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Effects of a bacterial trehalose lipid on phosphatidylglycerol membranes

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ABSTRACT

Bacterial trehalose lipids are biosurfactants with potential application in the biomedical/healthcare industry due to their interesting biological properties. Given the amphiphilic nature of trehalose lipids, the understanding of the molecular mechanism of their biological action requires that the interaction between biosurfactant and membranes is known. In this study we examine the interactions between a trehalose lipid from *Rhodococcus* sp. and dimyristoylphosphatidylglycerol membranes by means of differential scanning calorimetry, X-ray diffraction, infrared spectroscopy and fluorescence polarization. We report that there are extensive interactions between trehalose lipid and dimyristoylphosphatidylglycerol involving the perturbation of the thermotropic gel to liquid-crystalline phase transition of the phospholipid, the increase of fluidity of the phosphatidylglycerol acyl chains and dehydration of the interfacial region of the bilayer, and the modulation of the order of the phospholipid bilayer. The observations are interpreted in terms of structural perturbations affecting the function of the membrane that might underline the biological actions of the trehalose lipid.

1. Introduction

Biological surface active compounds are a diverse group of amphiphilic surface-active molecules synthesized by different prokaryotic and eukaryotic organisms [1]. From the chemical point of view biosurfactants present a wide structural diversity, the compounds with the most interesting properties being lipopeptides and glycolipids [2]. Glycolipids of bacterial origin are widespread biosurfactants which contain carbohydrates in combination with long-chain aliphatic or hydroxyl aliphatic acids. An important group of biosurfactant glycolipids includes the rhamnolipids secreted by *Pseudomonas aeruginosa*, which have been shown to affect the structural properties of membrane phospholipids [3,4]. Another important group of glycolipid biosurfactants is formed by trehalose-containing glycolipids [5]. 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 [6]. At present, the main applications are found in the hydrocarbon bioremediation and oil and petroleum industry. A very important emerging field of application is the biomedical/healthcare industry, since some biosurfactants have been demonstrated to be suitable alternatives to synthetic products as antimicrobial and therapeutic agents [7,8]. Trehalose lipids have been reported to have antiviral properties [9,10]. It has been shown that trehalose lipids have excellent growth inhibition and differentiation-inducing activities against human leukaemia cells [11–13]. They also inhibit the activity of phospholipids- and calcium-dependent protein kinase C of HL60 cells [14] and show immunomodulating activity [15]. Interestingly, some trehalose lipids have shown antibacterial activity [16]. Although the amphiphilic nature of trehalose lipids points to the membrane as their hypothetical site of action, very little is known about the interaction between these biosurfactants and biological membranes. We have found that trehalose lipid permeabilizes phospholipid membranes [17] and induces hemolysis of human red blood cells [18]. In order to get insight into the molecular interaction between these biosurfactants and the lipidic component of biological membranes, we have studied the effect of trehalose lipid on the most important membrane phospholipids. We have shown that trehalose lipid increases the fluidity of phosphatidylcholine membranes forming domains in the fluid state [19], and that it exhibits an important dehydrating effect on the interfacial region of saturated phosphatidylethanolamines and greatly promotes the formation of the inverted hexagonal H_{II} phase in unsaturated phosphatidylethanolamines [20]. Recently we have shown that trehalose lipid was also able to affect the thermotropic membrane phase transition of phosphatidylserine in the absence and presence of calcium [21]. This work focuses on the effects of trehalose lipid on the gel and fluid phases of phosphatidylglycerol, a predominant phospholipid of the cytoplasmic membrane of bacteria. Phosphatidylglycerol has been used extensively as model for acidic phospholipid membranes [22–24]. The physico-chemical properties of phospholipids present in membranes of microorganisms are of interest because it has been suggested that the lipid composition of bacterial

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membranes plays an important role in the interaction with antimicrobial compounds [25]. Combining differential scanning calorimetry, X-ray diffraction, infrared spectroscopy and fluorescence polarization, we have studied the interaction between a purified trehalose lipid (Fig. 1) and dimyristoylphosphatidylglycerol in order to understand the influence of this biosurfactant on the thermotropic and structural properties of phosphatidylglycerol membranes.

2. Materials and methods

2.1. Materials

Dimyristoylphosphatidylglycerol, sodium salt (DMPG), was purchased from Avanti Polar Lipids Inc. (Birmingham, AL). All the other reagents were of the highest purity available. Purified water was deionised in a Milli-Q equipment from Millipore (Bedford, MA), and filtered through 0.24 μm filters prior to use. Stock solutions of DMPG and the trehalose lipid were prepared in chloroform/methanol (8:1) and stored at −20 °C. Phospholipid concentrations were determined by phosphorous analysis [26].

2.2. Trehalose lipid production and purification

Strain 51T7 was isolated from an oil-contaminated soil sample after culture enrichment with kerosene, and was identified as *Rhodococcus* sp. [27]. 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 structurally characterized as described before [27,28].

