

Calcium-Induced Changes in Oxidized Cholesterol Bilayers

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Calcium is known to play a significant role in biological systems, e. g. as a modifier of membrane processes of activation and inactivation of ionic channels (Khodorov 1975). A strong interaction of Ca^{2+} with negatively charged lipids is well documented (Papahadjopoulos 1968; Triggle 1972; Tocanne et al. 1974). However, the only studies on planar lipid bilayers available are those of Papahadjopoulos and Ohki (1969) and Ohki and Papahadjopoulos (1970). These have shown that an asymmetric addition of Ca^{2+} to phosphatidylserine black films produces an increase in membrane conductance and, at a given concentration (depending on pH), it induces breakdown of the film. Laclette and Montal (1977) found that CaCl_2 , when added to a solvent-free membrane made from phosphatidylserine of cardiolipine to only one of its sides appreciably does not change its physical stability and conductance. In contrast, black films (which contain a solvent) are unstable to this asymmetric addition of CaCl_2 . These authors have shown that the destabilizing effect of Ca^{2+} can be attributed to a variation in the composition between the two types of bilayer membranes, namely to the solvent content of the bilayer, rather than to a surface charge difference. A spin label study of biological membranes and liposomes by Ohniski (1975) and Ohki et al. (1981) has shown calcium induced lateral phase separation in these systems.

To our knowledge, only one paper has so far dealt with the problem of the influence of Ca^{2+} ions on the mechanical properties of vesicles. It is a work by Mitaku and Aruga (1982) who registered a value about 20 % increase in the volume elasticity modulus K , for vesicles made of dipalmitoylphosphatidylcholine under the influence of Ca^{2+} ions in a concentration range of 10—100 mmol/l.

It is known, however, that for elastic materials, such as membranes, following relationship may be accepted: $K \gg E_{\parallel} > E_{\perp} \gg G$ (Passechnik 1980), where E_{\parallel} , E_{\perp} are the Young moduli of elasticity in direction parallel and perpendicular to the membrane plane respectively, and G is the modulus of lift. Therefore we can expect the value of E_{\perp} , which characterizes the mechanical properties of the hydrophobic region of the membrane, to be more susceptible to the influence of Ca^{2+} ions.

In accordance with the facts mentioned above, we estimated the value of E_{\perp} to study the influence of Ca^{2+} ions on the hydrophobic region of the bilayer. Our studies were performed on two types of membranes, namely: i) membranes made from oxidized cholesterol in *n*-octane (BLM), ii) membranes of the same type made solvent-free. Membranes were formed in the aqueous phase which contained 0.1 mol/l NaCl: various concentrations of CaCl_2 were added.

The method published by Passechnik and Hianik (1977) was used to measure the electrical capacity and elasticity of BLM. An alternating voltage $U = U_0 \cdot \sin 2\pi ft$ (U_0 —amplitude of the voltage, f —frequency, t —time) was applied to the membrane, inducing a pressure $p = C_m U^2 / 2h$ (C_m —specific electrical capacity of the membrane, h —its thickness), and leading to a decrease in the membrane thickness *phenomenon of electrostriction*.

The compression of the membrane by an alternating electrical field induced modulation of the alternating current flowing across the membrane. The latter is a current of a basic frequency, f , and amplitude, A_1 ; it is equal to the capacity of the membrane, C , $A_1 = 2\pi f C U_0$. In addition, a current component of a frequency of $3f$ and an amplitude $A_1 \gg A_3$, was evoked. The ability of the membrane to change its thickness under the influence of an external force is characterized by the Young modulus of elasticity in the direction perpendicular to the membrane plane, E_{\perp} . In the case of membranes made from oxidized cholesterol (Wobschall 1972) and in solvent-free membranes (Passechnik 1980), an isotropic model of membrane deformation can be taken into consideration. In this case (Passechnik and Hianik 1977), following relationship was used:

