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Piezoelectricity of phospholipids: a possible mechanism for mechanoreception and magnetoreception in biology

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We show that phospholipids, which are the main constituents of cell membranes, are piezoelectric. This was done by periodically shearing and compressing films of hydrated L-α-phosphatidylcholine, inducing a tilt of the molecules with respect to the bilayer's normal, which produced an electric current perpendicular to the tilt plane, corresponding to a polarisation of about 300 nC cm^{-2} at 5° of tilt. We also measured electric currents induced by an alternating magnetic field of less than 100 G in hydrated phospholipids doped with 0.5 wt% of ferrofluid of magnetite (Fe_3O_4) nanoparticles. A discussion of possible implications of these effects on biophysical processes is also provided.

Keywords: lipids; piezoelectricity; magnetoreception; mechanoreception

1. Introduction

Lipids are important components in biological systems (*1–2*), specifically when dealing with the structure of cellular membranes. The cellular membrane fulfils the following functions, which are critical to cellular survival. It acts as a flexible, self-healing barrier between the cell and its environment and also acts as a structural unit for functional proteins (*4–7*). However, the membrane does not have a purely passive role. There is now recognition of the importance that lipid organisation plays within the cell membranes in controlling protein function (*8*), and many disease states have been associated with aberrations of these lipid/protein interactions. A number of these interactions occur via electric signals. For example, some membranes swell (*9*) or become birefringent (*10, 11*) in response to voltage changes, which was interpreted as a converse piezoelectric (*12*) or electroclinic effect (*13*), respectively. Ion channels sensitive to membrane stretch have been observed in muscle cells (*14*) and piezoelectric models of the outer hair cell composite membranes have been considered (*15*). These latter models, based on the flexoelectric properties of the lipid bilayers (*16*), relate the membrane curvature to polarisation normal to the lipid bilayers, as discussed extensively by Petrov (*5–7*).

Using liquid crystal terminology, a stack of lipid bilayers can be considered as a SmA* phase (Sm stands for smectic, which indicates a layered structure; A means that the average molecular orientation (director) is normal to the layers; and * indicates that the constituent molecules are chiral). Such a phase has D_∞ symmetry with no net polar order in its unstressed configuration. However, when a tilt is induced, for example, by shear and/or layer compression, a SmC* phase forms with electrically polar C_2 symmetry, with the polar axis normal to the tilt plane (*17*). This means that in the SmA* phase there is a linear coupling between electric charges and mechanical deformation, known as piezoelectricity. It is important to note that this symmetry argument is valid for any number of bilayers, including single or double lipid bilayers, which are the major components of cellular membranes.

In this article, we describe piezoelectric properties of over 10-μm-thick stacks of phospholipid L-α-phosphatidylcholine bilayers, both by direct mechanical deformations and when the director tilt is enforced by a magnetic field. After describing the experimental observations we argue that piezoelectricity of phospholipids might explain previously observed ferroelectric-like interactions between lipid bilayers and ion channels (*18*) and may have consequences in various sensory mechanisms, such as in mechanoreception or magnetoreception.

2. Experiments

We studied a phospholipid extract from dried egg yolk purchased from SIGMA (catalogue number P5394, CAS number: 8002-43-5) without further chemical processing. The 95% component of this mixture is L-α-phosphatidylcholine (see Figure 1). This phospholipid forms a stable SmA* phase with the base layer structure illustrated in Figure 1.

