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Flexoelectricity of lipid bilayers

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The direct flexoeffect in single lipid bilayers in the form of black lipid membranes has been investigated experimentally by the oscillating pressure technique in the regime of voltage measurement. Black lipid membranes of various composition have been studied in order to check the effect of lipid surface charge on the curvature-electric response and its frequency dependence; these include egg yolk lecithin (low negative charge); egg yolk lecithin plus phosphatidyl serine (high negative charge); egg yolk lecithin with surface adsorbed ions of uranyl acetate (high positive charge). An increase of the response has been found by increasing the surface charge and a reversal of the sign of the flexoelectric coefficient from positive to negative has been obtained by changing the sign of the surface charge from negative to positive. These results underline the leading role of the contribution of the surface charge to the flexoelectricity of lyotropics. Their theoretical interpretation provides further insight into the molecular mechanism of this phenomenon.

1. Introduction

The flexoelectricity of lipid bilayers (and structurally related biomembranes) in their liquid crystal state was first postulated in 1974 [1]. One of the possible mechanisms of lyotropic flexoelectricity namely the dipolar mechanism at free lipid flip-flop, was analysed as early as 1975 [2]. The case of free flip-flop was considered in analogy to the dipole flexoelectricity of thermotropic liquid crystals [3], but already at this stage the possible existence of flexoelectricity not only with wedge-like, but also with sterically symmetric (cylindric) lyomesogens was clarified as being related to the specific bilayer (and antiparallel) type of organization of lipid dipoles. The situation of blocked flip-flop considered later [4] lead to the invention of a new dipolar contribution resulting from the curvature-induced variations of the longitudinal components of the molecular dipoles. Such a possibility was later analysed for thermotropic nematics and was called conformational flexoelectricity [5].

In late 1970s experiments with black lipid membranes started to be made based on the flexoelectric theory. They yielded, from dipolar viewpoint, an unexpectedly high magnitude for the direct flexoeffect. At this time the role of the surface charge of lyomesogens started to be revealed [6]. The next step was to formulate a new contribution namely the charge contribution, having no analogue in thermotropics. This charge contribution was further analysed in 1984 [7] for free and blocked flip-flop and/or lateral diffusion and its leading role in black lipid membrane experiments was demonstrated. The quadrupolar contribution was also considered [8] and, unlike thermotropics [9], it was shown to be negligible. Some recent reviews on this topic [10, 11] and the original theoretical calculations [12] should be mentioned. Very recently the oscillating pressure technique was extended to microscopic lipid bilayers

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and native membrane patches located at the tips of patch micropipettes. By this combination with the patch-clamp method the flexoelectric response of a native membrane of locust muscle was measured for the first time [13].

While in most of the previous experiments the current measurement regime (a shorted-out external circuit) was employed, in the present work the voltage measurement regime (an open external circuit) was applied by using a high impedance nanovoltmeter. The results with black lipid membranes with different values and signs of the surface charge and their theoretical interpretation underline the importance of the charge contribution to the flexoelectricity of the lipid bilayer and membrane.

2. Theoretical remarks

We now give some expressions from the phenomenological theory of the direct flexoelectric effect. Layered lyotropic systems like lipid bilayers, black lipid membranes and biomembranes were characterized [1, 2, 8] by an area flexoelectric coefficient f, relating the polarization per unit area P_S to the local bilayer curvature $c_1 + c_2 = 1/R_1 + 1/R_2$, via

$$P_S = f(c_1 + c_2). \tag{1}$$

For a spherical curvature of radius R equation (1) becomes

$$P_S = f\frac{2}{R}. \tag{2}$$

The sign of f is assumed positive, if $\mathbf{P}_S$ points outward from the centre of curvature and negative if $\mathbf{P}_S$ points inward. The units of f is coulombs and the observed order of magnitude is 10^{-18} C.

