NOTES

Influence of Cholesterol on the Surface Charge Density and Surface Potential of Lipid Bilayer Membranes

The surface charge density and potential of asolectin membranes with and without cholesterol were measured. When the cholesterol content was at a 2:1 molar ratio of asolectin/cholesterol, there was a 25% increase in both magnitudes. The possible influence of this on the explanation of other phenomena is discussed.

INTRODUCTION

A great proportion of biological membranes are known to present a surface charge density due to charged groups in their constituent lipid molecules. Many of these membranes are known to contain cholesterol.

Since Bernard's pioneering paper about molecular associations of lecithin and cholesterol in 1958 (1), several authors have extensively studied the effects of cholesterol on many physicochemical properties of lipid monolayers and bilayers (2–7). Others have performed structural analyses of these mixtures by X-ray diffraction (8, 9); part of this knowledge was considered by Presti *et al.* (10), who proposed a packing model for the molecular interaction between cholesterol and phospholipids, combining the strong van der Waals attraction and hydrogen bonding, and thereby permitting the hydrocarbon chains to come in close contact. As a consequence, cholesterol would produce an increase in the number of charged head groups per unit area and hence a corresponding increase in the surface charge density. In the present experiment we tried to determine the magnitude of this effect.

MATERIALS AND METHODS

(a) Membrane Formation and Electrical Measurements

The lipids used were L-α-phosphatidylcholine (Asolectin) from soybean, Type II-S, Sigma Chemical Company, and cholesterol, analytical grade, min 99% (GC), Serva, Feinbiochemica, Heidelberg. We chose a natural lipid instead of a pure or synthetic one in order to be closer to a natural system. Brain phospholipids could be used as well. Asolectin is a charged lipid since it contains an average of 8.8% cardiolipin (phosphatidylglycerol), which has one negative charge per molecule (11). Membranes without cholesterol were formed from a solution containing 10 mg of Asolectin dissolved in 500 μl of *n*-decane. Membranes with cholesterol were formed from a solution of 7 mg of Asolectin plus 2 mg of cholesterol dissolved in 500 μl of *n*-decane. Membranes were formed by the brush technique (12) and were supported by a quartz partition in cup configuration (13).

The aqueous solutions at the beginning of each experiment were 10 m*M* KCl + 2 m*M* EDTA ($Na_2H_2Y \cdot 2H_2O$) + 2 m*M* Tris ($C_4H_{11}NO_3$), at pH 7.2. The contents of both compartments were continuously stirred after membrane formation.

The steady-state membrane conductance was determined by means of a four-electrode system. Two Ag/AgCl electrodes were used to pass current and another pair of similar electrodes, placed nearer the membrane, read the voltage V_m across the membrane. The voltage electrodes were connected to a Keithley 602 electrometer having a high input impedance. The current was provided by a simple battery/potentiometer assembly in series with a 10^8 ohm resistor in order to function as a current-clamp device and was read using a 43K Analog Devices op-amp in a current-to-voltage configuration. The output voltage (V_{out}) of the op-amp was fed into a Tektronix 5115 oscilloscope. Membrane voltage deflections never exceeded ±20 mV.

The membrane conductance, G, was calculated as

$$G = \frac{1}{R_{fb}}\left(\frac{V_{out}}{V_m}\right), \qquad [1]$$

where R_{fb} is the feedback resistance of the op-amp. The unmodified membrane conductance was quite variable, as is the rule with lipid bilayer preparations, but membranes with conductances higher than 10^{-8} mho $\times$ cm^{-2} were discarded. Valinomycin was added to both sides of the aqueous solutions at a dose of 1×10^{-8} g/cm^3 and steady-state membrane conductances were typically 1–3 $\times 10^{-3}$ mho $\times$ cm^{-2} after valinomycin addition.

(b) Surface Charge Measurements

The membrane conductance due to the valynomicin–potassium complex was used to probe the membrane surface potential, according to the procedure used by Muller and Finkelstein (14). This method is based on the ratio of

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two conductance measurements of the same membrane, under two different conditions: First, the membrane conductance, G_1, is measured in a KCl aqueous solution containing no trace of divalent cations (2 mM EDTA is present); a short time later a precise quantity of Mg^{2+} ions is added to both sides of the membrane in order to screen most of the negative charges present on the membrane surface. The new steady-state conductance, G_2, is measured after 2 min and the change in surface potential is calculated from

$$Z_{02} - Z_{01} = \ln(G_1/G_2), \qquad [2]$$

where

$$Z_{0i} = \frac{e\Psi_{0i}}{kT}, \quad i = 1, 2,$$

and Ψ_{0i} is the surface potential.