2.3. Differential scanning calorimetry

Samples for DSC were prepared by mixing the appropriate amounts of DMPG and trehalose lipid 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 100 mM NaCl, 0.1 mM EDTA, and 10 mM Hepes pH 7.4 was added, and vesicles were formed by vortexing the mixture, always keeping the temperature above 40 °C. Experiments were performed using a MicroCal MC2 calorimeter (MicroCal, Northampton, USA). The final phospholipid concentration was 1 mg ml^{-1}. The heating scan rate was 60 °C h^{-1}. The construction of the partial phase diagram was based on the heating thermograms of mixtures of DMPG and trehalose lipid at various trehalose lipid concentrations. The onset and completion temperatures for each transition peak were plotted as a function of the mol fraction of trehalose lipid. These onset and completion temperatures formed the basis for defining the boundary lines of the partial temperature-composition phase diagram.

2.4. X-ray diffraction

Simultaneous small (SAX) and wide (WAX) angle X-ray diffraction measurements were carried out as described previously [29] using a modified Kratky compact camera (MBraum-Graz-Optical Systems, Graz Austria), which employs two coupled linear position sensitive detectors (PSD, MBraum, Garching, Germany). Nickel-filtered Cu Kα X-rays were generated by a Philips PW3830 X-ray Generator operating at 50 kV and 30 mA. Samples for X-ray diffraction were prepared by mixing 15 mg of DMPG and the appropriate amount of trehalose lipid in chloroform/methanol (8:1). Multilamellar vesicles were formed as described above. After centrifugation at 13,000 rpm, the pellets were placed in a steel holder, which provided good thermal contact to the Peltier heating unit, with cellophane windows. Typical exposure times were 10 min, allowing 10 min prior to the measurement for temperature equilibration.

Background corrected SAXS data were analyzed using the program GAP (global analysis program) written by Georg Pabst and obtained from the author [30,31]. This program allowed to retrieve the membrane thickness, $dB = 2(Z_H + 2\sigma_H)$ from a full q-range analysis of the SAXS patterns [32]. The parameters Z_H and σ_H are the position and width, respectively, of the Gaussian used to describe the electron-dense headgroup regions within the electron density model.

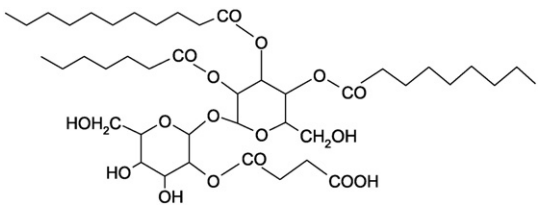

Fig. 1. The chemical structure of *Rhodococcus* sp. trehalose lipid.

2.5. Infrared spectroscopy (FT-IR)

For the infrared measurements multilamellar vesicles were prepared, as described above, in 50 μl of the same buffer prepared in D_2O. Samples were placed between two CaF_2 windows (25 mm × 2 mm) separated by 25 μm Teflon spacers and transferred to a Symta cell mount. Infrared spectra were acquired in a Nicolet 6700 Fourier-transform infrared spectrometer (FT-IR) (Madison, WI). Each spectrum was obtained by collecting 256 interferograms with a nominal resolution of 2 cm^{-1}. The equipment was continuously purged with dry air in order to minimize the contribution peaks of atmospheric water vapor. The sample holder was thermostated using a Peltier device (Proteus system from Nicolet). Spectra were collected at 2 °C intervals, allowing 5 min equilibration between temperatures. The D_2O buffer spectra taken at the same temperatures were subtracted interactively using either Omnic or Grams (Galactic Industries, Salem, NH) software.

2.6. Steady-state fluorescence polarization

Steady-state fluorescence polarization measurements were 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 during a 60 s interval. 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:

$$P = (I_{VV} - GI_{VH}) / (I_{VV} + GI_{VH})$$

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 factor, calculated as the ratio of the efficiencies of the detection system for vertically and horizontally polarized light, and is equal to I_{HV}/I_{HH}.

4. Results and discussion

The heating thermograms corresponding to pure DMPG and DMPG/trehalose lipid systems are presented in Fig. 2. The thermogram of pure DMPG shows two transitions attributed to the weakly energetic

