,54]. Both PE dispersions were annealed by seven thermal cycles, where 1 thermal cycle comprises high-temperature

storage at 60 °C (16:0-PE) or 70 °C (18:0-PE) for one hour, and freezing storage at -15 °C for more than 12 h. All PE dispersions were sonicated again in a very short time at a designated temperature to adjust the size of multilamellar vesicles suitable for the phase-transition observation of each lipid before experiments.

2.2. DSC measurements

The phase transitions of PE bilayer membranes under atmospheric pressure were observed by use of high-sensitivity differential scanning calorimeters, MCS-DSC (MicroCal, Northampton, MA, USA) and VP-DSC (Malvern Instrum. Ltd., Worcestershire, UK). After a degas treatment of 10–15 min for sample and reference solutions, the measurements were started with a heating rate of 0.75 K min⁻¹. The endothermic peaks in the DSC thermograms were analyzed by use of software Origin 7.0 (Lightstone Corp., Tokyo, Japan). DSC measurements of the 18:1(*cis*)-PE bilayer membrane at low temperatures below the freezing point of water were also performed by using a SSC 5200-DSC 120 calorimeter (SII Nanotechnology Co. Ltd., Chiba, Japan). The prepared sample and reference solutions were sealed up to the amount of 60 μl in DSC silver cells. After reaching thermal equilibrium, the measurements were carried out under a heating rate of 0.3 or 0.5 K min⁻¹. The endothermic peaks were analyzed by attached software for the apparatus. The thermal quantities were averaged with the standard deviations from at least triplicate measurements.

2.3. High-pressure light transmittance measurements

Since the refractive index of a dilute vesicle solution noticeably changes at a phase transition, we adopted light-transmittance measurements as the phase-transition observation under high pressure. The phase transitions under high pressure were observed by a light transmittance technique using a high-pressure cell assembly PCI-400 (or PCI-500) (Syn. Corp., Kyoto, Japan) attached to U-3010 (or U-3900) spectrophotometer (Hitachi High-Technology Corp., Tokyo, Japan). Two kinds of light-transmittance measurements developed in our laboratory were made; one is an isobaric thermotropic observation by scanning temperature at constant pressure and the other is an isothermal barotropic observation by scanning pressure at constant temperature. Pressures were generated by a hand-operated HP-500 hydraulic pump (Syn. Corp., Kyoto, Japan) and monitored using a Heise gauge with an accuracy of 0.2 MPa. The temperature of the pressure cell was controlled by circulating thermostated water from a water bath through a jacket enclosing the cell. Abrupt changes in transmittance of monochromatic light (wavelength 560 nm) were observed at phase transitions and the phase-transition temperatures or pressures were determined from the inflection points in the transmittance vs. temperature or pressure curve. The heating rate at a given pressure in the isobaric thermotropic measurements and the pressurizing rate at a given temperature were 0.5 K min⁻¹ and 20 MPa (5 MPa in the vicinity of the phase transition) with a 15-min interval, respectively. The measurements were performed at least three times under the isobaric or isothermal conditions. The detailed procedures for the light transmittance measurements were described elsewhere [15,17,20].

3. Results

3.1. Thermal behavior of diacyl-PE bilayer membranes under atmospheric pressure

The DSC thermograms of all diacyl-PE bilayer membranes obtained by a heating scan are presented in Fig. 1. We observed two kinds of endothermic peaks in the thermograms of the saturated PE bilayer membranes depending on the thermal history of a lipid sample. One large endothermic peak was obtained in the first heating scan after freezing storage. But in the subsequent scan (second scan), which was reheated

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