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Ca^{2+} adsorption to lipid membranes and the effect of cholesterol in their composition

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ABSTRACT

The aim of this work is to determine the binding of ionic calcium (Ca^{2+}) to lipid membranes in which the availability of the phosphate groups to the aqueous phase is modified by the degree of saturation of the lipids and the inclusion of cholesterol.

The shifts in the phosphate bands observed in the Fourier transform infrared spectroscopy (FTIR) spectra are direct evidence of the interaction of Ca^{2+} with phosphate groups.

The binding analysis was done by determining the changes in the zeta potential of liposomes suspended in buffer at controlled temperature. The changes produced by the ion on the zeta potential of dioleoylphosphatidylcholine (DOPC); dipalmitoylphosphatidylcholine (DPPC); distearoylphosphatidylcholine (DSPC); dimyristoylphosphatidylethanolamine (DMPE) and their mixers with cholesterol were measured, showing a Langmuir isotherm behavior in all the lipid composition assayed.

The results show that the interaction of Ca^{2+} to lipid membranes depends on the exposure and the density of phosphate groups at the membrane interphase.

1. Introduction

There is increasing interest in the binding of metal cations to biological membranes because it affects the stability and the properties of phospholipid bilayers. Specifically, the adsorption of calcium to the surface of biological membranes has important physiological consequences such as the modulation of the insertion of proteins (for reviews see [1,2]). Studies on model membranes have demonstrated that these physiological effects may be due not only to a binding of ionic calcium (Ca^{2+}) but also to a screening effect in the aqueous diffuse double layer at the lipid–water interface [3]. In this regard, it is also important to take into account that Ca^{2+} may act as a second messenger in the transduction of biologically relevant functions.

Numerous binding sites can buffer the ion concentration in cells. For example, retinal membranes have been suggested to bind a considerable fraction of the intracellular Ca^{2+} ions [4]. Most likely, the membranes buffering capacity is an important factor determining the distribution of ions within living cells [5].

Ions interact with charged phospholipids *via* Coulombic forces. The apparent association of metal cations with lipid membranes is distinctly more intense for anionic lipids than for neutral, zwitterionic ones. This behavior can be explained taking into account that the net negative surface charges of membranes of acidic lipids increases cation concentration near the lipid–water interface according to the Gouy–Chapman theory of the electrical double layer [6].

The respective intrinsic association constant of Ca^{2+} was, however, shown to be virtually independent of the net surface charge after correction for differences in electric surface potential [7,8]. It reflects specific lipid–ion interactions such as orientation dependent charge–dipolar interactions with neutral residues.

Ions can deeply penetrate into the polar region of the membrane, which is an interphase rather than an interface because the polar residues are distributed throughout a mesh-like region [9].

Thus, it is likely that there may be regions playing the role of Ca^{2+} reservoir and with the ability to exchange it with the aqueous media. To achieve this function, the binding capacity should be modulated by the properties of the surface. In this regard, lipid membranes present different domains composed by lipids in different phase state that may have different affinities for Ca^{2+}. In an attempt to get an insight on the binding properties of lipid membranes composed by mixtures commonly found in biological

Abbreviations: Ca^{2+}, ionic calcium; PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; PE, phosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; Chol, Cholesterol; MLVs, Multilamellar Vesicles; ζ, Zeta potential; μ, electrophoretic mobility; FTIR, Fourier transform infrared spectroscopy; T_c, transition temperature; T_w, work temperature.

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systems, we have measured the binding curve to lipid membranes in the gel and fluid state composed by different kinds of phosphatidylcholines and phosphatidylethanolamines and its mixtures with cholesterol. The effect of cholesterol on the phase state of the lipids is an important variable. As it has been already reported in the bibliography, cholesterol has a dual nature — in the physiologically important fluid state it promotes ordering and rigidity, while in the gel state its effects are the opposite [10].

As Ca^{2+}–phospholipids' interaction is due to electrostatics between the phosphate groups, the aim of this work is to determine the binding of Ca^{2+} to lipid membranes in which the exposure of the phosphate to the aqueous phase has been modified by the degree of saturation of the lipids and the inclusion of cholesterol.

A direct evidence of the interaction of Ca^{2+} with phosphate groups was evaluated by the changes in the phosphate bands of Fourier transform infrared spectroscopy (FTIR) spectra. The binding analysis was done by determining the changes in the zeta potential of liposomes suspended in buffer at controlled temperature.

2. Materials and methods

Distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylethanolamine (DMPE) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL). The lipids were 99% pure and used without any further purification, after being checked by thin layer chromatography (TLC), using the solvent mixture corresponding to each type of phospholipid. Single spots after exposure to the suitable developers were obtained.

