Capacitance–voltage relationship in phospholipid bilayers containing gangliosides

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Changes in the position of the minimum of the parabolic capacitance–voltage curve allow the measurement of the amount of ganglioside present in artificial bilayers made with phosphatidylcholine–ganglioside mixtures and asymmetrically shielded with Ca^{2+}. The screening effect of the ionic solution must be considered. With ganglioside/phospholipid molar ratios of up to 15%, all glycolipids can be found at the membrane surfaces.

Ganglioside *Lipid bilayer* *Membrane capacitance* *Surface charge*

1. INTRODUCTION

Gangliosides are sialoglycosphingolipids mainly located at the outer surface of the mammalian cell membrane. The presence of the sialic acid in the oligosaccharide chain is the main distinctive feature of these membrane glycoconjugates. Though a detailed knowledge of the role of this class of lipids in the membrane organization is not yet established, there is much evidence concerning their implication in several biological processes involving membrane-mediated information [2–4]. The composition and location of gangliosides are often dramatically modified in neoplastic cells [3,5,6].

To investigate the role played by the glycoconjugates in membrane phenomena, we are studying the influence of gangliosides on the electrical properties of artificial black lipid membranes made with dioleoyl-phosphatidylcholine. GM_1, GD_{1a} and GT are the mono-, di- and tri-sialogangliosides used.

Experiments have been performed by different authors on gangliosides containing lipid bilayers [7–9]. However, the preparation of the binary mixtures with DOPC in *n*-decane presents some difficulties owing to the solubility peculiarities of gangliosides. This makes necessary a careful control of the presence of gangliosides at the BLM surfaces. Our findings show that the electrical capacity properties of the membrane allow the measurement of the amount of gangliosides present in the BLM.

2. THEORY

Several investigators have demonstrated that the electrical capacity of solvent-containing BLM is voltage-dependent [10–14], the capacity–voltage relationship being given by the following equation:

$$[C(V) - C(0)]/C(0) = \alpha(U + V)^2 + \beta \tag{1}$$

where:

$C(0)$ = the capacity at zero applied voltage;

Abbreviations: BLM, black lipid membranes; DOPC, dioleoylphosphatidylcholine; PS, phosphatidylserine; GM_1, Galβ1$\longrightarrow$3GalNAcβ1$\longrightarrow$4Gal (3$\longleftarrow$2αNeuAc) β1$\longrightarrow$4Glcβ1$\longrightarrow$1Cer; GD_{1a}, NeuAcα2$\longrightarrow$3Galβ1$\longrightarrow$3GalNAcβ1$\longrightarrow$4Gal (3$\longleftarrow$2αNeuAc)β1$\longrightarrow$4Glcβ1$\longrightarrow$1Cer; GT, NeuAcα2$\longrightarrow$3Galβ1$\longrightarrow$3GalNAcβ1$\longrightarrow$4Gal(3$\longleftarrow$ 2αNeuAc8 $\longleftarrow$ 2αNeuAc)β1 $\longrightarrow$ 4Glcβ1$\longrightarrow$1Cer (abbreviations recommended by Svennerholm [1])

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V = the applied voltage;
U = the potential across the membrane due to surface charge asymmetry. In symmetrically charged membrane $U = 0$.

The parameter α depends on the percentage and type of the organic solvent used to form the bilayer, and the value of β is determined by the choice of the reference capacity. The dependence of the electrostatic potential ψ at the membrane surface on the charge density σ and electrolyte concentration $\tilde{C}$ is predicted by the Gouy equation [15]:

$$\sigma = (\sqrt{\tilde{C}}/A)\sinh(ze\psi/2kT) \qquad (2)$$

where:
e = the electronic charge;
z = the valence of the electrolyte solution;
k = the Boltzmann constant;
T = the absolute temperature.

