Chirality of lipids makes fluid lamellar phases piezoelectric

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The role of chirality in membrane-forming lipids is not well appreciated at present. Here we demonstrate that the chirality of phospholipids makes fluid lipid bilayers piezoelectric. Thus, chiral lipids would play a central role in the functioning of cell membranes as active mechanotransducers. By periodically shearing and compressing nonaqueous lamellar phases of left (*L*-α-phosphatidylcholine), right (D-α-phosphatidylcholine), and racemic (DL-α-phosphatidylcholine) lipids, we induced a tilt of the molecules with respect to the bilayer's normal and produced electric current perpendicular to the tilt plane, with the chiral lipids only. This effect is due to the Sm-A^* phase liquid crystal structure of the bilayers, which under molecular tilt becomes a ferroelectric Sm-C^* phase, where the polarization is normal to the tilt plane. This coupling allows for a wide variety of sensory possibilities of cell membranes such as mechanoreception, magnetosensitivity, as well as in-plane proton membrane transport and related phenomena such as adenosine triphosphate (ATP) synthesis, soft molecular machine performance, etc.

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INTRODUCTION

Chirality of membrane-forming lipids, and phospholipids in general, is usually regarded as weak and insignificant. Phase transition studies fail to reveal any significant difference between left and right lipid enantiomers. Although lipids do exist *in vivo* in a left form only, the far-reaching consequences of this enantiomorphic purity are still not appreciated in membrane biophysics. On the contrary, liquid crystal physics traditionally pays a close attention to mesogens' chirality. Therefore, the application of the liquid crystal approach to living matter physics calls for an elucidation of the chirality effects in membranes.

The symmetry principles of liquid crystal ferroelectricity [1] or strictly speaking, helielectricity [2], can be validated with lyotropics [3,4], by taking just a common membrane-forming phospholipid, lecithin, that is weakly chiral. The synthetic lecithins exist in the form of left or right enantiomers, or racemates, all of them are commercially available (Sigma). The low-temperature gel phase $L_{\beta'}$ that is tilted, will be helielectric, very similar to a Sm-C^* thermotropic. The high-temperature fluid phase L_α then will be analogous to Sm-A^*. Both these phases should display a piezoelectric response under viscous shear stress. The gel phase is expected to be piezoelectric because of distorting the rotational symmetry of the polarization helix (see Refs. [5,6] for thermotropic case). The fluid phase is expected to be piezoelectric because of the director tilting. Tilting combined with chirality may lead to a macroscopic polarization normal to the tilt plane provided the molecules have nonzero dipole moments [7,8].

A long-standing problem of polarization measurements with lipid-water lyotropics is their high ionic conductivity. To reduce the conductivity, i.e., the screening of the spontaneous polarization, a nonaqueous lamellar phase could be prepared by replacing water with another hydrophilic solvent, e.g., ethylene glycol [9–11]. Observations of piezoelectricity of nonaqueous, cholesterol-containing lyotropic phases were performed with one of us in 1988 by using the oscillation drop method [12]. Cholesterol was employed because of its marked chirality and a substantial molecular dipole. Subsequently, these measurements were carried out with hydrated lecithin gel phases containing cholesterol [13,3]. There was also some earlier evidence for shear-induced electric signals in a shear cell with an oscillating top electrode filled up by a lecithin-water lamellar phase, without cholesterol [14].

The first detailed observation of shear piezoelectricity of the fluid L_α phase was published only very recently by some of us [15]. By periodically shearing and compressing films of hydrated *L*-α-phosphatidylcholine in a cell with oscillating top electrode a tilt of the molecules with respect to the bilayers' normal was induced, which produced an electric current perpendicular to the tilt plane. This corresponded to a polarization of about 300 nC/cm^2 at 5° of tilt. Due to the lack of D-α-phosphatidylcholine and their racemates, however, it has not been proven that the signal is due to chirality. To prove it undoubtedly one needs to show that the direction of the induced polarization is opposite on the materials with opposite handedness, and disappears in the racemic mixtures. The purpose of this paper is to show that the shear induced electric current in the L_α phase of lipids is indeed due to their chirality.

