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Electric field-induced orientation of L- and DL-phosphatidylcholine bilayers

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Abstract

Membrane orientation induced by an alternating electric field has been examined for the L-enantiomer and racemic dipalmitoylphosphatidylcholine (DPPC) bilayers. The orientation effect was measured by bending curvature of hairpin-like deformation of the multilamellar cylindrical tubes with varying field-strength, frequency and tube size. It has been observed that both L- and DL-DPPC tubes are similar in the profiles of field-strength dependence and frequency dependence on the curvature deformation, but different in the deformed curvatures. DL-DPPC tubes deform largely as compared with L-DPPC tubes. The square of the deformed curvature of DL-DPPC tubes is larger than that of L-DPPC by about 37% on average. The result indicates that the racemic membrane is responsive to the electric field as compared with the L-enantiomer membrane. This suggests that a hybrid arrangement of head groups of the racemic lipid leads an effective response of the membrane due to the head group orientation.

Keywords: Phosphatidylcholine; Head group packing; Stereoisomer; Curvature elasticity; Electric field effect

1. Introduction

Phospholipid is a major constituent of biological membranes. Knowledge of the molecular arrangement in the bilayers is fundamental to understanding the behavior of the system. We have a lot of information on the hydrocarbon chain packing [1], whereas insufficient knowledge of the head groups, in particular, of the effects on physical properties of the bilayer membranes. Many of the lipids have a zwitterionic head group with a consequent large dipole moment, and there are steric isomers of L- and D-type due to the asymmetric carbon atom in the glyceryl group. The formation of different crystal structures by the L- and the racemic modifications was observed for the phosphatidylcholines [2,3] and the phosphatidylethanolamines [4,5]. Dielectric property of the bilayer membranes is related with arrangement and motion of the charged head groups. The dielectric property is of significance in relevance to non-thermal effects of ionic strength and an electric field on biological cells as pearl chain formation [6].

As is well known, an alternating electric field induces orientation of lipid vesicles [7,8]. Cylindrical lipid vesicles orient with the long axis parallel to the field line, where the normal of the lipid membranes is perpendicular to the field except for ones at the edge of the tubes. Due to the orientation, slightly bent tubes deform with a hairpin-like conformation [8]. Electro-orientation of particles was theoretically studied by Schwarz et al. [9–11], based on the minimum energy principal, and by Miller and Jones [12], based on the effective dipole moment. According to these theories, a nonspherical particle suspended in a liquid is oriented by an alternating electric field in a medium with a different dielectric property, even if the particle is homogeneous in the dielectric property. The orientation depends on the shape of the particle, on the dielectric properties of the particle and the medium, and also on the frequency of the applied field. In the case of lipid vesicles, the stable direction of the orientation is parallel to the electric field. Previously, we measured curvature deformation of the cylindrical tubes induced by an electric field and analyzed the data using the energy theory of electro-orientation [8,13]. The results indicate that the field-induced orientation of membranes is mainly caused by a difference in dielectric

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property between the vesicle and the surrounding medium. In addition, the membrane orientation is partially driven by electric field-induced orientation of the polar head groups [8]. In this work, we study the effect of the polar head groups on field-induced membrane orientation using the L- and the racemic dipalmitoylphosphatidylcholines (DPPC). The degree of membrane orientation is measured by bending curvature of hairpin-like deformation of the cylindrical multilamellar vesicles. It is shown that DL-DPPC membranes tend to be easily oriented by an electric field as compared with L-DPPC ones. We attempt to explain this behavior in consideration of the head group arrangement at the surface of the membranes.

