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Curvature induced conductive and displacement currents through lipid bilayers

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Résumé. — On présente une discussion sur les courants électriques produits par la courbure des bicouches lipidiques. Quatre sources de courants sont étudiées, celles dues aux charges électriques transportées par les groupements de têtes ou celles provenant des dipôles de ces groupements. Deux de ces sources sont actives quand le flip-flop est lent, et les deux autres quand le flip-flop est rapide. On donne une expression générale de ces courants pour chaque fréquence d'oscillations de la courbure.

Abstract. — A discussion of the curvature generated currents in lipid bilayers is presented. Four sources of currents are recognized as due to the charges carried by or bonded to the lipid head groups and to the head group dipoles. Two of these sources are operating at slow flip-flop, while the other two operate at fast flip-flop. A general expression for the current valid at any frequency of the curvature oscillations is derived.

Recently some experiments demonstrated that by curving periodically bilayer lipid membranes (BLM), one can observe an electric current in the external circuit even without applying an external electric field [1, 2, 3]. It was supposed that a phenomenon similar to the piezoelectric effect exists in BLM [1], connected with the periodical membrane stretching. Later on, realizing that a membrane is a liquid crystalline (LC) structure, this phenomenon was ascribed to the flexoelectric effect, connected with the periodical membrane bending [4]. The flexoelectric effect is very likely of great biological significance in the process of transformation of mechanical energy, into electric energy, and *vice versa*, in native LC structures like biomembranes [4].

The flexoelectric effect emphasizes the role of dipoles and quadrupoles of lipid molecules. Dipoles may operate either at free flip-flop (dipole reorientation) [5] or at blocked flip-flop (bending induced change of the dipole magnitude) [6, 7]. The quadrupoles do not require any flip-flop in general [7]. The dipolar and quadrupolar currents have a pure displacement character.

Unfortunately, although providing a qualitatively consistent picture, these mechanisms cannot explain the true value of the current amplitude ($\sim 10^{-10}$ A [7]). They provide values one order of magnitude lower than the experimentally observed ones. This does not mean that the flexoeffect is not of importance for native membranes, but some other mechanism(s) should give the essential contribution(s) at the conditions used in the experiments with model systems (BLM).

An elucidation of the role of charges seems necessary. It was started with the recent papers of Paschenik *et al.* [8, 9]. The authors stressed the fact that, in the experiment performed earlier [1], periodical deformation was achieved by means of oscillating hydrostatic pressure. The pressure gradient drives water flow through the membrane. Lateral lipid density fluctuations [10] represent an effective way for water and ion transport. Due to the difference in reflection coefficients for positive and negative ions, selective drag of positive ions by water flow may result in a conductive current through the membrane [11, 8, 9]. But our own estimates, using the

measured value of the hydraulic conductivity L_p of BLM and realizing that the difference of the reflection coefficients (both being almost equal to 1) cannot be bigger than 10^{-2} , have given a value even 3-4 orders of magnitude lower than the experimental current amplitude.

Two other contributions of charges will be discussed in the present letter. The experiments under consideration were performed with BLM made from egg yolk lecithin (containing also cholesterol in the case of [2]). The lecithin head group is zwitterion and its total charge is zero at normal pH. But some lecithin molecules could bind positive ions like K^+ or Na^+ [12]. The presence of some amount of impurities like charged lipids cannot be excluded either.

At free flip-flop conditions, some of the lipid molecules which pass from the compressed inner monolayer of the bent membrane to the expanded outer one may serve as carriers of a dipole and a charge simultaneously. This would result in a conductive current of positive charges through the membrane.

At blocked flip-flop conditions, the change of the surface density of charges at both surfaces of the bent membrane is equivalent to a fast displacement current.

In general, we will characterize the flip-flop rate by the half lifetime, τ . This time may be rather long in model systems but is rather short in native membranes. Let us note also that in the experiments under consideration here, it is more correct in fact to speak about « forced flip-flop » driven by the density gradient in the bent membrane. The half lifetime, τ , may be strongly reduced if metastable pores in the membrane are formed [13, 14]. The hydrophilic edges of these pores bridge the two monolayers of the BLM, forming in this way a path for transbilayer exchange of lipid molecules by means of fast lateral diffusion [15]. One can imagine now that a continuous monolayer is flowing through the edge(s) like a conveyor belt to adjust the transbilayer density difference (or, more correctly, the elastic energy difference [5]). At very low frequencies, exchange of lipid material with the border may take place as well. But it is known that this process is ineffective above several Hz [1].