The general relation between the surface charge density, σ, and the surface potential, Ψ_0, is given by the Grahame equation (15–16):

$$\sigma^2 = \frac{\epsilon RT}{2\pi} \sum_j |j|_\infty \{\exp(-\nu_j Z_0) - 1\}. \qquad [3]$$

If σ is given in units of esu/cm^2, the following parameters have to be considered: ϵ is the dielectric constant of water (ϵ = 78.54 at 298°K); R is the gas constant (R = 8.3143 erg (°K)$^{-1}$ mole^{-1}); T is the absolute temperature (T = 298°K in our experiment); $|j|_\infty$ is the concentration in moles/ml of the jth ion at infinity, i.e., very far from the membrane surface; ν_j is the valence of the ion j; e is the elementary charge ($e = 4.80298 \times 10^{-10}$ esu); and k is the Boltzmann constant ($k = 1.38054 \times 10^{-16}$ erg (°K)$^{-1}$).

We have applied Eq. [3] to two situations:

Situation 1. Before adding Mg^{2+} ions to the KCl bathing solutions:

$$\frac{\sigma^2 2\pi}{\epsilon RT} = |K^+|_\infty \{\exp(Z_{01}) - \exp(-Z_{01}) - 2\} + \{|Na^+|_\infty + |Tris^+|_\infty\}\{\exp(-Z_{01}) - 1\} + |HY^{3-}|_\infty \times \{\exp(3Z_{01}) - 1\} + |H_2'Y^{2-}|_\infty \{\exp(2Z_{01}) - 1\}. \qquad [4]$$

In this situation we have considered $|K^+|_\infty = |Cl^-|_\infty = 10^{-5}$ moles/ml, $|Na^+|_\infty = 4 \times 10^{-6}$ moles/ml, $|Tris^+|_\infty = 1.85 \times 10^{-6}$ moles/ml, $|HY^{3-}|_\infty = 1.82 \times 10^{-6}$ moles/ml, $|H_2'Y^{2-}|_\infty = 0.18 \times 10^{-6}$ moles/ml. Where $|HY'^{-}|_\infty$ (ν = 2, 3) are the ions given out by the EDTA molecule.

Situation 2. After adding Mg^{2+} ions to both bathing solutions (Mg^{2+} ions were provided by adding 1-μl aliquots of a 0.5 g/ml $MgSO_4 \cdot 7H_2O$ stock solution), until the conductance reached a new steady state. Putting the new values of the ion concentrations into Eq. [3], and replacing Z_{01} in Eq. [3] by Z_{02} given by Eq. [2], we have

$$\frac{\sigma^2 2\pi}{\epsilon RT} = |K^+|_\infty \left\{\frac{G_1}{G_2}\exp(Z_{01}) + \frac{G_2}{G_1}\exp(-Z_{01}) - 2\right\} + |Mg^{2+}|_\infty \left\{\left(\frac{G_2}{G_1}\right)^2 \exp(-2Z_{01}) - 1\right\} + |SO_4^{2-}|_\infty \left\{\left(\frac{G_1}{G_2}\right)^2 \exp(2Z_{01}) - 1\right\} + \{|Na^+|_\infty + |Tris^+|_\infty\}\left\{\frac{G_2}{G_1}\exp(-Z_{01}) - 1\right\}. \qquad [5]$$

In Eq. [5] we have considered: $|K^+|_\infty = |Cl^-|_\infty = 10^{-5}$ moles/ml, $|Na^+|_\infty = 4 \times 10^{-6}$ moles/ml, $|Tris^+|_\infty = 1.85 \times 10^{-6}$ moles/ml, $|Mg^{2+}|_\infty = |MgSO_4|_{added} - 2$ mM (because the Mg^{2+} ion complexes with EDTA negative ions; see Table I), $|SO_4^{2-}|_\infty = |Mg^{2+}|_\infty + 2$ mM = $|MgSO_4|_{added}$.

Since the membrane surface charge density, σ, remains unchanged, expressions [4] and [5] should be identical and were estimated by varying Z_{01} ($-2 < Z_{01} < -1$, $Z_{01}(I) = -2 + I$ with $0 < I < 0.95$ in steps of 0.005) and calculating the difference between the right-hand sides of expressions [4] and [5]. If this difference is less than 10^{-7} the agreement is considered to be satisfactory.

