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SCIENCE DIRECT
Chemistry and Physics of Lipids 138 (2005) 50–59
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

1,2-Dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) monolayers: influence of temperature, pH, ionic strength and binding of alkaline earth cations

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Received 15 June 2005; received in revised form 11 August 2005; accepted 15 August 2005
Available online 6 September 2005

Abstract

Ion binding and lipid ionization of the acidic phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) in monolayers was studied by measuring the lateral pressure Π as a function of the molecular area A at the air/water interface at different temperatures. The pH of the subphase (pH 2 and 7) and the ionic strength (NaCl) was varied. In addition, different divalent cations (1 mM $MgCl_2$, $CaCl_2$ and $SrCl_2$, pH 7) were added. DMPG is partly protonated on pure water at pH 7. An increase in the NaCl concentration in the subphase leads to film expansion. This effect is caused by an ionization of the headgroup of DMPG, i.e. a shift of the apparent pK. More condensed films are obtained on pure water at pH 2, due to the reduction of electrostatic repulsion by headgroup protonation and the possibility for the formation of a hydrogen bonding network. The divalent cations Mg^{2+}, Ca^{2+} and Sr^{2+} interact differently with a DMPG monolayer in pure water at pH 7. In the presence of 1 mM $CaCl_2$ a condensation of the DMPG film is induced, whereas an expansion of the monolayer is observed in the presence of Mg^{2+} and Sr^{2+}. Two counteracting effects are operative: (a) ionization of the headgroup due to electrostatic screening leads to film expansion and (b) binding of the divalent cations to the lipid headgroups leads to condensation. The latter effect is more pronounced in the case of Ca^{2+}, whereas the binding of Mg^{2+} and Sr^{2+} to DMPG is weaker. Site-specific cation binding has to be assumed in addition to electrostatic effects.

Keywords: Lipid monolayers; DMPG; Cation binding; Salt effects; pH effects

1. Introduction

Phospholipid monolayers are used as model systems for lipid bilayers, because they represent one half of a lipid membrane. Therefore, many studies have been conducted studying the binding of various proteins to lipid monolayers (Lahdo and De La Fornière-Bessueille, 2004; Theretz et al., 1983; Trommeshauser and Galla, 1998; Whitehouse et al., 2004), or the binding of cations (Akashi et al., 1998; Bonté et al., 1986; Kaznessis et al., 2002; Mashak et al., 1982; Park et al., 1999). Lipid monolayers are, however, also present in biological systems and are involved in different biological processes. The process of respiration is such an example (Schief et al., 2003; Smith et al., 2003; Yu and Possmayer, 2003). Therefore, the interactions of pulmonary surfactant proteins with lipid monolayers has been studied

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0009-3084/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2005.08.001

in great detail (Bangham et al., 1979; Goerke, 1974, 1998; Kramer et al., 2000; Panda et al., 2004; Post et al., 1995; Ruano et al., 1998a,b; Taneva and Keough, 1994a,b,c; Veldhuizen et al., 1998; Walters et al., 2000). For humans and many animals, the formation of a functioning monolayer film at the alveolar/air interface plays a vital role. The film covers the alveolar surface, thus reducing the surface tension of the air–liquid interface facilitating the respiratory mechanism. The interfacial phospholipid monolayer is composed of phosphocholine (PC) enriched with 10–25 wt.% phosphoglycerol (PG). This lipid film is primarily responsible for the tensioactive properties of lung surfactant, whereas PC and surfactant-associated proteins are required for the formation and proper dynamics of the surfactant monolayer in the airways (Amrein et al., 1997; Bangham et al., 1979; Goerke, 1974, 1998; Ruano et al., 1998a,b; Veldhuizen et al., 1998; Yu and Possmayer, 2003). Avery and Mead have shown that PG is not present in the pulmonary surfactant of infants with respiratory distress syndrome (RDS), a potentially fatal disease of the newborn (Avery and Mead, 1959). At present, it is not clear how PG interacts with other pulmonary surfactant components including calcium to maintain alveolar stability (Nag et al., 1994).

The interactions of proteins with PC/PG membranes can be mediated by the presence of divalent cations (Tocanne et al., 1994; Trommeshauser and Galla, 1998; Yu and Possmayer, 2003). In addition, other phenomena, like the induction of domain formation are observed by altering the electrostatics of the lipid molecules (Creuwels et al., 1996; Garidel et al., 1997; Ma et al., 1998; Tocanne et al., 1994; Vaz, 1994). Changes in the electrostatic surface charge have large implications for the lipid–protein interactions. Nordera et al. (1997) have shown that the adsorption of Pseudomonas aeruginosa exotoxin A to an anionic phospholipid monolayer is controlled by pH and the surface potential of the film. In addition, Wu et al. (1999) demonstrated that annexin V, a protein that exhibits functionally relevant Ca^{2+} dependent binding to anionic phospholipid membranes, can be protected from denaturation in a ternary Ca^{2+}/phospholipid/protein complex. These examples show that pH variation and the presence of divalent cations play a crucial role in optimal lipid–protein interaction.

Furthermore, in recent studies we have shown that the interaction of PG with different alkaline earth cations is cation specific (Garidel and Blume, 1999; Garidel et al., 1998, 2000a,b,c). These investigations were performed using lipid vesicles at high ionic strength. However, very little information is available for PG monolayer divalent cation interactions at very low ionic strength.

The advantage of monolayer studies with phospholipids spread at an air/water interface is that they can be performed in a well-defined way. The two-dimensional molecular film density, the ionic conditions of the subphase, as well as the temperature and composition of the subphase can easily be varied. The simplest way to characterize a surfactant monolayer is by measuring the lateral pressure Π as a function of the molecular area A. Π is defined as the difference in surface tension in the absence and presence of surfactant at the air/water interface (Albrecht et al., 1978). The detailed features of the Π/A curve depend on the molecular composition of the film (nature and charge of the headgroup, length and saturation degree of the hydrocarbon chain) and the subphase composition, e.g. different ionic strength, different cations, pH, temperature of the subphase, etc. (Möhwald, 1995; Pétriat et al., 2004).

We investigated monolayer films composed of 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), which has a dissociable headgroup. Altering either the pH of the subphase or the concentration of the monovalent or divalent cations in the subphase can change the degree of dissociation of the headgroup and thus the charge density of the film. We have triggered the electrostatic changes of the DMPG molecules in the film (1) by altering the pH of the subphase (pH 7 and 2), (2) by altering the sodium chloride concentration of the subphase at pH 7 and (3) by the presence of divalent cations ($MgCl_2$, $CaCl_2$ and $SrCl_2$, 1 mM at pH 7) in the subphase. The isotherms of DMPG monolayers were recorded at different temperatures. Special attention was devoted to the liquid condensed (LC) to liquid expanded (LE) phase transition and to the shifts in this phase transition caused by altering the subphase composition.

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Lipoid KG (Ludwigshafen, Germany) and Enzymatix Ltd. (Cambridge, UK). Stearic and arachidic acids were from Sigma. Calcium chloride dihydrate, magnesium chloride hexahydrate, strontium chloride hexahydrate and sodium chloride of analytical grade were obtained from E. Merck, Darmstadt (Germany).