Chemistry and Physics of Lipids 213 (2018) 1–12

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

DPPC monolayer response to non-spanning cobalt-cage metallosurfactants: Electrostatic complex formation

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ARTICLE INFO

Keywords:
DPPC
Langmuir monolayers
Langmuir-Blodgett
Cobalt-cage complexes
Metallosurfactant
Atomic force microscopy

ABSTRACT

A novel series of amphiphilic cobalt-cage derivatives (ACCD), bearing a diaza-crown bridge and varying alkyl chains, facilitate ion transport across biomembrane models via self-aggregation. In this study, compression isotherm analyses and atomic force microscopy (AFM) were used to assess the interactions of these amphiphiles with Langmuir monolayers of dipalmitoylphosphatidylcholine (DPPC) in order to elucidate electrostatic and steric contributions to ion transport. The stability and compressibility of DPPC monolayers are disrupted by ACCD molecules with short (C_{12}) alkyl chains. These top-heavy amphiphiles (large cone angles) create voids at the interface of the hydrophobic/aqueous layer leading to monolayer expansion and packing efficiency of the aliphatic chains is disrupted. Long-tailed analogues (C_{16}, C_{18}) are cohesively integrated into DPPC monolayers due to their smaller cone angles at the interfacial region and increased hydrocarbon compatibility in the hydrophobic region. Thermodynamic data indicate the formation of electrostatic complexes between DPPC and longer-tailed amphiphiles consistent with AFM observations of aggregate structures at the corresponding concentrations.

1. Introduction

The development of metallosurfactants (Griffiths et al., 2006; Kaur and Mehta, 2014) has received sustained interest due to their diverse applications in fields such as medicine (Kaur et al., 2017; Jagadeesan et al., 2013; Riyasdeen et al., 2014), drug delivery (Parera et al., 2016; Zha et al., 2016) and catalysis (Mancin et al., 2009; Li et al., 2012). Of particular interest are metallosurfactants derived from cobalt (III) cage complexes which exhibit potent antimicrobial and antibacterial properties (Veeralakshmi et al., 2015; Gahana and Harrowfield, 2015; Kumar et al., 2009). Amphiphilic cobalt (III) cage complexes, were first shown by Walker et al. (2003) and (Behm et al., 1993) to elicit biological activity on nematodes due to membrane rupture via unregulated transmembrane ion-flux. Since typical cell membranes are too hydrophobic to be easily traversed by charged/or highly polar species, understanding the mechanisms by which this class of molecules interact with cell membranes is of fundamental importance to studies of cellular biology and drug design (Jagadeesan et al., 2013).

In previous papers (Jaggernauth and Fairman, 2011; Lalgee et al., 2014), we reported the synthesis and characterization of a series of cobalt-cage metallosurfactants, **CC-12**, **CC-16**, and **CC-18** (Fig. 1), where the head group is connected to the aliphatic tail through a diaza crown spacer. The amphiphilicity of **CC-12**, **CC-16**, and **CC-18** in vitro is much reduced compared to sodium dodecylsulphate (SDS), with critical micelle concentrations (CMC) values of 1.08, 0.94 and 0.37 mM respectively, compared to 8.1 mM for SDS.

We also demonstrated ion transport by **CC-12**, **CC-16**, and **CC-18** across planar bilayer membranes of 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC), which was ascribed to a possible toroidal pore formation (Lalgee et al., 2014). Such unusual ion-channel-type behavior without a specific membrane protein-substrate interaction is further investigated in this report. We propose that critical factors include (i) the electrostatic and spatial disruption caused by the triply-charged cobalt-cage component of the head group, (ii) the conformational flexibility of the aza-crown component which may facilitate selective guest-ion transport and (iii) the variable length aliphatic tails (12, 16, or 18 carbons) that penetrate the bilayer. Furthermore, Langmuir Blodgett isotherm experiments on **CC-12**, **CC-16**, and **CC-18** demonstrated the ion selectivity of the molecules for transport of Ca^{2+}, Mg^{2+} and K^{+} ions across the interfacial region between the monolayer and the aqueous subphase (Jaggernauth and Fairman, 2011).

The systematic variation in the aliphatic chain length (12, 16, or 18 carbons), allows partitioning the ion transport behavior among steric, Coulombic, or dipolar interaction of the head group of the surfactant with the interface. Possible alterations of the bulk fluidity of the phospholipid model membrane structure to accommodate the

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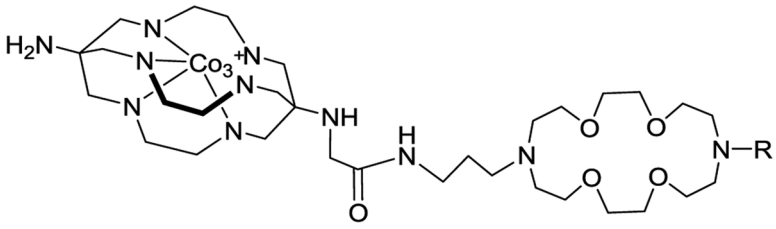
https://doi.org/10.1016/j.chemphyslip.2018.02.003
Received 7 August 2017; Received in revised form 23 February 2018; Accepted 26 February 2018
Available online 27 February 2018
0009-3084/



CC-12, R = $C_{12}H_{25}$; **CC-16**, R = $C_{16}H_{33}$; **CC-18**, R = $C_{18}H_{37}$

Fig. 1. Structures of Compounds **CC-12, CC-16,** and **CC-18**.

amphiphiles are also likely to occur. We have therefore investigated the physical interaction of amphiphiles **CC-12**, **CC-16**, and **CC-18** with DPPC monolayers.

