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Osmotic stress induces a phase transition from interdigitated gel phase to bilayer gel phase in multilamellar vesicles of dihexadecylphosphatidylcholine

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Abstract

We have investigated the effects of poly(ethylene glycol) (PEG) on the structure and phase behavior of multilamellar vesicles of dihexadecylphosphatidylcholine (DHPC-MLVs) using an X-ray diffraction method. At low concentrations of PEG-6K (MW = 7500), DHPC-MLVs were in an interdigitated gel ($L_{\beta}I$) phase, a gel phase with interdigitated hydrocarbon chains. At around 24% (w/v) PEG 6K, a phase transition from the $L_{\beta}I$ phase to a bilayer gel phase occurred in the DHPC-MLVs, and above this concentration, they were in a bilayer gel phase. On the other hand, ethylene glycol (EG), the monomer of PEG, did not induce this phase transition in the DHPC-MLVs. A mechanism of this phase transition is proposed and discussed; a decrease in the repulsive interaction between the head groups of the phospholipids in the bilayer gel phase with an increase in PEG concentration, which is due to a decrease in the cross-sectional area of the head group region by osmotic stress, may be the main reason for this phase transition.

Keywords: Interdigitated gel phase; Phospholipid membranes; Phase transition; X-ray diffraction; Osmotic stress; Poly(ethylene glycol)

1. Introduction

Biomembranes and phospholipid membranes usually form bilayer structures where two phospholipid monolayers contact each other via their hydrophobic alkyl chains and their hydrophilic surfaces contact with water. These bilayer structures are very stable due to hydrophobic interaction [1]. Recently, interdigitated gel ($L_{\beta}I$) structures in phospholipid membranes have attracted much attention [2]. In these structures, lipid molecules from opposing monolayers are interpenetrated or interdigitated and terminal segments of their alkyl chains face the aqueous phase and contact with water. Multilamellar vesicles (MLVs) and large unilamellar vesicles of diacyl phosphatidylcholines, such as DPPC (dipalmitoylphosphatidylcholine), can form $L_{\beta}I$ structures in the presence of alcohols such as ethanol [3–7], ethylene glycol (EG) [8,9] and short-chain alcohols [10]. On the other hand, MLVs of an ether-linked dialkyl phosphatidylcholine such as DHPC (dihexadecylphosphatidylcholine) are in the $L_{\beta}I$ phase in water [11–13]. A mismatch between the head group cross-section and the alkyl chain cross-section has

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been considered as the main reason for the formation of the $L_\beta I$ phase in DHPC-MLVs in water [2,14].

Recently, we proposed a novel concept of osmoelastic coupling in biomembranes [15,16,9]. In a suspension of phospholipid vesicles containing a high-molecular-weight substance such as poly(ethylene glycol) (PEG), the PEG molecules are preferentially excluded from the region adjacent to the vesicle surface (exclusion layer). Such exclusion induces an osmotic stress onto the membranes of the vesicles, which increases with an increase in PEG concentration. In an equilibrium state, the membranes of the vesicles are compressed to produce elastic pressure which counterbalances the osmotic stress (osmoelastic coupling). Shrinkage of Sephadex gels by the addition of PEG was observed, which demonstrates the osmoelastic coupling as a model system [17].

In this report, we have investigated effects of PEG on the structure and phase behavior of DHPC-MLVs by using X-ray diffraction. At high concentrations of PEG-6K (MW = 7500), a phase transition from $L_\beta I$ phase to bilayer gel phase occurred. We have also proposed a mechanism for this phase transition.

2. Materials and methods

1,2-Dihexadecyl-*sn*-glycero-3-phosphatidylcholine (DHPC) was purchased from Fluka Chemie AG. Polyethylene glycol 6000 (PEG 6K; $(CH_2CH_2O)_n$) (average MW = 7500, $n = 170$) and ethylene glycol (EG) were purchased from Wako Chemical Co. Multilamellar vesicles (MLVs) were prepared by adding appropriate amounts of various concentrations of PEG 6K or EG in PIPES buffer (10 mM PIPES, pH 7.0) to dry lipids in excess water, and the suspension vortexed for about 30 s at around 55°C several times. We also prepared samples of MLVs by a slightly different way; we added solutions of high-concentration PEG 6K to the preformed MLV solution in PIPES buffer, and the mixture was incubated for 1 h at 60°C. We obtained almost the same experimental results for both the samples made in the different ways, which suggests that in the former samples, PEG 6K molecules do not enter inside the MLVs as well as in the latter samples.