Peroxidation levels in unsaturated phospholipids (DOPC) were checked by ultraviolet spectroscopy, and it was found negligible under the conditions employed.

Cholesterol (Chol), Hepes, calcium chloride ($CaCl_2$), potassium chloride (KCl), potassium bromide (KBr) and sodium chloride (NaCl) were obtained from Sigma Chemical Co. Inc. (St. Louis, MO).

All other chemicals were of analytical grade and ultrapure water (conductivity 0.09 μS/cm, pH 6 ± 0.3) was obtained in an Osmoion equipment (Apema, Buenos Aires, Argentina).

2.1. Multilamellar vesicle preparation

Lipids in a chloroform solution were dried under N_2 stream. Lipid mixtures of PC:Chol were prepared by co-dissolving the appropriate proportions in chloroform, expressed as mol/mol ratios.

Multilamellar liposomes (MLVs) were prepared by dispersing the dry lipid film in Hepes 100 μM, KCl 1 mM, pH 7, buffered solution. In all cases, the relation lipid to water was maintained in order to make the preparation more reproducible.

2.2. FTIR in liposomes

Lipids were dispersed in H_2O and sealed in a cell with AgCl windows at constant temperature (22 °C). The FTIR spectra were obtained using a Nicolet™ 380 infrared spectrophotometer, equipped with a deuterated triglycine sulphate (DTGS) detector with 1 cm^{-1} spectral resolution. Values corresponding to non-hydrated lipids were measured in KBr disks. A total of 200 scans were done in each condition and the spectra were analyzed using the mathematical software WinFirst 3.5. A number of different samples (no less than four) were processed.

In order to study the interaction of phosphate groups with Ca^{2+}, spectra of MLVs of DPPC and DPPC:Chol (4:1) in absence and presence of ionic calcium were done. The main bands corresponding to the phosphate groups were analyzed.

2.3. Zeta potential in liposomes

The zeta potential (ζ) of MLVs liposomes at different calcium chloride concentrations was determined in Zeta-Meter System 3.0 equipment (Staunton, VA, USA) at 22 °C.

The electrophoretic mobility (μ) of multilamellar liposomes was determined in a capillary cell in which two electrodes were connected to a dc source. The total lipid concentration in all cases was 46 μM.

Data reported are the average of the measurements done, for each condition, with, at least, three different batches of liposomes. A total of 20 measurements were done for the different batches.

The zeta potential (ζ) was calculated from the mobility using the Helmholtz–Smoluchowski equation:

$$\zeta = \frac{\mu\eta}{\varepsilon\varepsilon^{\circ}}$$

where ε and ε^{o} are the dielectric permitivity of the aqueous solution and the permitivity of the free space; η is the dynamic viscosity of the suspension [1].

Therefore, the binding of Ca^{2+} was determined by measuring the electrophoretic mobility of liposomes of DPPC, DPPC:Chol (4:1), DOPC, DOPC:Chol (4:1), DMPE and DMPE:Chol (4:1) from which the zeta potential was calculated.

2.4. Mathematical analysis for the zeta potential experimental data

The percentage of coverage of a surface can be described by ratio

$$\theta = \frac{x}{x_{\max}}$$

where x is the amount of adsorbant at a given Ca^{2+} concentration in the bulk solution and x_{max} is the maximum amount that the surface can adsorb.

The ratio (θ) can be expressed as a function of Ca^{2+} concentration (C) in the solution in contact with the membrane by a Langmuir–Freundlich isotherm (LF) [11] as:

$$\theta = \frac{(KC)^n}{1 + (KC)^n}$$

The constant K is the Langmuir adsorption constant and increases with an increase in the binding energy of adsorption and with a decrease in temperature; n is the heterogeneity parameter describing the width of energy distribution function. It is 1 for a Langmuir behavior.

The value θ can be determined by the changes in the zeta potential of the lipid vesicles due to the presence of Ca^{2+}:

$$\theta = \frac{\Delta\zeta}{\Delta\zeta_{\max}} = \frac{(K[Ca^{2+}])^n}{1 + (K[Ca^{2+}])^n}$$

Taking into account that Ca^{2+} interaction is favored by the electrostatic force with the phosphate groups, the adsorption features can be analyzed in terms of the change of zeta potential *vs.* Ca^{2+} concentration. Therefore, this analysis allowed calculating the parameters of the following equation:

$$\Delta\zeta = \frac{\Delta\zeta_{\max}(K[Ca^{2+}])^n}{1 + (K[Ca^{2+}])^n} \quad (1)$$

where $\Delta\zeta_{max}$ is the adsorption capacity, K the equilibrium constant related to the adsorption energy distribution maximum and n the heterogeneity parameter describing the width of energy distribution function.