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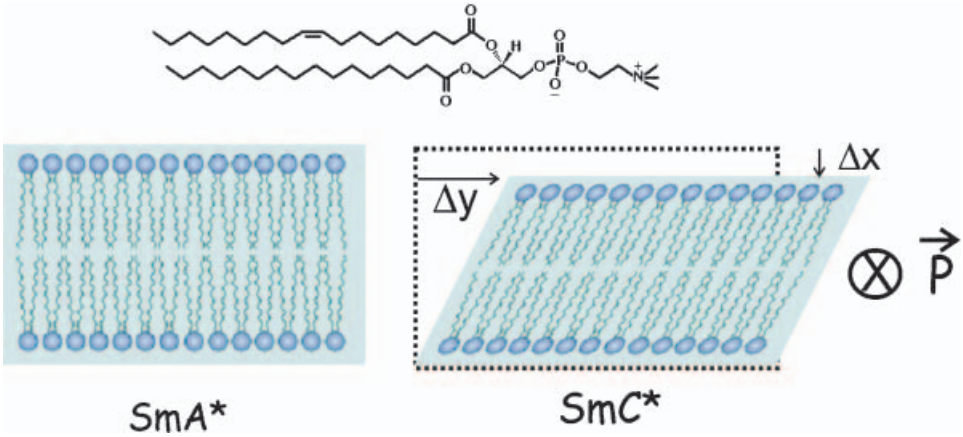
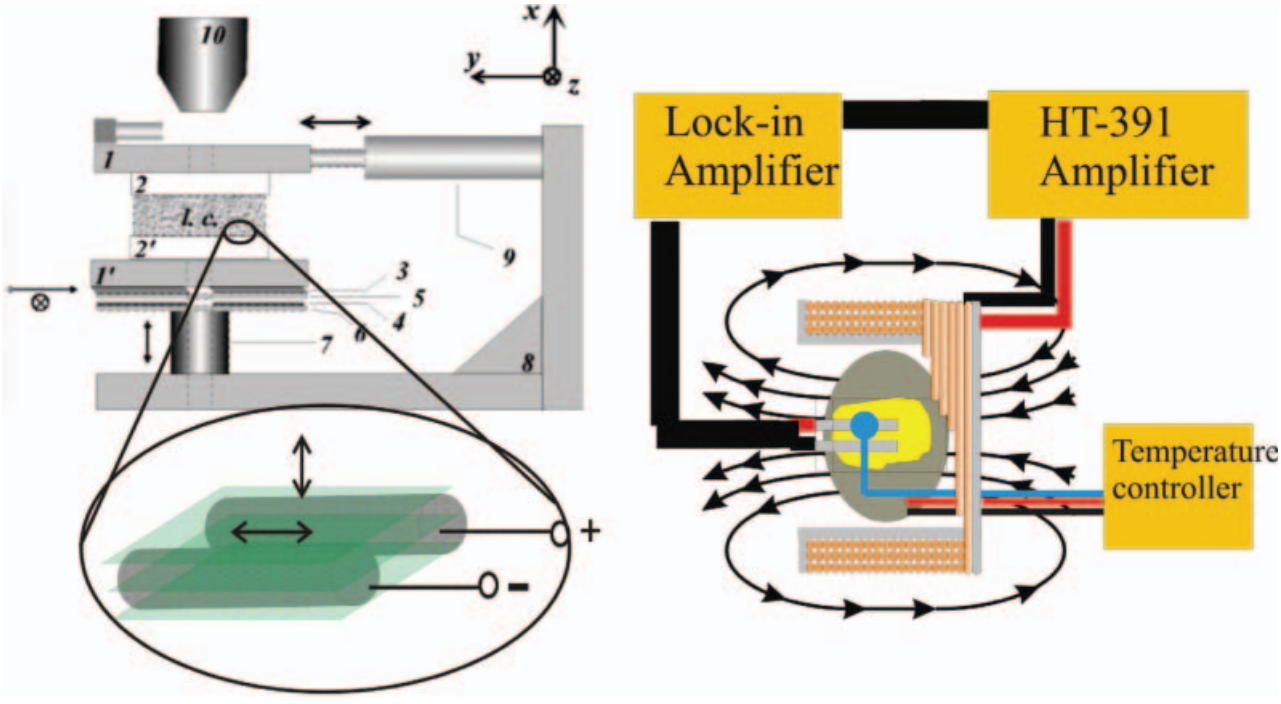


Figure 1. Illustration of the molecular structure of phospholipid L-α-phosphatidylcholine and the piezoelectricity of a lipid bilayer. A tilt of the average molecular orientation (director) with respect to the layer normal, induced by mechanical shear and/or layer compression, leads to a SmC* configuration with polarisation normal to the tilt (shear) plane.

For the piezoelectric measurements (Figure 2(a)), the liquid crystal material is sandwiched between two glass plates (2 and 2′) that are firmly fixed to temperature-stabilised heaters (1 and 1′). A pair of piezoelectric plates (5 and 6), sensitive to forces in the lateral directions, are attached to the bottom of the lower heated plate, which is connected to a rigid frame (8) via three layers of piezo-sensors (5, 6 and 7) sensitive in orthogonal directions. The frame also holds a piezoelectric actuator (9; PSt 500/10/5 from



(a) (b)

Figure 2. Illustration of the experimental arrangements. (a) The setup used for piezoelectric measurements. The upper part illustrates the sample holder with the piezo-actuators and piezo-detectors. The lower part illustrates the alignment and electrode arrangement compared with the smectic bilayers and mechanical deformation directions. The electrodes running in the y direction have diameters of 60 μm (this determines the film thickness) and are separated by 2 mm. The lateral dimension in the y direction is 1 cm. This means that the area where the induced charge is collected is $A = 60\,\mu\text{m} \times 1\,\text{cm} = 0.6\,\text{mm}^2$. (b) Schematics of the experimental setup used to detect magnetic-field-induced electric signals in L-α-phosphatidylcholine doped with 0.5 wt% ferrofluid. The magnetic field, shear direction and the electrode wires are all parallel to each other. The top wire from the temperature controller is a thermistor. The circle in the centre of the magnet represents the bottom heating element (top not shown).

