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ScienceDirect

Chemistry and Physics of Lipids 150 (2007) 117–124

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

www.elsevier.com/locate/chemphyslip

Effect of trehalose on the contributions to the dipole potential of lipid monolayers

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Received 31 May 2007; received in revised form 27 June 2007; accepted 28 June 2007
Available online 1 July 2007

Abstract

The dipole potential and the area changes induced by trehalose on dimyristoyl phosphatidylcholine (DMPC), 1,2-di-*O*-tetradecyl-sn-glycero-3-phosphocholine (dietherPC), dimyristoyl phosphatidylethanolamine (DMPE), 1,2-di-*O*-tetradecyl-sn-glycero-3-phosphoethanolamine (dietherPE) monolayers have been studied at different temperatures. The insertion of trehalose into DMPC monolayers in the fluid and gel states requires of the presence of carbonyl groups. The area increase observed at 0.15 M trehalose is congruent with the decrease in the dipole potential. However, in dietherPC, in which trehalose does not affect the area, a decrease in the dipole potential is also observed. This is interpreted as a result of the displacement of water from the phosphate groups exposed to the aqueous phase. In DMPE, trehalose also decreases the dipole potential without affecting the area of saturated monolayers and in dietherPE no effect on dipole potential and area was observed. It is concluded that the spacer effect of trehalose depends on the specific interaction with CO, which is modulated by the strength of the interaction of the PO groups with lateral NH groups. However, it is not the only contribution to the dipole potential decrease.

Keywords: Lipid monolayers; DMPC; DMPE; DietherPC; DietherPE; Trehalose; Dipole potential; Area per lipid; Surface pressure

1. Introduction

The understanding of the interfacial properties of lipid membranes requires information on the arrangement and properties of water localized at the polar head group region.

It is known that phosphatidylcholines hydrates with 14–18 water molecules per lipid the fluid state and with around 7–8 in the gel state. The first 14 tightly bound water molecules are considered a hard-core hydration shell, as derived from studies in reversed micelles and molecular simulation (Essman et al., 1995; Nagle and Tristam-Nagle, 2000; Luzardo et al., 2000; Lairion et al., 2002).

In this regard, several H bonding compounds, such as sugars, polyphenols, and phenolic sugars, have been used to study the distribution of water around the hydration sites, i.e. carbonyl and phosphate groups, and its effects on the interfacial properties, such as dipole potential and area per lipid (Disalvo et al., 2002, 2004; Lairion and Disalvo, 2007; Frías et al., 2006). Among sugars, trehalose has been extensively investigated due to its special properties for the preservation of the structure of lipid membranes, in comparison to others. For these reasons, trehalose–membrane interaction is an interesting system to study, due to its potential technological applications as a preservative of cellular structures and functions in

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doi:10.1016/j.chemphyslip.2007.06.221

freeze-drying and heat-drying processes (Crowe et al., 1984, 1987, 1997).

The studies carried out until now, in relation to the effects of trehalose on lipid membranes and its protective properties can be divided in three groups.

One of them focuses on the structural properties of the lipid membranes dried in the presence of trehalose (Crowe et al., 1987; Crowe and Crowe, 1995; Sun et al., 1996). In this condition, the transition temperature of the solid is greatly decreased and a new phase has been described (Tsvetkova et al., 1998).

Another approach has dealt with the properties of bilayers in the gel state, formed after the rehydration of lipids dried in the presence of trehalose (Viera et al., 1992). Hydrophobic probes as Merocyanine 540 gave, in this condition, a peak at 570 nm, characteristic of membranes in the fluid state, at temperatures corresponding to the gel state. The results of these studies concluded that trehalose remains anchored in the lipid membrane after rehydration, giving a gel phase with particular surface properties. As the magnitude of the peak ascribed to fluid phases appeared together with those featuring the gel state, it was argued that trehalose was able to expand some regions of the lipid interphase acting as a spacer of the lipid head groups.

A third line of research has studied the effect of trehalose in lipids dispersed in aqueous solutions without further dehydration and rehydration. In this condition, two experimental models have been employed: the multi and unilamelar vesicles and lipid monolayers spread on the air–water interface. FTIR measurements in MLV's, showed that trehalose interacts strongly with the phosphate and the carbonyl groups of phosphatidylcholines (Luzardo et al., 2000). Concomitant with this interaction, a decrease in the amount of water molecules per lipid was calculated, which decreased from about 18 to 10. It was suggested that, at the concentration used, trehalose was not able to displace the water strongly bound to the first shell of hydration, i.e. the first 10 water molecules, probably bound to the phosphate. In addition, the measurements in PC monolayers showed a decrease of the dipole potential congruent with the water displacement caused by the interaction with the hydration sites PO and CO groups (Luzardo et al., 2000).

Trehalose insertion has also been studied by simulation techniques (Skibinsky et al., 2005; Sum et al., 2003; Villarreal et al., 2004). All of them show that this sugar intercalates between the phospholipid head groups concerting H-bonds with the head groups in the absence of water. However, different features merge from the different studies. Skibinsky et al. (2005) predicts an expansion of the area per lipid, Sum et al. (2003) claims for no changes and Villarreal et al. (2004) suggest a small decrease. In spite of the differences in the simulation conditions, several of these results are coincident with experimental findings in monolayers and bilayers.

The calculations of Skibinsky et al. (2005) are in nice agreement with the evaluation of the number of water molecules determined by water activity in DMPC (Luzardo et al., 2000; Disalvo et al., 2002). They found that trehalose is able to replace the same number of hydrogen bonds than those concerted by the displaced water molecules. Thus, around 10 trehalose molecules displace 14 water molecules (Skibinsky et al., 2005; Pereira and Hunenberger, 2006). The data of water activity in DMPC, at different trehalose ratio, indicates that four trehalose molecules per lipid displace eight water molecules per lipid.

In contrast to these predictions and experimental results, the simulations carried out by Sum et al. (2003) showed that the addition of trehalose to DPPC bilayers does not alter the bilayer structure. The dissimilar consequences were explained suggesting that trehalose may adopt several dynamical spatial conformations that allow it to conform to the topology of the nearest lipids.

Villarreal et al. (2004) suggest an intercalation of the sugar with its main axis parallel to the normal of the bilayer, thus suggesting a small decrease in the dipole potential.

Although it is accepted that trehalose intercalates in the polar head group region causing a partial displacement of water, details of the effects on the dipole potential in relation to the topological features of carbonyl and phosphate groups, the effects on area and the hydration properties have not been reported. In order to compare the different contributions to the changes in the dipole potential, mainly: hydration of constitutive dipoles, presence/absence of constitutive dipoles and area per lipid (amount of dipoles per area), we have undertaken a comparative study between DMPC and DMPE and its ether derivatives ditetradecyl-PC (dietherPC) and ditetradecyl-PE (dietherPE).

The studies using these lipids would allow the inspection of how the lateral interaction due to the presence of CO, PO, choline and amine groups can affect the trehalose interaction with lipids, giving place to different interfacial properties.

The influence of CO and PO on the insertion of polyhydroxilated compounds have been reported with other polyhydroxilated compounds, such as arbutin and phloretin (Lairion and Disalvo, 2004, 2007). In contrast, to our knowledge, studies of the dipole potential and the area changes of lipid interfaces, in which the accessibility of CO and the PO groups are modulated, have