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Influence of temperature, anions and size distribution on the zeta potential of DMPC, DPPC and DMPE lipid vesicles

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ABSTRACT

The purpose of the work is to compare the influence of the multilamellarity, phase state, lipid head groups and ionic media on the origin of the surface potential of lipid membranes.

With this aim, we present a new analysis of the zeta potential of multilamellar and unilamellar vesicles composed by phosphatidylcholines (PC) and phosphatidylethanolamines (PE) dispersed in water and ionic solutions of polarizable anions, at temperatures below and above the phase transition.

In general, the adsorption of anions seems to explain the origin of the zeta potential in vesicles only above the transition temperature (T_c). In this case, the sign of the surface potential is ascribed to a partial orientation of head group moiety toward the aqueous phase. This is noticeable in PC head groups but not in PEs, due to the strong lateral interaction between PO and NH group in PE.

1. Introduction

Electrostatic forces are one of the main interactions taking place in the insertion of peptides and proteins with biological membranes. The fixation and conformational stabilization of proteins and peptides in the membrane has been ascribed to coulombic interactions via charged phospholipids in the membrane and charged amino acids in the protein surface [1–4].

However, most natural membranes are mainly composed of neutral lipids, such as phosphatidylcholines and phosphatidylethanolamines. At the critical lipid concentration, the zwitterionic lipids arrange in a bilayer structure giving a head group lattice on the surface resulting in a surface charge distribution.

Several studies have reported that multilamellar liposomes of these lipids are negatively charged when the electrophoretic mobility of the particles is measured in an ionic media [5–12].

This result has been explained by means of two models. In one of them, it has been argued that the resulting negative charge is due to an exposure of the phosphate groups lying in an outer plane with respect to the choline groups [8,9,13,14]. This proposal is based on the assumption that choline, being partially hydrophobic due to the methyl groups at the nitrogen end, is buried in the membrane interphase, trying to avoid water. Several experiments with ^{2}H NMR by Seelig [15] and Seelig et al. [16] support this interpretation.

The problem with this hypothesis is that, as the experimental determinations of zeta potential are done with lipid particles moving in an electric field, the plane of shear is far enough in comparison to the distance of separation of phosphate and choline so that they can be considered as overlapped. Then, surface potential should be near zero. For this reason, the negative surface charge was ascribed to the specific adsorption of counter anions on the surface. In this context, several works showed that negative ions adsorb according to its volume i.e. the degree of polarization of their electron clouds [17,18].

The analysis of the validity of these hypotheses has been limited to phosphatidylcholines in the liquid crystalline state.

Although specific ionic effects have become ubiquitous in both chemical and biological literature, a comprehensive theoretical description of the physical interactions responsible for these effects is still elusive [11].

In this work, we present a new analysis of the origin of the charge on the lipid membranes by comparing multilamellar and unilamellar vesicles composed by phosphatidylcholines and phosphatidylethanolamines dispersed in water and in ionic solutions of polarizable anions, at different temperatures.

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2. Materials and methods

2.1. Materials

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL) and used as received. Chloroform, KCl and $KClO_4$ were of analytical grade.

Solutions were prepared by weight in distilled and deionized water (Super Q Millipore system, conductivity lower than 18 $\mu S\,cm^{-1}$). The pHs of the solutions used for zeta potential measurements were pH 7.4.

2.2. Liposome preparation

Lipids were dissolved in chloroform that was removed by evaporation with N_2 stream to obtain a dry lipid film. The residual amounts of the organic solvent were removed maintaining the films under high vacuum for additional 2 h in Thermo Scientific Speed Vac SPD11V. The resulting dry lipid films were then hydrated with 5 mL of three different media: 1 mM KCl, 1 mM $KClO_4$ and water, pH 7.4, and homogenized with cycles of vigorous vortexing at around 10 °C above the transition temperature of the lipids. This combination of vortexing and heat yields a polydispersed population of MLVs. The final conductivity and pH of the resulting suspension were the same as that found for pure water. These values were not altered during the determination of zeta potential. Then, samples were equilibrated at the working temperatures [19–22] inside the Zetasizer equipment.

Unilamellar vesicles (LUVs) were obtained by a sequential extrusion of the MLVs dispersions in an Avanti Mini-Extruder, through a polycarbonate membrane of 100 nm pore size at around ten degrees centigrades above the transition temperature of each lipid. Then, samples were equilibrated at the working temperatures [19–22] inside the Zetasizer equipment. Sizes were measured before and after temperature stabilization with good reproducibility.

2.3. Methodologies

The size distribution of liposomes before and after extrusion and the zeta potentials (ζ) of DMPC, DPPC and DMPE liposomes were determined in Zetasizer Nano ZS90 equipment (Malvern Instruments Ltd., UK). Measurements with DMPC vesicles were done at 35 °C, 18 °C and 8 °C. In all cases, samples were incubated at each temperature during 1 h before measurement. In the same way, measurements were made for DPPC at 48, 38 and 18 °C and for DMPE at 18, 38 and 60 °C. Reported data are averaged over three different batches of liposomes.

