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Lateral pressure change on phase transitions of phosphatidylcholine/diolein mixed membranes

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ABSTRACT

In this work, the changes in the lateral pressure in mixed membranes of egg yolk phosphatidylcholine (EPC) and a nonlamellar-forming lipid diolein (DO) were investigated with respect to increasing DO content. Several fluorescence techniques were employed to probe transitions of EPC/DO lipid mixtures from lamellar to inverted hexagonal via bicontinuous cubic phases. Excimer fluorescence of dipyrenyl phospholipids revealed that the lateral pressure in the acyl chain region of the EPC/DO mixed membrane increased with the mole fraction of DO (X_{DO}) in the lamellar phase and further increased almost linearly up to $X_{DO} = 0.2$ through the lamellar-to-cubic phase transition. Water penetration into the acyl chain region, as determined by fluorescence lifetime experiments, decreased linearly with increasing X_{DO} until the cubic phase was formed. These results suggest that the acyl chain packing becomes tighter by the incorporation of DO in the lamellar phase and that it does not alter during the lamellar-to-cubic phase transition. Only the packing of the headgroup region was found to increase during the transition to the cubic phase, as detected by the fluorescence lifetime of dansyl phosphatidylethanolamine, which localizes in this region. Through the cubic-to-hexagonal transition, headgroup packing was increased further, and the acyl chain packing was in turn loosened. These results suggest that the acyl chain packing stress induced by the incorporation of DO is released not by the lamellar-to-cubic phase transition but by the cubic-to-hexagonal transition.

1. Introduction

Amphiphilic lipids assemble and form various liquid crystalline phases by hydration. The particular phases that are formed depend on the molecular shape, that is, the size balance between the hydrophilic headgroup and the hydrophobic acyl chains [1]. Nonlamellar-forming lipids with a negative spontaneous curvature assemble into nonlamellar phases, such as an inverted hexagonal phase or a bicontinuous cubic phase. Mixtures of lamellar- and nonlamellar-forming lipids are frequently observed to form planar lipid bilayers (lamellar phase), even with a relatively large fraction of nonlamellar-forming lipids [2–4]. When cone-shaped, nonlamellar-forming lipids are forced to form a planar bilayer structure, the monolayers constructing the bilayer undergo elastic deformation because of differences between membrane curvature and spontaneous curvature of the lipid. This leads to an increase in the lateral pressure in the acyl chain region [5,6] and concomitantly brings about unfavorable hydrophobic hydration at the bilayer surface [7,8]. These physical changes in the interior and at the surface of membranes are considered to be the driving force for lamellar–nonlamellar phase transition.

Biological membranes maintain a planar bilayer structure, even though they contain many nonlamellar-forming lipids. The lateral pressure and hydration of biological membranes are thought to change depending on the fraction of these lipids. The lateral pressure is assumed to control the function and structure of membrane proteins [5,6,9,10] and to affect membrane-binding propensity and enzymatic activity of soluble proteins functioning at the membrane surface [11–15]. Therefore, detailed observations of the physicochemical properties of membranes containing nonlamellar-forming lipids are required to better understand lipid–protein interactions. Diacylglycerols (DGs) are metabolic products of phospholipase C-mediated phospholipid hydrolysis to phosphatidylcholine [16] and phosphatidylinositol 4,5-bisphosphate [17]. In addition to their biochemical role as an allosteric activator of protein kinase C (PKC) [18], DGs are considered to have a

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biophysical function in modifying the physicochemical properties of biological membranes, because of their negative spontaneous curvature.

We have previously demonstrated that lipid mixtures of egg yolk phosphatidylcholine (EPC) and diolein (DO) form lamellar, bicontinuous cubic, and inverted hexagonal phases [19]. In the present study, the lateral pressure and water penetration in the EPC/DO mixed membranes were investigated using fluorescence measurements.

2. Experimental methods

2.1. Materials

EPC (purity > 99%) was provided by Asahi Kasei Co. (Tokyo, Japan). DO (purity > 99%, mixed isomers with ca. 85% 1,3- and 15% 1,2-isomer) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Monoolein (1-Monooleoyl glycerol, MO, purity > 99%) was supplied by NOF Corp. (Tokyo, Japan). 1,2-Bis-(1-pyrenebutanoyl)-*sn*-glycero-3-phosphocholine (C_4dipyPC), 1,2-bis-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine (C_{10}dipyPC), 2-(9-anthroyloxy)stearic acid (2-AS), and 2-(3-(diphenylhexatrienyl)propanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (DPH-PC) were purchased from Invitrogen (Eugene, OR, USA). 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(5-dimethylamino-1-naphthalenesulfonyl) (dansyl-PE) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). These products were used without further purification. All other chemicals used were of the highest reagent grade.

2.2. Sample preparation

EPC/DO mixed membranes were prepared as follows. Stock solutions of EPC and DO in methanol were mixed in the required proportions. For the samples labeled with a fluorescence probe, a methanol solution of C_4dipyPC, C_{10}dipyPC, DPH-PC, dansyl-PE, or 2-AS was mixed with the lipid solution to yield 0.1 mol% (for C_4dipyPC and C_{10}dipyPC), 0.25 mol% (for dansyl-PE and DPH-PC), or 0.5 mol% (for 2-AS) of total lipid. After the solvent was evaporated, the sample was dried under vacuum. Following this, Tris-buffered saline (10 mM Tris–HCl, 150 mM NaCl, pH 7.0) was added to the sample at 50 wt% of the lipid concentration. The hydrated sample was vortexed, repeatedly freeze-thawed in a 1.5 mL sample tube, and kept at room temperature prior to performing the measurements. Mixed membranes of POPC/MO were prepared using a similar procedure.