$$E_{\perp} = \frac{3}{4} \frac{C_m \cdot U_0^2}{h} \frac{A_1}{A_3}$$

The method of measurement of the modulus E_{\perp} consists in recording the values A_1 and A_3 . Some of the real values for membranes used in present work were as follows: capacity $C_0 = 5 \cdot 10^{-10} \text{F}$; voltage on the membrane $U_0 = 150 \text{mV}$; amplitude of the capacity current at a frequency of $f = 990 \text{Hz}$ $A_1 = 4.7 \times 10^{-7} \text{A}$; pressure $p = 10^4 \text{N/m}^2$; $A_3 = 1.6 \times 10^{-9} \text{A}$; and the ratio $A_1/A_3 = 2.9 \times 10^2$, being equal to the modulus $E_{\perp} = 4.3 \times 10^6 \text{N/m}^2$.

BLM containing 40 mg/ml *n*-octane in were formed by the method of Mueller et al. (1963) on a hole (0.3 mm in diameter) in the wall of a teflon bowl. Bilayers were formed by the method of Rudnev et al. (1981) using a mixture of *n*-octane and dioxane.

The concentration of the membrane-forming solution was 5 mg/ml, the volume composition of the solvent was 2 % *n*-octane and 98 % dioxane. Dioxane gets out easily to the aqueous phase. Consequently, a membrane containing practically no solvent originates. Oxidized cholesterol was obtained by the method

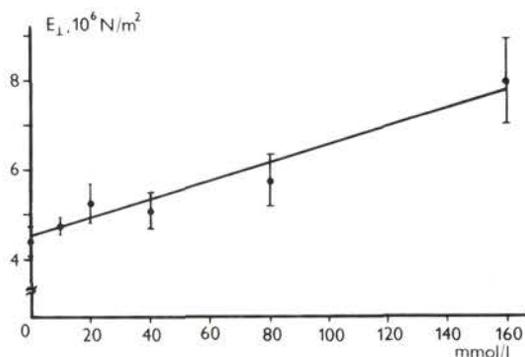


Fig. 1. Dependence of the value of E_{\perp} (ordinate) on the concentration of Ca^{2+} ions (abscissa), BLM made from oxidized cholesterol in *n*-octane.

of Tien et al. (1966). All the preparations were chemically pure; monodistilled water was used. All experiments were made at room temperature ($T = 20^{\circ}\text{C}$). In each series of experiments, measurements were performed on 10–15 membranes, and mean quadratic errors were calculated. Results obtained with extreme concentrations of Ca^{2+} ions were subjected to statistical analysis using the Student criterion.

A linear increase in the value of E_{\perp} with increasing Ca^{2+} concentration was characteristic for BLM (Fig. 1). Based on a study of BLM stability at the presence of Ca^{2+} ions (Laclette and Montal 1977) the effect of calcium ions may be brought into association with expulsion of the solvent from the bilayer region of the membrane. The increase in the modulus E_{\perp} may be a result of this effect. This was also observed in membranes made from egg lecithin in *n*-heptane at the presence of 20 mmol/l to 40 mmol/l of calcium. (Passechnik et al. 1981).

On the other hand, bilayers practically do not contain solvent. They have a considerable value of the specific capacity, C_m , and of the modulus, E_{\perp} . We obtained $C_m = (0.4 \pm 0.1) \mu\text{F} \cdot \text{cm}^{-2}$ for BLM made from oxidized cholesterol in *n*-octane, and $C_m = (0.81 \pm 0.04) \mu\text{F} \cdot \text{cm}^{-2}$ for bilayers. In addition, the value of E_{\perp} for bilayers was 16 times higher than that for BLM (Fig. 2). Moreover, bilayers were more stable than BLM. On a continuous application of a voltage $U_0 = 150$ mV for 10 minutes, the voltage of the electrical breakdown was 1.5 times higher as compared with that for BLM, and it reached a value of about 500 mV.