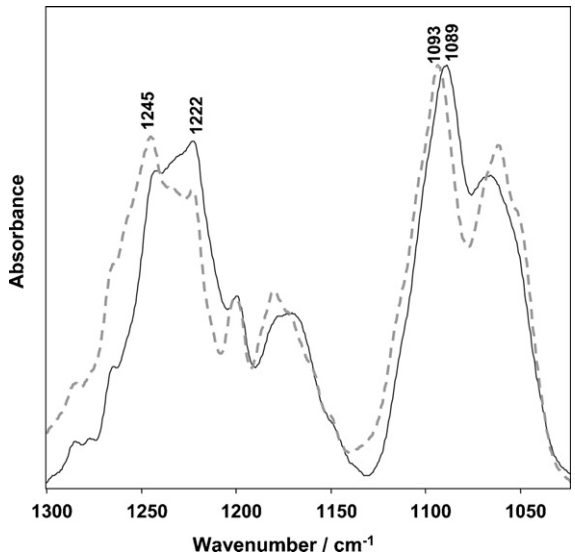

Fig. 1. FTIR absorbance spectra of DPPC fully hydrated (—) and DPPC KBr disk (– – –), in the region between 1000 and 1300 cm^{-1}.

In order to quantify the goodness of the fits, R^2 was calculated in each fit. It is a fraction between 0.0 and 1.0, and has no units. The higher this value, the better the model fits.

R^2 is computed from the sum of the squares of the point distances from the best-fit curve determined by non-linear regression. This sum-of-squares value is called SS_{reg}, which is in the units of the *Y*-axis squared. To turn R^2 into a fraction, the results are normalized to the sum of the square of the point distances from a horizontal line through the mean of all Y values. This value is called SS_{tot}. If the curve fits the data well, SS_{reg} should be much smaller than SS_{tot}.

R^2 is calculated using this equation:

$$R^2 = 1.0 - \frac{SS_{reg}}{SS_{tot}}$$

3. Results

Fig. 1 shows the FTIR spectra of the phosphate bands of aqueous (—) and dry (– – –) DPPC below the transition temperature (T_c = 41.5 °C). These two experimental spectra reproduced what is described in bibliography–that three absorption bands have been assigned to various vibrational modes of the hydrated DPPC phosphate group–with maximum wavenumbers at 1060, 1086 and 1222 cm^{-1}, corresponding respectively to a R–O–P–O–R′ vibration and to the symmetric and asymmetric PO_2^- stretching vibration [12,13]. These vibrational modes are not sensitive to lipid phase transitions since their values remain constant above and below the phase transition temperature of the phospholipid. Dehydration produces blue-shifts from 1086 to 1091 cm^{-1} for the symmetric PO_2^- stretching vibration and from 1222 to 1245 cm^{-1} for the asymmetric PO_2^- vibrational band [12–14].

This is consistent with our results, which present a shift from aqueous to dry DPPC (Fig. 1) maximum value of asymmetric PO_2^- band from 1222 to 1245 cm^{-1}. The symmetric PO_2^- vibrational band values of our spectra present a slight blue-shift, as mentioned above, from 1089 to 1093 cm^{-1}.

Table 1 shows the maximum wavenumbers of symmetric and asymmetric PO_2^- stretching vibration of hydrated DPPC in the absence and the presence of 1 M $CaCl_2$ and the same for DPPC:Chol (4:1). There are significant blue-shifts of both bands, the symmetric and asymmetric PO_2^- stretching vibration when the membranes are in the presence of Ca^{2+}.

Table 1
Maximum wavenumbers of symmetric and asymmetric PO_2^- stretching vibration of hydrated DPPC in the absence and the presence of 1 M $CaCl_2$ and the same conditions for DPPC:Chol (4:1).

Condition	Assignment of maximum wavenumbers (cm^{-1})	
	Asymmetric PO_2^- st.	Symmetric PO_2^- st.
DPPC	1222 ± 1	1089 ± 1
DPPC (1 M $CaCl_2$)	1243 ± 1	1096 ± 1
		1110 ± 1 (shoulder)
DPPC:Chol (4:1)	1224 ± 2	1088 ± 1
DPPC:Chol (4:1) (1 M $CaCl_2$)	1240 ± 1	1093 ± 1
		1108 ± 1 (shoulder)