By crossing a polarized plane with surface polarization P_S we can observe an electric potential jump (transmembrane voltage) U given, according to the Helmholz equation, by

$$U = \frac{P_S}{\varepsilon\varepsilon_0}. \tag{3}$$

Here ε is the dielectric constant of the bilayer interior and ε_0 is the absolute dielectric permittivity of free space. If the membrane curvature $c_1 + c_2$ oscillates in time like

$$\frac{2}{R} = \frac{2}{R_m}\sin\omega t, \tag{4}$$

then the flexoelectric polarization will also oscillate. Depending on the regime of lateral and transversal lipid exchange either blocked (B) or free (F), the polarization will follow instantaneously the curvature with the corresponding flexocoefficient f^B or will be phase shifted and will have a frequency dependent amplitude. Consequently, we can expect to measure in the blocked case

$$U^B = \frac{f^B}{\varepsilon\varepsilon_0}\left(\frac{2}{R_m}\right)\sin\omega t, \tag{5}$$

and in the free case

$$U^F = \frac{f^F}{\varepsilon\varepsilon_0}\left(\frac{2}{R_m}\right)\frac{1}{\sqrt{(1+\omega^2\tau^2)}}\sin(\omega t - \varphi), \tag{6}$$

where $\tan\varphi = \omega\tau$ and τ is the relaxation time of the polarization at a step-wise curvature change.

The free lipid exchange regime is expected at low frequencies and the blocked regime at higher. The aim of the present experiment can be realized by measuring the frequency dependence of the curvature-generated transmembrane voltage from a black lipid membrane subjected to an oscillating pressure difference. To keep a constant value of the curvature amplitude $2/R_m$ at each frequency the so-called capacitance microphone effect can be used, that is essentially a calibration of the amplitude of the second harmonic of the capacitance current, generated by the oscillating membrane under constant voltage difference [7].

3. Experiment

3.1. *Chemicals*

Black lipid membranes were basically formed from chromatographically pure lecithin extracted from egg yolks (EYL) (the lipid was prepared in Centre Paul Pascal, Talence, France and stored in evacuated ampules). 40 mg/ml lecithin in *n*-decane was used as a membrane-forming solution. A drop of this solution was applied with a glass pipette to a 1 mm pretreated orifice in a Teflon plate, partitioning a Teflon chamber into two compartments (see later). In some experiments mixed black lipid membranes of lecithin with negatively charged phosphatidyl serine (PS) (Serva, 20 mg/ml solution in chloroform) were formed. The necessary amount of PS solution was dried on a piece of glass coverslip and then dipped in the membrane-forming solution EYL/*n*-decane until complete dissolving.

Lecithin black lipid membranes modified by uranyl acetate (Merck) were prepared by the addition of various amounts of concentrated uranyl acetate solution in water to the bathing electrolyte. As electrolyte solution an unbuffered saline (0.1 M NaCl in distilled water) or a buffered one with phosphate buffer (pH 6) was used. Strong adsorption of UO_2^{2+} ions on the bilayer lipid surface is expected, thus resulting in a large positive surface charge depending on the volume concentration of uranyl acetate [14, 15].

The purity of EYL was controlled by thin layer chromatography on Merck plates. The surface charge was estimated by measurements of the electrophoretic mobility of large multilamellar liposomes prepared from lecithin in 0.1 mM NaCl using the method described by Bangham. Measurements were performed by a Mark II equipment (Rank Brothers) in the Institute of Physical Chemistry of the Bulgarian Academy of Sciences.

3.2. *Experimental set-up*

The scheme of the experimental set-up is shown in figure 1, essentially, a Teflon chamber consisting of two compartments is used. The front compartment is open to the air and on the front face there is a glass window enabling the observation of membrane formation and thinning by one of the eye-pieces of a low power binocular microscope; a lamp is inserted in the place of the second eye-piece to provide normal illumination of the membrane. The rear compartment is closed by a Teflon cap carrying the two electrodes (Ag/AgCl) and the pipeline transmitting the oscillating air pressure generated by a loudspeaker. A Teflon plate with a cone-shaped orifice is pressed between the two compartments and used as a black lipid membrane support. A static pressure difference between the two compartments may often occur after closing the rear compartment with the cap; it results in a static bulging of the membrane. Membrane planarity is restored by a Teflon leveling piston attached to a