Furthermore:

$$A = 1/\sqrt{8N\epsilon_r\epsilon_0 k_T} \qquad (3)$$

where:
N = Avogadro's number;
ϵ_0 = the permittivity of free space;
ϵ_r = the dielectric constant of the water solution
(ϵ_r = 78.3, dielectric constant of the water at 25°C [15])

If ψ and ψ' are the electrostatic potentials at the interfaces due to the charge densities σ and σ', respectively, we have $U = \psi - \psi'$. Under our experimental conditions $\psi' = 0$ ($\sigma' = 0$) and $U = \psi$, therefore the knowledge of the value of U allows the calculation of σ.

3. MATERIALS AND METHODS

DOPC was purchased from SERVA Feinbiochemica (Heidelberg). Phosphatidylserine and gangliosides, extracted from beef brain, were generously provided by FIDIA Res. Labs. All lipids were > 98% pure and organic solvents were of analytical grade. Binary mixtures of DOPC and gangliosides were obtained dissolving appropriate amounts of each in chloroform:methanol (2:1, v/v), were then dried extensively under vacuum to remove traces of solvent and finally dissolved in *n*-decane (20 mg/ml). Molar ratios of mixtures GM_1–DOPC, GD_{1a}–DOPC and GT–DOPC were, respectively, 15.3%, 12.9% and 14.8%. Mixtures with concentrations of gangliosides > 15% formed membranes which presented very high mechanical and electrical instability.

Bilayer lipid membranes were prepared as in [16]. They were formed through a 0.7-mm hole in a thin teflon septum dividing two compartments containing buffered 0.025 M NaCl (pH 7) at a fixed temperature (25°C). To create a surface charge asymmetry, a solution of 1.1 mM $CaCl_2$ was added to one compartment to screen charged groups (sialic acid and serine), as in [14]. The experiments were carried out in voltage clamp and the stationary capacity measured by comparing the electrical signals from BLM and from a variable RC circuit.

Experimental points in fig.1–5 are mean values of at least 4 measurements performed on each of 8 membranes. Continuous curves represent the best fit of the experimental points with eq. (1).

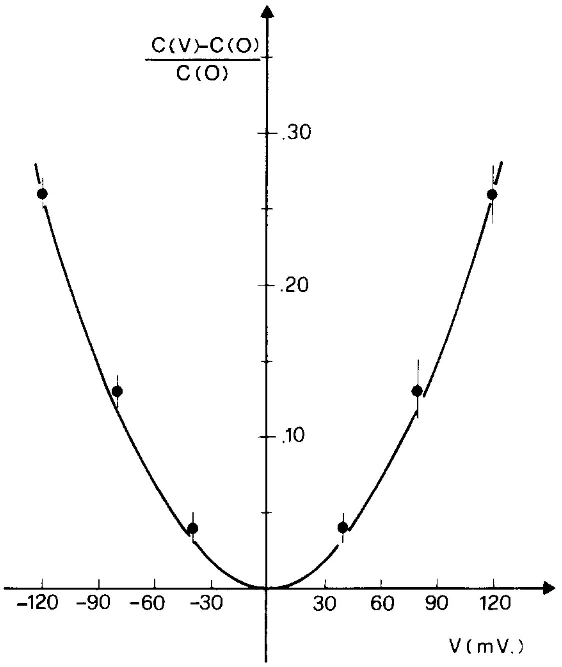

Fig.1. Electrical capacitance *vs* applied potential of a pure DOPC membrane, after the addition of 1.1 mM $CaCl_2$ in a single compartment (asymmetrical conditions). The minimum of the curve is at $V = 0 \pm 2$ mV.

4. RESULTS AND DISCUSSION

Fig.1 shows the $[C(V-C(0)]/C(0)$-dependence on applied voltage V for pure DOPC membranes in asymmetrical conditions. The values of α, β and U for this curve are, respectively, $\alpha = 18 \pm 1\ \mathrm{V}^{-2}$, $\beta = 0 \pm 0.01$, $U = 0 \pm 2$ mV. The minimum of the curve is at zero applied potential, therefore we can exclude the presence of an electrostatic surface potential in pure DOPC bilayers. Accordingly, it has been reported [15] that dipole potentials of membranes made of zwitterionic phospholipids do not vary for changes in the external ionic solutions.

