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Structure and properties of phospholipid–peptide monolayers containing monomeric SP-$B_{1–25}$ I. Phases and morphology by epifluorescence microscopy

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Abstract

Epifluorescence microscopy was used to study the structure and phase behavior of phospholipid films containing a human-sequence monomeric SP-$B_{1–25}$ synthetic peptide (mSP-$B_{1–25}$). Measurements were done directly at the air–water (A/W) interface on films in a Langmuir–Whilhelmy balance coupled to a fluorescence microscope and real-time detection system to yield an approximate optical resolution of 1 μm. Fluorescence was achieved by laser excitation of 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-PC (BODIPY-PC, concentration ≤1 mol%). The presence of mSP-$B_{1–25}$ in films of 4:1 (mol/mol) 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)/1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) (DOPG) had a substantial effect on lipid morphology and phase behavior that depended on both surface pressure and peptide concentration (10, 5, and 1 wt.%). The mSP-$B_{1–25}$ peptide tended to fluidize phospholipid monolayers based on expanded molecular areas and reduced collapse pressures. In addition, epifluorescence measurements revealed the formation of solid-phase domains apparent as three-armed counter-clockwise spirals separated from regions of fluid liquid-expanded phase domains in compressed phospholipid–peptide films. The appearance of these separated solid-phase domains resembled pure *L*-DPPC rather than the ensemble-type solid domains found in films of DPPC/DOPG alone and were most apparent when 10 wt.% mSP-$B_{1–25}$ was present. In contrast, films containing lower, more physiological mSP-$B_{1–25}$ contents of 5 and 1 wt.% exhibited a prominent intermediate 'dendritic' phase that increased in extent as surface pressure was raised. This phase was characterized by branching structures that formed a lattice-like mesh network with fluorescence intensities between a dye-depleted solid domain and a dye-enriched liquid phase. These results indicate that mSP-$B_{1–25}$ at near-physiological levels produces morphological changes in phospholipid monolayers analogous to those observed for native SP-$B_{1–79}$.
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Keywords: mSP-$B_{1–25}$; Epifluorescence microscopy; Air–water interface; Lung surfactants; Surfactant protein (SP)-B; Synthetic peptides

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; A/W, air–water; BODIPY-PC, 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt); HEPES, *N*-(2-hydroxyethyl)piperizine-*N*′ -(2-ethanesulfonic acid); HMP, 4-hydroxymethylphenoxyacetyl-4′ -methylbenzyhydrylamine resin; RDS, respiratory distress syndrome; SP-B/C, mixture of hydrophobic surfactant peptides B and C; SP-B, pulmonary surfactant protein B; SP-$B_{1–25}$, synthetic peptide containing the first 25 amino acids of the N-terminus of SP-B; SP-C, pulmonary surfactant protein C; TFA, trifluoroacetic acid; TFE, trifluoroethanol.

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1. Introduction

Pulmonary surfactant forms a lipid–protein film that lowers and varies surface tension at the alveolar air–water (A/W) interface in the mammalian lungs. One of the most important components of this film is surfactant protein B (SP-B). SP-B is a small (MW~8700 Da), lipid-associating protein that belongs to the Saposin superfamily that includes Saposin B, Saposin C, granulysin, and NK-lysin. These proteins share a common disulfide connectivity that constrains their overall backbone to form an amphipathic helix hairpin structure [1], which has been conserved in SP-B for an estimated 300 million years [2]. The mature SP-B protein exists not only as a monomer, but also as oligomers, such as a covalently linked homodimer that is prevalent in humans. The presence of endogenous SP-B is crucial for normal lung function, since gene knockouts for this protein are lethal in mice [3] and hereditary SP-B deficiency is fatal in humans [4]. In addition, organic solvent extracts of animal lung surfactant that contain SP-B are the basis for highly active exogenous lung surfactants used to treat premature infants with the respiratory distress syndrome (RDS) as well as patients with clinical acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS) [5,6].