pretransition from the lamellar-gel phase ($L_{\beta'}$) to the ripple phase ($P_{\beta'}$) at 10.2 °C and the highly energetic main phase transition from the $P_{\beta'}$ to the liquid-crystalline phase (L_α) at 22.4 °C. The values of transition temperatures agree well with previous reports [33,34]. This pattern of thermotropic phase behaviour is similar to that exhibited by saturated phosphaticylcholines [35,36]. The presence of rather low amounts of trehalose lipid, such as 0.02 molar fraction, makes the pretransition to disappear, in a similar way to that observed for the interaction of trehalose lipid with phosphatidylcholine membranes [19]. Increasing concentrations of trehalose lipid lower the phase transition temperature and decrease the cooperativity of this transition. Fig. 3 shows that the enthalpy change associated with the gel to liquid-crystalline phase transition of DMPG progressively decreases as more trehalose lipid is present in the system reaching near 40% of the value of the pure system in the case of the most concentrated sample. These effects on the gel to liquid-crystalline phase transition endotherm of DMPG are qualitatively similar to those previously observed on the thermotropic behaviour of different diacylglycerophospholipids like phosphatidylcholine [19], phosphatidylethanolamine [20] and phosphatidylserine [21], indicating that the hydrophobic moieties of trehalose lipid incorporate into the phosphatidylglycerol acyl chain bilayer and, like in the case of the other glycerophospholipids, perturb the acyl chains, reduce the cooperativity of the transition and shift the phase transition to lower values. Similarly to other anionic phospholipids like phosphatidylserine [21], the thermograms are asymmetric, suggesting the presence of trehalose lipid enriched domains. However, these domains are different from those formed in zwitterionic phospholipids like phosphatidylcholine [19] and phosphatidylethanolamine [20] were, in the presence of trehalose lipid, new peaks or shoulders were present in the lower part of the thermograms.

From the calorimetric data obtained form the thermograms shown in Fig. 2, partial phase diagram was constructed for DMPG in mixtures with trehalose lipid. Fig. 4 shows that both fluid and solid lines display near ideal behaviour, the temperature decreasing as more trehalose lipid is present in the system, which indicates miscibility both in the gel and the liquid-crystalline phases. The mixtures evolve from a lamellar gel phase (G) to a coexistence region and then to a lamellar liquid-crystalline phase (F). The broadening of the thermograms

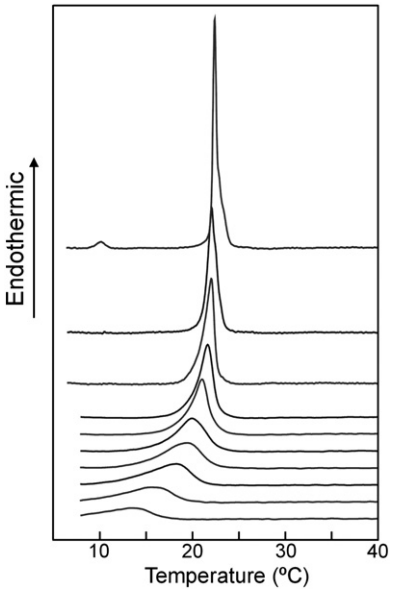

Fig. 2. DSC heating thermograms for DMPG containing trehalose lipid at different concentrations. Molar fraction of trehalose lipid from top to bottom: 0, 0.02, 0.05, 0.07, 0.10, 0.15, 0.20, 0.30, 0.40 and 0.50.

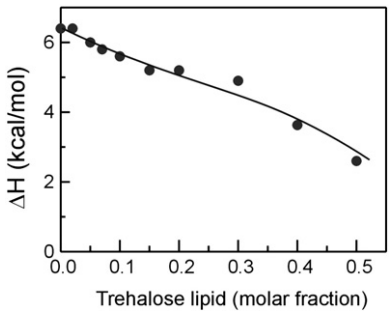

Fig. 3. Enthalpy change for the gel to liquid-crystalline phase transition of mixtures of DMPG/trehalose lipid at different molar fractions.

observed in Fig. 2 is evidenced by the widening of the coexistence region (G + F) as more trehalose lipid is present in the system.

X-ray diffraction was used to address the effect of trehalose lipid on the overall structural properties of DMPG. To obtain information about the packing of the DMPG acyl chains in the presence of trehalose lipid, we made experiments in the wide angle region (WAX), which report on the chain lattice. Fig. 5 shows the WAX pattern corresponding to pure DMPG and DMPG containing trehalose lipid at different temperatures. As shown in Fig. 5, pure DMPG at 6 °C, i.e. below the pretransition, gives a sharp reflection at 4.18 Å and a broad one at 4.10 Å, which is representative for lipids being in the $L_{\beta'}$ gel phase in an orthorhombic packing with hydrocarbon chains tilted to the membrane surface [24,37]. At 15 °C, i.e. above the pretransition, a symmetric reflection appears around 4.15 Å which is attributed to a lipid phase with hydrocarbon chains being oriented normal to the bilayer plane in a two-dimensional hexagonal lattice as described for the $P_{\beta'}$ phase [37,38]. Eventually, at 35 °C, i.e. above the main phase transition, a diffuse scattering reflection is observed, which is representative for the liquid-crystalline L_α phase. At 6 °C, in the presence of trehalose lipid, the characteristic pattern corresponding to the $L_{\beta'}$ is replaced by one corresponding to the $P_{\beta'}$, which is consistent with the disappearing of the pretransition observed above in the DSC experiments. At 15 °C, all the samples show the symmetric reflection characteristic of the $P_{\beta'}$ phase. However in the presence of 0.2 mol fraction of trehalose lipid the reflection is very broad, suggesting that part of the lipid has already undergone the transition to the L_α phase, which is consistent with the decrease of the phase transition temperature found in the DSC experiments. Finally at 35 °C, the presence of trehalose lipid does not affect the packing of the DMPG acyl chains in the liquid-crystalline phase.

