ELSEVIER

Biophysical Chemistry 113 (2005) 233–244

Biophysical Chemistry

www.elsevier.com/locate/bpc

Structure and properties of phospholipid–peptide monolayers containing monomeric SP-$B_{1–25}$ II. Peptide conformation by infrared spectroscopy

Saratchandra Shanmukh[a], Nilanjana Biswas[a], Alan J. Waring[b,c], Frans J. Walther[c], Zhendong Wang[d], Y. Chang[d], Robert H. Notter[d], Richard A. Dluhy[a,*]

[a]*Department of Chemistry, University of Georgia, Athens, GA 30602-2556, United States*
[b]*Department of Medicine, Division of Infectious Diseases, Center for the Health Sciences, UCLA, 10833 Le Conte Avenue, Los Angeles, CA 90095, United States*
[c]*Department of Pediatrics, Research and Education Institute at Harbor-UCLA Medical Center, 1124 West Carson Street, Building F-5 South, Torrance, CA 90502, United States*
[d]*University of Rochester, Department of Pediatrics, Box 850, Neonatology, 601 Elmwood Avenue, Rochester, NY 14642, United States*

Received 20 July 2004; received in revised form 15 September 2004; accepted 15 September 2004
Available online 18 October 2004

Abstract

The conformation and orientation of synthetic monomeric human sequence SP-$B_{1–25}$ (mSP-$B_{1–25}$) was studied in films with phospholipids at the air–water (A/W) interface by polarization modulation infrared reflectance absorption spectroscopy (PM-IRRAS). Modified two-dimensional infrared (2D IR) correlation analysis was applied to PM-IRRAS spectra to define changes in the secondary structure and rates of reorientation of mSP-$B_{1–25}$ in the monolayer during compression. PM-IRRAS spectra and 2D IR correlation analysis showed that, in pure films, mSP-$B_{1–25}$ had a major α-helical conformation plus regions of β-sheet structure. These α-helical regions reoriented later during film compression than β structural regions, and became oriented normal to the A/W interface as surface pressure increased. In mixed films with 4:1 mol:mol acyl chain perdeuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine/1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (sodium salt) (DPPC-d_{62}:DOPG), the IR spectra of mSP-$B_{1–25}$ showed that a significant, concentration-dependent conformational change occurred when mSP-$B_{1–25}$ was incorporated into a DPPC-d_{62}:DOPG monolayer. At an mSP-$B_{1–25}$ concentration of 10 wt.%, the peptide assumed a predominately β-sheet conformation with no contribution from α-helical structures. At lower, more physiological peptide concentrations, 2D IR correlation analysis showed that the propensity of mSP-$B_{1–25}$ to form α-helical structures was increased. In phospholipid films containing 5 wt.% mSP-$B_{1–25}$, a substantial α-helical peptide structural component was observed, but regions of α and β structure reoriented together rather than independently during compression. In films containing 1 wt.% mSP-$B_{1–25}$, peptide conformation was predominantly α-helical and the helical regions reoriented later during compression than the remaining β structural components. The increased α-helical structure of mSP-$B_{1–25}$ demonstrated here by PM-IRRAS and 2D IR correlation analysis in monolayers of 4:1 DPPC:DOPG containing 1 wt.% (and, to a

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; A/W, air–water; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, 1,2-dipalmitoyl-d_{62}-*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; IRRAS, infrared reflection absorbance spectroscopy; PM-IRRAS, polarization–modulation infrared reflectance–absorption spectroscopy; RDS, respiratory distress syndrome; SP-B/C, naturally isolated mixture of hydrophobic surfactant peptides B and C; SP-B, pulmonary surfactant protein B; SP-$B_{1–25}$, synthetic peptide containing first 25 amino acids of the N-terminal of SP-B; SP-C, pulmonary surfactant protein C; TFA, trifluoroacetic acid; 2D IR, two-dimensional infrared correlation analysis; TFE, trifluoroethanol.

* Corresponding author. Tel.: +1 706 542 1950; fax: +1 706 542 9454.
E-mail address: dluhy@chem.uga.edu (R.A. Dluhy).

doi:10.1016/j.bpc.2004.09.009

lesser extent, 5 wt.%) peptide may be relevant for the formation of the intermediate order 'dendritic' surface phase observed in similar surface films by epi-fluorescence.

