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Interactions of a bacterial trehalose lipid with phosphatidylglycerol membranes at low ionic strength

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ABSTRACT

Trehalose lipids are bacterial biosurfactants which present interesting physicochemical and biological properties. These glycolipids have a number of different commercial applications and there is an increasing interest in their use as therapeutic agents. The amphiphilic nature of trehalose lipids points to the membrane as their hypothetical site of action and therefore the study of the interaction between these biosurfactants and biological membranes is critical. In this study, we examine the interactions between a trehalose lipid (TL) from *Rhodococcus* sp. and dimyristoylphosphatidylglycerol (DMPG) membranes at low ionic strength, by means of differential scanning calorimetry, light scattering, fluorescence polarization and infrared spectroscopy. We describe that there are extensive interactions between TL and DMPG involving the perturbation of the thermotropic intermediate phase of the phospholipid, the destabilization and shifting of the DMPG gel to liquid crystalline phase transition to lower temperatures, the perturbation of the sample transparency, and the modification of the order of the phospholipid palisade in the gel phase. We also report an increase of fluidity of the phosphatidylglycerol acyl chains and dehydration of the interfacial region of the bilayer. These changes would increase the monolayer negative spontaneous curvature of the phospholipid explaining the destabilizing effect on the intermediate state exerted by this biosurfactant. The observations contribute to get insight into the biological mechanism of action of the biosurfactant and help to understand the properties of the intermediate phase display by DMPG at low ionic strength.

1. Introduction

Glycolipids of bacterial origin are widespread biosurfactants which contain carbohydrates in combination with long-chain aliphatic or hydroxyl aliphatic acids. An important group of glycolipid biosurfactants is formed by trehalose-containing glycolipids (Asselineau and Asselineau, 1978). These trehalose lipids are mainly produced by rhodococci and present such interesting physicochemical and biological properties that a number of different commercial applications have been proposed for them (Lang and Philp, 1998).

There is an increasing interest in the use of biosurfactants as therapeutic agents (Banat et al., 2000; Rodrigues et al., 2006). Trehalose lipids have been reported to have antiviral properties (Azuma et al., 1987; Hoq et al., 1997), and it has also been shown that trehalose lipids have excellent growth inhibition and differentiation-inducing activities against human leukemia cells such as myelogenous leukemia cell K562, promyelocytic ceukemia cell HL60 and basophilic leukocyte KU812 (Isoda et al., 1996, 1997b; Sudo et al., 2000). In addition, trehalose lipids inhibit the activity of phospholipids- and calcium-dependent protein kinase C of HL60 cells (Isoda et al., 1997a), and show immunomodulating activity (Kuyukina et al., 2007).

The amphiphilic nature of trehalose lipids points to the membrane as their hypothetical site of action, thus, the study of the interaction between these biosurfactants and biological membranes is very important. We have found that a trehalose lipid from *Rhodococcus* sp. (TL) (Fig. 1) permeabilizes phospholipid membranes (Zaragoza et al., 2009) and induces red blood cells hemolysis (Zaragoza et al., 2010). We have studied the effect of TL on the most important membrane phospholipids in order to get insight into the molecular interaction between these biosurfactants and the lipidic component of biological membranes. We have shown that TL increases the fluidity of phosphatidylcholine membranes forming domains in the fluid state (Aranda et al., 2007), and that it exhibits and important dehydrating effect on the interfacial region of saturated phosphatidylethanolamines (PE) and greatly promotes the formation of the inverted hexagonal H_{II} phase in

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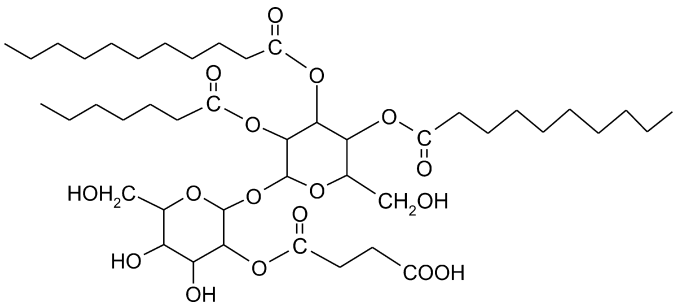

Fig. 1. The chemical structure of the most abundant trehalose lipid produced by *Rhodococcus* sp. (TH–succ–C_{11}–C_{10}–C_7).