RESULTS

The results are summarized in Table I. The effect of cholesterol (in a 2:1 Asolectin/cholesterol molar ratio) added to the lipid bilayer was an increase of approximately 25% in the surface charge density and surface potential of the membrane.

DISCUSSION

The present study was done to determine whether it is possible to detect changes in membrane surface charge density induced by the inclusion of cholesterol in the lipid matrix. According to Szabo (6), cholesterol induces an increase in the dipolar surface potential of neutral lipid membranes. This was determined on the basis of changes in orientation, strength, and packing density of molecular dipoles near the membrane surface. The analysis and interpretation of our results are certainly complicated by the fact that the membranes we studied carry a fixed charge density at their surfaces. These complications will not, however, affect our interpretation as we only determined changes in surface potential induced by Mg^{2+}.

The effect of cholesterol can therefore be interpreted as a packing effect caused by the interspersed cholesterol molecules approximating the charged polar head groups

TABLE I

Surface Charge Densities and Surface Potentials of Membranes with and without Cholesterol

Membranes without cholesterol[a]				Membranes with cholesterol[b]			
$\lvert Mg^{2+}\rvert_\infty$ (moles/ml × 10^6)	G_1/G_2	σ_i ((−) esu/cm^2)	Ψ_{0i} ((−) mV)	$\lvert Mg^{2+}\rvert_\infty$ (moles/ml × 10^6)	G_1/G_2	σ_i ((−) esu/cm^2)	Ψ_{0i} ((−) mV)
0.91	8.92	4042	29.6	3.71	15.62	5841	41.3
1.12	7.95	3841	28.2	3.70	16.00	5904	41.7
3.66	7.14	3947	29.9	2.69	12.00	5029	36.2
1.86	5.71	3261	24.1	1.77	11.11	4679	33.9
1.77	7.50	3816	28.0	1.73	11.36	4737	34.3
0.90	8.93	4041	29.6	3.53	12.50	5247	37.6
2.61	8.00	4078	29.8	2.72	11.36	4892	35.3
1.75	7.50	3451	25.4	2.61	12.00	5009	36.1
1.86	7.40	3805	27.9	1.86	12.50	4970	35.8
1.77	6.50	3518	25.9	2.66	14.00	5388	38.5

[a] $\sigma = (-3780 \pm 88)$ esu/cm^2; $\Psi_0 = (-27.8 \pm 0.6)$ mV.

[b] $\sigma = (-5170 \pm 135)$ esu/cm^2; $\Psi_0 = (-37.1 \pm 0.8)$ mV.

Note. The results are reported as $\sigma = \bar{\sigma} \pm \epsilon_{\bar{\sigma}}$ and $\Psi_0 = \bar{\Psi}_0 \pm \epsilon_{\bar{\Psi}_0}$, where $\bar{\sigma}$ and $\bar{\Psi}_0$ are the mean values for the surface charge density and surface potential and $\epsilon_{\bar{\sigma}}$ and $\epsilon_{\bar{\Psi}_0}$ their corresponding standard mean deviations.

and thereby increasing the charge density at the membrane surface.

It is known that cholesterol influences the conductance of bilayers to valynomicin–potassium complexes, as reported by Szabo (6). This effect, however, is not relevant in the interpretation of our results, since we calculate the charge density from the ratio of conductances of the same membrane modified solely by the addition of divalent ions to the aqueous solutions. Therefore, changes in membrane fluidity or other parameters that do not directly affect the surface charge density are cancelled. The effect of Mg^{2+} ions per se on membrane parameters other than surface potential cannot be ruled out in our protocol.

The effect of cholesterol on the membrane surface charge density could be related to the observations of Sebba (17) that the interfacial tension was reduced by the presence of cholesterol in oil–lamella biliquid foams. Variations in surface tension, γ, are related to variations in surface charge density by the Lippmann equation (18), namely,

$$\gamma = \gamma_0 - \frac{S}{2C}\sigma^2, \qquad [6]$$

where γ_0, S, and C are the interfacial tension in the absence of the charge, the area, and the capacitance of the interface, respectively. We observe in Eq. [6] that an increase in σ produces a diminution of γ.

Also, if the effect of increasing the membrane surface charge density by the presence of cholesterol is maintained in natural (or pathological) conditions it could be responsible for the breaking away of cells in metastasis by decreasing the intercellular adhesion forces (19).

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