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Influence of a new amphiphilic peptide with phospholipid monolayers at the air–water interface

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Abstract

Langmuir monolayer (surface pressure (π)–area (A), surface potential (ΔV)–area (A), and dipole moment ($\mu_\perp$)–area (A) isotherms) and fluorescence microscopy techniques were used to investigate a new-designed 18-mer amphiphilic α-helical peptide (Hel 13-5) which consists of 13 hydrophobic and 5 hydrophilic amino acid residues. We present here a study of the surface behavior of Hel 13-5 against dipalmitoylphosphatidylcholine (DPPC) and/or Egg-phosphatidylcholine (Egg-PC), which are major components in artificial pulmonary surfactant. A temperature dependence of pure DPPC and Hel 13-5 was examined using Langmuir isotherm techniques over the temperature range of 298.2–310.2 K. Basic interfacial behavior of Hel 13-5 was investigated by adding Hel 13-5 to pure DPPC and the DPPC/Egg-PC (1:1, mol:mol) mixture on a substrate solution of 0.02 M Tris buffer (pH 7.4) with 0.13 M NaCl at 298.2 K. The cyclic compression–expansion isotherms of these systems were obtained to confirm the spreading and respreading abilities of Hel 13-5. In addition, fluorescence microscopy measurements were carried out to understand the interactions of Hel 13-5 with pure DPPC or the DPPC/Egg-PC mixture. From the fluorescent images, the distinct differences between these systems were observed. Adding a small amount of Hel 13-5 to DPPC induced the "moth-eaten" disaggregation of liquid-condensed (LC) domains made of DPPC. On the other hand, the phenomenon that the LC domains were shrunk in size by adding a small amount of Hel 13-5 occurred for the there-component system (DPPC/Egg-PC/Hel 13-5).

Keywords: Pulmonary surfactant; Respiratory distress syndrome; Langmuir monolayer; π–A isotherm; ΔV–A isotherm; $\mu_\perp$–A isotherm; DPPC; Egg-PC; Hel 13-5; Fluorescence microscopy

1. Introduction

Pulmonary surfactant (PS) secreted by the alveolar type II cell is complex mixture of lipids and proteins. PS lining at the air/alveolar interface of the mammalian lung mainly contributes to maintaining the structural stability of the alveolus during respiration. This function in vivo reduces the surface tension down to almost zero on expiration, facilitating the work of breathing and preventing alveolar collapse at low volumes [1]. Deficiency of PS causes neonatal respiratory distress syndrome (NRDS) in premature infants, resulting in extremely high mortality rates.

PS consists of multiple lipids (∼90 wt.%) and four surfactant proteins (SP-A–SP-D; ∼10 wt.%). Although the compositions of PS are quite different among diverse species [2–5], it contains practically phospholipids (80–90 wt.%). Furthermore, the phospholipids largely include phosphatidylcholines (main component is dipalmitoylphosphatidylcholine, DPPC of ∼50%) in human PS. Pulmonary surfactant proteins play a decisive role as well, even though their content is less than 10 wt.% of the surfactant mass.

A single monolayer of the most abundant molecular species, DPPC, can reduce the surface tension down to almost zero under the excess compression beyond the collapse of the monolayer states. However, pure DPPC film does not sufficiently keep the surface tension low due to the space caused by a bulky head group of DPPC and is also sensible to mechanical disturbance and collapses irreversibly when compressed

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beyond the minimum surface tension [6]. In addition, it is very slow to adsorb from the aqueous suspension and to respread when compression is relieved. Hydrophobic proteins (SP-B and SP-C) concerned with interfacial functions can resolve these defects in native PS [2,7,8]. While, hydrophilic proteins (SP-A and SP-D) play an important role in the first line defense against inhaled pathogens [9]. Furthermore, SP-A regulates surfactant homeostasis [10–12]. Although hydrophobic proteins may collapse at relatively high surface tensions where the ejection of materials from the monolayer occurs, they facilitate the adsorption and spreading of surfactant molecules [13,14]. This specific phenomenon has been known as "squeeze-out" theory [15]. The theory describes that the fluidizing lipids are selectively removed from the interface on compression, leaving behind the monolayer enriched in lipids that possess abilities of lowering surface tension [16–18]. The "squeeze-out" theory includes an idealized immiscible interaction between the LC phase (rigid) and LE phase (fluid) of lipid components.

Pulmonary surfactant (PS) works in three-dimensional alveoli. However, Langmuir monolayer system characterized by two-dimensional surface films serves as a good model for biophysical studies of PS. At the air–liquid interface, direct visualization of lipid or lipid–protein monolayers doped with fluorescent probes and fluorescence microscopy (FM) measurement now provide the powerful information about structural transitions on dynamic compression [14,19–21].