Fig. 6 presents the scattering pattern of pure DMPG and DMPG/trehalose lipid systems in the small angle region (SAX) at temperatures both below and above the main phase transition. The SAX pattern of all samples exhibits pure diffuse scattering at all temperatures originating

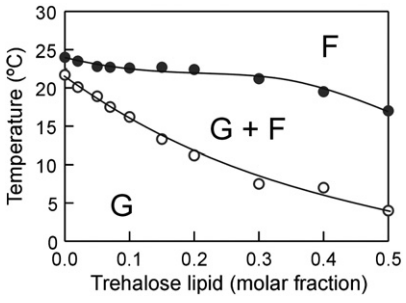

Fig. 4. Partial phase diagrams for DMPG in DMPG/trehalose lipid mixtures. Open and solid circles were obtained from the onset and completion temperatures of the main gel to liquid-crystalline phase transition. The phase designations are as follows: G, gel phase; F, liquid-crystalline phase.

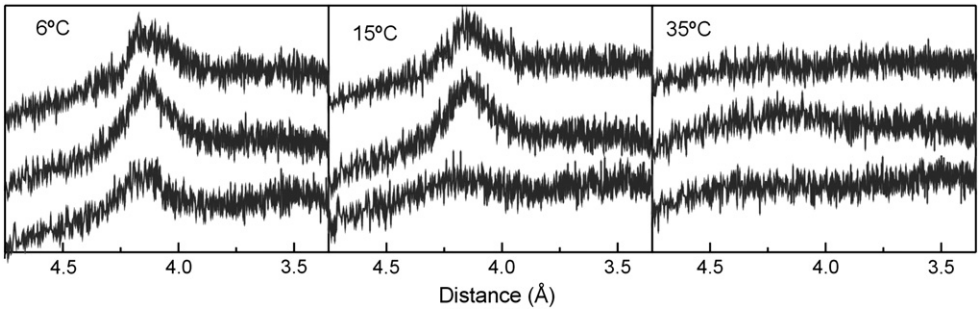

Fig. 5. Wide angle X-ray diffraction profiles of DMPG system containing different concentrations of trehalose lipid at different temperatures. From top to bottom: pure DMPG, DMPG containing 0.05 mol fraction trehalose lipid, and DMPG containing 0.20 mol fraction trehalose lipid.

from positionally uncorrelated bilayers. This can be explained by the overall negative surface charge that leads to the formation of positionally uncorrelated bilayers, most likely large unilamellar vesicles, because of electrostatic repulsion [33,39]. The analysis of the SAX patterns using the global analysis program (GAP) allowed us to calculate the membrane thickness (d_B) of the different systems. For pure DMPG a membrane thickness of 50.0 Å was calculated for the gel phase and 44.7 Å for the liquid-crystalline phase (these values being in agreement with previous reports [24,40]), while a decrease of membrane thickness both in the gel (47.8 Å) and the liquid-crystalline phase (43.0 Å) was found for the system containing 0.2 molar fraction of trehalose lipid.

The infrared spectra of glycerophospholipid contain useful information regarding the intermolecular interactions that occur in the different domains of the molecule. In this way, the CH_2 stretching region includes information about the conformational disposition of the hycrocarbon chains, whereas the C = O stretching region includes information about lipid interfacial hydration–hydrogen bonding interaction. The CH_2 symmetric stretching band near 2850 cm^{-1} is of remarkable importance because of its sensitivity to changes in the mobility and in the conformational disorder of the hydrocarbon chains [41]. Fig. 7 presents the temperature dependence of the frequency at the absorbance maximum of the symmetric CH_2 stretching vibration band of the infrared spectra of pure DMPG and DMPG/trehalose lipid systems. For pure DMPG the data show a discontinuous increase of about 2 cm^{-1} in the frequency of the band maximum at a temperature that coincides with the gel to liquid-crystalline phase transition, in agreement with previous results [34]. This frequency increase is characteristic of the chain melting transition of hydrated phospholipids [42] and results from the increased conformational disorder in the hydrocarbon as a consequence of the introduction of a high population of *gauche* conformers [43]. In the presence of low concentrations of trehalose lipid the chain melting phase transition is broadened and starts at lower temperatures than that of pure DMPG, in accordance with the DSC experiments shown above. At high trehalose lipid concentration a further broadening and shift of the transition to lower temperatures is observed together with an increase of the frequency of the band both below and above the phase transition, the latter indicating and increase in *gauche* conformers and thus an increase in hydrocarbon chain conformational disorder both in the gel and in the liquid-crystalline phase. A similar increase in hydrocarbon chain conformational disorder was found in the case of phosphatidylserine [21], and suggests that the decrease of about 2 Å in the bilayer thickness that we found in the SAX measurements shown above could be due to an effective increase in the *gauche* conformers leading to an increase in conformational disorder.