2. Materials and methods

L-α-DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine) and DL-α-DPPC(1,2-dipalmitoyl-*rac*-glycero-3-phosphatidylcholine) with a purity of 99.9% were obtained from Sigma Chemical Co., and used without further purification. Water was purified by deionizing and filtering to give the specific resistance of 10 MΩ cm. These lipids were suspended in the purified water at a temperature (about 20 °C) well below the chain-melting transition temperature (T_m). The lipid concentration was 10 mg/ml. In order to obtain the lipid crystals with an appropriate size (some 10 μm), the suspension was sonicated with a bath type sonicator for several seconds at a temperature well below T_m. About 30 μl of the suspension was injected into a sample chamber. The sample chamber is made of a glass objective slide and a covered glass slip. Two parallel electrodes of stainless steel (20 mm long, 10 mm wide and 3 mm thick) are built in the chamber with a distance of 500 μm (inner volume: 30 μl). The electrodes are connected to a sine wave voltage source (Model 4502, Kikusui Electric Co., Tokyo) for generation of an alternating electric field. After injection of the dispersions, the chamber was well sealed with silicon grease against evaporation and placed on a temperature-controlled heater. The samples were monitored with a microscope connected to a CCD video camera (2400C, Hamamatsu Photonics, Hamamatsu).

Cylindrical vesicles were obtained by swelling the lipids above the chain-melting transition. Just above T_m (~ 42 °C), the lipids spontaneously swelled forming cylindrical multilamellar tubes at the edge of the crystals. The cylindrical tubes grew straight into excess water connecting with the crystals at the root. After the initial growth stopped (about 10 min), an alternating electric field was applied at a frequency range of 100 kHz–2 MHz. In order to avoid a temperature increase by Joule's heat, the electric field was applied for a short time period of about 10 s. Bending curvature of tubes with a hairpin-like deformation was measured with and without the electric field on the photographs. To avoid an underestimation of the curvature due to the three-dimensional size, we made sure that the curved region of the tube was in the same focus plane. The curvature measurement was performed at a temperature range of 45–46 °C.

3. Results and discussion

No visible difference in morphology was observed between L- and DL-DPPC cylindrical tubes. When an electric field is applied, these tubes orient with the long axis parallel to the electric field. Due to the field-induced orientation, tubes with a slight crook before the field application bend with hairpin-like deformation as shown in Fig. 1. The deformed tubes return to the initial figure of each tube by turning off the electric field. This behavior is reversible under a short-time application of the field. Some tubes were observed to be less responsive to the field as seen in this figure. This is due to adhesion to the lipid aggregates or the glass slide. Also, bending curvature varies within one tube. Therefore, we measured the maximum curvature of the responsive tube. When a tube bends from an initial curvature c_o to a curvature c, elastic energy stored in the tube is

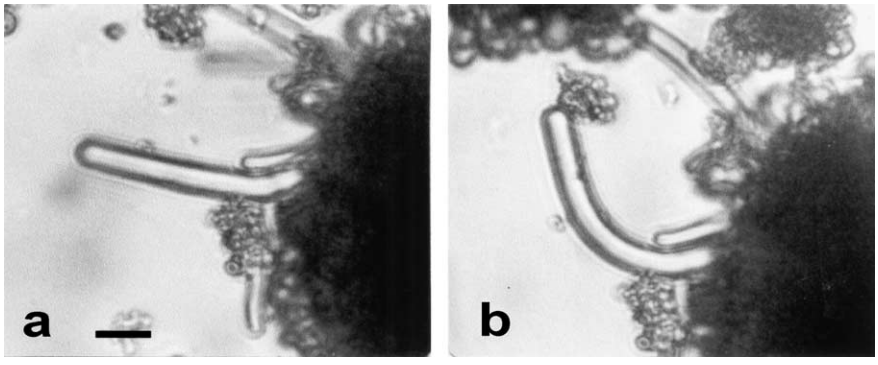

Fig. 1. Hairpin-like curvature deformation of DL-DPPC multilamellar tubes induced by an alternating electric field of strength 75 V/cm and frequency of 200 kHz. (a) Before, and (b) after application of the field. The direction of the field is vertical. The distance between the electrodes is 500 μm. Temperature is 45 °C. The bar represents 20 μm.

proportional to the difference in the square of the curvature $c^2 - c_o^2$ [8,13]. Thus the curvature difference was measured as a measure of membrane orientation induced by the electric field.