Piezomechanik GmbH), which can shift the top plate with a maximum amplitude of 5 μm. The actuator is driven by a high-voltage amplifier (LE 430/015 from Piezomechanik GmbH). The motion of the top plate is monitored using a piezoelectric accelerometer (BK 4375 from Bruel & Kjaer, sensitivity 0.1 mm s^{-2}). The sample holder is placed in a polarising microscope (10), which enables textural observations of the sample during the measurements. Controlled periodic shear deformations of a given frequency can be made that detect the induced corresponding current using a lock-in amplifier (7265 DSP from PerkinElmer).

As shown in Figure 3(a), an important feature of the vibration created by this setup is that it only occurs along the y direction at frequencies below 200 Hz, and at higher frequencies there are a number of resonances where the vertical vibration becomes generated with basically similar amplitudes.

Comparing Figures 3(a) and (b), it can be seen that the peak positions measured in the vibration of the top plate and of the induced currents correspond to each other (the correspondence is more evident at low frequencies, $f<400$ Hz). The frequency dependence of the induced polarisation (see Figure 3(b)) shows that the response is decreasing towards lower temperatures as the material becomes stiffer. The amplitude of the current measured at different runs increases after the initial frequency scans. Simultaneous textural observations revealed improved homeotropic alignment during vibration of the upper plate. This alignment dependence clearly shows that the measured currents are related to piezoelectricity. Here we note that the phospholipid samples tend to degrade at higher temperatures preventing verification of the fact that the piezoelectric signal vanishes in the isotropic phase (unfortunately, owing to the lack of D-isomers, the racemic samples could not be observed). However, our recent measurements on synthetic glycolipids (Jákli, A.; Hashim, R. unpublished) in exactly the same geometries show piezoelectrically similar signals, which disappeared in the isotropic phase, confirming that the measured electric current is a result of piezoelectricity.

It is important to note that the induced polarisation at low frequencies is much smaller than at the resonances, where, in addition to the horizontal vibration, vertical motion also takes place. This suggests that the induced tilt angle is determined more by the vertical than the horizontal vibrations. Indeed a vertical displacement Δx causes a decrease of the layer spacing l by $\Delta l = l \cdot \Delta x/d$, where $d=60$ μm is the film thickness. Assuming rigid molecules, the induced tilt angle θ is determined from the relation $\cos\theta = 1 - \Delta l/l = 1 - \Delta x/d$. Taking the largest $\Delta x \sim 0.5$ μm measured at 200 Hz (see Figure 3(a)), this provides $\theta \sim 7^\circ$. However, the maximum contribution of the shear (horizontal vibration by Δy) to the induced tilt would be $\theta_y \sim \Delta y/d$, which is 1.2 μm/60 μm = 1/50 ~ 1.1°. The actual director tilting effect of the shear along the layers probably is much weaker, since in this case (at least at low frequencies) the bilayers can simply slide across each other without causing director reorientation. However, under layer compression the director must tilt and the role of horizontal shear is only to uniformly direct the buckling of the molecules induced by the vertical vibration.

The tilt angle dependence of the induced polarisation at room temperature is plotted in Figure 4. It can be seen that there is a hysteresis, probably related to the hysteresis in alignment. As we mentioned above, textural observations indicated that there is an improvement in homeotropic alignment with increasing amplitude of vibrations. This improving uniform alignment leads to the increasing slope as the function