EXPERIMENTAL APPROACH

The synthetic right enantiomer 2,3-dihexadecanoyl-sn-glycero-1-phosphocholine (D-DPPC) and the synthetic left enantiomer 1,2-dihexadecanoyl-sn-glycero-3-phosphocholine (L-DPPC) were purchased from Sigma, and used as received. We also prepared their 50:50 mixtures (DL-DPPC) to serve as the racemate control material. Nonaqueous phases were prepared by adding 30 or 50 wt. % ethylene glycol

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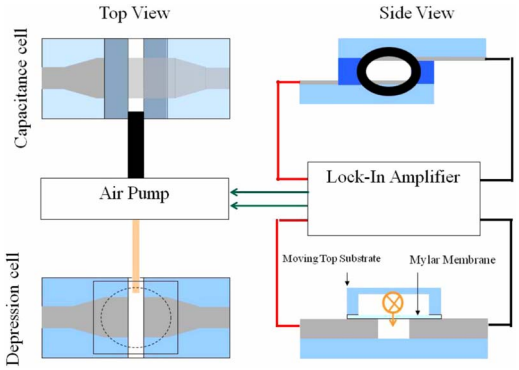


FIG. 1. (Color online) Illustration of the two experimental setups used in this study. Top: Capacitance cell design. Bottom: Depression cell design. Medium gray illustrates the electrode areas.

(EG, Aldrich, 99.9% purity) to the dry lipids in screw cap vials. The samples were then stirred, heated, and centrifuged a few times at 5000 rpm for equilibration. The final EG concentration in the cell, however, maybe slightly below due to evaporation of EG during preparation of the experimental cells, although this should be much smaller than for aqueous solutions due the higher (196 °C) boiling point of EG.

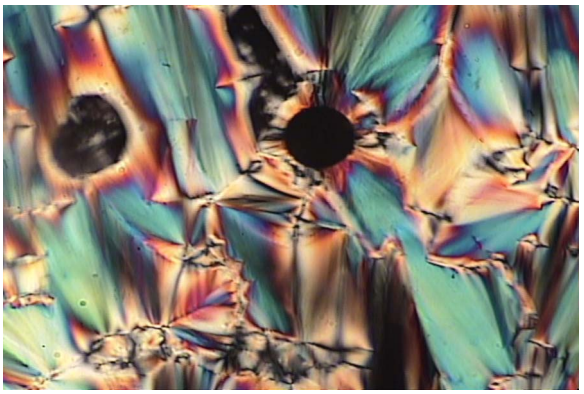
Resistance of experimental cells of 100 μm after filling was measured to be typically about 50 MΩ or higher at room temperature (22 °C) and about 1 MΩ at 80 °C. Brief application (1 min.) of 10–20 VDC to the cell electrodes was able to increase this resistance 5–10 times higher than before by sweeping out ions.

To investigate the effect of chirality on the tilt-induced electric polarization (piezoelectric effect), we have studied two types of sample geometries, which we will refer to as capacitance and depression cell designs (see Fig. 1).

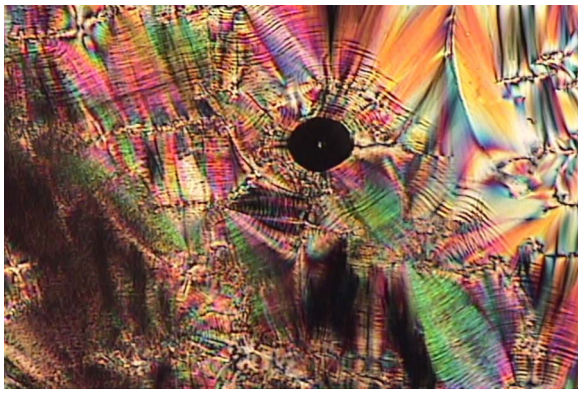
The capacitance cell design comprised of two transparent conductive indium tin oxide (ITO) electrodes and an attached plastic pipeline [see Fig. 1(a)]. The earlier design of these cells was perfected here by using glass spacers of 100-μm thickness and patterned ITO electrodes. In this way the uv cured NOA 71 adhesive holding the substrates and spacers never touched the electrodes. All these ensured the absence of any artificial piezosignal from polymer spacers or from the adhesive. Cells were placed in an Instec Hot stage HS 2000 regulated by an Instec Heat Controller. Oscillations of the lyotropic liquid crystal material with respect to the electrodes were excited by a compression loudspeaker fed by the lock-in internal oscillator. The oscillation frequency (80 Hz) was chosen near the broad resonant frequency of the pipeline/loudspeaker (65 Hz) measured by a piezocrystal sensor fixed at the end of the pipeline. In addition, the relative phase of the 80 Hz oscillating air pressure at the end of pipeline with respect to the internal oscillator providing speaker driving voltage, was set to 0°, which is convenient for comparison of the phases. Piezoelectric response (amplitude and phase) was measured by a computer-interfaced lock-in amplifier (7265 DSP from PerkinElmer) in a current mode. To induce a director tilt, the pressure of the air in contact with the liquid crystal was oscillated by a loudspeaker through a flat glass capillary of typically 3 mm width and 100 μm thickness. This "oscillating drop method" [12,13] relies on an asymmetry of the induced tilt along the planes of the electrode substrates, and requires at least partially bookshelf alignment (layers are not parallel to the substrates). The alignment was predominantly planar and the tilt was never averaged to zero because of some dissymmetry between upper and lower half of the 100 μm layer. Although the results are only qualitative and do not provide the magnitude of the piezoelectric constant, the sample holder does not have moving parts, so the signals are stable and well reproducible, thus enabling to compare materials with different chirality.