Fig. 2 shows the result of the curvature difference $c^2 - c_o^2$ as a function of the square of field strength. It is clear that the curvature difference is proportional to the square of the electric field strength for both L- and DL-DPPC tubes, in agreement with the result previously observed for egg yolk phosphatidylcholine [8]. This result reflects that the electric energy approximately converts to the bending energy, because the electric energy is proportionally related to the square of the field strength. It is noted that values of the curvature difference vary with DL and L types. Since the electric field-induced orientation depends on the frequency [11,12], there is a possibility that the difference in two lipids is due to a difference in the frequency dependence of the orientation. However, as shown in Fig. 3, these tubes resemble each other in the frequency profiles. Nevertheless, they vary in magnitude of the curvature difference, i.e., the deformed curvature of DL-DPPC tube is larger than that of L-DPPC tube in the whole frequency range.

To confirm this difference in magnitude of the deformed curvatures, we measured various tubes in diameter at constant field strength of 75 V/cm (maximum amplitude value) and frequency of 200 kHz. In the measurements, we selected tubes with similar ratios of an outer tube radius r_o and length of the curved region L in order to reduce effects of the tube shape. The result is summarized in Fig. 4. Although the points are scattered, it is seen that DL-DPPC tubes are large in the square of the deformed curvature

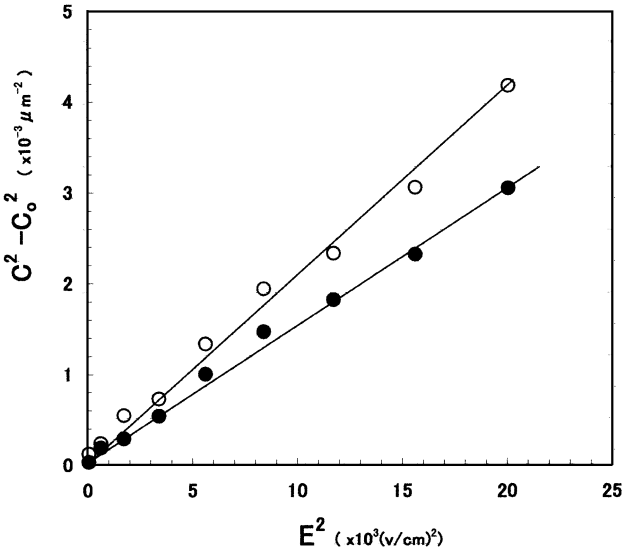

Fig. 2. Field strength dependence of curvature deformation induced by the electric field at constant frequency of 200 kHz for (○): a DL-DPPC tube (outer diameter ϕ; 7.7 μm, Ratio of ϕ to the curved length ϕ/L; 0.08) and (●): a L-DPPC tube (ϕ; 8 μm, ϕ/L; 0.08). The curvature deformation is represented by an increase in the square of curvatures $c^2 - c_o^2$, where c and c_o are curvatures with and without electric field application, respectively.

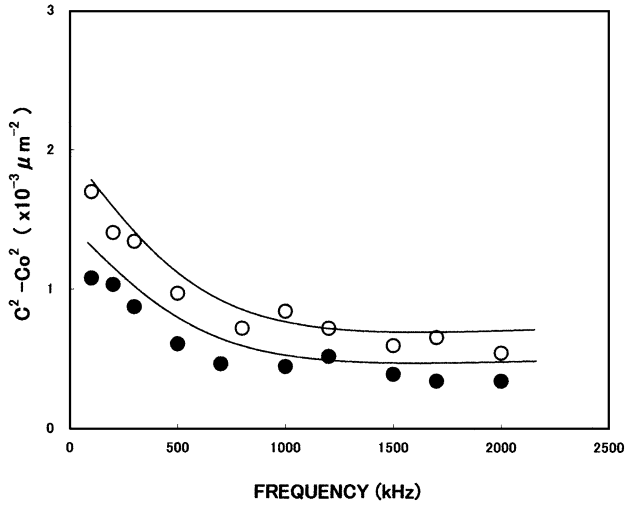

Fig. 3. Frequency dependence of curvature deformation induced by the electric field at a constant of field strength of 75 V/cm for (○): a DL-DPPC tube (ϕ; 8 μm, ϕ/L; 0.1) and (●): a L-DPPC tube (ϕ; 7.7 μm, ϕ/L; 0.12).

