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Surface and hysteresis properties of lipid interphases composed by head group substituted phosphatidylethanolamines

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ABSTRACT

This work analyzes the surface properties of PE-containing membranes modified at the head group region by the addition of methyl and ethyl residues at or near the amine group. These residues alter the lipid–lipid and lipid–water interactions by changes in the hydrogen bonding capability and the charge density of the amine group thus affecting the electrostatic interaction.

The results obtained by measuring the dipole potential, the zeta potential, the area per lipid and the compressibility properties allow to conclude that the H-bonding capability prevails in the lipid–lipid interaction. The non polar groups attached to the C_2-carbon of the ethanolamine chain introduces a steric hindrance against compression and increases the dipole potential. The analysis of areas suggests that lipids with methylated head groups have a much larger compressibility at expense of the elimination of hydration water, which is congruent with the broader extent of the hysteresis loop.

1. Introduction

Phosphatidylethanolamine (PE) is one of the most abundant lipids in eukaryotic cell membranes unevenly distributed between the inner and the outer leaflets of the bilayer [1]. The higher ratio of PEs in the membrane leaflet facing the inner media in comparison to the external one has called the attention to the topological properties of those surfaces with the expectative that they may have a key functional role [2,3]. The hydration of solid dimyristoylphosphatidylethanolamine (DMPE) produces a negligible shift in the asymmetric stretching frequency of the phosphate groups in contrast to that in dimyristoylphosphatidylcholine (DMPC). This accounts for the fact that the strong lateral interactions between the phosphate (PO_4) and amine (NH_3) groups, present in the solid PEs, still remain when the lipids are fully hydrated [4]. The lower mobility of the head group is reflected in a higher energy to translocate the phosphoethanolamine (P—N) dipoles in an electrical field, which decreases in the presence of increasing ratios of PCs of saturated chains in phosphoethanolamine monolayers [5]. In addition, it has been proposed that in PC – PE mixtures the propensity of the membrane to abandon the bilayer structure is determined by changes in the hydration of the polar head group [6,7]. This suggests that the interaction of the amine group with adjacent phosphate groups is hindered by the presence of methyl groups of the PCs.

On the other hand, subtle changes in the thermotropic behavior of substituted PEs were found when methyl groups were covalently attached to the amine group of PEs [8,9]. The addition of only one methyl group to the amine of PE results in thermotropic properties similar to those found in fully methylated amines such as in PCs. That is, transition temperatures shift in the order DMPE (52 °C), *N*-monomethyl DMPE (42 °C), *N, N*-dimethyl DMPE (26 °C) and DMPC (24 °C). In addition, methylation completely eliminates the hysteresis between the heating and the cooling thermograms observed in DMPE. This lipid does not show pretransition, but it appears with one methylation at 20 °C, with two methylations at 8 °C and with three methylations at 16 °C. The enthalpy of the pretransition, which is associated with hydration, also increases in the order *N*-methyl DMPE, *N,N* -dimethyl DMPE, DMPC (Frías et al., to be published). These results are in agreement with those reported for *N*-methylated DPPE's [27].

Abbreviations: DMPE, dimyristoylphosphatidylethanolamine; N-methyl DMPE, *N*-mono methyl dimyristoylphosphatidylethanolamine; C_2 ethyl DMPE, C_2 ethyl dimyristoylphosphatidylethanolamine; DMPC, dimyristoylphosphatidylcholine.

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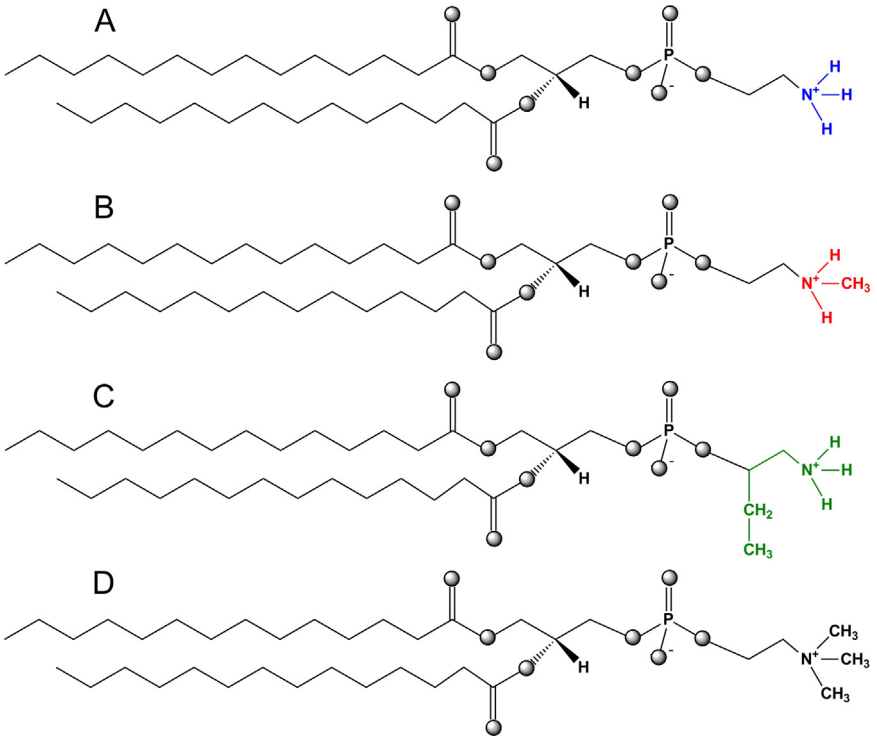