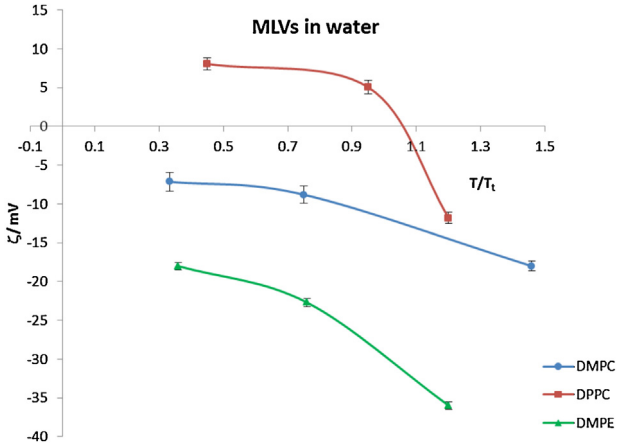

Fig. 1. Zeta potential at different reduced temperatures of MLVs composed by DMPC, DPPC and DMPE prepared and dispersed in water. In all cases, the standard deviation was lower than 10%. Reported data are averaged over three different batches of liposomes.

3. Results

The zeta potential at different reduced temperatures of MLVs of lipids dispersed in pure water is shown in Fig. 1. As an additional data, the size distribution of these preparations is shown in Table 1.

In this condition, it is observed that all lipids show a negative zeta potential at temperatures above the phase transition. This behavior is also found with DMPC and DMPE below the phase transition, while DPPC represents an exception since it yields a positive value.

Similar results were obtained for liposomes prepared with the same lipids dispersed in 1 mM KCl or $KClO_4$ (Fig. 2). It is immediately evident that zeta potential of DPPC MLVs above the transition temperature is negative in the presence of KCl and becomes more negative in $KClO_4$. This is congruent with the proposal that negative charges are due to the adsorption of polarizable anions.

This behavior is also observed in DMPC but the differences between the anionic species are within the experimental error. Also, the size distribution of the lipid dispersions is shown in Table 1.

At temperatures below the phase transition, all dispersions become less negative and in the case of DMPC, the zeta potential is

Table 1
Zeta potential and size particle of MLV and LUVS Of DMPC, DPPC and DMPE dispersed in different anionic media and temperatures.

T/°C	H_2O			KCl 1 mM			$KClO_4$ 1 mM		
	MLVs size ± SD[a] (μm)	MLVs ζ ± SD[a] (mV)	LUVs ζ ± SD[a] (mV)	MLVs size ± SD[a] (μm)	MLVs ζ ± SD[a] (mV)	LUVs ζ ± SD[a] (mV)	MLVs size ± SD[a] (μm)	MLVs ζ ± SD[a] (mV)	LUVs ζ ± SD[a] (mV)
DMPC									
8	2.5 ± 0.12	−7.1 ± 0.70	−7.6 ± 0.80	4.3 ± 0.22	7.9 ± 0.70	3.1 ± 0.30	6.2 ± 0.31	15.0 ± 0.90	0.61 ± 0.10
18	2.4 ± 0.12	−8.8 ± 0.80	−9.4 ± 0.90	5.1 ± 0.25	6.1 ± 0.50	0.50 ± 0.10	3.1 ± 0.15	11.0 ± 0.80	−0.72 ± 0.10
35	1.6 ± 0.80	−18.0 ± 1.1	−20.0 ± 1.4	2.5 ± 0.13	−7.5 ± 0.60	−10.0 ± 0.70	2.5 ± 0.13	−10.0 ± 0.70	−11.0 ± 0.70
DPPC									
18	9.3 ± 0.50	8.1 ± 0.60	2.7 ± 0.30	7.4 ± 0.37	−3.5 ± 0.30	0.41 ± 0.10	9.3 ± 0.46	0.91 ± 0.10	−2.3 ± 0.20
38	3.2 ± 0.16	5.1 ± 0.50	2.3 ± 0.20	4.3 ± 0.22	−4.8 ± 0.40	−2.6 ± 0.20	3.3 ± 0.17	−4.1 ± 0.30	−9.1 ± 0.70
48	0.85 ± 0.04	−11.0 ± 0.80	−10.0 ± 0.70	1.3 ±	−16.0 ± 0.80	−16.0 ± 1.1	0.91 ± 0.05	−23.0 ± 1.1	−30 ± 1.5
DMPE									
18	4.6 ± 0.23	−18.0 ± 0.90	−29.0 ± 1.7	2.7 ± 0.14	−25.0 ± 1.3	−30.0 ± 1.8	2.5 ± 0.12	−25.0 ± 1.3	−24.0 ± 1.4
38	2.6 ± 0.13	−22.0 ± 1.1	−33.0 ± 1.6	2.4 ± 0.12	−28.0 ± 1.4	−34.0 ± 1.9	2.1 ± 0.11	−28.0 ± 1.4	−30.0 ± 1.7
60	0.55 ± 0.03	−36.0 ± 1.8	−39.0 ± 1.8	0.75 ± 0.04	−34.0 ± 1.5	−42.0 ± 2.1	0.86 ± 0.04	−35 ± 1.4	−38.0 ± 1.9

[a] SD: standard deviation. $n = 3$.