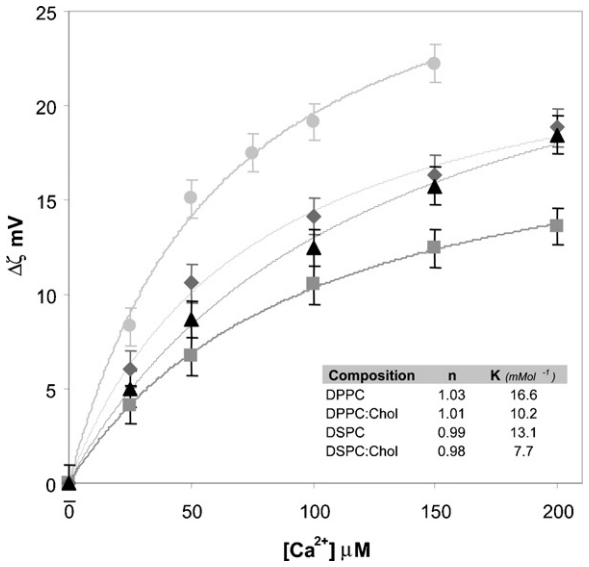

Fig. 2. Changes on zeta potential of MLVs with different lipid composition, due to the presence of Ca^{2+}: (●) DPPC; (■) DPPC:Chol (4:1); (♦) DSPC; (▲) DSPC:Chol (4:1).

A blank with the same lipids in the presence of 1 M NaCl (data not shown) was performed to validate that the shifts are due to ionic calcium, and not to the presence of any positive ion.

The inclusion of cholesterol into the lipid composition did not generate significant effects in the absence of the Ca^{2+}. The presence of that ion evidences blue-shifts in the same direction as in the case of DPPC liposomes, but in lower magnitude.

Figs. 2–5 show the experimental relative changes of the zeta potential due to the adsorption of ionic calcium (Ca^{2+}) to lipid membranes of the different compositions described (represented by points). The line representation corresponds to the non-linear regression fitting the experimental data to Eq. (1) (see Section 2).

The parameters obtained from Eq. (1) (Table 2) show significant differences between the *K* parameter of the different lipid composition assayed. However, we obtained in all the cases a similar value for $n \cong 1$, which validates a Langmuir isotherm behavior for the

Table 2
Parameters of Eq. (1) obtained for each lipid composition.

Composition	*n*	*K* ($mMol^{-1}$)	R^2
DOPC	0.97	42.4	0.995
DOPC:Chol	0.96	28.2	0.996
DPPC	1.03	16.6	0.995
DPPC:Chol	1.01	10.2	0.999
DSPC	0.99	13.1	0.997
DSPC:Chol	0.98	7.7	0.997
DMPE	1.02	0.1	0.970
DMPE:Chol	0.97	0.6	0.956
DMPE:DPPC	0.97	24.8	0.979

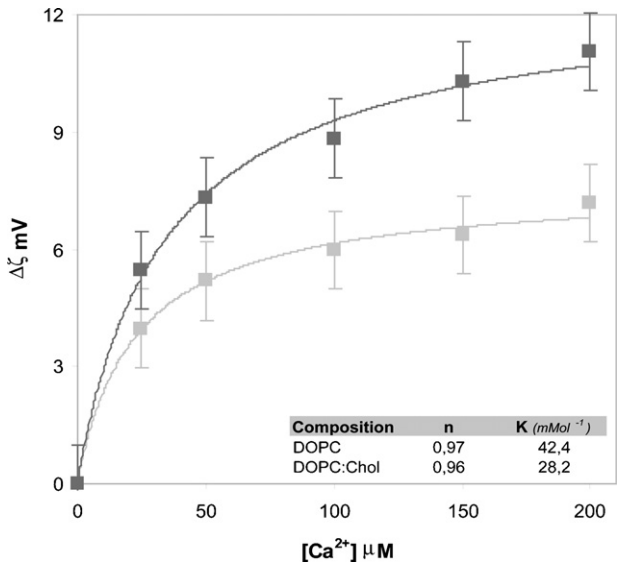

Fig. 3. Changes on zeta potential of MLVs with different lipid composition, due to the presence of Ca^{2+}: (□) DOPC and (■) DOPC:Chol (4:1).

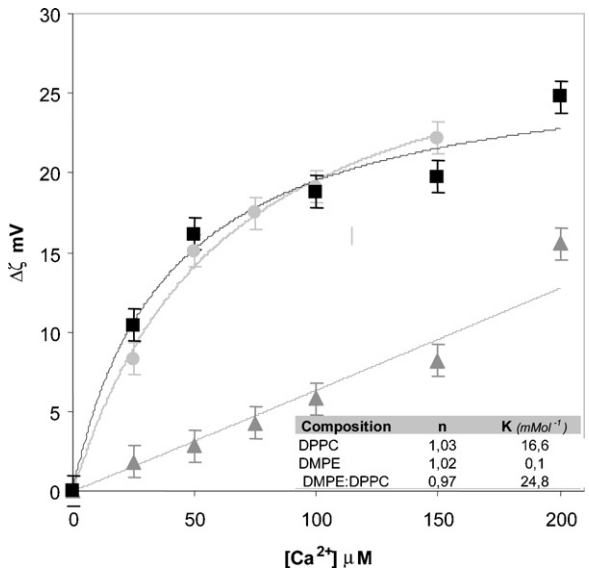

Fig. 5. Changes on zeta potential of MLVs with different lipid composition, due to the presence of Ca^{2+}: (●) DPPC; (▲) DMPE and (■) DPPC:DMPE (1:1).