Fig. 1. Schematic description of A) dimyristoylphosphatidylethanolamine (DMPE) (T_c: 52 °C), B) *N*-mono methyl dimyristoylphosphatidylethanolamine. (*N*-methyl DMPE) (T_c: 45 °C), C) C_2 ethyl dimyristoylphosphatidylethanolamine (C_2 ethyl PE) (T_c: 35 °C) and D) dimyristoylphosphatidylcholine, (DMPC) (T_c: 24 °C).

A simple explanation of those results is that the presence of one, two or three methyl groups affects the head to head interaction and thus the phase behavior. However, there are several properties that may change concomitantly due to the presence of those voluminous groups, such as: the area per lipid, the polarization of water at the exposed methyl groups to water, the H-bonding network between adjacent PEs and the electrostatic interactions between PO_4 and NH_3 groups.

Considering that the size of the polar head group could be related to the amount of water immobilized around it. As PE hydrates less in a bulk phase than PC [7,28], the interaction with water would be different for PE than for PC due to the higher positive charge density of ethanolamine in comparison to choline [10]. Water and polar head group arrangements resulting from the lateral interaction determines the free energy of the interphase necessary for the adsorption of additives present in the aqueous environment. In this regard, differences in the insertion of aminoacids have been found when methyl groups are covalently bounded to the amine group of phosphatidylethanolamines [11].

However, it is not clear how the strong lateral interactions of PO_4 and NH_3 groups of adjacent molecules is governed by net hydrogen bonds or by electrostatic interactions, both contributing to the lateral cohesion forces.

Systematic information on the surface properties of head group substituted phosphatidylethanolamine regarding the surface properties and the intermolecular forces is not available. For this reason, this work analyzes the surface properties of PE-containing membranes in terms of the lipid–lipid and lipid–water interactions of different phosphoethanolamines and their modifications by substitution in the ethanolamine group. The structural changes in the polar head group of the PE by the insertion of methyl and ethyl groups at or near the amine group may alter the hydrogen bonding capability and change the charge density of the amine group thus affecting the electrostatic interaction. The methyl group blocks the ability to form hydrogen bonds, while the introduction of a bulky group in the C-chain of the ethanolamine should introduce a steric hindrance for lateral packing without affecting hydrogen-bonding groups. To enhance the steric hindrance an ethyl group was introduced in the C-chain of the ethanolamine leaving the NH groups of the PE free to interact by hydrogen bonding.

With this aim, the dipole potential, zeta potential, area per lipid and the compressibility properties of the lipids schematically described in Fig. 1 were measured in monolayers and bilayers.

2. Materials and methods

2.1. Lipids and solutions

The sources of commercially available chemicals, solvents, and chromatographic adsorbents were ACS grade and were redistilled before use [12].

1,2-dimyristoylphosphatidylcholine (DMPC) and 1,2- dimyristoylphosphatidylethanolamine (DMPE) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Purity of the lipids was found to be >99% by thin layer chromatography and used without further purification.

N-monomethyl DMPE and C_2 ethyl DMPE were synthesized from their respective phosphatidylcholines by transphosphatidylation using savoy cabbage phospholipase D [13] and purified by silicic acid column chromatography [12–16].

2.2. Liposome preparation

Multilamellar liposomes (MLVs) were prepared dispersing the lipids by vortexing in 1 mM KCl at temperatures higher than that of the phase transition, for 60 min. Large unilamellar vesicles (LUVs) were prepared by extruding the liposome dispersions through a polycarbonate membrane (pore size 1000 nm) above the transition temperature of the lipids. After several tests, this size of the unilamellar vesicles was chosen in order to ensure optical visibility