$c^2 - c_o^2$ as compared with L-DPPC tubes. The mean value of DL-DPPC tubes is larger by about 37% than that of L-DPPC tubes.

Let us consider the origin of the difference in the curvature deformation between these tubes. When they are isotropic particles in the dielectric property, electric energy given by the field can be derived by the energy theory of electro-orientation [11]. Since the stable orientation of the tubes is parallel with respect to the field, electric energy of transition from the initially perpendicular orientation is given as Eq. (2) of Ref. [13]. We have also previously derived the deformed curvature on the assumption that the electrical energy converts to the bending energy without

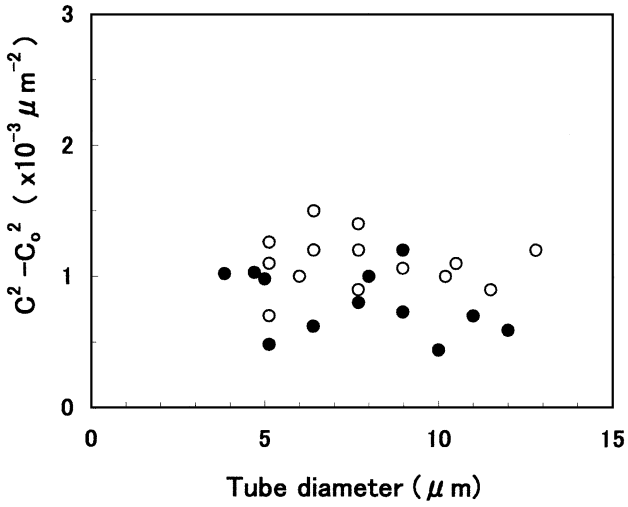

Fig. 4. Electric field-induced curvature deformation as a function of tube diameter for (○): DL-DPPC and (●): L-DPPC tubes. Values of ϕ/L range from 0.08 to 0.17. Strength and frequency of the electric field are 75 V/cm and 200 kHz, respectively. Temperature is 45–46 °C.

thermal dissipation at an equilibrium configuration of the tube [8]. The derived curvature is

$$c^2 - c_o^2 = \frac{4}{3}\frac{d\varepsilon_o E^2 \Delta u}{\kappa_c}, \quad (1)$$

where d is the interlamellar repeat distance of the membrane, κ_c the curvature elastic modulus of single bilayers, ε_o the permittivity of the surrounding medium, E the field strength and Δu the dimensionless energy of the transition from the initially perpendicular orientation to the parallel orientation with respect to the field. The dimensionless energy is related to the frequency, the tube shape and the dielectric parameters of the system. The assumption of an equality of the electrical and bending energies hold well for the system, since the elastic modulus determined from the equation is in reasonable agreement with values by other experimental methods [14,15].

Note that the curvature difference $c^2 - c_o^2$ of Eq. (1) is explicitly independent on tube diameter and the length of the curved region L, but depends implicitly on the ratio $2r_o/L$ through the dimensionless energy Δu. The data shown in Fig. 4 are for tubes ranged in the ratio $2r_o/L$ from 0.08 to 0.17. The variation of the ratio induces a change in the electric energy only by about 8%. This reflects the observed result of a few correlations between the curvature difference and the tube diameter, but leads to scattering of the data.

The square of curvatures presented in Eq. (1) depends on the interlamellar repeat distance of the membrane. However, the interlamellar repeat distances are identical in two lipids of DL and L types [16,17]. Also, the square of curvatures in Eq. (1) are inversely related to the curvature elastic modulus. Many investigators have measured curvature elasticity of DPPC membranes by various experimental methods [14,18,19]. The measured values are widely ranged. This wide range is probably due to the different experimental methods. It reasonably seems that both lipid membranes are also similar in the curvature elasticity because the elastic property is mainly related with the acyl-chain packing. Therefore, the observed difference of the response to the electric field between L- and DL-DPPC cannot be explained completely by the energy theory for homogeneous particles in the dielectric property. In addition, this hardly explains the observed result from the effective moment-based theory because the model particle is homogeneous in the dielectric property.