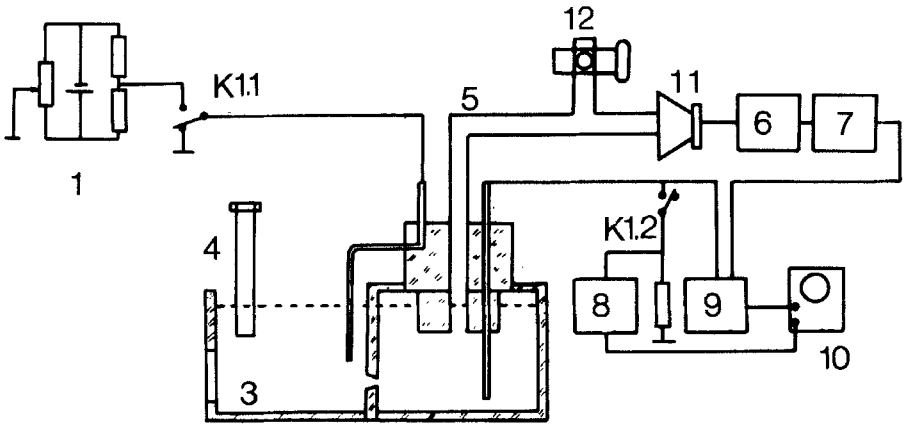

Figure 1. Experimental set-up: 1, DC voltage source, 2, coupled switches (K1.1 and K1.2), 3, measuring cell, 4, levelling piston, 5, vibration system, 6, amplifier, 7, function generator, 8, selective nanovoltmeter UNITRA Type 237, 9, lock-in nanovoltmeter UNITRA Type 232, 10, oscilloscope Textronix-2246A, 11, loudspeaker, 12, valve.

micrometer screw and immersed in the front compartment. The same piston is used deliberately to produce an initial membrane curvature when making experiments to determine the sign of the flexoelectric coefficient (see later).

Membrane thinning is followed electrically by the membrane capacitance. It is measured by a circuit consisting of a 1 kHz triangular voltage and a differentiator, where the membrane serves as an input capacitor to the differentiator. the amplitude of the output rectangular voltage was calibrated with known capacitors. As the dynamics of the membrane oscillation under the oscillating pressure difference depends on the ratio between the stretching elasticities of the membrane and its torus (see later) [16, 17] the aim was to study black lipid membranes with possible smaller tori. Observed visually such membranes displayed a capacitance larger than 2.5 nF, therefore, black lipid membranes of lesser capacitance were not studied further.

Electric potentials generated by the oscillating membrane curvature were registrated by a lock-in nanovoltmeter (Unitra, type 232) referenced by a function generator. A preamplifier of input resistance larger than 100 MΩ was employed in order to obtain a regime of voltage measurement; the AC resistance of the membrane was less than 1 MΩ. Measurements were usually made in the range 70–500 Hz. The second harmonic used for curvature calibration was measured by a selective nanovoltmeter (Unitra, type 237).

3.3. *Measuring procedure*

3.3.1. *Curvature calibration*

After thinning of the black lipid membrane, measuring its capacitance C_0 and visually ensuring its planarity (corresponding also to a capacitance minimum) the loudspeaker was set into operation at a particular frequency by carefully increasing from zero the driving voltage from the function generator. The input of the selective nanovoltmeter, shorted out by a 1 MΩ resistor, was connected to the rear electrode by the key K 1.2, the selective nanovoltmeter was adjusted to the second harmonic of the driving frequency with 40 dB selectivity and a small DC voltage ($\Delta\varphi$ = 100 mV) was applied across the black lipid membrane by the key K 1.1. In this situation we

measured the drop of the capacitance current across the 1 MΩ resistor produced by the oscillation of the membrane capacitance coming from the change of its area (and thickness) in the course of its bulging. (This current is known to be a second harmonic with initially flat membrane [7], having an amplitude