A considerable increase in the value of E_{\perp} with increasing Ca^{2+} concentration was typical for bilayers (Fig. 3). This considerable increase in E_{\perp} for bilayers was recorded in a concentration range of Ca^{2+} ions (20–40 mmol/l) lower than that used for the measurements with BLM. At the same time, the specific capacity of

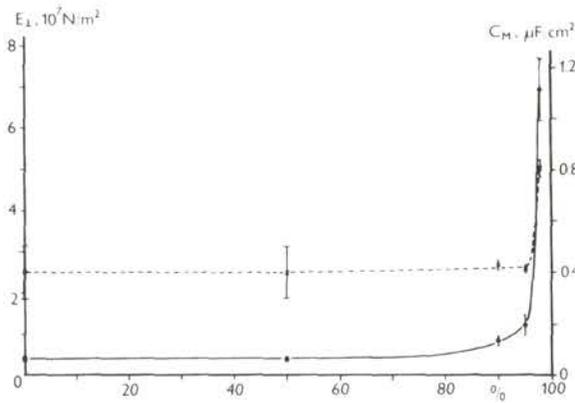


Fig. 2. Dependence of the value of E_1 (●) and C_m (×) on various n-octane: dioxane ratio in membrane-forming solutions at 20°C; m: abscissa: dioxane content in %.

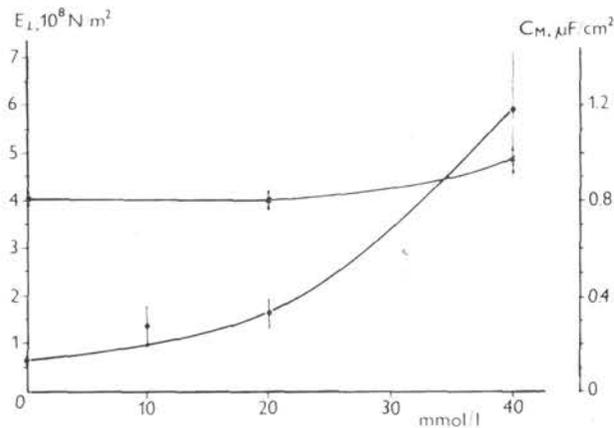


Fig. 3. Dependence of the value of E_1 (●) and C_m (×) on the concentration of Ca^{2+} ions; solvent-free membranes made from oxidized cholesterol.

the bilayer did not appreciably change i. e. the value of A_3 changed considerably, and the compressibility of the hydrophobic region of the membrane changed accordingly. Statistical evaluation of experimental results using by the Student criterion for the extreme range of Ca^{2+} concentrations: (0—160 mmol/l for BLM, and 0—40 mmol/l for bilayers) showed statistically significant differences in values of E_1 ($p \geq 99\%$).

The interaction of calcium with the membrane may be due to specific adsorption of the former on to the membrane surface from oxidized cholesterol. Cholesterol oxidation produces five products. Three of them are stable and differ

from each other by oxygen binding on the head groups of the hydrocarbon cycles of cholesterol Gibbons et al. (1982). Calcium likely does not form ionic bonds with oxygen, forming weak complexes of the chelate type.

A similar phenomenon of chelation — formation of complexes of polyvalent cations with polar groups of phospholipids of monolayers — was reported in papers by Papahadjopoulos (1968), Shah (1982). Mitaku and Aruga (1982) have explained the influence of Ca^{2+} ions in the same way, i. e. as a result of chelation — formation of complexes of Ca^{2+} ions with vesicles from dipalmitoyl-phosphatidylcholine using NMR spectroscopy. Yabusaki and Wells (1975) showed that calcium probably forms complexes with oxygen of the head groups of negatively charged phospholipids. Calcium may therefore be assumed to form chelates with the oxygen head groups of oxidized cholesterol.

