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Synthesis, characterization and inclusion into liposomes of a new cationic pyrenyl amphiphile

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ABSTRACT

The aggregation properties of a new cationic fluorescent amphiphile tagged on the hydrophobic tail with a pyrene moiety and bearing two hydroxyethyl functionalities on the polar headgroup were investigated by fluorescence experiments as pure components or in mixed liposomes containing an unsaturated phospholipid, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, at different molar ratios. The obtained results put in evidence that the conformation and the miscibility of the lipids in the aggregates strongly influence the excimer/monomer ratio. Mixed monolayers at the same composition were investigated by Langmuir compression isotherms to deepen the understanding of lipid organization and miscibility, both in the polar and in the hydrophobic regions. The presence of two hydroxyethyl functionalities on the polar headgroup of the newly synthesized amphiphile exerts a shielding effect of the charge of the amphiphile increasing the compressibility of lipid components in contrast with the disturbing effect of the unsaturated acyl chains of the phospholipid.

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1. Introduction

Biological membranes are quasi-two-dimensional complex fluids composed of mixtures of phospholipids and other biomolecules such as glycolipids, sterols, proteins and sphingolipids (Alberts et al., 2007). To understand the organization of these highly heterogeneous and ordered architectures it is generally necessary to study the role of each membrane component. An approach to the comprehension of lipid organization in biological membranes is investigating the properties of membrane models such as micelles and liposomes of increasing complexity, formulated with different natural lipids and/or synthetic components. Often, when liposomes are used as artificial membranes, fluorescent lipids are included in the formulation to investigate lipid organization and interactions using fluorescence spectroscopy and fluorescence microscopy techniques (Klymchenko and Kreder, 2014). The fluorescent moiety can be linked to the polar headgroup or to the hydrophobic tail. A large variety of lipid molecules tagged with different types of dyes have been used to study physicochemical features of membranes such as the packing and polarity of lipid bilayer (Bernik et al., 2001), lipid segregation (Klymchenko and Kreder, 2014) or surface potential (Zuidam and Barenholz, 1997). A further important and useful application of fluorescent lipids concerns the sensoristic field. Many fluorescent sensors based on pyrene derivatives displayed good selectivity and reliability in aqueous media (Wang et al., 2007; Fan et al., 2015; Kim et al., 2005).

The pyrene moiety is a hydrophobic dye commonly used in the synthesis of fluorescent lipids, the first ω-pyrene derivatives of long-chain fatty acids having been described in 1975 (Galla and Sackmann, 1975). Pyrene features a long lifetime of the excited state (Bombelli et al., 2011) and its derivatives can form excited-state dimers (excimers) with red-shifted fluorescence emission (Hartmann and Galla, 1980). Excimer formation is frequently exploited for investigating different processes such as the formation of lipid domains (Barenholz et al., 1996a), membrane fusion (Stegmann et al., 1993), lipid-protein interactions (Mach and

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Middaugh, 1995) and membrane fluidity and lipid trafficking in living cells (Repakova et al., 2006).

Herein we report the synthesis and the physicochemical characterization of a novel cationic amphiphile tagged on the hydrophobic tail with pyrene *via* an ether bond, **1** (Chart 1). The presence of two hydroxyethyl groups on the polar headgroup makes it quite large, polar and hydrated thus can influence the balance between the hydrophilic (headgroup) and the hydrophobic (tail) portion thus influencing its properties. We investigated its optical features and its aggregation properties with the aim of evaluating its potentiality as fluorescent probe for the study of lipid aggregates. Moreover, this surfactant could be also useful for diagnostic purposes because, due to its positive charge, it confers to the aggregates in which is included a high tendency to interact with negatively charged cell membranes.

The absorption and emission features of the fluorescent amphiphile were investigated in aqueous and in organic solvent (methanol and octanol) as a function of concentration and temperature. Its aggregation properties were investigated as a pure component and when included in 1,2-dioleoyl-*sn*-glycero-phosphocholine (DOPC) liposomes (Chart 1). Mixed DOPC/**1** monolayers at water/air interface were also investigated to understand the influence of the molecular structure of the components and of their relative amount on lipid organization, these aggregates being considered as two-dimensional membrane models.

2. Experimental

2.1. Instrumentation

^{1}H and ^{13}C spectra of compounds **4** were performed on a Varian Mercury 300 at 300.13 and 75.48 MHz, respectively; whereas ^{1}H and ^{13}C spectra of compounds **3**, **5**, **1** were performed on a Bruker AVANCE 400 at 400.13 and 100.61 MHz respectively; δ in ppm relative to the residual solvent peak of $CDCl_3$ at 7.26 and 77.0 ppm for ^{1}H and ^{13}C, respectively. *J* in Hz.