$$I_{2\omega} = \Delta\varphi \Delta C_m \omega, \tag{7}$$

where ΔC_m is the amplitude change of the membrane capacitance.) Now the rms value of the voltage drop across the 1 MΩ resistor was adjusted by changing the driving voltage to obey the relationship

$$U_{2\omega}^{rms}/V = \frac{1}{\sqrt{2}} RI_{2\omega} = \frac{\nu/\text{Hz}}{10^7}, \tag{8}$$

which meant practically 10 μV at 100 Hz; 50 μV at 500 Hz and so on. This, in accordance with equation (7) meant to set up a frequency-independent amplitude of the capacitance change

$$\Delta C_m = \frac{\sqrt{2}}{2\pi} \frac{1}{10^7 R \Delta\varphi} = 0{\cdot}225\,\text{pF}$$

for $R = 1\,\text{M}\Omega$ and $\Delta\varphi = 100\,\text{mV}$.

Now the relation between the capacitance change and the area change (from which the actual curvature radius R_m can be calculated [7]) depends on the bulging regime [16]

$$\frac{\Delta C_m}{C_0} = \frac{\Delta S_m}{S_0} Q(\omega). \tag{9}$$

In the so-called medium bulging regime $Q(\omega)$ takes the frequency independent value of 2α, where α is the ratio of the torus stretching elasticity to the sum of the torus and membrane elasticity. At higher frequencies where the torus viscosity completely prevents its stretching we can reach the limit of fast bulging regime with $Q(\omega)$ of 2. Our data with unmodified EYL membranes suggested the realization of the medium bulging regime over the whole frequency range 50 to 500 Hz like [16, 17], but the uranyl acetate modified membranes probably entered into the fast bulging regime above 300 Hz (see later). The actual curvature was calculated from [7]

$$\frac{1}{R_m} = \sqrt{\left[\frac{\Delta C_m}{C_0} \frac{1}{Q(\omega)}\right]} \cdot \frac{2}{r}, \tag{10}$$

where C_0 is the measured black lipid membrane capacitance and r is its radius, calculated from C_0 by using the flat capacitor expression with a membrane thickness $d = 4{\cdot}8\,\text{nm}$ for EYL/*n*-decane black lipid membranes [18]. Provisionally taking $\alpha \approx 0{\cdot}05$ [16] we could estimate R_m as 9 mm in the medium bulging regime for a black lipid membrane with C_0 of 3 nF. We note that the actual α of the black lipid membrane was not known, but the square root in equation (10) reduced its influence on the uncertainty of the final estimate. In the fast bulging regime the calculation gave a definite value for R_m of 40 nm for the same black lipid membrane (d is not significantly changed by uranyl acetate modification [15].)

3.3.2. *Flexoelectric measurements*

After having adjusted the second harmonic amplitude in accord with equation (8) the selective nanovoltmeter was disconnected, the transmembrane voltage was set to

zero and the lock-in nanovoltmeter was set into operation. Because of its high input impedance measurement of the flexoelectric voltage generated by the oscillating black lipid membrane under open circuit conditions was possible. The phase of the response was adjusted to maximize the in-phase component of the voltage. When a frequency independent response was found (for blocked exchange) the flexocoefficient f^B was calculated from

$$f^B = \varepsilon\varepsilon_0\sqrt{2}U^B_{rms}\frac{R_m}{2}. \tag{11}$$

3.3.3. *Determination of the sign of the flexoelectric coefficient*

In order to determine the sign of f we estimated the phase difference between the capacitive and flexoelectric current with initially curved membranes [7]. If the static membrane curvature is larger than the oscillation amplitude so that the planar state is never reached the membrane oscillations already have the fundamental frequency. The capacitance current is

$$I_C = \omega\Delta\varphi\Delta C\cos\omega t. \tag{12}$$

Its phase depends on the sign of ΔC, which is related to the sign of the static curvature: $\Delta C > 0$ for positive static curvature, i.e. membrane bulged towards the observer and $\Delta C < 0$ for negative static curvature. Reversal of the sign of the curvature reverts the phase of I_C by 180°. $\Delta\varphi$ is also considered positive if the electric field points towards the observer, i.e. if the rear electrode is positive.