Recently, we synthesized a de novo-designed 18-mer amphiphilic α-helical peptide (Hel 13-5), consisting of 13 hydrophobic and 5 hydrophilic amino acid residues [22]. In addition, we have already reported that Hel 13-5 could induce neutral liposomes to adopt long nanotubular structures and that the interaction of specific peptides with specific phospholipid mixtures could induce the membrane structures to resemble cellular organelles, such as Goldi apparatus [23–27]. Furthermore, we found that the size and shape of such nanotubes depend on lipid compositions [26]. Incidentally, Hel 13-5 (MW ∼2200) is considerably similar to SP-B (79 amino acid residues, MW ∼8700) and SP-C (35 amino acid residues, MW ∼4200) in terms of α-helical structure that is important for lipid–protein interactions in the monolayer state. These confirmations suggest that synthesized Hel 13-5 substitutes for native SP-B and SP-C to fulfill the pulmonary function at the alveolar liquid–protein interface.

In this study, the behavior of spread monolayers for multicomponent systems of new synthesized Hel 13-5 and pure DPPC or DPPC/Egg-PC (1:1, mol:mol) was investigated by surface pressure (π)–, surface potential (ΔV)–, surface dipole moment ($\mu_\perp$)–area (A) isotherms, and fluorescence microscopy (FM). DPPC is the main phospholipid in mammalian PS and the main surface-active constituent, too. In addition, two-component system (DPPC/Egg-PC) is applied to mimic the phosphatidylcholine components of the alveolar membranes [2–5]. Monolayers mainly spread on a 0.02 M Tris buffer (pH 7.4) with 0.13 M NaCl at 298.2 K were investigated at the air/water interface. The study was made on the temperature dependence of pure DPPC and Hel 13-5 in order to understand the monolayer stability, the solubility into aqueous subphase, and the structure change over the temperature range of 298.2–310.2 K. In addition, the cyclic compression–expansion isotherms, known as a hysteresis curve, of these two- or three-component systems were carried out to examine the spreading and respreading abilities of Hel 13-5.

2. Experimental

2.1. Materials

Hel 13-5 (MW 2203 Da) was synthesized by Fmoc strategy based on the solid phase technique starting from Fmoc-Leu-PEG-PS resin (0.1 m mol scale) with Perseptive 9050 automatic peptide synthesizer and purified by HPLC with reversed-phase column (20 mm × 250 mm, YMC C8) as described previously [22]. Dipalmitoyl phosphatidylcholine (DPPC, purity > 99%) and egg-phosphatidylcholine (Egg-PC, purity > 99%) were obtained from Avanti Polar Lipids Inc. (Birmingham, Alabama, USA). 3,6-bis(diethylamino)-9-(2-octadecyloxycarbonyl) phenyl chloride (R18) was obtained from Molecular Probes as a fluorescent probe. They were used without further purification or characterization. *n*-Hexane and ethanol (specially prepared reagent) used as spreading solvents were from Merck (Uvasol) and Nacalai Tesque, respectively. Tris (hydroxymethyl) aminomethane (Tris) and acetic acid (HAc) of guaranteed reagent grade for the preparation of a subphase were purchased from Nacalai Tesque. Sodium chloride (Nacalai Tesque) was roasted at 1023 K for 24 h to remove any surface-active organic impurities. We used two model surfactant lipids; pure DPPC [28] and DPPC:Egg-PC [23] (DPPC:Egg-PC = 1:1 by molar ratio).

2.2. Methods

2.2.1. Surface pressure–area isotherms

The surface pressure (π) of the monolayer was measured by using an automated home-made Wilhelmy balance, which was the same as that used in the previous studies [29]. The surface pressure balance (Mettler Toledo, AG-64) has a resolution of 0.01 mN m^{-1}. The pressure-measuring system was equipped with a filter paper (Whatman 541, periphery 4 cm). The trough was made from a Teflon-coated brass (area of 15 cm × 50 cm). The π–A isotherms were recorded over the temperature range from 298.2 to 310.2 K. The subphase and the ambient air temperature were precisely controlled by the thermostat and the clean room-grade ribbon heater, respectively. Solutions of DPPC (1.35 mM) and Egg-PC (1.35 mM) were prepared in *n*-hexane/ethanol (9/1, v/v), and that of Hel 13-5 was made in *n*-hexane/ethanol (4.5/5.5 v/v). The spreading solvent was allowed to evaporate for 15 min prior to compression. The monolayer was compressed at a speed of < 0.45 nm^2 molecule^{-1} min^{-1}.