To obtain a good estimate of the magnitude of the piezoelectric response, we have constructed the depression cell design (see lower part of Fig. 1) as well. Here the top plate is movable and consist of an air chamber connecting to a rectangular capillary where the air is pumped in periodically by a loudspeaker. The top and three side walls of the chamber is constructed from rigid glass plates, whereas the bottom is a relatively flexible Mylar sheet. This design assures a simultaneous horizontal and vertical motion of the upper substrate. Such a motion is basically the same as used in Ref. [15] and provides periodic tilting of the lipid layers in homeotropically aligned stacks of bilayers by silanized top and bottom substrates. In this geometry and in the L_α phase, the shear along the layers alone would not necessarily provide tilt, but the synchronous shear and compression is needed: the compression facilitates the tilt and the uniform shear makes the tilt direction uniform. (We note that in the L_β phase the compression is probably not needed, because there the director is already tilted and the shear just unwinds the helix.) The 100-μm-thick side walls of the liquid crystal containing cells are coated with Ni electrodes, thus serving both as spacers and the electrodes normal to the tilt plane and the induced electric polarization. Piezoelectric signals (amplitude and phase) are measured by a computer-interfaced lock-in amplifier (7265 DSP from PerkinElmer). Although this arrangement allows quantitative measurement of the induced polarization, the complex movement of the top substrate leads to pumping out of the material. For this reason, the signals usually decrease in time and we had to fill multiple cells and average over the signals to get reliable estimates of the induced polarization. The magnitude of the induced director tilt was estimated as described in Ref. [15] after measuring the vertical depression of the top substrate. For this a Leitz Mirau Interferometer mounted to the objective port of an Olympus BX51 Microscope (used in reflection mode) was employed to measure the upward or downward deflections of the sample induced by the air flow within the air chamber of the top plate. A green filter (532 nm) was used to improve the fringe resolution. The theoretical limit of the accuracy of the interferometer is 3 nm [16]; however, vibrations and thermal fluctuations in the set up extended this to about 5 nm even when the microscope was mounted on a vibration damped optical table. This error is still much smaller than the experimentally observed sample deflections, which were typically in the 20–100 nm range depending on the voltage applied on the speaker.

Textural observations and registration of the intensity of transmitted light between crossed polarizers were done by a polarizing microscope (Olympus BX51) equipped with a



(a)



(b)

FIG. 2. (Color online) Polarizing microscopy textures of 100-μm-thick D-DPPC/EG (50/50 wt. %). (a) In the fluid phase at 61.3 °C after cooling from the isotropic phase (above 100 °C). (b) In the gel phase at 35.5 °C after cooling from the fluid phase. Note that the focal conic domains are decorated by a fine striation pattern due to chirality.

CCD camera and a photodiode. This setup was used for detection of phase transitions according to a method described earlier [17].