The capacitance–voltage relationship for membranes made of the GM_1–DOPC mixture is given in fig.2. Curve a, whose axis is coincident with the ordinate axis, shows that, in absence of $CaCl_2$, the charge density is the same on both membrane surfaces ($U = 0$). Addition of 1.1 mM $CaCl_2$ shifts the minimum of $[C(V) - C(0)]/C(0)$ from zero voltage to 18 mV (curve b). Therefore -18 mV is the potential U across the membrane due to the charge asymmetry. The values of the parameters resulting from the fit are: $\alpha = 18 \pm 3\ \mathrm{V}^{-2}$, $\beta = -0.02 \pm 0.01$, $U = -18 \pm 2$ mV. Further increases of $[CaCl_2]$ do not affect curve b indicating that 1.1 mM of Ca^{2+} is enough to screen entirely the charge on the selected surface. Inserting the value $U = -18 \pm 2$ mV on eq. (2) we obtain for the charge density $\sigma_{GM_1} \simeq 1$ charge/2500 Å^2. Because the mean area of the membranes is 0.31×10^{14} Å^2, it follows that the total number of charges q_{GM_1} is $\simeq 1.3 \times 10^{10}$.

We verified also that the addition of $CaCl_2$ in the opposite compartment abolished the observed surface potential.

Similar experiments are reported in fig.3 for GD_{1a}–DOPC and in fig.4 for GT–DOPC mixtures. We summarize here the results. The values of the parameters for the curve in fig.3 are $\alpha = 12 \pm 1\ \mathrm{V}^{-2}$, $\beta = -0.02 \pm 0.01$, $U = -30 \pm 3$ mV. With the value $U = -30 \pm 3$ mV we obtain from eq. (2) the charge density $\sigma_{GD_{1a}} \simeq 1$ charge/1400 Å^2 and, for the number of charges, $q_{GD_{1a}} \simeq 2.1 \times 10^{10}$ (mean area of the membranes $= 0.30 \times 10^{14}$ Å^2).

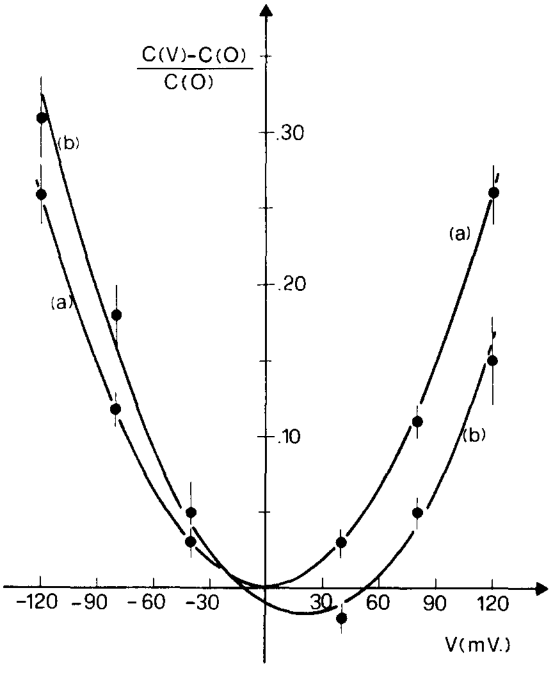

Fig.2. Electrical capacitance *vs* applied potential of mixed membranes made with GM_1 and DOPC in *n*-decane (20 mg/ml): (a) membranes formed between symmetrical ionic solutions; (b) after the addition of 1.1 mM $CaCl_2$ in a single compartment. The minimum of (b) is at $V = 18 \pm 2$ mV; $T = 25°C$; NaCl = 25 mM.