adsorption isotherms of Ca^{2+} to all the lipid composition assayed (Figs. 2–5). That means a semi-empirical isotherm based on four assumptions [15]:

1. The surface of the adsorbent is uniform, that is, all the adsorption sites are equivalent.
2. Adsorbed molecules do not interact.
3. All adsorption occurs through the same mechanism.
4. At the maximum adsorption, only a monolayer is formed: molecules of adsorbate do not deposit on other, already adsorbed, molecules of adsorbate, only on the free surface of the adsorbent.

The different *K* parameters (Table 2) show that the membranes of phosphatidylcholine (PC) have more affinity to the calcium binding than the membranes of phosphatidylethanolamine (PE). Thus, the change of the polar head group (choline to ethanolamine) reduces the affinity of the ion to the surface (Fig. 4) showing a linear behavior in the range of Ca^{2+} concentrations used in this work, and a very low value for the *K* parameter.

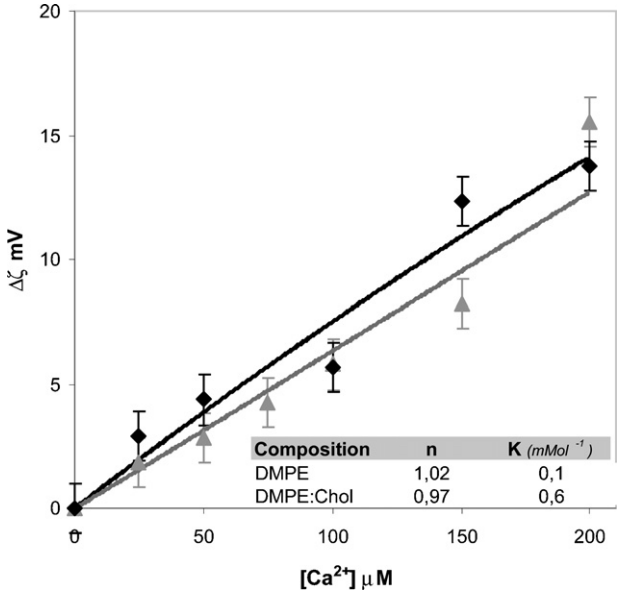

Fig. 4. Changes on zeta potential of MLVs with different lipid composition, due to the presence of Ca^{2+}: (▲) DMPE and (♦) DMPE:Chol (4:1).

The inclusion of cholesterol in PCs membranes produces a net change in the adsorption, which shows a decrease in *K* between 40 and 30%, depending on the PC used, indicating a decrease in the strength of Ca^{2+} adsorption (Figs. 2 and 3).

The MLVs of DPPC:DMPE (1:1) present an increase in the affinity of Ca^{2+}, when compared with the Ca^{2+} affinity of both lipid membrane components individually (Fig. 5).

4. Discussion

Fig. 1 depicts the strong sensitivity of the PO_2^- bands to the lipid hydration process. This is especially true for the antisymmetric mode [12,14,16–18]. Among phospholipids, phosphate groups are sterically, as well as electronically, more exposed to interact with water molecules establishing hydrogen bonds.

The presence of cholesterol in the membrane composition does not cause significant changes on the maximum wavenumbers on the stretching bands due to the PO_2^- moiety (Table 1). The main result of the spectra analysis is the observed effect of calcium on vesicles in both compositions.

The shifts observed in both symmetric and antisymmetric stretching bands due to the presence of ionic calcium evidence a dehydration of the phosphate group due to the presence of the ion.

The effect of the calcium on membranes with cholesterol seems to be lower.

Taking into account these results, where the perturbation of the ionic calcium to the phosphate groups of the phospholipids is remarkable, we are going to analyze the changes in the zeta potential of vesicles of different compositions due to the Ca^{2+}.

The addition of ionic calcium to the lipid interphase modified the zeta potential of the lipid vesicles. The analysis of these experiments and the parameters obtained may indicate the degree of exposure of the phosphate group to the aqueous media of the different lipid composition assayed.