RESULTS AND DISCUSSION

The temperature dependence of the transmitted optical signal reveal a transition between a fluid and gel phases at around 40–45 °C depending on the lipid-water composition and the direction and magnitude of temperature variation. At 1 °/min heating and cooling rates there is a 2 °C hysteresis between the phase transitions, which is quite normal for liquid crystals. No sign of the pretransition at 37 °C, typical for DPPC/water phases, could be seen on both traces.

Typical textures of 100-μm-thick films between crossed polarizers are shown in Fig. 2. Figure 2(a) shows the fluid phase (61.3 °C) after cooling from the isotropic phase (above 100 °C), and Fig. 2(b) illustrates the texture in the gel phase (35.5 °C) after further cooling from the fluid phase. One can see periodic striped texture very similar to helical smectic C^* type textures [7,8] demonstrating that, although in L_β phase the bilayers are separated by isotropic

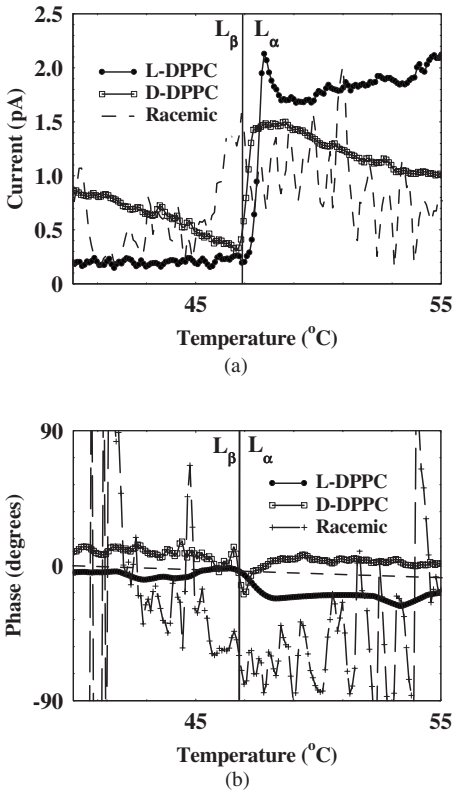


FIG. 3. Temperature dependences of the piezoelectric signals measured at 80 Hz mechanical excitations in heating for the left (L-DPPC/EG), right [D-DPPC/EG/ (50/50 wt. %)] and racemic mixtures. (a) The amplitude of the signal. (b) The phase of the signal.

fluid EG layers, the direction of the tilt is correlated in mesoscopic range, and lipid structure is chiral even without cholesterol (see Ref. [12]). The helical structure does not appear in the fluid L_α phase, because there the director is not tilted with respect to the layer normal.

Figure 3 shows the amplitude and phase of the piezoelectric current vs temperature in heating for L, D, and LD-DPPC/EG (50/50 wt. %) in a capacitance cell. In the gel phase the piezoelectric response is smaller in the L-DPPC than in the D-DPPC, probably due to the difference in alignment. The amplitudes of the responses increase upon melting to the L_α phase, then decrease slowly at further increasing temperatures. This is probably related to the decrease of the order parameter. This behavior excludes a possible origin of the response related to electrokinetic effects (streaming potentials). Streaming potentials in capacitance cell may, in principle, arise if an electric dissymmetry between the top and bottom electrode or lyotropic interfaces is present. Although the signal of the racemic mixture is not zero, it is an order of magnitude noisier than in the enantiomers. In control measurements with pure EG in the capacitance cell, the current response was similar to that of the racemic DPPC mixture: very noisy and notably exponentially increasing with the temperature (not shown). The disappearance of the piezosignal in the racemic mixture is more evident from the temperature dependences of the phases. For the enantiomers the phases measured in the D and L components are stable

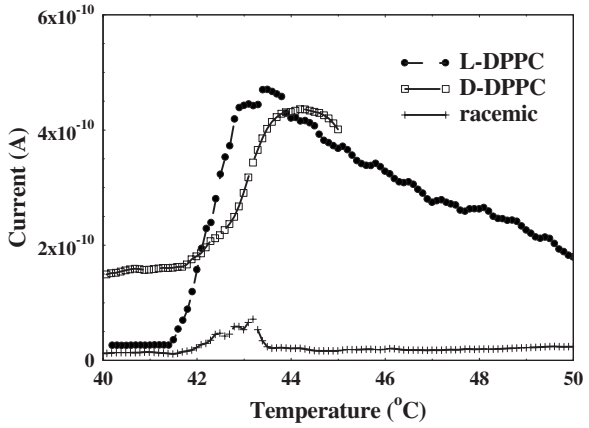


FIG. 4. Temperature dependences of the average signals in the L, D, and LD-DPPC/EG systems as measured in the depression cells.