2.3. Fluorescence measurements

Front-face illumination was applied to take fluorescence measurements of the prepared lipid mixtures [20]. The sample was placed between quartz slide glasses, set in a cell holder, and oriented at an angle of 30° from the incident light to reduce the scattered excitation light entering the monochromator. The foreground emission light was then detected.

Steady-state fluorescence emission spectra were measured on a Jasco FP-6200 spectrofluorometer (Tokyo, Japan). The emission spectra of C_4dipyPC and C_{10}dipyPC were recorded with an excitation wavelength of 345 nm, and emission wavelengths of 378 nm (monomer) and 478 nm (excimer) were chosen for determining the excimer-to-monomer fluorescence intensity ratio.

Fluorescence lifetimes were measured on a HORIBA NAES-550 nanosecond fluorometer (Kyoto, Japan) with a pulsed hydrogen lamp (full width at half maximum: ~2 ns). Samples labeled with 2-AS were excited through a HOYA U360 band-pass filter and TOSHIBA UV-34 cutoff filter, and detected through a $CuSO_4$ solution (250 mg/mL) and a HOYA L42 cutoff filter. Samples labeled with DPH-PC were excited through a U360 filter and detected through a $CuSO_4$ solution and an L42 cutoff filter. Samples labeled with dansyl-PE were excited through a HOYA U350 band-pass filter and detected through a $CuSO_4$ solution and a HOYA Y48 cutoff filter.

Total fluorescence decay $I(t)$ is expressed as the summation of exponential decay functions with the fractional amplitude α_i and the fluorescence lifetime τ_i for the ith component:

$$I(t) = \sum \alpha_i \ \exp\left(\frac{-t}{\tau_i}\right) \quad (1)$$

The mean lifetime $\langle\tau\rangle$ is defined as follows:

$$\langle\tau\rangle = \frac{\sum_{i=1}^{n} \alpha_i \tau_i^2}{\sum_{i=1}^{n} \alpha_i \tau_i} \quad (2)$$

Experimentally obtained fluorescence decay $I^*(t)$ is a convolution of $I(t)$ and an intensity profile of pulsed excitation light $P(t)$,

$$I^*(t) = \int_0^\infty P(t')I(t-t')dt' \quad (3)$$

The values of τ_i were obtained by fitting the experimental fluorescence decay using Eq. (1) and assuming double-exponential decay ($i = 1, 2$).

3. Results

In a previous study, we investigated the phase behavior of EPC/DO mixtures using small-angle X-ray scattering (SAXS) and ^{31}P NMR measurements at 25 °C [19], and we found that they predominantly formed a bicontinuous cubic phase of primitive type (C_P), bicontinuous cubic phase of diamond-type (C_D) and inverted hexagonal phase, at DO fractions (X_{DO}) of 0.2, 0.3, and 0.4, respectively. We further verified by ^{31}P NMR that the mixture forms a lamellar phase at $X_{DO} = 0.15$ (data not shown). These results verify that lamellar-to-cubic and cubic-to-hexagonal phase transitions occur between 0.15 and 0.2 and between 0.3 and 0.4, respectively.

3.1. Excimer formation of dipyrenyl phospholipids

We utilized the fluorescence of dipyrenyl phospholipids for the detection of lateral pressure changes in the acyl chain region. An increase in the lateral pressure brings two pyrene moieties of the fluorescent probe close and thus enhances intramolecular excimer formation, resulting in an increase in the excimer-to-monomer fluorescence intensity ratio (I_e/I_m) [21]. We previously examined changes in the lateral pressure in acyl chain regions near the bilayer center and the interface of the mixed membranes of POPC and MO using long-chain C_{10}dipyPC and short-chain C_4dipyPC, respectively [8], and we have applied this method to the EPC/DO mixed membranes in the present study. Experiments were performed at two different temperatures (25 and 30 °C) to visualize changes in the I_e/I_m ratio unambiguously through the phase boundary. At each lipid composition, no phase transition was observed within this temperature range (data not shown).

For C_{10}dipyPC (Fig. 1A), I_e/I_m increased with an increase in X_{DO} in the lamellar phase (X_{DO} = 0–0.15) and increased further almost linearly up to $X_{DO} = 0.2$ through the lamellar-to-cubic phase transition. The behavior at this transition differs from that observed for the POPC/MO mixtures, in which I_e/I_m decreased at the transition [8]. In the cubic phase (X_{DO} = 0.2–0.3), I_e/I_m was constant irrespective of the type of cubic phase (C_P at $X_{DO} = 0.2$ and C_D at $X_{DO} = 0.3$). The I_e/I_m ratio was also independent of X_{DO} in the inverted hexagonal phase (X_{DO} = 0.4–0.5) but was lower than that in the cubic phase, suggesting a decrease in lateral pressure during the cubic-to-hexagonal