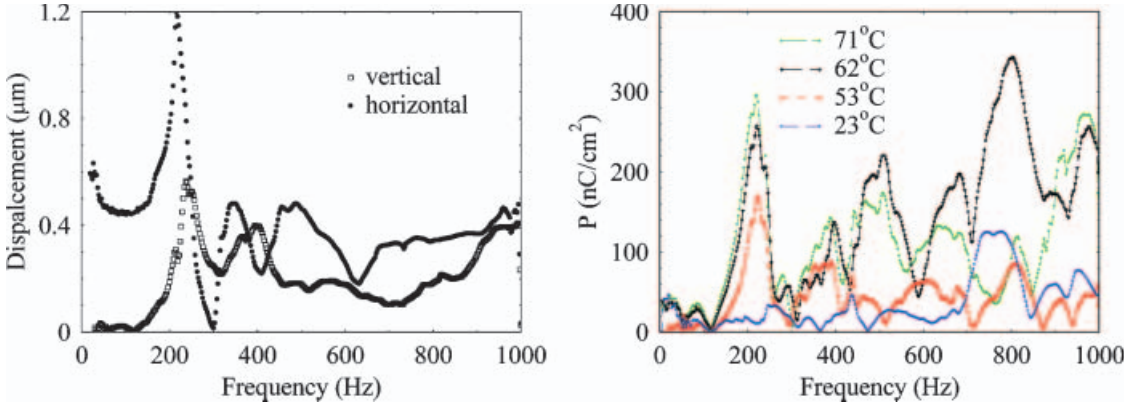


Figure 3. (a) Frequency dependences of the amplitudes of the vibrations of the top plate in vertical (Δx) and horizontal (Δy) displacements. (b) The frequency dependence of the induced electric polarisation P calculated from the piezo-current I as $P = I/(A \cdot \omega)$, where $A \sim 0.6$ mm^2 is the area of the electrode and ω is the angular frequency of the applied distortion.

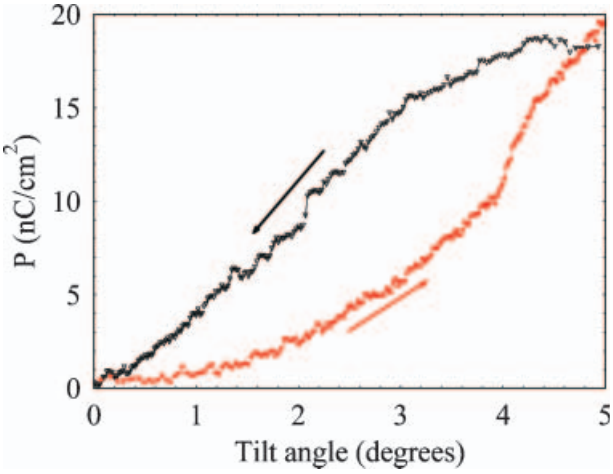


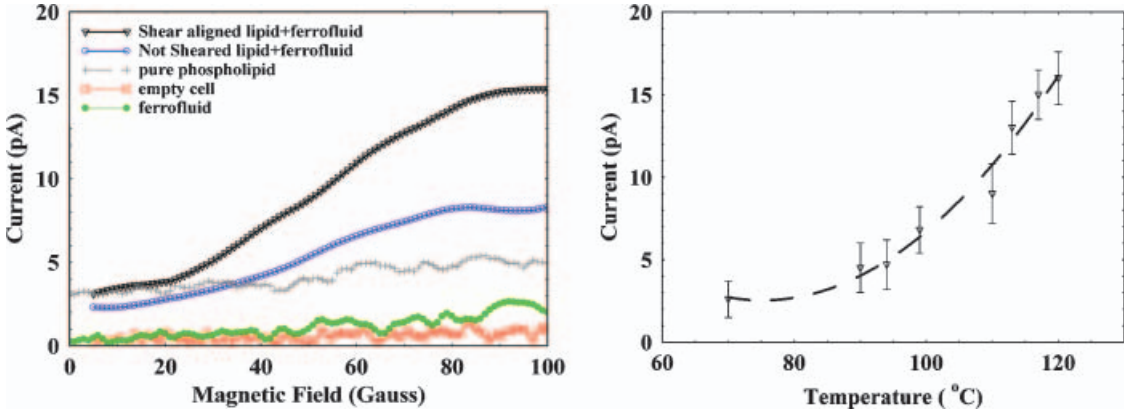
Figure 4. Tilt angle dependence of the induced polarisation at room temperature.

of tilt angle. In decreasing amplitudes the slope is almost constant below 3°, showing that the homeotropic alignment basically stays unchanged. The saturation above 4° may indicate that in this range the polarisation is no longer a linear function of the tilt angle.

After achieving these unambiguous piezoelectric responses, we tested whether tilt of the director (i.e. charge generation) could also be induced by a magnetic field. For this we doped L-α-phosphatidylcholine with ferrofluid, which contain magnetite (Fe_3O_4) nanoparticles in oleic acid surfactant solution, and the generation of electric currents has been measured by periodically varying the magnetic fields (see the setup in Figure 2(b)). The role of the surfactant is to prevent aggregation by steric repulsion (*19*). In spite of this, it is known that ferrofluids in lyotropic systems, especially in the presence of magnetic fields, show particle aggregations (see (*20*) and references therein), so we have tested the uniformity before and after measurements. Above 1 wt% ferrofluid concentrations, we indeed observed aggregates in polarising microscope textures when the sample was cooled from elevated temperature in the presence of fields stronger than 500 G. For this reason, in our measurements we have set the concentration to 0.5 wt%, and limited the magnetic field to 100 G. In these low concentrations and small fields, the texture appeared to be homogeneous and could be aligned similarly to the undoped lipid.