Although pure DPPC is known to form relatively rigid and well organized bilayers, we focus on the response of a single monolayer component on water subphase to gain insight into the earliest stages of membrane interaction which eventually lead to selective ion transport and permeation by guest ions. In particular, the variations in membrane packing and rearrangement that occur as the compounds are partitioned into the monolayer may be correlated with the bulk stability and miscibility of the resultant mixed amphiphilic-lipid monolayers at the air/water interface (Maget-Dana, 1999). Therefore, measurement of the π-A isotherms relative to the respective pure lipid films can be related to the physical state of the DPPC molecules at the air/water interface (Maget-Dana, 1999). Additionally, the extent of film stabilization in the resulting mixed monolayer can be evaluated from the isotherm data by calculating excess Gibbs free energies of mixing (ΔG_{ex}), excess molecular areas (A_{ex}) and isothermal elasticities (C_s^{-1}) as a function of concentration and surface pressure (Maget-Dana, 1999; Castelli et al., 2007; Eftaiha and Paige, 2011). These data provide an estimate of the disruption of membrane lateral organization and packing as well as the relative thermal barriers to the processes of reorganization in each case.

We also examine the mixed amphiphile-lipid monolayers, prepared via Langmuir-Blodgett deposition, for direct observation of amphiphile-induced membrane defects and possible dynamic formation of pores or other aggregates by atomic force microscopy (AFM) (Deleu et al., 2014; Picas et al., 2012). Changes in surface morphology observed in these supported mixed monolayers are expected to highlight the role of amphiphile molecular structure on membrane interactions at the molecular level.

2. Materials and methods

2.1. Materials

Amphiphiles **CC-12**, **CC-16**, and **CC-18** were prepared and supplied, as described in a previous report, by one of the authors (Jaggernauth and Fairman, 2011). DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) was purchased from Avanti Polar Lipid Inc. (Birmingham, Al, USA). HEPES buffer (*N*-(2 hydroxyethyl) piperazine-*N*-2-ethanesulphonic acid) was obtained from Sigma Aldrich (St. Louis, MO) and used as received. Ultrapure water (resistivity of 18.2 MΩ cm) obtained from a Thermo Scientific Barnstead Easypure II was used to prepare subphase solutions. Distilled chloroform was used to prepare lipid-amphiphile mixtures. Mixed lipid-amphiphile solutions were prepared in chloroform using 1 mg/ml stock solution of the lipid (DPPC) at different molar fractions 0, 0.003, 0.006, 0.009, 0.019, 0.038, 0.075, 0.15, 0.30, 0.50, 0.80 and 1.00 of amphiphile/lipid ratio.

2.2. Langmuir monolayers

The surface pressure-area (π-A) isotherms were measured at room temperature at 24 °C with a platinum Wilhelmy plate attached to the microbalance of a KSV Minimicro Trough (Teflon symmetric Delrin, 100 cm^2 surface area). Before each measurement, the trough and barriers were washed thoroughly with ethanol and ultrapure water. The instrument was housed in a dust-free cabinet to prevent surface contamination. To confirm that the surface of the trough and sub phase were adequately cleaned before each experiment, the barriers were compressed over the entire surface area range to ensure that surface pressure fluctuations were less than ± 0.2 mN/m. Subsequently, 10–12 μL of amphiphile/lipid mixtures were spread with a Hamilton™ microsyringe onto the sub phase (10 mM HEPES, pH = 7.4) and left for 20 mins to allow evaporation of the solvent and equilibration before compression. Symmetric compression was achieved by moving the barrier at a constant rate of 5 mm/min. Each experiment was performed at least twice to ensure reproducibility.

2.3. Langmuir-Blodgett monolayers

For AFM analysis, Langmuir–Blodgett (LB) monolayers were deposited at a constant surface pressure of 12 mN/m, by vertically withdrawing freshly cleaved mica sheets through the air–water interface at a rate of 0.1 mm/min. The mica was initially half-dipped into the subphase before monolayer deposition. Films relative to surfactant molar fractions of 0, 0.003, 0.006, 0.009, 0.019, 0.038 were studied.

2.4. Atomic force microscopy

The surface morphology of the films was analyzed by Atomic force microscopy (Veeco Multimode V Atomic Force Microscope). The scans were performed in Tapping mode under ambient conditions. Data acquisition was achieved using commercial silicon cantilevers (RTESPA-300, Bruker AFM Probes, Santa Barbara, CA) with 8 nm nominal radius, 40 N/m elastic force constant, and resonance frequency around 300 kHz for high sensitivity. The scan rate ranged between 1 and 3 Hz and a resolution of 256 pixels per line. At least three separate areas were imaged for each sample (monolayer composition).

2.5. Data analysis

2.5.1. Phase transitions

The effect of the model series of compounds on the physical state of DPPC lipid monolayer π-A isotherms can be inferred from the effective compression modulus C_s^{-1} for mixed films (Arczewska and Gagoś, 2011) according to Eq. (1):

$$C_s^{-1} = -A\left(\frac{d\pi}{dA}\right) \quad (1)$$

where A is the effective cross sectional area of the molecule at the indicated surface pressure, π. High values of C_s^{-1} reflect fluidity of the monolayers and more organized structures produce the converse. Each phase transition is indicated by a static surface pressure where the area per molecule is changing. The characteristic minimum of the C_s^{-1} versus