and have opposite signs both in the gel and fluid phases, clearly showing that they must compensate each other in the racemic mixture. We note here that the phase shift we plotted in Fig. 3(b) is between the voltage driving the speaker and the induced electric current. The strain has a phase shift ϕ with respect to the driving voltage (depending on the electronics of the speaker, the frequency and the length of the air column between the speaker and the sample), and the current has a phase shift of $\pm 90°$ with respect the induced charge depending on the sign of the chirality. For this reason the measured phase shift is $\pm(90°-\phi)$, which is symmetric with respect to zero, just as we observe.

As mentioned above, the measurements in the depression cells can, in principle, provide the magnitude of the polarization, but the measurements had to be repeated several times and averaged to decrease the error below 20%. The average signals under about 50 nm maximum deflections are shown in Fig. 4 in cooling at 80 Hz.

It is clearly seen that the signals in the racemic phase are much smaller than in the enantiomers. From the measured 50-nm depression, we estimate that the induced tilt in the center of the film is up to $\theta \approx \sim \cos^{-1}[(100\ \mu\text{m} - 0.05\ \mu\text{m})/100\ \mu\text{m}] \sim 2°$. From the about 0.5 nA current induced on $0.1 \times 5\ \text{mm}^2$ area at 80 Hz we estimate that the maximum polarization is about 1 nC/cm^2. This corresponds to about an order of magnitude smaller polarization per unit tilt than measured for the neat phospholipid, probably related to alignment and screening ion issues.

The small signal near the phase transition to the gel phase in the racemic sample shown in Fig. 4, can be caused by several factors that will deserve separate studies in the future. One possibility is the increase of the induced tilt near the transition, where the director tilt softens just as near the Sm-A–Sm-C^* transitions [8]. This would then magnify the effect of even small enantiomeric excess. Although the measurement error of the concentration of the D and L DPPC is only about 1%, the helical twisting powers of the different enantiomers may not be exactly the same. We note that in de Vries-type Sm-A^* materials it was shown by Walba *et al.* [18] that electroclinic (which is basically the inverse of the piezoelectric effect) is very sensitive to detect small enantiomeric excess.

CONCLUSION

Our findings unambiguously show that chiral lipids display piezoresponses while their racemic mixture does not. It demonstrates an important role played by lipid chirality in lyotropic phases and in membranes: it makes lamellar lyotropic phases piezoelectric. We emphasize that the symmetry arguments we borrowed from thermotropic liquid crystal physics [1] used are robust, and valid even if the fine structures of the L_α and L_β phases are different from the Sm-A^* and Sm-C^*, respectively. Concerning the specific microscopic mechanism, we suggest that the tilt of the tails lead to a coordinated changing of the conformation of polar head, thus causing the transversal polarization.

Biological membranes are based on bilayers of chiral lipids. In addition, they contain substantial amounts of cholesterol. In living conditions membranes typically are in the fluid state and the molecules are normal to the bilayers. This is because the chains are flexible and occupy about the same area as of the heads. When the chains freeze, they occupy smaller area, thus leading to tilt. However, a coordinated tilt of lipid molecules could be induced even in the fluid phase by external agents such as shear deformation, electric or magnetic torque, steric field by embedded proteins, etc. Thus, our findings suggest the existence of some piezoelectric phenomena in biomembranes, with a vector of the spontaneous polarization parallel to the membrane plane, not normal to it. If this is the case, ion transport processes, especially proton transport along the membrane surface [19–22] and related phenomena such as ATP synthesis [20,23] should be interpreted by paying attention to the membrane piezoelectricity.

Furthermore, the capabilities of piezoelectric or nanoparticle membrane composites as sensors of weak magnetic fields have already been demonstrated [12]. The involvement of lipid tilting as a further mechanical degree of freedom in membranes, and its inherent piezocoupling to the electrical degree of freedom due to chirality will further enrich the concept and the understanding of membranes as soft mechanoelectrical machines [24,25].

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