The C = O stretching band of pure DMPG is a fairly broad band around 1750–1700 cm^{-1}, and seems to be a summation of subcomponents centered near 1741 and 1727 cm^{-1}[34]. The relative intensities of these component bands reflect the contribution of subpopulations of dehydrated and hydrated carbonyl groups [44]. Fig. 8 shows the temperature dependence of the frequency at the absorbance maximum of the C = O stretching band of the infrared spectra of pure DMPG and DMPG/trehalose lipid systems. For pure DMPG, the gel to liquid-crystalline phase transition produced a shift of the maximum frequency to lower values, reflecting the increase in intensity of the underlying component band at 1727 cm^{-1}, attributed to a higher amount of hydrogen bonded carbonyl groups resulting from a phase state-induced increase in the hydration of the polar–apolar interface [34]. The broadening and shift of the phase transition to lower temperatures produced by the presence of trehalose lipid can be also observed following the maximum of the C = O band depicted in Fig. 8. It is interesting to note that the presence of high concentration of trehalose lipid produced a shift of the maximum of the C = O band to higher frequencies as compared to pure phospholipid, both in the gel and liquid-crystalline phases. This increase in frequency indicates an increase in the proportion of dehydrated C = O component

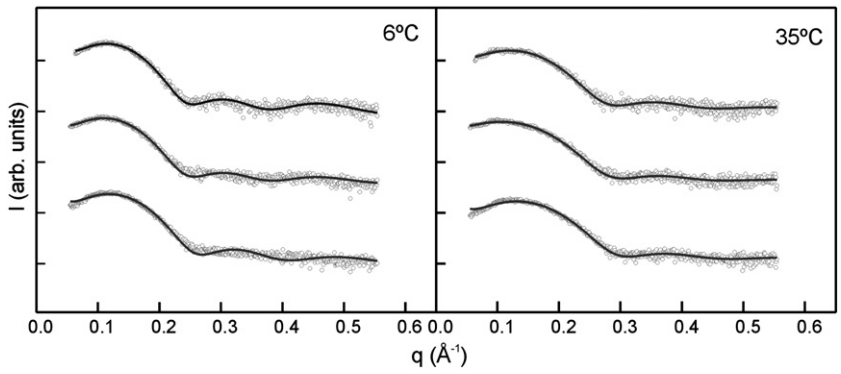

Fig. 6. Experimental (open symbols) and best fitting (bold lines) small angle X-ray diffraction profiles of DMPG system containing different concentrations of trehalose lipid at different temperatures. From top to bottom: pure DMPG, DMPG containing 0.05 mol fraction trehalose lipid, DMPG containing 0.20 mol fraction trehalose lipid.

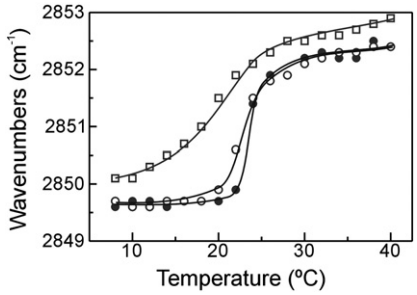

Fig. 7. Temperature dependence of the maximum of the symmetric CH_2 stretching absorption band exhibited by pure DMPG (●) and DMPG/trehalose lipid mixtures at 0.05 (○) and 0.20 (□) molar fraction.

at all temperatures and suggests that trehalose lipid interacts with the interfacial region of the DMPG bilayer, decreasing the hydrogen bonding of the C = O groups with the water molecules of the hydration layer. The latter is in line with the dehydrating effect of trehalose lipid observed in other diacylglycerophospholipids systems [19–21] which was attributed to the ability of free hydroxyl groups of trehalose lipid to participate in hydrogen bonds, leaving less water molecules available to interact with the phospholipids. It seems that the presence of trehalose lipid produces different effects on the conformational order of the DMPG molecule: it causes conformational disorder in the methylene region while dehydrates the interfacial region, which is correlated with a decrease of lipid mobility [45].

Fluorescence polarization measurements, using DPH and TMA-DPH probes, were carried out to report changes in membrane order both at the deep inner phospholipid bilayer and at the interfacial region of the membrane, respectively. Fig. 9 shows the fluorescence polarization of DPH (Fig. 9A) and TMA-DPH (Fig. 9B) incorporated into pure DMPG systems and those containing trehalose lipid, as a function of temperature. The sharp drop in polarization values detected in the pure DMPG sample reflects the decrease in membrane order taking place during the gel to liquid-crystalline phase transition. The presence of trehalose lipid caused a broadening and shifting of the transition to lower temperatures, in line with the evidence shown above. Interestingly, at temperatures below the phase transition, when the phospholipids are in the ordered gel phase, the presence of trehalose lipid decreases the polarization values of both probes indicating a decrease in the membrane order, and emphasizing the perturbation of the methylene region exerted by the biosurfactant. However, at temperatures above the phase transition, when the phospholipids are in the disordered liquid-crystalline phase, the presence of trehalose lipid produces a small increase in the polarization values indicating an ordering effect of the fluid phase. This latter effect is more prominent in the case of the TMA-DPH probe which is located near the aqueous interface, and underlines the influence of the dehydrating effect of trehalose lipid on the fluid DMPG membrane.