Keywords: mSP-$B_{1–25}$; Surfactant protein (SP)-B; Polarization–modulation IR spectroscopy; 2D IR correlation analysis; Lung surfactants; Synthetic peptides

1. Introduction

Pulmonary surfactant protein B (SP-B) is a highly active component of endogenous lung surfactant, with an amphipathic molecular structure capable of interacting strongly with both hydrophobic and hydrophilic regions of phospholipids to increase adsorption and overall dynamic surface tension lowering [1–5]. SP-B is also a functionally crucial constituent in clinical exogenous surfactants used to treat diseases of surfactant deficiency or dysfunction such as the neonatal respiratory distress syndrome (RDS), clinical acute lung injury (ALI), and the acute respiratory distress syndrome (ARDS) [5,6]. Because of the functional importance of SP-B in endogenous and exogenous surfactants, its molecular biophysical behavior has been of significant research interest. Although a good deal is now known about the structure and activity of SP-B, its specific interactions and molecular orientations directly in interfacial films with phospholipids are not yet completely defined. The full-length SP-B protein is thought to have at least five to six distinct domains [7–10], including an N-terminal region with a short insertion sequence that can assume an extended β-sheet conformation and is adjacent to a stable amphipathic helix.

One approach to elucidating the contributions to activity of different structural regions of SP-B involves the study of synthetic peptides such as SP-$B_{1–25}$, which incorporates the 25 amino acids in the important N-terminal region of the native protein [11–16]. In a companion study, we have used epifluorescence techniques to investigate the morphology and phase behavior of compressed interfacial films containing human sequence monomeric SP-$B_{1–25}$ (mSP-$B_{1–25}$) plus 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) [17]. The present paper examines the molecular behavior of mSP-$B_{1–25}$ in films with 4:1 acyl chain perdeuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC-d_{62}:DOPG at the air–water (A/W) interface using polarization–modulation IR reflection–absorption spectroscopy (PM-IRRAS). PM-IRRAS has several advantages over conventional polarized IR reflectance spectroscopy for studying molecular properties in films at the A/W interface. These include the ability to discriminate isotropic water and water vapor absorptions, as well as to analyze spectra directly for molecular orientations and conformations in the interfacial film in situ [18].

We have previously used conventional monolayer IR spectroscopy as well as PM-IRRAS to examine the molecular interactions of bovine naturally isolated mixture of hydrophobic surfactant peptides B and C (SP-B/C) in monolayers with synthetic phospholipids at the A/W interface [19,20]. In addition, the conformation of SP-$B_{1–25}$ has been investigated in experimental studies [12,13] and in computer simulations [14,16]. However, there is little or no information on the orientation and conformation of this peptide in interfacial monolayers containing DPPC plus an unsaturated anionic component (DOPG) as occurs in native lung surfactant. The results presented here provide the first direct spectroscopic analyses of the structure and orientation of mSP-$B_{1–25}$ in a phospholipid matrix at the A/W interface. They also complement epi-fluorescence experiments carried out in a companion study on morphological and phase changes in monolayers of 4:1 DPPC:DOPG with 1, 5, and 10 wt.% mSP-$B_{1–25}$ [17].

2. Materials and methods

2.1. Synthetic materials

The synthetic acyl chain perdeuterated phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC-d_{62}) as well as 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. High-performance liquid chromatography (HPLC)-grade methanol and chloroform were obtained from J.T. Baker (Phillipsburg, NJ). Ultrapure NaCl was obtained from Fluka. Ultrapure H_2O used for film balance 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 balance subphases in all experiments were 120 mM NaCl adjusted to pH 7.

2.2. Peptide synthesis and purification

SP-$B_{1–25}$ (NH_2-FPIPLPYCWLCRALIKRIQAMIPKG-COOH) was made by solid-phase peptide synthesis employing *O*-fluorenylmethyl-oxycarbonyl (Fmoc) chemistry. Fmoc amino acids and coupling agents were from AnaSpec (San Jose, CA). Solvents and other reagents used for peptide synthesis and purification were HPLC grade or better (Fisher Scientific, Tustin, CA; Aldrich Chemical, Milwaukee, WI). The peptide was synthesized on a 0.25 mmol scale with an ABI 431A peptide synthesizer configured for FastMoc™ double-coupling cycles [21] utilizing a prederivatized *N*-α-Fmoc-glycine 4-hydroxymethylphenoxyace-