The experimental arrangement contains two tungsten shims enclosing the phospholipids sandwiched between two glass slides. The tungsten shims act as both 25 μm spacers and as the electrodes. This forms a cell with an effective area of 2 cm × 25 μm (0.5 mm^2). For control measurements, an empty cell, pure ferrofluid and undoped phospholipid were also tested. The measurements could be carried out at various temperatures controlled by an Omega CN8500 temperature controller, and the setup allowed shearing of the cell for alignment purposes. The electromagnet was made by reworking a 110 V to 220 V transformer by removing the iron core and reconfiguring the primary and secondary coils to act as one large coil. The electromagnet was driven by a Regent home theatre system model HT-391 amplifier allowing the magnetic field to vary up to 100 G. Similar to the piezoelectric measurements, the input signal was provided and the current produced by the sample was measured by the lock-in Amplifier 7265 DSP from PerkinElmer, allowing phase-sensitive detection of the electric current at the frequency of the applied magnetic field.

The main results of the magnetic measurements are summarised in Figure 5, where the magnetic field



(a) (b)

Figure 5. Electric current induced by a 1.1 Hz periodic magnetic field that causes reorientation of the director in the plane along the electrode wires. (a) Magnetic field dependence of the phospholipids: 0.5 wt% ferrofluid mixtures in different alignments and of the control groups. (b) The temperature dependence of the current of the aligned mixture at 100 G.

dependence of the induced currents are shown for the shear aligned and non-sheared phospholipid doped with 0.5 wt% ferrofluid in comparison with the control groups. It can be seen that the effect is largest for the aligned mixture and is much smaller for the control group. All of the phospholipids-containing samples have a constant background of about 3 pA, which is most likely a result of thermally driven director fluctuations. Not surprisingly, the response is independent of the magnetic field in the pure phospholipids and increases with the magnetic field in the mixtures doped with ferrofluid. We also note that the slight linear increase for the pure ferrofluid up to about 2 pA at 100 G is probably a result of magnetic induction. The response in the aligned mixture is about one order of magnitude larger than the background and it can be related to the magnetic-particle-induced director realignment, which was detected optically. The director tilt induces the electric current by the same piezoelectric mechanism that we have illustrated in figure 1 and measured above.

The temperature dependence of the induced electric signal is shown in Figure 5(b). Similar to the piezoelectric observations on the pure phospholipids (see Figure 5(b)), the response decreases at lower temperatures, indicating that the material becomes increasingly rigid against mechanical deformation.

3. Discussion

We have unambiguously demonstrated the piezoelectricity of the SmA* phase of the phospholipid L-α-phosphatidylcholine, both when the director tilt was caused by direct mechanical vibrations and when it was induced by a periodic magnetic field acting on dispersed magnetic nanoparticles.

Although we did not measure the piezoelectric effect in single lipid bilayers, the simple symmetry arguments shown in Figure 1 dictate that single bilayers are also piezoelectric. As phospholipids are the major components of the biological cell membranes, we further hypothesise that the cell membranes are also piezoelectric and electric charges are generated along the membrane when the lipids become tilted as a result of mechanical stimuli. As opposed to the flexoelectric polarisation, here the tilt-induced polarisation occurs within the insulating chains of the bilayers and therefore cannot be screened by free ions of the surrounding aqueous plasma. We believe that cell membrane piezoelectricity might have numerous applications in biological processes and maybe be utilised in mechanoreceptors. For example, our magnetic measurements suggest that piezoelectricity might have a role in magnetoreception where animals use magnetite (Fe_3O_4) particles to sense local changes in magnetic fields (*21, 22*). Magnetoreception of migratory animals is well established experimentally, but its biophysical mechanism is not yet clear (*19*). Although direct coupling of the particles (*23*) linked by strands to ion-channel proteins in the membrane might look like a sufficient explanation, we argue that lateral communication of the ion-channel proteins is needed to provide both orientational and positional information that is necessary for navigation (*19*). In view of our results presented in this article, this effect can be clearly provided via the mechanical induction of lateral piezoelectric charges.

Understanding piezoelectric properties in biology may shed light on a number of biophysical processes involving signalling within cell membranes. Although detecting piezoelectric signals within real biological cell membranes requires much more sophisticated techniques than those we used here, our results clearly show how important those studies could be. The main aim of this article is to stimulate future studies of piezoelectricity of biological cell membranes.

Acknowledgements

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