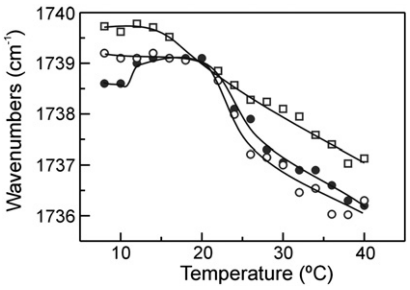

Fig. 8. Temperature dependence of the maximum of the carbonyl stretching absorption band exhibited by pure DMPG (●) and DMPG/trehalose lipid mixtures at 0.05 (○) and 0.20 (□) molar fraction.

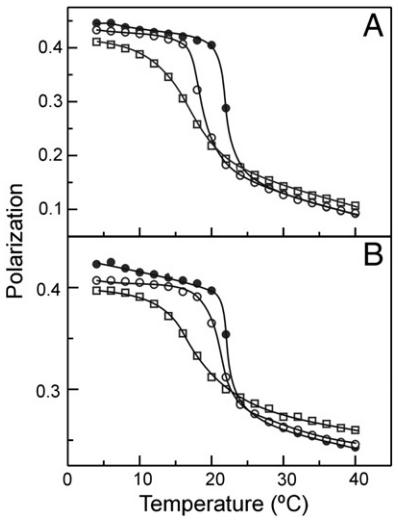

Fig. 9. Steady state fluorescence polarization as a function of temperature of DPH (A) and TMA-DPH (B) incorporated into membranes composed of pure DMPG (●) and DMPG/trehalose lipid mixtures at 0.05 (○) and 0.20 (□) molar fraction.

5. Conclusions

The aim of this work was to characterize the interactions between a trehalose lipid biosurfactant and phosphatidylglycerol membranes. Our DSC data supported that trehalose lipid is able to incorporate into DMPG membranes and to intercalate between the phospholipids molecules, where it can reduce the cooperativity and lower the transition temperature of the gel to liquid-crystalline phase transition. The partial phase diagram showed good miscibility between trehalose lipid and DMPG, both in the gel and liquid-crystalline phases. X-ray diffraction measurements indicated that trehalose lipid did not affect the macroscopic bilayer organization of DMPG, but the presence of the biosurfactant produced a small decrease of the bilayer thickness. Infrared experiments revealed that the biosurfactant increased the fluidity of the phospholipids acyl chains and decreased the hydration of the interfacial region of the membrane. Finally, fluorescence polarization of membrane probes provided evidence that trehalose lipid disordered the DMPG membrane in the gel phase while producing a small ordering effect in the liquid-crystalline phase. In view of the crucial structural and functional importance of phosphatidylglycerol in membranes, particularly bacterial ones, and the broad diversity of biological actions played by trehalose lipids, the results presented here contribute to the knowledge of the molecular mechanisms underlying the membrane-related biological actions of this bacterial trehalose lipid biosurfactant.

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References

[1] S. Lang, Biological amphiphiles (microbial biosurfactants), Curr. Opin. Colloid Interface Sci. 7 (2002) 12–20.
[2] E.Z. Ron, E. Rosenberg, Natural roles of biosurfactants, Environ. Microbiol. 3 (2002) 229–236.
[3] A. Ortiz, J.A. Teruel, M.J. Espuny, A. Marqués, A. Manresa, F.J. Aranda, Effects of dirhamnolipid on the structural properties of phosphatidylcholine membranes, Int. J. Pharm. 325 (2006) 99–107.
[4] M. Sánchez, J.A. Teruel, M.J. Espuny, A. Marqués, F.J. Aranda, M. Sánchez, J.A. Teruel, M.J. Espuny, A. Marqués, A. Manresa, M. Sánchez, J.A. Teruel, M.J. Espuny, A. Marqués, A. Ortiz, Modulation of the physical properties of dielaidoylphosphatidylethanolamine membranes by a dirhamnolipid biosurfactant produced by *Pseudomonas aeruginosa*, Chem. Phys. Lipids 142 (2006) 118–127.