It is our aim to derive a general expression for the current, valid for any value of the flip-flop rate and for arbitrary frequency of the curvature oscillations. Thus we need to know the transbilayer density difference as a function of time. Let us denote the number of molecules per unit area in the external monolayer by v^e , in the internal layer by v^i and their difference by Δv :

$$\Delta v = v^e - v^i. \quad (1)$$

Both v^e and v^i are expressed with respect to unit area of the middle surface of the bilayer, which we consider as nonextended at the bending. For Δv we can write an equation analogous to that used in the theory of dielectric relaxation [16]:

$$d(\Delta v)/dt = (\Delta v_m - \Delta v)/\tau \quad (2)$$

where Δv_m is the equilibrium density difference.

For spherically curved surfaces, the total curvature $c_1 + c_2$ is expressed by the radius of curvature in the following way:

$$c_1 + c_2 = 2/R. \quad (3)$$

If we induce a curvature $2/R$ initially and wait long enough so that $d(\Delta v)/dt = 0$, Δv will become equal to the equilibrium density difference Δv_m . In general [5]:

$$\Delta v_m = (1/2) v_0 d(2/R) \frac{2k_h + k_c}{k_h + k_c} \quad (4)$$

where $v_0 = (v^e + v^i)/2$ is the mean lipid density, d is the membrane thickness and k_h and k_c are the elastic constants for area compression for the heads and the chains of the lipid molecules, respectively. In the case $k_h \ll k_c$, our result becomes:

$$\Delta v_m = (1/2) v_0 d(2/R) \quad (5)$$

which is identical with the Helfrich's result [17], obtained with the assumption that the flip-flop proceeds until the lipid densities with respect to the middle surfaces of each monolayer (situated at distances $\pm (d/4)$ from the middle surface of the bilayer) become equal. In what follows, we will use the simpler form (5).

Consider a curvature, oscillating in time according to the law:

$$c(t) = (2/R_m) \cos \omega t \quad (6)$$

where R_m is the minimal value of the radius of curvature. Then the equilibrium density difference will oscillate as well:

$$\Delta v_m = v_0(d/R_m) \cos \omega t. \quad (7)$$

Putting (7) into (2), we get for the steady state solution of the differential equation the expression:

$$\Delta v(t) = \frac{v_0(d/R_m)}{\sqrt{1 + \omega^2 \tau^2}} \cos(\omega t - \varphi) \quad (8)$$

where $\tan \varphi = \omega \tau$.

Let us assume now that κv_0 lipid heads are carrying a charge e ($0 < \kappa < 1$). The surface density of charges at planar state of the membrane will be:

$$\sigma_0 = \kappa e v_0 \quad (9)$$

where e is the electron charge. Due to the oscillating membrane curvature, a difference of surface densities of charges at both surfaces will exist at any time:

$$\Delta \sigma(t) = \kappa e \left(\frac{v^e}{1 + (d/2)c} - \frac{v^i}{1 - (d/2)c} \right) \approx \kappa e [\Delta v(t) - v_0 d c(t)]. \quad (10)$$

We see that $\Delta\sigma$ consists of two terms. The first is connected with real transport of charges and the second is due to stretching and compressing the membrane surfaces. Substituting (6) and (8) into (10), we can calculate the current density as follows :

$$j_q(t) = \frac{d}{dt} \Delta\sigma = -\kappa e v_0 (d/R_m) \frac{\omega}{\sqrt{1 + \omega^2 \tau^2}} \times \\ \times \cos(\omega t - \varphi) + 2\kappa e v_0 (d/R_m) \omega \sin \omega t. \quad (11)$$

Consequently, two components of the current are obtained — a conductive one and a displacement one. Before discussing the result (11) in detail, we will calculate, in an analogous way, the displacement current due to the dipoles.