In order to explain the variance of the curvature deformation, it is important to note contributions of the polar head group orientation. Head groups of phosphatidylcholine molecules are located relatively parallel to the bilayer plane and almost freely rotate about the glycerol bond [20,21]. Since the head group is a dipole due to the positive charge of the choline group and negative charge of the phosphate group, the head group orients by application of an alternating electric field. When the bilayer plane is parallel with respect to the field line, i.e., the hydrocarbon chains are perpendicular to the field line, the head groups orient together with the field. However, when the bilayer plane is not parallel to the field, not all the head groups can orient in the direction to the field line because of a steric barrier of the chains. Two typical examples of head group orientation are schematically shown in Fig. 5. In the case of conformation (a), when the field is upward, the head group turns to the field line and directs the chains toward the perpendicular respect to the field. However, when the field is downward, the head group directs the chains toward the parallel with the field. In this case, the head group orientation does not contribute to orientation of the bilayer membrane. However, the other conformation (b) permits the head group to direct the chains toward the perpendicular for both field directions. In this case, the head group orientation contributes to the membrane orientation.

A reasonable packing of head groups at the bilayer surface of DL-DPPC is a hybrid arrangement considering minimization of the electric potential, similar with DL-dilauroylphosphatidylethanolamine [1,22], where D- and L-head groups orient alternately. Since this hybrid packing alternately includes more than two head group conformations, the effect of the orientation of conformation (b) is provided in the whole membrane. This leads to an effective response of the membrane to an electric field as observed for the DL-DPPC tubes.

On the other hand, the head groups of L-DPPC lie parallel to each other. It is possible that there are two kinds of domains; one of them is a domain with conformation (a) and the other is a domain with conformation (b). The L-DPPC membranes probably consist of these domains. Note that the effect of head groups on the chain orientation is limited only to the domain of conformation (b). Since the curvature elastic modulus is very small, the chain orientation induced by the head groups at this domain does not link

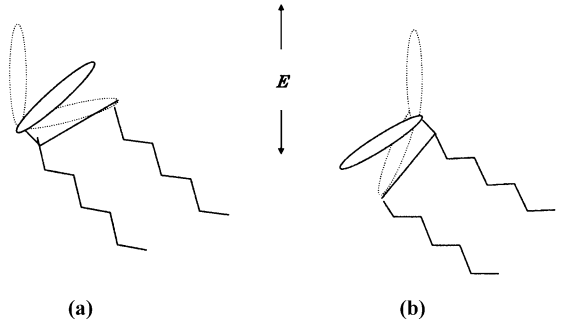

Fig. 5. Schematic representation of head group orientation induced by an alternating electric field. The oval and zigzag lines represent the head group and the chains, respectively. In conformation (a), for the upward field, the head group turns to the field line and directs the chains toward the perpendicular aspect of the field. But, for the downward field, the head group directs the chains toward the parallel with the field. Thus the head group orientation does not contribute to orientation of the bilayer membrane. In conformation (b), the head group directs the chains toward the perpendicular for both field directions. In this case, the head group orientation is effective in the membrane orientation.

to other domains. The effect of the orientation of conformation (b) is localized in the membrane. This leads to less contribution of head groups on the membrane orientation than that of DL-DPPC head groups. The field-induced orientation of the L-DPPC membranes is mainly caused by the difference in the dielectric properties between the membranes and the surrounding medium as with the orientation of isotropic particles in the dielectric property. In the above explanation, the arrangement of the head groups at the bilayer surface is simplified. The head group arrangement in the real membranes seems to be more complicated. Further, an explanation considering the hydration structure at the bilayer surface should be performed.

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