X-ray diffraction experiments were performed by using nickel-filtered Cu Kα X-radiation ($\lambda = 0.154$ nm) from a rotating anode-type X-ray generator (Rigaku, Rotaflex, RU-300, 50 kV, 300 mA). Small-angle X-ray scattering (SAXS) data were recorded using a position-sensitive proportional counter (Rigaku, PSPC-5) with camera length of 350 mm and associated electronics (multichannel analyzer, etc., Rigaku). Wide-angle X-ray scattering (WAXS) patterns were recorded by a flat plate film cassette loaded with a high-sensitive X-ray film (Fuji Medical X-ray Film) with a camera length of 66.0 mm. Samples were sealed in a thin-walled glass capillary tube (outer diameter 1.0 mm) and mounted in a thermostatable holder whose stability was ± 0.2°C [9].

SAXS data were processed by a standard method [18]. Integrated intensities of various diffraction peaks, $I(h)$, where h is the order number, were determined after background subtraction. Measured intensities are corrected by multiplying by the square of the order number (h^2) for a powder pattern (unoriented samples) and a correction factor due to the geometry of the PSPC, $P(h)$. Hence, the structure amplitude, $F(h)$, equals $\sqrt{h^2 I(h) P(h)}$. Electron density distributions, $\rho(x)$, were calculated by the following formula:

$$\rho(x) \propto \Sigma \sqrt{h^2 I(h) P(h)}\, j(h) \cos(2\pi h x/d) \qquad (1)$$

where $j(h)$ is the phase information for each order h, and d is the spacing. For a centrosymmetric $\rho(x)$ function, $j(h)$ must be either $+1$ or -1 for each order h.

3. Results and discussion

DHPC-MLV in excess water at 20°C is known to be in the interdigitated gel ($L_\beta I$) phase [12–14]. To investigate the effects of osmotic stress on the phospholipid membrane in the $L_\beta I$ phase, we have obtained structural information on DHPC-MLV in various PEG-6K concentrations by using X-ray diffraction methods such as SAXS and WAXS. As shown in Fig. 1, the spacing (lamellar repeat period) (d) of DHPC-MLV at 20°C rapidly increased from 4.6 to 6.6 nm at around 24% (w/v) PEG-6K. A sudden

and large increase of the spacing suggests that a phase transition occurred in the DHPC-MLV. At the PEG concentrations of the phase transition (24–26%), two kinds of first-order diffraction peaks were observed. The spacing (d) contains contributions of both the membrane thickness (d_m) and the fluid spacing between adjacent membranes (d_f); $d = d_m + d_f$. To obtain information on the membrane thickness and structure, we have determined the electron density profiles, $\rho(x)$, of the DHPC-MLV in various concentration of PEG-6K by using Eq. (1). The phases, $j(h)$, were determined by graphs of the structure amplitudes $F(h)$ plotted versus reciprocal space coordinate [19,12], and the $j(h)$ for low (< 20%) and high (> 30%) concentrations of PEG were $(-1, -1, +1)$ for orders $h = 1$–3 and $(-1, -1, +1, -1)$ for orders $h = 1$–4, respectively. The phase of the DHPC-MLV in the absence of PEG-6K is the same as that in [12]. Fig. 2 shows electron density profiles of DHPC-MLV in 0 and 50% (w/v) PEG 6K. They show that the distance between head group peaks across the bilayer, d_{p-p}, is 3.2 nm at 0% and 5.0 nm at 50% (w/v) PEG 6K. A WAXS pattern at 0% PEG 6K at 20°C consisted of a sharp reflection at 0.41 nm, showing that alkyl chains were packed in a hexagonal arrangement without any inclination. A WAXS pattern at 50% PEG 6K consisted of a strong broad reflection at 0.42 nm. The results of the d_{p-p} and the WAXS patterns indicate that DHPC-MLV in 0% PEG 6K was in the $L_\beta I$ phase, but was in the bilayer gel phase in 50% PEG 6K . Therefore, Fig. 1 shows that DHPC-MLVs at low concentrations of PEG 6K (below 24% w/v) were in the $L_\beta I$ phase, and those at high concentrations of PEG 6K (above 26% w/v) were in the bilayer gel phase, and at the intermediate concentration ($\approx$ 24–26%) both the phases coexisted in DHPC-MLV. Hence, a phase transition from the $L_\beta I$ phase to the bilayer gel phase occurred at around 24% (w/v) PEG 6K.