In the case of PC and PE, the difference between the groups esterified to the phosphate group shows an important change in the affinity of the ion for the surface. It is known from the bibliography [19–22] and works of our group [23,24], that PEs create strong

head to head interaction between phosphate and amine groups of adjacent PE molecules, generating a network of hydrogen-bonded inter-phospholipids, which decreases the availability of the phosphate group and explains the low value of the affinity constant (Table 2).

On the other hand, PCs present a weaker interaction between phospholipids neighbors. They interact *via* water bridges, which result in a greater exposure of the phosphate and its concomitantly higher binding of the ion to this surface.

Fig. 5 shows a significant increase in the affinity of Ca^{2+} in relation to both component of lipid membrane individually. This behavior can be explained considering a disruption of the hydrogen-bonded network of PE, induced by the presence of PCs [22]. This disruption allows a higher interaction of ionic calcium to the surface.

Figs. 2 and 3 show differences between the *K* parameter of the PCs assayed. There is a significant difference when the phase state of the lipid changes, considering that DOPC membrane is in the fluid state and the saturated PCs are in gel phase at working temperature (T_w). However, one quantity that describes the bilayer microstructure with regard to molecular packing is the average interfacial area per lipid molecule [25].

The main variable considered here to interpret the results of affinity of ionic calcium to the different surfaces is the exposure of the phosphate groups. There is another important variable related to the same group that is the proximity of one group to another, meaning the density of the phosphate groups in each surface.

In the case of PE, what is evaluated is the availability of the group. In the case of PCs, the differences in the area per lipid molecule could be related to the differences in the calcium affinity. As it is known from the bibliography, PCs with unsaturated acyl chains present a greater area per lipid molecule than PCs with saturated acyl chains [26–28], caused by the spacer effects produced by the wobbling of the acyl chains with unsaturations.

The smaller area per lipid molecule of PCs with saturated acyl chains determines a higher density of the phosphate groups in the surface. The proximity of these groups could favor the electrostatic repulsion of the calcium ions.

The difference between DPPC and DSPC could be explained in the same way (Fig. 2 and Table 2). At the same temperature, the area per lipid decreases with the acyl length. In other words, longer chains keep the headgroups closer together [29,30]. The reason is that longer chains have an increased interchain van der Waals attraction that generates a reduction in the area per lipid. These observations are in agreement with the fact that longer chains have higher melting temperatures.

Thus, the differences between PCs could be related to changes in the average interfacial area per lipid molecule, more than to the difference in the phase state.

Figs. 2 and 3 also show that the inclusion of cholesterol to phosphatidylcholine membranes acts as a negative modulator for the ion–lipid interaction, the decrease on the affinity of Ca^{2+} is around 40% when the structural lipids are saturated PCs, like DPPC and DSPC. The decrease is 33.5% when the structural phosphatidylcholine is unsaturated.

The advancing understanding of cholesterol effects on lipid bilayers has been reviewed over the years by several authors ([31–38], for review see [39]). As for the specific interactions occurring between phospholipids and cholesterol, there are several studies on these kinds of mixes. In particular, Pandit et al. [39] explain a model of interaction between DPPC and cholesterol, which explains the behavior observed in our experiments. They showed the existence of an anomalous type of hydrogen-bonded network between the methyl hydrogens of the phospholipid choline group and the hydroxyl oxygen atom of cholesterol [40]. Such a CH···O interaction might come as a surprise; however, the idea of the CH···O hydrogen bond is well established [41–43]. In addition, Gu et al. demonstrated that the strength of the CH···O interaction increases substantially upon the presence of an electronegative atom next to the donor C–H group. In the case of the choline group of dipalmitoylphosphatidylcholine (DPPC), the $N(CH_3)_3$ group could be responsible for a situation where methyl groups can strongly interact with the hydroxyl group of cholesterol [40]. Furthermore, the CH···O interaction dies off much more slowly than the conventional OH···O hydrogen bond imparting a larger range of influence to this specific interaction [42,40].

What is important to explain our results is a derivative from this hydrogen-bonded network established between cholesterol and PC (of at least 16 hydrocarbon chain). Pandit et al. [40] show that larger capacity for complexation is concurrent with a larger angle made by PN with the outward bilayer normal. This coupling between the headgroup orientation and the capacity to participate in a CH···O binding mode arises because PC–cholesterol binding requires that the –CH from choline of one PC molecule should be placed in a position to donate a proton to an acceptor oxygen atom of a cholesterol molecule. The MD studies of Tu et al. [44] and of Pasenkiewicz-Gierula et al. [45] also revealed that the strong $N–CH_3···OH$ interaction was coupled with an inward orientation of the headgroup.

Thus, the lower interaction observed in the binding of Ca^{2+} to PC membranes with the inclusion of cholesterol can be explained by an inward orientation of the headgroup, determining a lower exposure of the phosphate group to interact with the ion.