The flexoelectric current with $\Delta\varphi = 0$ in the case of blocked exchange is

$$I_P = \omega\frac{S_0}{d}\Delta P\cos\omega t, \tag{13}$$

where S_0 and d are the membrane area and thickness, respectively. Its phase is determined by the sign of the flexocoefficient ($\Delta P = f^B\Delta c$) and does not depend on the sign of the static curvature C_0. Since I_C and I_P have an identical time dependent part simply by comparing the amplitude of I_P at $\Delta\varphi = 0$ and the amplitude of $I_P + I_C$ at a definite sign of $\Delta\varphi$ and ΔC we can obtain the sign of ΔP, i.e. to determine the sign of f^B. In the free exchange case an additional phase difference may arise between the curvature and the polarization precluding a definite conclusion about the sign of f^F from such a simple method.

4. Results

We shall start with the electrophoretic measurements of the surface charge of EYL bilayers. They yield a negative sign of the surface charge and a rather small value of the electrophoretic charge density at the hydrodynamic slipping surface $\sigma_\zeta = -0{\cdot}009\ \mu C/cm^2$ for lecithin from a freshly opened ampule. This value represents the actual surface charge σ_S compensated to a great extent by the counterions in the diffuse Gouy layer confined in the space between the bilayer surface and the hydrodynamic slipping surface. σ_S is about one order of magnitude larger than σ_ζ. Representing it as $\sigma = \beta e/A_0$, where β is the partial charge per polar head, A_0 is the area per polar head and e is the proton charge, we can estimate a value for β of about $-0{\cdot}4$ per cent for $A_0 = 0{\cdot}7\ nm^2$. In the course of time this value increased, mostly due to the oxidation of the double bonds of EYL resulting in charged products. For a

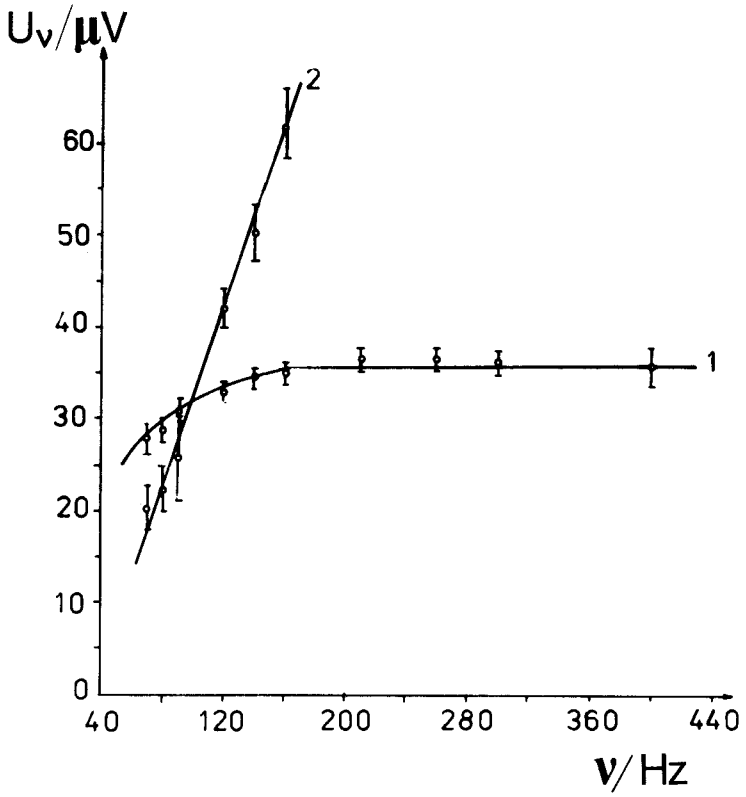

Figure 2. Frequency dependence of the flexoelectic signal of black lipid membranes: curve 1, from egg yolk lecithin/*n*-decane, curve 2, from egg yolk lecithin +2 wt phosphatidyl serine/*n*-decane, electrolyte 0·1 M NaCl.

three-month old probe $\sigma_\zeta = -0{\cdot}104\,\mu C/cm^2$ was measured, i.e. with the same suppositions β of about $-4{\cdot}6$ per cent could be estimated.