[5] C. Asselineau, J. Asselineau, Trehalose-containing glycolipids, Prog. Chem. Fats Other Lipids 16 (1978) 59–99.
[6] S. Lang, J.C. Philp, Surface-active compounds in *rhodococci*, Antonie Van Leeuwenhoek 74 (1998) 59–70.
[7] I.M. Banat, R.S. Makkar, S.S. Cameotra, Potential commercial application of microbial surfactant, Appl. Microbiol. Biotechnol. 53 (2000) 495–508.
[8] A. Franzretti, I. Gandolfi, G. Bestetti, T.J.P. Smyth, I.M. Banat, Production and applications of trehalose lipid biosurfactants, Eur. J. Lipid Sci. Technol. 112 (2010) 617–627.
[9] M. Azuma, T. Suzutani, K. Sazaki, I. Yoshida, T. Sakuma, T. Yoshida, Role of interferon in the augmented resistance of trehalose 6,6′-dimycolate-treated mice to influenza virus infection, J. Gen. Virol. 68 (1987) 835–843.
[10] Md. Hoq, M. Suzutani, T. Toyoda, T. Horijke, G. Yoshida, I.M. Azuma, Role of γδ TCR lymphocytes in the augmented resistance of trehalose 6,6′-dimycolate-treated mice to influenza virus infection, J. Gen. Virol. 78 (1997) 1597–1603.
[11] H. Isoda, H. Shinmoto, D. Kitamoto, M. Matsumura, T. Nakahara, Succinoyl trehalose lipid induced differentiation of human monocytoid leukemic cell line U937 into monocyte-macrophages, Cryotechnology 19 (1996) 79–88.
[12] H. Isoda, H. Shinmoto, D. Kitamoto, M. Matsumura, T. Nakahara, Differentiation of human promyelocytic leukaemia cell line HL60 by microbial extracellular glycolipids, Lipids 32 (1997) 263–271.
[13] T. Sudo, X. Zhao, Y. Wakamatsu, M. Shibahara, N. Nomura, T. Nakahara, A. Suzuki, Y. Kobayashi, C. Jin, T. Murata, K.K. Yokohama, Induction of the differentiation of human HL60 promyelocytic leukaemia cell line by succinoyl trehalose lipids, Cryotechnology 33 (2000) 259–264.
[14] H. Isoda, D. Kitamoto, H. Shinmoto, M. Matsumura, T. Nakahara, Microbial extracellular glycolipid induction of differentiation and inhibition of the protein kinase C activity of human promyelocytic leukemia cell line HL60, Biosci. Biotechnol. Biochem. 61 (1997) 609–614.
[15] M.S. Kuyukina, I.B. Ivshina, S.V. Gein, T.A. Baeva, V.A. Chereshnev, In vitro immunomodulating activity of biosurfactant glycolipid complex from *Rhodococcus rubber*, Bull. Exp. Biol. Med. 144 (2007) 326–330.
[16] E. Vollbrecht, U. Ra, S. Lang, Microbial conversion of vegetable oils into surface-active di-, tri-, and tetrasaccharide lipids (biosurfactants) by bacterial strain *Tsukamurella spec*, Lipids 101 (1999) 389–394.
[17] A. Zaragoza, F.J. Aranda, M.J. Espuny, J.A. Teruel, A. Marqués, A. Manresa, A. Ortiz, Mechanism of membrane permeabilization by a bacterial trehalose lipid biosurfactant produced by *Rhodococcus* sp. Langmuir 21 (2009) 7892–7898.
[18] A. Zaragoza, F.J. Aranda, M.J. Espuny, J.A. Teruel, A. Marqués, A. Manresa, A. Ortiz, Hemolytic activity of a bacterial trehalose lipid biosurfactant produced by *Rhodococcus* sp.: evidence for a colloid-osmotic mechanism, Langmuir 26 (2010) 8567–8572.
[19] F.J. Aranda, J.A. Teruel, M.J. Espuny, A. Marqués, A. Manresa, E. Palacios-Lidón, A. Ortiz, Domain formation by a *Rhodococcus* sp. biosurfactant trehalose lipid incorporated into phosphatidylcholine membranes, Biochim. Biophys. Acta 1768 (2007) 2596–2604.
[20] A. Ortiz, J.A. Teruel, M.J. Espuny, A. Marqués, A. Manresa, F.J. Aranda, Interactions of a *Rhodococcus* sp. biosurfactant trehalose lipid with phosphatidylethanolamine membranes, Biochim. Biophys. Acta 1778 (2008) 2806–2813.
[21] A. Ortiz, J.A. Teruel, M.J. Espuny, A. Marqués, A. Manresa, F.J. Aranda, Interactions of a bacterial biosurfactant trehalose lipid with phosphatidylserine membranes, Chem. Phys. Lipids 158 (2009) 46–53.
[22] K.K. Eklund, P.K. Kinnunen, Effects of polyamines on the thermotropic behaviour of dipalmitoylphosphatidylglycerol, Chem. Phys. Lipids 39 (1986) 109–117.
[23] J.M. Alakoskela, P.K. Kinnunen, Thermal phase behavior of DMPG: the exclusion of continuous network and dense aggregates, Langmuir 10 (2007) 4203–4213.
[24] G. Pabst, S.L. Grage, S. Danner-Pongratz, W. Jing, A.S. Ulrich, A. Watts, K. Lohner, A. Hickel, Membrane thickening by the antimicrobial peptide PGLa, Biophys. J. 95 (2008) 5779–5788.