Our work shows that the exposure of the phosphate group is perturbed. Moreover, there is another fact that could explain the effect of cholesterol, which is the so-called condensing effect. It has been re-defined as a decrease of the surface area occupied by PC molecules in mixed lipid bilayers containing cholesterol [31,39]. This effect is weaker in the case of di-unsaturated DOPCs than on fully saturated PCs [46]. A straightforward analysis showed immediately that a lesser degree of membrane condensation and a lower degree of ordering was induced by cholesterol in membranes consisting of unsaturated lipids.

This last evidence could explain the difference in the magnitude of the decrease of bonding between the DOPC and the saturated PCs as structural lipids of the membranes containing cholesterol.

The effect of cholesterol presence in the membrane did not seem to be the same in the case of DMPE as the structural lipid. The low affinity of ionic calcium continues in this surface, although it is higher than in the case of DMPE alone.

Studies of the organization of cholesterol/phosphatidylethanolamine (PE) mixtures are limited in scope and number, despite the fact that PEs are a major component of the plasma membranes of eukaryotic cells [47–49].

McMullen et al. [49] concluded that the limited lateral miscibility of cholesterol in PE relative to PC bilayers is probably due to the relatively strong inter-headgroup hydrogen bonding and electrostatic interactions of the former phospholipid, favoring phospholipid–phospholipid contacts over cholesterol–phospholipid interactions. Thus, below the transition temperature, where the tight packing of neighboring PE molecules permits extensive non-polar and polar intermolecular interactions, the intercalation of cholesterol between neighboring PEs is unfavorable. As a result, the number of cholesterol–PE contacts, and thus the effective cholesterol–PE interaction stoichiometry, is considerably lower than in corresponding cholesterol/PC mixtures.

Therefore, the higher value of the *K* parameter in the case of DMPE:Chol may be related to a very slight disruption of PE-PE interactions due to the presence of cholesterol.

5. Conclusion

The importance of phosphate groups on the surface properties of lipid bilayer has been suggested before [14]. However, no correlation between the exposure and modulation has been previously analyzed in terms of the interaction of ionic calcium.

The present study provides evidence that the exposure of PO_2^- groups affects the interaction of ionic calcium with the lipid membrane. This property is directly determined by the lipid species in accordance with the lateral interaction strength of the phosphate with the adjacent head groups and the density of this group on the surface.

Strong interactions would hinder phosphate exposure, while the area per lipid molecule is determinant in the density of this group.

The variety of lipids found in a cell membrane would gain relevance because in relation to its composition, lateral interactions and the nature of lipid head groups would modulate the interaction of Ca^{2+} normally found in physiological aqueous space. This leads to speculation on the capacity of lipid membranes to generate microdomains with differential affinity for ionic calcium.

The presence of cholesterol significantly reduces the calcium affinity to PC's membranes, something that could be relevant in microdomains like the so-called "rafts", where cholesterol is found in great proportion.

This is not trivial, considering the important role of this divalent ion in the interphase: conscription of certain cytosolic proteins, modulation of ionic-channels, or participation into receptor activation, to mention just some of its functions.