Elemental analyses for C, H, N and S were performed on an EA 1110 CHNS-O instrument.

Steady-state and time-resolved fluorescence experiments were carried out on a Fluoromax-4 Horiba-Jobin Yvon spectrofluorimeter.

UV measurements were carried out on a Cary 300 UV–vis double beam spectrophotometer (Varian Australia PTY Ltd., Mulgrave, Vic., Australia).

Surface pressure (π) measurements were carried out by means of a Wilhelmy plate (39.240 mm of perimeter) technique using a Langmuir Minitrough, KSV Instruments Ltd, Helsinki in Teflon with 325 mm of length, 75 mm of width and 24,380 mm^2 of total area. The instrument was enclosed in a box to reduce surface contamination.

Dynamic Laser light scattering (DLS) measurements were performed with a Malvern Nano-ZetaSizer, equipped with a 5 mW HeNe laser (wavelength = 632.8 nm) and a digital logarithmic correlator.

High-resolution electrospray ionisation mass spectrometry (HRESIMS) spectra were recorded using a Micromass Q-TOF Micromass spectrometer (Waters) in the electrospray-ionization mode.

2.2. Materials

DOPC was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Phosphate-buffered saline (PBS, 0.01 M phosphate buffer; 0.0027 M KCl; 0.137 M NaCl; pH 7.4), dialysis tubing cellulose membrane D 9527 and all reagents employed for the synthesis of **1** were purchased from Sigma-Aldrich. Anhydrous acetone was prepared by distillation after reflux over dry K_2CO_3; anhydrous CH_2Cl_2 was prepared by distillation after reflux over P_2O_5; other solvents and chemicals were used as purchased without further purification. For thin layer chromatography (TLC) silica gel 60 glass plates coated with fluorescent indicator F254 were used. For column chromatography silica gel 60, 70–230 mesh ASTM or aluminium oxide 90 active or neutral (activity I), 70–230 mesh ASTM was used.

2.3. Synthesis of amphiphile *1*

2.3.1. Synthesis of compound *3*, 1-hydroxypyrene

1-hydroxypyrene was prepared from pyrene-1-carboxaldehyde **2** according to a known procedure by making changes in the purification procedure (Sehgal and Kumar, 1989). To a stirred solution of commercially available pyrene-1-carboxaldehyde **2** (2.0 g, 8.6 mmol) in anhydrous CH_2Cl_2 (80 mL) *m*-chloroperbenzoic acid (*m*-CPBA, 2.2 g, 13 mmol) was added under an atmosphere of Ar. The resulting solution was refluxed under Ar atmosphere until TLC monitoring (SiO_2, AcOEt/*n*-hexane 1/9, x2) showed the complete disappearance of the starting material (24 h). The reaction mixture was treated with 10% $NaHCO_3$ until effervescence ceased and the organic solvent was evaporated under reduced

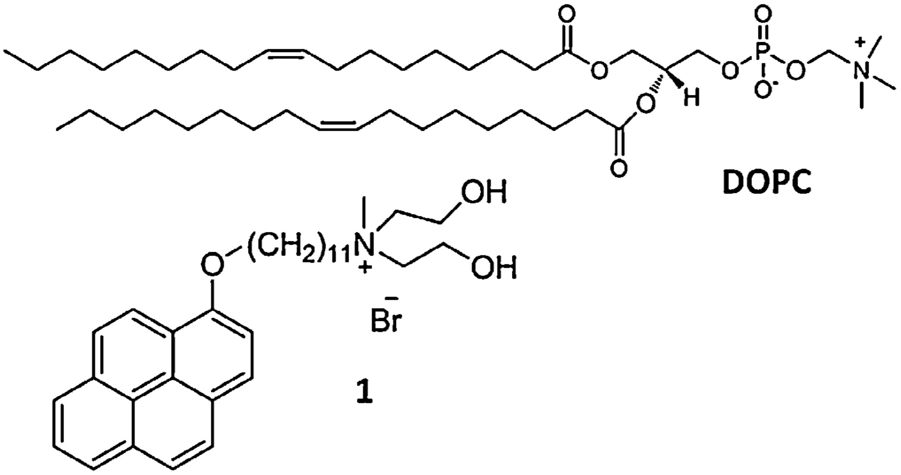

Chart 1. Molecular structure of pyrenyl amphiphile 1 and DOPC.

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