[25] K. Lohner, The role of membrane lipid composition in cell targeting of antimicrobial peptides, in: K. Lohner (Ed.), Development of Novel Antimicrobial Agents: Emerging Strategies, Horizon Scientific Press, Wymondham, Norfolk, UK, 2001, pp. 149–165.
[26] C.J.F. Böttcher, C.M. Van Gent, C. Pries, A rapid and sensitive sub-micro phosphorus determination, Anal. Chim. Acta 24 (1961) 203–204.
[27] M.J. Espuny, S. Egido, M.E. Mercadé, A. Manresa, Characterization of trehalose tetraester produced by a waste lube oil degrader *Rhodococcus* sp 51T7, Toxicol. Environ. Chem. 48 (1995) 83–88.
[28] M.J. Espuny, S. Egido, I. Rodón, A. Manresa, M.E. Mercadé, Nutricional requirements of a biosurfactant producing strain *Rhodococcus* sp 51T7, Biotechnol. Letters 18 (1996) 521–526.
[29] J.A. Teruel, A. Ortiz, F.J. Aranda, Influence of organotin compounds on phosphatidylserine membranes, Appl. Organometal. Chem. 18 (2004) 111–116.
[30] G. Pabst, M. Rappolt, H. Amenitsch, P. Laggner, Structural information from multilamellar liposomes at full hydration: full q-range fitting with high quality X-ray data, Phys. Rev. E 62 (2000) 4000–4009.
[31] G. Pabst, R. Koschuch, B. Pozo-Navas, M. Rappolt, K. Lohner, P. Laggner, Structural analysis of weakly ordered membranes stacks, J. Appl. Crystallogr. 63 (2003) 378–1388.
[32] G. Pabst, Global properties of biomimetic membranes on molecular features, Biophys. Rev. Lett. 1 (2006) 57–84.
[33] G. Pabst, S. Danner, S. Karmakar, G. Deutsch, V.A. Raghunathan, On the propensity of phosphatidylglycerols to form interdigitated phases, Biophys. J. 93 (2007) 513–525.
[34] Y.-P. Zhang, R.N.A.H. Lewis, R.N. McElhaney, Calorimetric and spectroscopic studies of the thermotropic phase behaviour of the *n*-saturated 1,2-diacylphosphatidylglycerols, Biophys. J. 72 (1997) 779–793.
[35] R.N.A.H. Lewis, N. Mak, R.N. McElhaney, Differential scanning calorimetry of the thermotropic phase behaviour of model membranes composed of phosphatidylcholines containing linear saturated fatty acyl chains, Biochem. 26 (1987) 6118–6126.
[36] R.N.A.H. Lewis, Y.-P. Zhang, R.N. McElhaney, Calorimetric and spectroscopic studies of the phase behaviour and organization of lipid bilayer model membranes composed of binary mixtures of dimirystoylphosphatidylcholine and dimiristoylphosphatidylglycerol, Biochim. Biophys. Acta 1668 (2005) 203–214.
[37] A. Tardieu, V. Luzzati, F.C. Reman, Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases, J. Mol. Biol. 75 (1973) 711–733.
[38] K. Lohner, A. Latal, G. Degovics, P. Garidel, Packing characteristics of a model system mimicking cytoplasmic bacterial membranes, Chem. Phys. Lipids 111 (2001) 177–192.
[39] H. Hauser, Some aspects of the phase behaviour of charged lipids, Biochim. Biophys. Acta 772 (1984) 37–50.
[40] R.M. Fernandez, K.A. Riske, L.A. Amaral, R. Itri, M.T. Lamy, Influence of salt on the structure of DMPG studied by SAXS and optical microscopy, Biochim. Biophys. Acta 1778 (2008) 907–916.
[41] R. Mendelson, H.H. Mantsch, Fourier transform infrared studies of lipid–protein interaction, in: A. Watts, J.J.H.H.M. De Pont (Eds.), Progress in Lipid Protein Interactions, vol. 2, Elsevier, New York, 1986, pp. 103–146.
[42] H.H. Mantsch, R.N. McElhaney, Phospholipid phase transition in model and biological membranes as studied by infrared spectroscopy, Chem. Phys. Lipids 57 (1991) 213–226.
[43] R.N.A.H. Lewis, R.N. McElhaney, Fourier transform infrared spectroscopy in the study of hydrated lipids and lipid bilayer membranes, in: H.H. Mantsch, D. Chapman (Eds.), Infrared Spectroscopy of Biomolecules, Wiley-Liss, New York, 1996, pp. 159–202.
[44] A. Blume, W. Hübner, G. Messner, Fourier transform infrared spectroscopy of $^{13}C=O$-labeled phospholipids hydrogen bonding to carbonyl groups, Biochemistry 27 (1988) 8239–8249.
[45] A.S. Ulrich, F. Volke, A. Watts, The dependence of phospholipids head-group mobility on hydration as studied by deuterium-NMR spin-lattice relaxation time measurements, Chem. Phys. Lipids 55 (1990) 64–66.