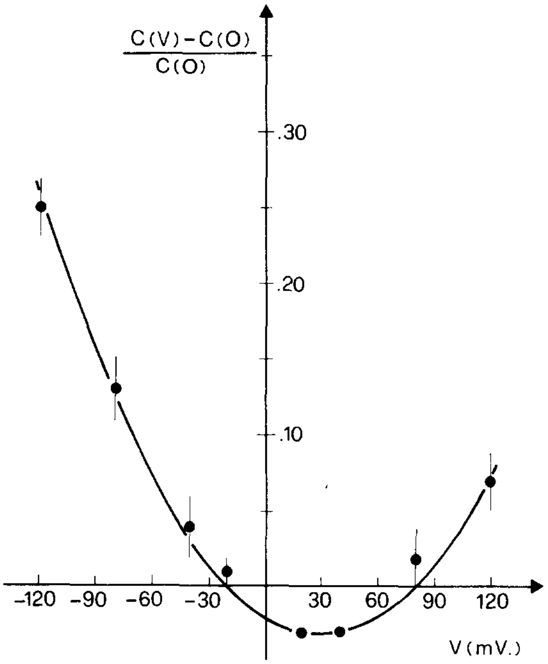

Fig.3. Electrical capacitance *vs* applied potential of mixed GD_{1a}–DOPC membranes. All conditions as in fig.1. Minimum at $V = 30 \pm 3$ mV.

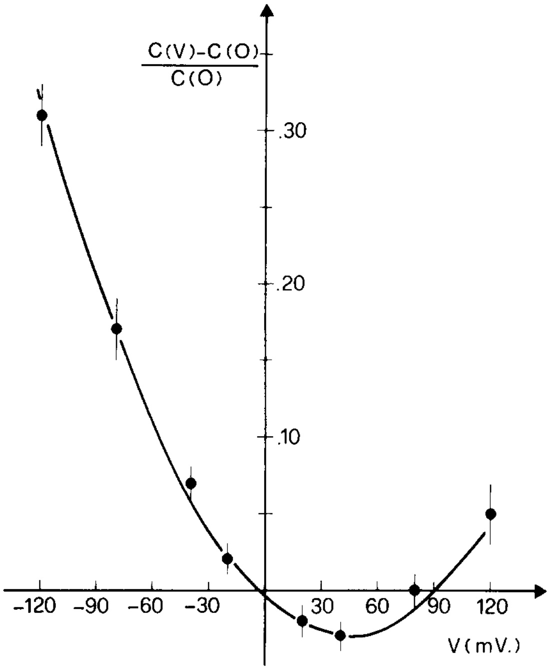

Fig.4. Electrical capacitance *vs* applied potential of mixed GT–DOPC membranes. All conditions as in fig.1. Minimum at $V = 45 \pm 4$ mV.

For the curve in fig.4 we have: $\alpha = 12 \pm 1\ V^{-2}$, $\beta = -0.02 \pm 0.01$ and $U = -45 \pm 4$ mV, therefore $\sigma_{GT} \simeq 1$ charge/900 Å^2 and $q_{GT} \simeq 4.1 \times 10^{10}$ (mean area = 0.35×10^{14} Å^2). Both σ- and q-values have uncertainties of ~10% in all experiments.

Values of α for all the 3 equations agree well with [11,17–19].

The values of q_{GM_1}, $q_{GD_{1a}}$ and q_{GT} cannot be simply correlated with the amount of GM$_1$, GD$_{1a}$ or GT contained in the membrane, but the screening effect exerted by the buffered NaCl solution on the surface charges has to be considered.