The frequency dependence of the amplitude of the flexoelectric response of pure EYL membranes is shown in figure 2. The graph represent the data averaged over 21 membranes. We can see that above 160 Hz the response is essentially frequency independent. This is in accord with equation (5) and makes it possible to draw the conclusion that the blocked lipid exchange case is realized above a frequency of 160 Hz. The same conclusion was reached in our previous investigation of the frequency dependence of the flexoelectric response of the bacterial phosphatidyl-etanolamine/*n*-decane black lipid membrane using a current measuring scheme [7]. In the present experiment measurements below 70 Hz were hindered by experimental difficulties and we were not able to reveal the free lipid exchange case, which should be characterized by a decrease in the response with frequency starting from a very low frequency plateau. In any case the observed increase of the signal in the range from 70 to 160 Hz could reflect the frequency dependence of f^B itself and, eventually, could imply that $f^F < f^B$, as the theory predicts. From the plateau value of the response of each membrane and its capacitance the flexocoefficient f^B is calculated from equations (10) and (11) (taking $Q(\omega) = 2\alpha = 0{\cdot}1$). The mean value of f^B from the 21 measurements is $(5{\cdot}3 \pm 1{\cdot}1)10^{-18}$ C. However, the uncertainty of the final result

depends on the provisional choice for α of 0·05. If we assume a relative error in α as high as 100 per cent then this source of error would only increase the relative error of f^B to 50 per cent (see [7]).

The data for the response of mixed EYL + PS membranes for 2 mol per cent phosphatidyl serine in the membrane forming solution are presented in the same figure 2; the points are averaged over four measurements. Unfortunately these measurements were very complicated because of the shorter lifetime of the black lipid membrane due to the presence of PS. The membranes did not last long enough to permit measurements above 160 Hz and the plateau was not revealed. However the increased response at higher frequencies is evident. A similar increase was obtained in our recent study using patch pipettes and lecithin + serine membranes as component to that for pure lecithin [13].

The data for uranyl acetate modified EYL membranes are shown in figure 3 for various concentrations of uranyl acetate in the bathing electrolyte in the range from 1 to 10 mM/l. Larger concentrations markedly stabilized the membranes and made it possible to extend the measurements to higher frequencies where with unmodified membranes the maximum power of the loudspeaker was insufficient to produce an oscillating curvature amplitude obeying the callibration condition in equation (8). The data with 1 and 3 mM/l of uranyl acetate differ from those with 5 and 10 mM/l. At lower concentrations an initial decrease of the signal is observed, passing a minimum at about 120 Hz, then increasing to a maximum and gradual decreasing in the range 200 to 300 Hz to a plateau. The minimum could reflect a transition from free