Let the dipolar moment of the lipids in the external monolayer be denoted by μ^e and that in the internal layer by μ^i . In general, $\mu^e \neq \mu^i$ due to the area dependence $\mu(A)$ [6]. The polarization per unit area in the outward direction is :

$$P_s = v^i \mu^i - v^e \mu^e = v_0 (\mu^i - \mu^e) - \\ - (\Delta v/2) (\mu^i + \mu^e) \approx v_0 (d\mu/dA) \Delta A - \mu \Delta v. \quad (12)$$

Here μ is the value of the dipolar moment at $A = 1/v_0$ and for ΔA , we have :

$$\Delta A = (1 - (d/2)c/v^i) - (1 + (d/2)c/v^e) \approx \\ \approx \Delta v/v_0^2 - cd/v_0. \quad (13)$$

The displacement current density is equal to the derivative of the polarization per unit volume :

$$j_d(t) = \frac{d}{dt} (P_s/d) = -\frac{v_0}{R_m} \left(\frac{1}{v_0} \frac{d\mu}{dA} - \mu \right) \times \\ \times \frac{\omega}{\sqrt{1 + \omega^2 \tau^2}} \sin(\omega t - \varphi) + \frac{d\mu}{dA} \frac{2}{R_m} \omega \sin \omega t. \quad (14)$$

Now we can compare both results for j_q and j_d . The first terms in (11) and (14), which are phase shifted with respect to the second ones, represent the low frequency contribution from the real transitions of lipid molecules or the free flip-flop contribution. The second terms, which are dominant at high frequencies, represent the blocked flip-flop contribution of charges and dipoles, respectively.

In the low frequency limit, $\omega \rightarrow 0$, we get :

$$j_q = \kappa e v_0 (d/R_m) \omega \sin \omega t. \quad (15)$$

The two contributions to j_q do not cancel each other because the charge density difference is established at the outer membrane surface while the equalization of the number density of lipids is taking place with respect to the middle surface of each monolayer, having two times lower relative change in area. Similarly,

$$j_d = (v_0/R_m) [(1/v_0)/(d\mu/dA) + \mu] \omega \sin \omega t. \quad (16)$$

The contribution of $d\mu/dA$ is still present at $\omega \rightarrow 0$ due to the same reasoning as above.

In the high frequency limit, we will have :

$$j_q = 2\kappa e v_0 (d/R_m) \omega \sin \omega t \quad (17)$$

$$j_d = 2(1/R_m) (d\mu/dA) \omega \sin \omega t. \quad (18)$$

The experimentally observed values of the amplitude of the electric current density at $(\omega/2\pi) = 50$ Hz are 10 statcoul/s.cm² [2] (egg lecithin-cholesterol-*n*-decan BLM, 0.1 M NaCl solution) and 12.5 statcoul/s.cm² [3] (egg lecithin-*n*-decan BLM, 0.1 M NaCl solution). The dipole moment of lecithin and its derivative are : $\mu = 0.6 \times 10^{-18}$ statcoul.cm, $d\mu/dA = 5 \times 10^{-5}$ statcoul/cm, respectively. The other parameters have the following values :

$$e = 4.8 \times 10^{-10} \text{ statcoul}, \quad v_0 \approx 2 \times 10^{14} \text{ cm}^{-2},$$

$$d = 7 \times 10^{-7} \text{ cm} \quad \text{and} \quad R_m = 0.2 \text{ cm}.$$

If we assume that oscillations at 50 Hz are in the low frequency limit, to achieve agreement of the sum of the amplitudes from (15) and (16) with the experimental data, it is necessary to put $\kappa = 9.2\%$. If, on the contrary, we are in the high frequency limit, it follows from (17) and (18) that we need $\kappa = 4.7\%$ for agreement. Both values seem reasonable, so it is not possible to judge from this alone what is really the flip-flop rate in these experiments. New careful studies of the frequency dependence of the current seem necessary.

Anyway, it appears that the ionic component of the electric current is rather high in comparison to the dipolar one which makes it difficult to use such experiments for measurements of the flexoelectric coefficients. For this purpose, the value of κ must be decreased, e.g. by means of taking electrolyte solutions with bigger atomic radii of the positive ions (like RbCl or CsCl). It is known that the binding constant of these ions to lecithin heads is smaller [12].

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