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A bacterial monorhamnolipid alters the biophysical properties of phosphatidylethanolamine model membranes

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ABSTRACT

This work presents a biophysical study on the interactions of a monorhamnolipid (monoRL) produced by *Pseudomonas aeruginosa* MA01 with model dielaidoylphosphatidylethanolamine (DEPE) membranes. Incorporation of monoRL into DEPE shifts the onset temperature of the $L_{\beta3}$-to-L_{α} and the L_{α}-to-H_{II} phase transitions toward lower values. Incorporation of monoRL into DEPE indicates the coexistence of lamellar and hexagonal–H_{II} phases in rhamnolipid-containing samples at 60 °C, at which pure DEPE is lamellar. Thus, both techniques show that monoRL facilitates formation of the hexagonal–H_{II} phase in DEPE, i.e. it destabilizes the bilayer organization. The phase diagram for the phospholipid component indicates a near-ideal behavior, with better miscibility of monoRL into DEPE in the fluid phase than in the gel phase. The various vibrational mode bands of the acyl chains of DEPE were studied by FTIR spectroscopy, focusing on the CH_2 symmetric stretching mode. Incorporation of monoRL into DEPE shifts the frequency of this band to higher wavenumbers, at temperatures both below and above the main gel to liquid–crystalline phase transition. Examination of the C═O stretching band of DEPE indicates that monoRL/DEPE interactions result in an overall dehydration effect on the polar headgroup of DEPE. These results are discussed in light of the possible role of rhamnolipids as bilayer stabilizers/destabilizers during cell membrane fluctuation events.

1. Introduction

Microorganisms are well known for the production of an increasing number of structurally diverse molecules of amphiphilic character [1–3], with very interesting potential applications [4]. Most of these compounds are known as biosurfactants, i.e., surface active molecules of biological origin. Currently, much effort is being dedicated to the search of new biosurfactants and to the application of biosurfactants in pharmaceutical formulations and in biomedicine [5,6], as antimicrobial agents [6,7], as additives in food and cosmetics [8,9], or in remediation technologies [10]. Within this context, it is clear that the characterization of the physicochemical and biological properties of biosurfactants is an essential step for the appropriate validation of these compounds in the abovementioned applications.

Pseudomonas aeruginosa, a Gram-negative bacterium well known for its environmental versatility, is able to cause disease in particular susceptible individuals. *P. aeruginosa* can utilize a wide range of organic compounds as substrates, thus conferring the microorganism an exceptional ability to colonize ecological niches, where nutrients are limited. Rhamnolipids constitute the main group of biosurfactants produced by *P. aeruginosa* when grown under appropriate conditions [11]. These glycolipid biosurfactants are composed of a hydrophilic head group constituted by one or two rhamnose molecules, called respectively monorhamnolipid (monoRL) (Fig. 1) and dirhamnolipid, and a hydrophobic tail formed by one or two fatty acids. The production of rhamnolipids shows high yields as compared to other biosurfactants [12], and used oils or wastes from the food industry can be used as carbon sources [8,13,14], the whole process being considered as a green process.

Studies on the interaction of the dirhamnolipid component, purified from the *P. aeruginosa* crude biosurfactant, with model phosphatidylcholine [15–18], and phosphatidylethanolamine membranes [19] have been recently carried out. Concerning the monoRL component, we have recently published on the physicochemical characteristics of the monomer-to-micelle transition of the *P. aeruginosa* monoRL [20], and its effect on model phosphatidylcholine membranes [21]. The abundance of phosphatidylethanolamine in biological membranes, and its capacity to promote non-bilayer structures have made this phospholipid a focus of attention for many years [22]. Dielaidoylphosphatidylethanolamine (DEPE), a major phospholipid found in an *Escherichia coli* fatty acid auxotroph, has been widely used as a model for unsaturated phosphatidylethanolamine species. The importance of nonbilayer lipids for protein function and the special packing properties of bilayers containing these lipids have been recently remarked [23]. This paper extends previous studies analyzing the molecular details on the interaction of monoRL

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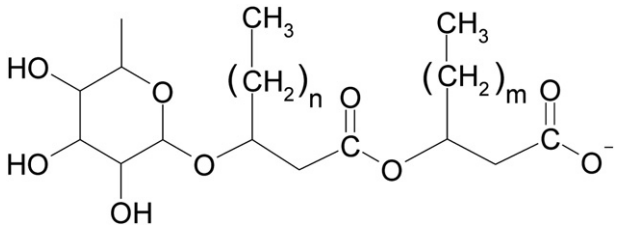

Fig. 1. The chemical structure of the monoRL compounds produced by *Pseudomonas aeruginosa* MA01. The predominant combinations of fatty acids found were: C_{10}–C_{10} (m, n = 6), C_{10}–C_{12} (m = 8, n = 6), and C_{10}–$C_{12:1}$ (m = 8, n = 6, one double bond).

with DEPE, establishing the relevant differences between dirhamnolipid and monoRL, and discussing the possible role of rhamnolipids in membrane stabilization/destabilization, and regulation of lipid polymorphism.