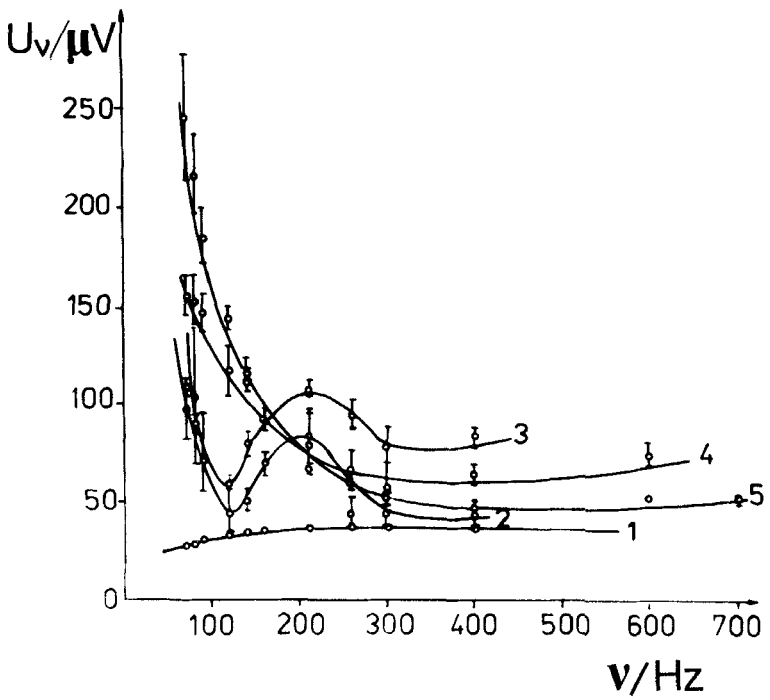

Figure 3. Frequency dependence of the flexoelectric signal of black lipid membranes modified by various concentrations of uranyl acetate in the electrolyte solution: curve 1, no UA (i.e. the same as figure 2, curve 1), curve 2, 1 mM/l curve 3, 3 mM/l curve 4, 5 mM/l curve 5, 10 mM/l.

to blocked exchange and the maximum could be due to a change of the bulging regime from medium to fast bulging because of the increase of the torus viscosity. At higher concentrations blocked exchange most probably prevails over the whole frequency range because of the crosslinking of lipid heads by UO_2^{2+} ions. The decrease of the signal with frequency up to 300 Hz is probably also due to the transition to fast bulging. If this is really the case we can see that this transition is gradually being shifted to lower frequencies with increasing uranyl acetate concentration, which is reasonable. As we have discussed in the fast bulging regime $Q(\omega)$ takes a 20 times higher value (2 instead of 0·1) thus greatly reducing the curvature amplitude necessary to satisfy equation (8); consequently the flexoelectric response will also be reduced. That this is indeed the case can be seen from the decrease of the necessary amplitude of the driving voltage (i.e. the mechanical amplitude of the loudspeaker) compared to that for the unmodified EYL membranes. In all cases the response was larger than the unmodified one. From the plateaux above 300 Hz under the condition of fast bulging in equation (10) flexocoefficients f^B in the range 24 to 40 $\times$ 10^{-18} C were calculated; the values had a maximum at 3 mM/l uranyl acetate.

Finally we describe the results for the sign of the flexocoefficient. They were performed at 300 Hz and $\Delta\varphi = 40$ mV (rear electrode positive). With the EYL membrane the comparison of the flexoelectric amplitude at positive (outward) curvature to that of the sum of flexoelectric and capacitance revealed an increase when switching on $\Delta\varphi$. According to equations (12) and (13) this means that the two currents have the same phase i.e. as $\Delta\varphi\Delta C > 0$ it follows that $\Delta P > 0$ or $f^B > 0$. The conclusion concerning the positive sign for the egg yolk lecithin membrane flexocoefficient is further supported by the experiment with negative (inward) static curvature. In that case a decrease of the total signal was observed when switching on $\Delta\varphi$. The positive sign is opposite to the sign of the surface charge established electrophoretically.

When the experiment was repeated with modified EYL with black lipid membrane in the electrolyte the results were just the opposite to those described, showing that $f^B < 0$ over the whole uranyl acetate concentration range studied. This negative sign is also opposite to the positive surface charge due to the strong UO_2^{2+} ion adsorbtion [14, 15].

5. Discussion

In order to discuss the results we need an expression for the contribution to the flexoelectric coefficient due to the surface charges, taking into account also the screening effect of counterions from the diffuse double layer. For this we could make use of the expression for the dipolar flexoelectric contribution at blocked exchange [10, 11]

$$f^{DB} = \left(\frac{\mu}{A_0} - \left(\frac{d\mu}{dA}\right)_{A_0}\right) d, \tag{14}$$

where μ is the normal component of the dipole moment per lipid head (considered positive if directed from the water to the hydrophobic core), $d\mu/dA$ is its derivative with respect to the area, A_0 is the area per head in the planar state and d is the membrane thickness. The flexocoefficient for the free case is easily obtainable by replacing d with $2\delta_H$ to give

$$f^{DF} = 2\left(\frac{\mu}{A_0} - \left(\frac{d\mu}{dA}\right)_{A_0}\right) \delta_H, \tag{15}$$

where δ_H is the distance between the neutral surface of each monolayer and the head group surface [10, 11]. Comparison of equations (14) and (15) shows that $f^{DF} < f^{DB}$ is always fulfilled. However, it has been established that the pure dipole contribution given by equations (14) and (15) is too small [11].