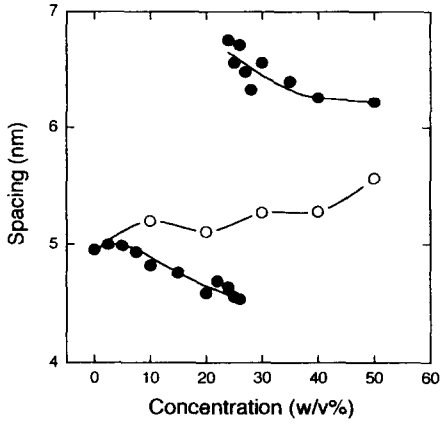

Fig. 1. Lamellar repeat periods (spacing) of DHPC-MLV in various concentrations of PEG-6K (●) and ethylene glycol (○) at 20°C.

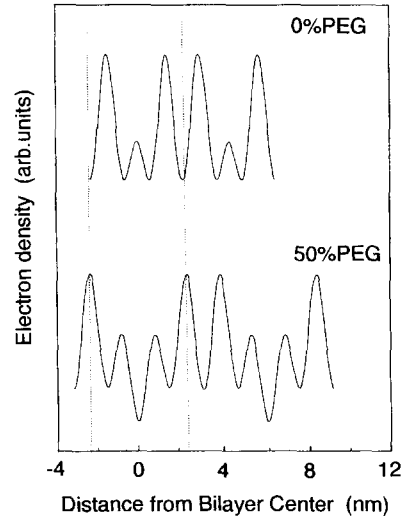

Fig. 2. Electron density profiles for DHPC-MLV in 0 and 50% (w/v) PEG-6K at 20°C. The distances from the bilayer center (nm) are the abscissae. For each profile, the geometric center of the bilayer is placed at the origin of the abscissae. The low-density region in the center of the profile corresponds to the phospholipid hydrocarbon chains and the high-density peaks on either side correspond to the lipid head groups.

As a control experiment, we have investigated the effect of ethylene glycol (EG), the monomer of PEG, on the structure of DHPC-MLV. The spacing of DHPC-MLV gradually increased with an increase in EG concentration (Fig. 1), and no phase transition occurred.

To elucidate a mechanism of the phase transition of a biomembrane, it is very important to evaluate the chemical potential of the membrane of each phase. The difference between the chemical potential of the membrane in the $L_\beta I$ phase ($\mu(L_\beta I)$) and that in the $L_{\beta'}$ phase ($\mu(L_{\beta'})$) is expressed as follows:

$$\Delta\mu = \mu(L_\beta I) - \mu(L_{\beta'}) = \Delta\mu_1 + \Delta\mu_2 + \Delta\mu_3 \quad (2)$$

where $\Delta\mu_1$ $(=\mu_1(L_\beta I)-\mu_1(L_{\beta'}))$ is due to the interaction of terminal methyl groups of the alkyl chains of the phospholipid with water or solvents [4,20], $\Delta\mu_2$ $(=\mu_2(L_\beta I)-\mu_2(L_{\beta'}))$ is due to the van der Waals interaction between the alkyl chains in the membrane [4], and $\Delta\mu_3$ $(=\mu_3(L_\beta I)-\mu_3(L_{\beta'}))$ is due to the repulsive interaction between the head groups of the phospholipids [21,22]. The terminal methyl groups of the alkyl chains are exposed to water in the $L_\beta I$ phase and to alkane (alkyl chains) in the $L_{\beta'}$ phase. Contact between the segment of the alkyl chains and water is unfavorable [1], and hence $\Delta\mu_1 > 0$. A van der Waals interaction energy between the alkyl chains has an attractive term which is proportional to r^{-5} [23,24] (r is the distance between the alkyl chains). In the case of diacyl-PC-MLV, the $L_\beta I$ phase is more stable than the $L_{\beta'}$ phase at this point, because X-ray diffraction data indicate that the average value of r of the membrane in the $L_\beta I$ phase is smaller than that in the $L_{\beta'}$ phase [4]. Therefore, $\Delta\mu_2 < 0$. Threshold concentrations of ethanol and EG to induce the $L_\beta I$ phase in diacyl-PC-MLV decreased with an increase in alkyl chain length [3,9]. This result demonstrates the large contribution of $\Delta\mu_2$ to the total $\Delta\mu$ and also the important role of $\Delta\mu_2$ (<0) in the induction of the $L_\beta I$ phase. $\Delta\mu_3$ is determined by the repulsive interaction between the head groups of the phospholipids due to steric hindrance and electrostatic repulsion in the $L_{\beta'}$ phase. In the case of DPPC-MLV and DHPC-MLV, which have no net charges, a steric effect due to a mismatch between the cross-sectional area of the head group region, which includes a head group segment and water molecules, parallel to the plane of the membrane and that of the alkyl chain may be a dominant factor [18]. The effective cross-section of the alkyl chains of DHPC-MLV is smaller than that of DPPC-MLV [2,14], and hence $|\Delta\mu_3|$ $(\Delta\mu_3 < 0)$ in DHPC-MLV is much larger than that in DPPC-MLV. Owing to this effect of $\Delta\mu_3$, DPPC-MLV in water is in the $L_{\beta'}$ phase since $\Delta\mu > 0$; DHPC-MLV in excess water is in the $L_\beta I$ phase since $\Delta\mu < 0$.