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References

[1] A. McLaughlin, C. Grathwohl, S. McLaughlin, Biochim. Biophys. Acta 513 (1978) 338.
[2] A. Scarpa, E. Carafoli (Eds.), Calcium Transport and Cell Function, New York Academy Of Sciences, New York, 1978.
[3] S.G.A. Mclaughlin, G. Szabo, G. Eisenman, J. Gen. Physiol. 58 (1971) 667.
[4] P.P.M. Schnetkamp, J. Membr. Biol. 88 (1985) 249.
[5] K. Ichikawa, Neurosci. Res. 25 (1996) 137.
[6] S. Mclaughlin, M. Nancy, G. Thomas, V. Gerard, A. Mclaughlin, J. Gen. Physiol. 77 (1981) 445.
[7] C. Altenbach, J. Seelig, Biochemistry 23 (1984) 3913.
[8] D. Huster, K. Arnold, K. Gawrisch, Biophys. J. 78 (2000) 3011.
[9] G. Cevc, J. Chem. Soc. Faraday Trans. 87 (1991) 2733.
[10] M.R. Vist, J.H. Davis, Biochemistry 29 (1990) 451.
[11] A.W. Marczewski, Appl. Surf. Sci. 253 (2007) 5818.
[12] J.L.R. Arrondo, F.M. Goñi, J.M. Macarulla, Biochim. Biophys. Acta 794 (1984) 165.
[13] U.P. Fringeli, H.H. Günthard, in: E. Grell (Ed.), Infrared Membrane Spectroscopy, Membrane Spectroscopy, Springer, Berlin, 1981, pp. 270–332.
[14] W. Hübner, A. Blume, Chem. Phys. Lipids 96 (1998) 99.
[15] I. Langmuir, J. Am. Chem. Soc. 38 (1916) 2221.
[16] C. Selle, W. Pohle, Biospectroscopy 4 (1998) 281.
[17] J. Grdadolnik, J. Kidric, D. Hadzi, Chem. Phys. Lipids 59 (1991) 57.
[18] M. de Oliveira Neto, J. Comput. Chem. 7 (1986) 617.
[19] T.J. McIntosh, A.D. Magid, in: G. Cevc (Ed.), Phospholipids Handbook, Marcel Dekker, New York, 1993, and papers cited therein.
[20] D.A. Pink, S. McNeil, B. Quinn, M.J. Zuckermann, Biochim. Biophys. Acta 1368 (1998) 289.
[21] W. Pohle, C. Selle, H. Fritzsche, M. Bohl, J. Mol. Struct. 408/409 (1997) 273.
[22] T. Róg, H. Martinez-Seara, N. Munck, M. Orešič, M. Karttunen, I. Vattulainen, J. Phys. Chem. B 113 (2009) 3413.
[23] M.F. Martini, E.A. Disalvo, Biochim. Biophys. Acta 1768 (2007) 2541.
[24] A.M. Bouchet, M.A. Frías, F. Lairion, F. Martini, H. Almaleck, G. Gordillo, E.A. Disalvo, Biochim. Biophys. Acta 1788 (2009) 918.
[25] K. Murzyn, T. Róg, G. Jezierski, Y. Takaoka, M. Pasenkiewicz-Gierula, Biophys. J. 81 (2001) 170.
[26] J. Li, R. Miller, H. Mfhwald, Colloids Surf. A: Physicochem. Eng. Asp. 114 (1996) 123.
[27] D. Marsh, Handbook of Lipids Bilayers, CRC Press, Boca Raton, FL, 1990.
[28] J.F. Nagle, S. Tristram-Nagle, Biochim. Biophys. Acta 1469 (2000) 159.
[29] W.J. Sun, S. Tristram-Nagle, R.M. Suter, J.F. Nagle, Biophys. J. 71 (1996) 885.
[30] H.I. Petrache, S.W. Dodd, M.F. Brown, Biophys. J. 79 (2000) 3172.
[31] P.L. Yeagle, Biochim. Biophys. Acta 822 (1985) 267.
[32] J. Finean, Chem. Phys. Lipids 54 (1990) 147.
[33] P.L. Yeagle, Biochimie 73 (1990) 1303.
[34] T.P.W. McMullen, R.N. McElchaney, Curr. Opin. Colloid Interf. Sci. 1 (1996) 83.
[35] R. Bittman, Subcell. Biochem. 28 (1997) 145.
[36] A. Wisniewska, J. Draus, W.K. Subczynski, Cell. Mol. Biol. Lett. 8 (2003) 147.
[37] W.K. Subczynski, A. Wisniewska, Acta Biochim. Pol. 47 (2000) 613.
[38] H. Ohvo-Rekilä, B. Ramsted, P. Leppimäki, J.P. Slotte, Prog. Lipid Res. 41 (2002) 66.
[39] T. Róg, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, Biochim. Biophys. Acta 1788 (2009) 97, and papers therein.
[40] S.A. Pandit, D. Bosticky, M.L. Berkowitz, Biophys. J. 86 (2004) 1345.
[41] G.R. Desiraju, Acc. Chem. Res. 24 (1991) 290.
[42] Y. Gu, T. Kar, S. Scheiner, J. Am. Chem. Soc. 121 (1999) 9411.
[43] P. Raveendran, S.L. Wallen, J. Am. Chem. Soc. 124 (2002) 12590.
[44] K. Tu, M.L. Klein, D.J. Tobias, Biophys. J. 75 (1998) 2147.
[45] M. Pasenkiewicz-Gierula, T. Róg, K. Kitamura, A. Kusumi, Biophys. J. 78 (2000) 1376.
[46] H. Martinez-Seara, T. Róg, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, R. Reigada, Biophys. J. 95 (2008) 3295.
[47] G.B. Ansell, S. Spanner, in: J.N. Hawthorne, G.B. Ansell (Eds.), New Comprehensive Biochemistry, Phospholipids, vol. 4, Elsevier Biomedical, Amsterdam, 1982.
[48] T.P.W. McMullen, R.N.A.H. Lewis, R.N. McElhaney, Biophys. J. 66 (1994) 741.
[49] T.P.W. McMullen, R.N.A.H. Lewis, R.N. McElhaney, Biochim. Biophys. Acta 1416 (1999) 119.