2. Materials and methods

2.1. Materials

Glucose, glycerol, sodium nitrate, potassium dihydrogen phosphate, magnesium sulfate, yeast extract, nutrient agar, ethyl acetate, hydrochloric acid, chromic acid, acetone, silica gel 60, chloroform, methanol and anthrone were purchased from Merck (Germany). Soybean oil was purchased from a local supermarket. Crude oil was obtained from the National Iranian Oil Company (NIOC). 1,2-Dielaidoyl-*sn*-glycero-3-phosphoethanolamine (DEPE) was from Avanti Polar Lipids Inc. (Birmingham, AL). All other reagents were of the highest purity available. Purified water was deionized using a Milli-Q equipment from Millipore (Millipore, Bedford, MA, USA) and had a resistivity of ca. 18 MΩ cm. Stock solutions of the phospholipid and the monoRL were prepared in chloroform/methanol (1:1) and stored at −20 °C. The buffers used through the work were aqueous 150 mM NaCl, 5 mM HEPES pH 7.4, and 150 mM NaCl, 5 mM HEPES pD 7.4 prepared in D_2O for the FTIR measurements. Water and all buffer solutions used in this work were filtered through 0.2 μm filters prior to use. The biosurfactant was produced, isolated and characterized as recently published [24].

2.2. Differential scanning calorimetry

Samples for DSC were prepared by dispersion of the required amounts of the phospholipid and the biosurfactant in the abovementioned aqueous buffer. Briefly, 3 μmol of DEPE and the corresponding amounts of monoRL, from stock solutions in chloroform/methanol (1:1), were mixed, and 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 the solvent were removed by a further minimum 3 h desiccation under vacuum. The abovementioned buffer (2 ml) was added to the dry samples and these were vortexed until a homogeneous suspension of multilamellar vesicles was obtained, always keeping the temperature above the gel to liquid–crystalline phase transition temperature of DEPE. Phospholipid phosphorus was determined by the method of Böttcher et al. [25]. Experiments were performed using a MicroCal MC2 calorimeter (MicroCal, Northampton, USA). The heating scan rate was 60 °C h^{-1}. The calorimeter was calibrated using pure phospholipids as standards.

Partial phase diagrams for the phospholipid component were constructed from the heating thermograms. The *solidus* and *fluidus* lines of the diagrams were defined by the onset and completion temperatures of the transition peaks obtained from heating scans. In order to avoid artifacts due to the thermal history of the sample the first scan was never considered. Second and further scans were carried out until a reproducible and reversible pattern was obtained, which usually occurred already with the second scan. The pretransitions were omitted from the diagrams for the sake of simplicity.

2.3. Fourier-Transform infrared spectroscopy

Samples for the Fourier-transform infrared (FTIR) spectroscopy measurements were prepared by mixing 10 μmol of DEPE and the corresponding amount of monoRL, from stock solutions in chloroform/methanol (1:1). The solvent was gently evaporated under a stream of dry N_2, to obtain a thin film at the bottom of a small glass tube, and last traces of the solvent were removed by a further minimum 3 h desiccation under vacuum. 100 μl of a 150 mM NaCl, 5 mM HEPES pD 7.4 buffer (in D_2O) was added to the dry samples and these were vortexed until a homogeneous suspension of multilamellar vesicles was obtained, always keeping the temperature above the gel to liquid–crystalline phase transition temperature of DEPE. An aliquot of the sample (approximately 20 μl), prepared as described above, was placed between two CaF_2 windows using 25 μm Teflon spacers, and the set was mounted in a thermostated cell holder. Infrared spectra were acquired in a Nicolet 6700 Fourier-transform infrared spectrometer (FTIR) (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. For examination of the C═O stretching band at various temperatures, the spectrum was zapped between 1780 and 1660 cm^{-1}, and the solvent baseline was subtracted. This C═O stretching band was fitted to its component bands by an iterative process using a Gaussian–Lorentzian function. The maxima of the component bands were set at 1742, 1728 and 1714 cm^{-1}, allowing a displacement of ±1 cm^{-1} during fitting.

2.4. Small-angle X-ray diffraction

Samples for X-ray diffraction analysis were prepared essentially as described above for DSC. Briefly, 10 μmol of DEPE and the corresponding amount of monoRL, from stock solutions in chloroform/methanol (1:1), were mixed. The solvent was gently evaporated under a stream of dry N_2, to obtain a thin film at the bottom of a small glass tube, and last traces of the solvent were removed by a further minimum 3 h desiccation under vacuum. 1 ml of a 150 mM NaCl, 5 mM HEPES pH 7.4 aqueous buffer was added to the dry samples and these were vortexed until a homogeneous suspension of multilamellar vesicles was obtained. The liposome suspensions were centrifuged in a bench microfuge and 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 5 min, allowing 10 min prior to the measurement for temperature equilibration. Small angle X-ray diffraction (SAXD) measurements were carried out using a Kratky compact camera (MBraum-Graz-Optical Systems, Graz, Austria) and a linear position sensitive detector (PSD; MBraum, Garching, Germany) monitoring the *s*-range ($s = 2 \sin\theta / \lambda$, 2θ = scattering angle, $\lambda = 1.54$ Å) between 0.0075 and 0.07 $Å^{-1}$. Nickel-filtered Cu K_α X-rays were generated by a Philips (Eindhoven, The Netherlands) PW3830 X-ray generator operating at 50 kV and 30 mA. The calibration of the detector position was performed by using silver stearate (d-spacing at 48.8 Å) as a reference material.