The phase transition from the $L_\beta I$ phase to the bilayer gel phase in DHPC-MLV at high concentrations of PEG 6K can be explained on the basis of the osmoelastic coupling theory [9,15,16]. In a suspension of phospholipid vesicles containing a high-molecular-weight substances such as PEG, the PEG molecules are preferentially excluded from the region adjacent to the vesicle surface (exclusion layer). The chemical potential of water in the exclusion layer, therefore, becomes higher than that in the bulk phase where PEG concentration is high. Such an imbalance of the chemical potential of water induces an osmotic stress onto the vesicle membranes, which increases with an increase in PEG concentration. In an equilibrium state, the membranes of the vesicles are compressed to produce elastic pressure to lower the chemical potential of water in the exclusion layer (osmoelastic coupling). The osmoelastic coupling theory is applicable to any kinds of vesicles, such as unilamellar vesicles, multilamellar vesicles and sheet-type membranes. In the case of MLV, PEG 6K molecules are excluded from the inside of the MLV owing to steric hindrance, so that the chemical potential of the water of the inside of the MLV becomes higher than that of the outside of the MLV. In an equilibrium state, the spacing and the fluid layer thickness of the MLV become shorter, and also all the membranes in the MLV are equally compressed to lower the chemical potential of water inside the MLV [15,16,9]. Fig. 1 shows that the spacing of the DHPC-MLV decreased with an increase in PEG 6K concentration, which supports the view that osmotic stress is exerted onto the DHPC-MLV in the presence of PEG 6K. In the compression of the membranes, a decrease of the cross-sectional area of the PC head group parallel to the plane of the membrane may be larger than that of the alkyl chain region due to a conformational change of the head group segments induced by a decrease of the water content in the head group region and a change of their interactions with water molecules. Therefore, in the bilayer gel phase in DHPC-MLV, the amount of mismatch between the cross-sectional area of the head group region and that of the alkyl chain may decrease with an increase in PEG 6K concentration, and hence the repulsive interaction between the head groups may decrease. Hence, the chemical potential of the membrane in the bilayer gel phase of DHPC-MLV may decrease, and thereby, $|\Delta\mu_3|$ may decrease with an increase in PEG concentration and, above its threshold concentration, the phase transition from the $L_\beta I$ phase to the bilayer gel phase may occur.

On the other hand, EG molecules can enter inside

the DHPC-MLV and are not excluded from the surface of the DHPC membrane owing to their small size, and thereby, do not induce an osmotic stress onto the membrane. Fig. 1 shows that the spacing of the DHPC-MLV increased slightly with an increase in EG concentration in contrast with the decrease in the spacing in the presence of PEG 6K, which supports the above conclusion. This is why EG does not induce a phase transition from the $L_{\beta}I$ to the bilayer gel phase in DHPC-MLV. In the interactions of EG or diethylene glycol (the dimer of EG) and DPPC-MLV, these molecules did not induce an osmotic stress onto the membrane and the spacings of the MLV increased with an increase in these molecules [9,25], which are the same results as the above. These molecules might decrease the bending modulus and thereby increase an undulation motion of the membranes, resulting in the increase in the spacing of the MLV [26,27]. A further investigation of the interaction of these molecules with phospholipid membranes is necessary.

Other large substances such as proteins and sugars, which are preferentially excluded from the surface of the DHPC membrane, may induce this kind of phase transition. In animal and plant cells, there are high-concentration proteins and large-size sugars, and thereby, an osmotic stress may be exerted on biomembranes of cell surfaces and various kinds of vesicles. In this situation, it may be important to consider the effects of osmotic stress on the structures and physical properties of the biomembranes.

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