Now we could introduce for charged lipids a dipole moment connected with the charge of the polar head and the double layer counterions charge, considering it as concentrated over a second plane of a capacitor of thickness λ_D, the Debye length. This is a popular approximation in the diffuse double layer theory. Then we could write

$$\mu = \beta e \lambda_D, \tag{16}$$

where

$$\lambda_D = \sqrt{\left[\frac{\varepsilon\varepsilon_0 RT}{2F^2 c}\right]} \tag{17}$$

and c is the ion concentration. This way, substituting equation (17) in equation (15) and taking λ_D to be independent of area A we find

$$f^{CB} = e\left(\frac{\beta}{A_0} - \left(\frac{d\beta}{dA}\right)_{A_0}\right)\lambda_D d. \tag{18}$$

This result can be derived rigorously by direct calculation of the potential jump across a curved bilayer bearing a surface charge and immersed in an electrolyte; this is to be published elsewhere.

Equation (18) demonstrates a direct correlation between the value and sign of the flexocoefficient and the value and sign of the first term of the right hand side, i.e. the value and sign of the surface charge. Concerning the second term with $d\beta/dA$ the situation is more complicated because depending on its value and sign it could even reverse the sign of f^{CB} observed in the present experiment. $d\beta/dA$ could be evaluated from monolayer measurements of the surface potential of the same partially charged egg yolk lecithin. Alternatively, it may be estimated theoretically on the basis of an adsorption/desorption model (to be published elsewhere) which gives

$$f^{CB} = \frac{b\alpha_0 e}{2A_0\sqrt{[(n_0 + b)^2 + 2Db\alpha_0 e/A_0]}}\lambda_D d, \tag{19}$$

where $D = (2_{ze}/kT)(n_0\lambda_D/\varepsilon\varepsilon_0)$, n_0 is the concentration of the ions, which can be adsorbed in the aqueous solution sufficiently far from the membrane, ze is their electric charge, b is the constant of the adsorption/desorption equilibrium and α_0 is the partial electric charge per polar lipid head when there was no adsorbtion. However, the sign of f^{CB} in this model is the same as the surface charge α_0, while the present experimental situation is just the opposite.

In fact, inspection of equation (18) shows that the first term even at complete charging of every head group ($\beta = 100$ per cent) gives a rather small contribution to f^{CB}. Indeed, with 0·1 M/l electrolyte, i.e. $\lambda_D = 0{\cdot}97\,\text{nm}$, $A_0 = 0{\cdot}7\,\text{nm}^2$, $d = 4{\cdot}8\,\text{nm}$, $f^{CB} \approx 1 \times 10^{-18}\,\text{C}$ is calculated, which is distinctly lower than the experimental value. This suggests the leading role of the $d\beta/dA$ term, whose value is not well known for the present lipid composition. Earlier evaluations of $d\beta/dA$ for phosphatidylethanolamine from monolayer measurements [7] really demonstrated its leading contribution. Recent theoretical calculations of $\beta(A)$ for dimyristoylphosphatic acid [19] could be used to calculate a similar value of $d\beta/dA$ as in [7] and in both cases

the sign of $d\beta/dA$ is the same as that of β. This could explain, on the basis of equation (18), the opposite signs of f^{CB} and β. Further experimental work is also necessary to check the effect of λ_D term in equation (18) i.e. the screening effect of the ionic strength. Our previous experiments with ethanolamine have shown that this effect really exists [7].

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