unsaturated phosphatidylethanolamines (Ortiz et al., 2008). We have also shown that TL was able to affect the thermotropic phase transition of phosphatidylserine in the absence and presence of calcium (Ortiz et al., 2009). Recently, we have studied the effects of TL on the gel and fluid phases of phosphatidylglycerol (PG), a predominant phospholipid of the cytoplasmic membrane of bacteria. Phosphatidylglycerol has been used extensively as model for acidic phospholipid membranes (Eklund and Kinnunen, 1986; Alakoskela and Kinnunen, 2007; Pabst et al., 2008). The physicochemical properties of phospholipids present in membranes of microorganisms are of interest because it has been suggested that the lipid composition of bacterial membranes plays an important role in the interaction with antimicrobial compounds (Lohner, 2001). We showed that TL did not affect the macroscopic bilayer organization of DMPG, but the presence of the biosurfactant produced a small decrease of the bilayer thickness together with an increase in the fluidity of the phospholipids acyl chains (Ortiz et al., 2011). DMPG has received particular attention because of its unusual phase properties. It appears to form single bilayers when dissolved in water at low lipid and low salt concentration (Gershfeld et al., 1986), exhibiting a very unusual thermal profile, with a broad transition with a width greater than 15 °C. The structure of DMPG aggregates along this remarkable transition region is still a matter of debate, recent studies suggest that DMPG forms leaky vesicles at both gel and fluid phases (Barroso et al., 2012) which are highly perforated with large holes (Enoki et al., 2012). In order to understand the influence of this biosurfactant on this unusual phase behavior of DMPG, we have purified TL from *Rhodococcus* sp. (Fig. 1) and carried out a study of the effect of the glycolipid on the thermotropic and structural properties of DMPG membranes at low ionic strength, using differential scanning calorimetry (DSC), light scattering, steady-state fluorescence polarization and infrared spectroscopy (FT-IR).

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-sn-glycero-3-phospo-rac-glycerol, sodium salt (dimyristoylphosphatidylglycerol, DMPG), was purchased from Avanti Polar Lipids Inc. (Birmingham, AL). 1,6-Diphenyl-1,3,5-hexatriene (DPH) and 1-[4-(trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) were from Sigma–Aldrich (Spain). All the other reagents were of the highest purity available. Purified water was deionized in a Milli-Q equipment from Millipore (Bedford, MA), and filtered through 0.24 μm filters prior to use. Stock solutions of DMPG and TL were prepared in chloroform/methanol (8:1) and stored at −20 °C. Phospholipid concentrations were determined by phosphorous analysis (Böttcher et al., 1961).

2.2. Trehalose lipids production and purification

Strain 51T7 was isolated from an oil-contaminated soil sample after culture enrichment with kerosene, and was identified as *Rhodococcus* sp. (Espuny et al., 1995). This strain was maintained by fortnightly cultures on Trypticase Soy Agar (Pronadisa, Spain) and preserved in cryovials at −20 °C. Biosurfactants were produced, purified and it structure was characterized as described before (Espuny et al., 1995, 1996). The TL surfactants are a mixture of several components containing four acyl substituents located at position 2, 3, 4 and 2′, these substituents were identified as succinic, heptanoic, decanoic and undecanoic acids. Fig. 1 depicts a representative most abundant component TH–succ–C_{10}–C_{11}–C_7.

2.3. Differential scanning calorimetry (DSC)

Samples for DSC were prepared by mixing the appropriate amounts of DMPG and TL in chloroform/methanol (8:1). The solvent was gently evaporated under a stream of dry N_2 to obtain a thin film at the bottom of a glass tube. Last traces of solvent were removed by a further 3 h desiccation under high vacuum. To the dry samples 2 ml of a buffer containing 10 mM Hepes, 0.1 mM EDTA pH 7.4 (the pH was balanced with small aliquots of 5 M NaOH, and the final [Na^+] was estimated to be approximately 2.2 mM) was added, and vesicles were formed by vortexing the mixture essentially as described previously (Alakoskela and Kinnunen, 2007). The suspensions were incubated for 90 min at 60 °C with continuous shaking and vortexed vigorously every 30 min three times. The lipid suspensions so obtained were used in the measurements during the same day without any cold incubation. Experiments were performed using a MicroCal MC2 calorimeter (MicroCal, Northampton, USA). The final phospholipid concentration was 2 mg ml^{-1}. The heating scan rate was 60 °C h^{-1}.

2.4. Light scattering

Samples containing 2 mg/mL DMPG prepared as described above were measured at 550 nm (angle of 90 °C) using a PTI Quantamaster spectrofluorometer (Photon Technology, NJ, USA). The cell holder was thermostated using a Peltier device, and the measurements were taken under continuous stirring.

2.5. Steady-state fluorescence polarization

Steady-state fluorescence polarization measurements were carried out with samples prepared as described above, containing 0.5 mg/mL DMPG and 1% fluorescence probes and performed with a PTI Quantamaster spectrofluorometer (Photon Technology, NJ, USA) equipped with motorized polarizers. Quartz cuvettes with a path length of 10 mm were used. The cell holder was thermostated using a Peltier device, and the measurements were taken under continuous stirring.

For monitoring DPH and TMA-DPH fluorescence, the excitation wavelength was set at 358 nm, and emission was monitored at 430 nm. The sample temperature was allowed to equilibrate for 5 min before fluorescence was recorded for 60 s, and then the excitation shutter was kept closed during heating to the next temperature, in order to minimize any photoisomerization of DPH and TMA-DPH. Steady-state fluorescence polarization values were calculated from the following equation:

$$P = \frac{I_{VV} - GI_{VH}}{I_{VV} + GI_{VH}}$$

where I_{VV} and I_{VH} are the fluorescence intensities with the excitation polarizer oriented vertically and the emission polarizer oriented vertically and horizontally, respectively. G is the grating