Although lung surfactant extracts containing SP-B are effective in treating RDS, less is known about the specific molecular mechanisms of action of this protein directly within lipid–protein monolayers. The full-length SP-B protein has multiple distinct domains, e.g., Refs. [7,8]. The N-terminal domain has a short insertion sequence that can assume an extended β-sheet conformation and is adjacent to a stable amphipathic helix. This is followed by a short hydrophobic sequence encompassing residues 26–34, which contain alanine–valine repeats having some α-helical/β-sheet conformational flexibility [8] terminated by a disulfide-stabilized bend region [9]. The bend domain is next to a surface helical sequence that allows disulfide connectivity to form the homodimer. These mid-protein sequences are followed by a short amphipathic leucine repeat that also can display α-helix/β-sheet conformational interconversion [10]. This sequence has served as a template for the synthetic KL4 peptide used in exogenous surfactant replacement applications [11]. Finally, recent structural NMR studies of the C-terminal domain of SP-B have shown that it can assume an amphipathic helical conformation in structure-promoting solvents and in micelles [12], suggesting that this domain optimizes interactions with surfactant lipids much like the N-terminal segment of the protein.

The distribution of cationic residues like lysine and arginine largely within the N- and C-terminal amphipathic helical segments of SP-B is functionally relevant, since these residues are important for interacting with anionic lipids [13,14]. This, along with the stable amphipathic helical structure of the N-terminal region, makes it particularly interesting for structural and functional investigations. A number of studies have investigated the biophysical actions of monomer and dimer human-sequence peptides incorporating the 25 amino acids at the N-terminus of SP-B (SP-$B_{1–25}$). SP-$B_{1–25}$ monomer or dimer peptides emulate a number of the biophysical actions of full-length SP-B, including associating with surfactant lipids [13,15], promoting lipid mixing between vesicles [16], and participating in surface pressure-dependent domain formation and buckling in lipid films [14,17–19]. Also, antibodies developed against SP-$B_{1–25}$ have been found to interact with the native full-length protein [15]. In addition, synthetic surfactants containing lipids combined with SP-$B_{1–25}$ have significant physiological activity in mitigating surfactant deficiency and/or dysfunction in animal models [20–22]. Although several experimental and computer investigations of the conformation of SP-$B_{1–25}$ have been done [23–26], there is little information on its properties in surface films containing the major lung surfactant phospholipid dipalmitoyl phosphatidylcholine (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC) plus a physiologically relevant anionic lipid like dioleoyl phosphatidylglycerol (1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt), DOPG). The present study examines in detail the molecular biophysical behavior of monomeric SP-$B_{1–25}$ (mSP-$B_{1–25}$) directly in spread films with 4:1 DPPC/DOPG at the air–water (A/W) interface. Emphasis in this paper is on the effects of mSP-$B_{1–25}$ on film domain and phase behavior by epifluorescence, while a companion paper examines the molecular interactions and interfacial orientation of the peptide in the film by polarization modulation–infrared reflectance-absorption spectroscopy.

2. Materials and Methods

2.1. Synthetic materials

The synthetic phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) (DOPG) were purchased from Avanti Polar Lipids (Alabaster, AL). These lipids were specified as >99% pure and were used as supplied. An acyl chain-labeled fluorescent lipid probe (2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-PC, BODIPY-PC) was obtained from Molecular Probes (Eugene, OR). ACS grade NaCl and high-performance liquid chromatography (HPLC) grade methanol and chloroform were obtained from J.T. Baker (Phillipsburg, NJ). Ultrapure H_2O used to form film subphases and in all cleaning procedures was obtained from a Barnstead (Dubuque, IA) ROpure/Nanopure reverse osmosis/deionization system and had a nominal resistivity of 18.3 MΩ cm. Film subphases in all experiments were 120 mM NaCl adjusted to pH 7 with a phosphate buffer.

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