It can be suggested that the formation of chelate complexes of Ca^{2+} ions with oxygen of the head groups of oxidized cholesterol would guide to a sterically better ordering of the lipid monolayer. Consequently, the membrane is less compressible by electrostriction.

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References

- Gibbons G. F., Mitropoulos K. A., Myant N. B. (1982): *Biochemistry of Cholesterol*. Elsevier, North-Holland
- Khodorov B. I. (1975): *General Physiology of Excitable Membranes*. Nauka, Moscow (in Russian)
- Laclette J. P., Montal M. (1977): Interaction of calcium with negative lipids in planar bilayer membranes. Influence of the solvent. *Biophys. J.* **19**, 199—202
- Mitaku S., Aruga S. (1982): Effect of calcium ion on the mechanical properties of lipid bilayer membrane. *Biorheology* **19**, 185—196
- Mueller P., Rudin D. O., Tien H. Ti, Wescott W. C. (1963): Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* **67**, 534—535
- Ohki K., Sekiya T., Yamauchi T., Nozawa Y. (1981): Physical properties of phosphatidylcholine--phosphatidylinositol liposomes in relation to a calcium effect. *Biochim. Biophys. Acta* **644**, 165—174
- Ohki S., Papahadjopoulos D. (1970): Asymmetric phospholipid membranes: effect of pH and Ca^{2+} . In: *Surface Chemistry of Biological Systems*. (Ed. M. Blank) pp. 155—174, Plenum Publishing Corporation, New York
- Ohnishi S. (1975): A spin-label study of biological membranes with special emphasis on calcium-induced lateral phase separation. *Adv. Biophys.* **8**, 35—82
- Papahadjopoulos D. (1968): Surface properties of acid phospholipids: interaction of monolayers and hydrated liquid — crystals with uni- and bi-valent metal ions. *Biochim. Biophys. Acta* **163**, 240—254
- Papahadjopoulos D., Ohki S. (1969): Stability of asymmetric phospholipid membranes. *Science* **164**, 1075—1077

- Passechnik V. I. (1980): On a model of elastic bilayer membrane. *Biofizika* **25**, 265—269 (in Russian)
- Passechnik V. I., Hianik T. (1977): Elastic properties of bilayer membranes in the direction perpendicular to the membrane plane. *Kolloid. Zh.* **39**, 1180—1185 (in Russian)
- Passechnik V. I., Hianik T., Artemova L. G. (1981): Change of bilayer lipid membranes elastic properties by Ca-ATPase incorporation. *Stud. Biophys.* **83**, 139—146
- Rudnev V. S., Bagaveev I. A., Rovin Yu. G. (1981): Dry planar bilayer lipid membranes. In: *Biophysics of the Membranes* (Ed. L. A. Blumenfeld) pp. 3—16, Nauka, Moscow (in Russian)
- Shah D. O. (1972): Monolayers of lipids in relation to membranes. *Prog. Surf. Sci.* **3—3**, 221—278
- Tien H. Ti., Carbone S., Dawidowicz E. A. (1966): Formation of "black" lipid membranes by oxidation products of cholesterol. *Nature* **212**, 718—719
- Tocanne J. F., Ververgaert P. H. J. Th., Verkleij A. J., Van Deenen L. L. M. (1974): A monolayer and freeze-etching study of charged phospholipids. II. Ionic properties of mixtures of phosphatidylglycerol and lysyl phosphatidylglycerol. *Chem. Phys. Lipids* **12**, 220—231
- Triggle D. J. (1972): Effect of calcium on excitable membranes and neurotransmitter action. *Progr. Surf. Membr. Sci.* **5**, 267—331
- Wobschall D. (1972): Voltage dependence of bilayer membrane capacitance. *J. Coll. Interface Sci.* **40**, 417—423
- Yabusaki K. K., Wells M. A. (1975): Binding of calcium to phosphatidylcholines as determined by proton magnetic resonance and infrared spectroscopy. *Biochemistry* **14**, 162—166

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