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Arbutin blocks defects in the ripple phase of DMPC bilayers by changing carbonyl organization

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Abstract

The effect of arbutin, a 4-hydroxyphenyl-β-glucopyranoside, on dimyristoylphosphatidylcholine (DMPC) bilayers was studied by turbidimetry, EPR and FTIR spectroscopies. The disruption of DMPC multilamellar vesicles (MLV's) with monomyristoylphosphatidylcholine (lysoPC), a product of hydrolysis of phospholipase A_2 (PLA_2), is more efficient at 18 °C, where DMPC MLV's are known to be in the ripple $P_{\beta'}$ phase, than at 10 °C ($L_{\beta'}$ flat gel phase). Disruption at 18 °C was inhibited by increasing concentrations of arbutin in the solution. This inhibition was correlated with the disappearance of the ripple phase in MLV's when arbutin is present. Shifts in FTIR carbonyl bands caused by arbutin or by temperature changes allow us to propose a model. It is interpreted that the changes in the water–hydrocarbon interface caused by arbutin, forcing a reaccommodation of the carbonyl groups, eliminate the topological defects in the lattice due to mismatches among regions with different area per lipid where lysoPC can insert.

Keywords: DMPC; Lipid membranes; Ripples; Defects; Arbutin; Carbonyl groups; Hydration; FTIR–EPR

1. Introduction

Lipid membranes composed by saturated fatty acids display, below the thermotropic chain-melting transition, a corrugated $P_{\beta'}$ phase characterized by the formation of periodic membrane ripples. A further decrease in temperature promotes a pretransition, indicating the onset of the flat $L_{\beta'}$ gel phase. It has been found that the ripples in the membrane surface of DMPC have a period of 141.7 Å (Wack and Webb, 1989). The area increase, occurring during the melting process, disrupts the lattice order if individual lipids melt independently. In this condition, defects are topologically possible to be formed in the lattice upon changes in the area per lipid.

Defects form as a result of geometric constraints in the formation of periodic patterns of gel and fluid domains (Heimburg, 2000). They are likely to occur in two opposing monolayers, and are dependent on hydration. The ripple phase seems to be better hydrated than the planar

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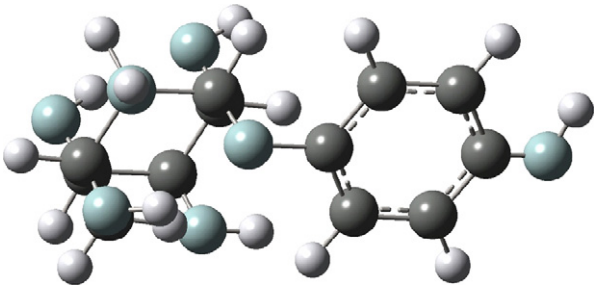

Fig. 1. Molecular structure of arbutin. Carbon atoms in black, oxygen atoms in blue.

membrane phases (Janiak et al., 1979; Doniach, 1979; Cevc, 1991; Le Bihan and Pezolet, 1998; Banerjee, 2002).

Below the pretransition and above the main transition, the membrane surface is flat, and thus, ripples and defects are less expected. However, it is known that defects, at the membrane surface, can be created when the liposomes are subjected to a hypertonic shock. The defects appear as an exposure of hydrophobic regions to the aqueous phase as measured by a fluorescent probe (Senisterra et al., 1988).

LysoPC monomer, a product of the hydrolysis of phospholipase A_2, has been shown to act as a molecular harpoon to sense defects in the molecular packing at the interface of DMPC bilayers. The monomer insertion in membranes in the corrugated $P_{\beta'}$ phase at 15 °C causes the disruption of the bilayer. It has been proposed that DMPC in this state presents holes or defects into which the amphiphilic compound lysoPC can fit (Disalvo et al., 1996).

Structurally, lysoPC monomers are compatible with the bilayer and are positioned such that the headgroup is in the same plane as the head groups of the diacyl lipid (McIntosh et al., 1995). Recently, infrared spectroscopy has shown that lysoPC interacts with the phosphate groups and with the non-hydrated population of the carbonyl groups (Díaz et al., 2003). In this regard, it must be noticed that several OH compounds interact with phosphates and carbonyl groups. Among them, arbutin (Fig. 1) interacts with DMPC bilayers affecting carbonyl and phosphate groups in the same way as lysoPC does (Frías et al., 2006; Hincha et al., 1999). This interaction increases the area per lipid of the phosphatidylcholines with a negligible change in the amount of hydration water (Lairion and Disalvo, 2007). The effect of arbutin on the carbonyl groups varies depending on the hydration state of the bilayer. Thus, the inhibitory effect of arbutin to the hydrolytic action of PLA_2 is a maximum when the membrane is dehydrated (Oliver and Crowe, 1996).

The formation of defects is a consequence of the compromise between the packing preference of the headgroups and that of the hydrocarbon chains. This results in an enhancement of the water–hydrocarbon contact area, an effect strongly disfavoured by the hydrophobic characteristics of the chains. Therefore, defects are indicative of an excess of surface energy, which can be decreased by the insertion of molecules that fit into the defect (Banerjee, 2002; Disalvo et al., 1996).

Considering the similar sites of interaction, we studied the effect of arbutin on the lytic effect promoted by lysoPC at 10 and 18 °C on DMPC vesicles. The changes were correlated with the effect of arbutin on the phase state of the lipids, mainly in the pretransition region, by EPR spectroscopy using paramagnetic probes. The results were correlated with the effects of arbutin or temperature changes on the stretching frequencies of carbonyl groups.

2. Materials and methods

2.1. Lipid sample preparation

Dimyristoylphosphatidylcholine (DMPC) and monomyristoylphosphatidylcholine (lysoPC) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). Purity of the lipids was checked by thin layer chromatography and they were used without further purification. Arbutin was purchased from Sigma–Aldrich, Inc. The purity was checked by FTIR spectra of solid samples.

2.2. Turbidity assays

Lipids in chloroform solution were dried to form a film under a stream of nitrogen. Then, they were rehydrated in solutions of different concentrations of arbutin (5–15 mM) heating above the transition temperature with gently agitation during 15 min to produce multilamellar vesicles (Bangham et al., 1974). LUV samples of DMPC were prepared by extrusion of MLV's through Nuclepore® membranes of 100 nm pore diameter. LysoPC solution was prepared in distilled water.

Turbidity was measured at 450 nm in a Beckman DU 7500 spectrometer. To determine the effect of arbutin on the transition temperature, turbidity was measured between 5 and 35 °C in intervals of 2 °C.

In order to investigate lytic effects, turbidity changes were followed by adding known amounts of lysoPC to DMPC dispersions in buffer or in arbutin solutions. The initial absorbance values (A_0) obtained with a lipid concentration of 26 mM in the cuvette were normalized