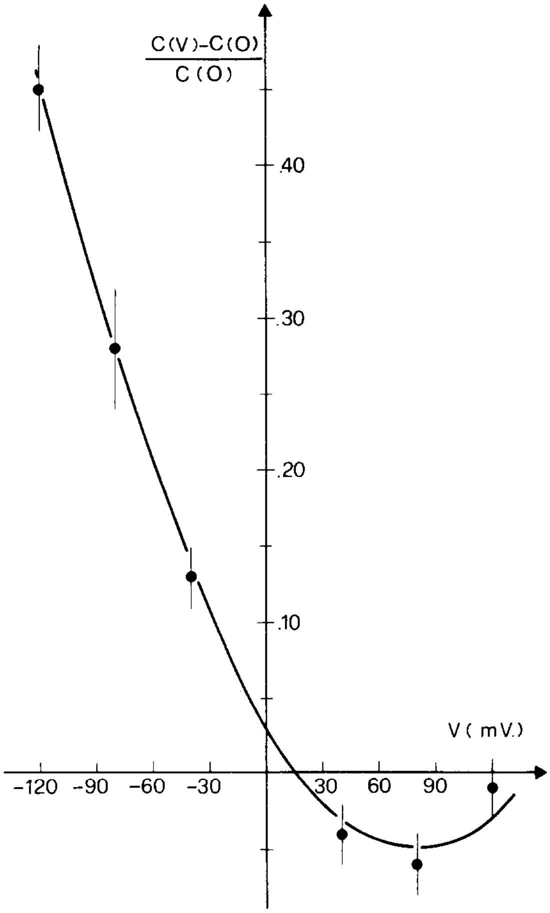

Fig.5. Electrical capacitance *vs* applied potential of pure PS membranes. All conditions as in fig.1. Minimum at $V = 80 \pm 5$ mV.

Fig.5 shows the results obtained with pure phosphatidylserine bilayers under the same experimental conditions. The values of the parameters of this curve are: $\alpha = 12 \pm 2\ V^{-2}$, $\beta = -0.05 \pm 0.01$ and $U = -80 \pm 5$ mV. Therefore we obtain $\sigma_{PS} \simeq 1$ charge/350 Å^2 and $q_{PS} \simeq 8 \times 10^{10}$ (mean area of the membranes = 0.29×10^{14} Å^2). As a phospholipid molecule in a BLM occupies a mean area of 60 Å^2 [15,20,21] the total number of charges on the membrane surface can be calculated and is equal to $q^*_{PS} \simeq 4.8 \times 10^{11}$. Then the screening effect of the electrolytes can be represented by the ratio $q_{PS}/q^*_{PS} = 0.17$ that we name 'screening coefficient' of the solution. As the electric charge is due to the presence of carbossilic groups at the polar head of PS and ganglioside molecules we can make the assumption that the same screening coefficient holds in both cases at the same value of pH. The knowledge of the value of the screening coefficient allows the calculation of the actual number of charges on the surface of the membranes and therefore of the number of ganglioside molecules present in the mixed bilayers. We derive $q^*_{GM_1} \simeq 7.5 \times 10^{10}$, $q^*_{GD_{1a}} \simeq 1.2 \times 10^{11}$ and $q^*_{GT} \simeq 2.4 \times 10^{11}$. Whereas GM$_1$ brings only one charge/molecule, GD$_{1a}$ 2 charges and GT 3 charges/molecule, the densities of ganglioside molecules are, respectively: $N_{\mathrm{GM}_1} \simeq 2.4 \times 10^{11}/\mathrm{mm}^2$, $N_{\mathrm{GD}_{1a}} \simeq 2.1 \times 10^{11}/\mathrm{mm}^2$ and $N_{\mathrm{GT}} \simeq 2.3 \times 10^{11}/\mathrm{mm}^2$. A BLM contains $\sim 1.6 \times 10^{12}/\mathrm{mm}^2$ phospholipid molecules [20,21] regardless of the length of the hydrophobic chain of the solvent [22]. Therefore the molar ratios we were looking for are GM_1:DOPC $\simeq$ 15%, GD_{1a}:DOPC $\simeq$ 13% and GT:DOPC $\simeq$ 14%, which are in very good agreement with the values indicated for the mixtures. We can conclude that up to ganglioside:phospholipid molar ratios of 15% all the glycolipids will be found in the lipid bilayer.

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