**Sequence Dependence Studies of SP-B1-25**

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**Introduction**

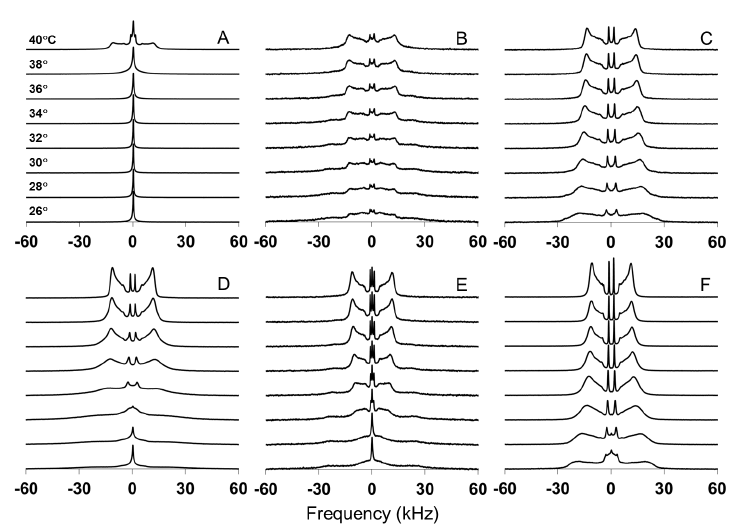
Pulmonary surfactant (PS) is a lipoprotein mixture found in the alveoli of the lungs and allows for proper lung function by lowering the surface tension at the alveolar air-water interface. It is composed of 80% phospholipids, 10% other lipids (primarily cholesterol), and 10% proteins, namely surfactant proteins A, B, C, and D. Of the four surfactant proteins, surfactant protein B (SP-B) is the only one required for survival. Current models of SP-B suggest its role in trafficking PS lipids from the aqueous hypophase to the air-water interface, however its mechanism of action is poorly understood. Previous 2H and 31P NMR experiments have indicated the presence of isotropic phases upon addition of SP-B1-25 to varying lipid systems, with pronounced effects in lipid mixtures containing deuterated dipalmitoylphosphatidylcholine (DPPC). This supports the model that SP-B1-25 selectively traffics DPPC lipids and may participate in the stabilization of PS at the air-water interface via a lipid fusion mechanism. It has been shown that only the first 25 residues of SP-B (SP-B1-25) are required to recapture most of the activity of full length SP-B, suggesting a critical role in the highly conserved N-terminus of SP-B. Point mutations in SP-B1-25 reveal different effects on lipid dynamics and morphologies in varying lipid systems. These results have prompted experiments aimed at delineating the sequence dependence on the mechanism of lipid trafficking.

**Experimental**

Variable temperature 2H NMR spectra of multilamellar vesicles with the indicated amount and variant of SP-B1-25 were collected on a 500 MHz Bruker DRX spectrometer utilizing a quadrupolar echo sequence in the AMRIS facility at UF. A 5 mm broadband observe (BBO) probe was used.

**Results and Discussion**

Previous results suggest that the behavior of SP-B1-25 may be sequence dependent as shown by the differences in lipid dynamics induced by the mutant and WT peptide in varying lipid systems. [1] This is shown in Figure 1a,d as the addition of mutant (C8S, C11S, M21I) and wild type (WT) SP-B1-25 induces isotropic phases in 4:1 DPPC-d62/POPG, while the isotropic phase is persistent through the temperature range only with addition of WT SP-B1-25. This behavior in lipid dynamics differs compared to that observed in calf lung surfactant (CLSE) and a synthetic lipid mixture mimicking CLSE (CLSESyn). In CLSE, the addition of mutant SP-B1-25 induces an isotropic phase in DPPC while WT SP-B1-25 does not (Figure 1b,e). In CLSESyn, addition of mutant and WT SP-B1-25 induce line shapes indicative of DPPC lamellar phases, however the line widths are more narrow with addition of mutant SP-B1-25, suggesting greater fluidity in DPPC dynamics.



**Conclusions**

These results suggest that SP-B1-25 may be sequence dependent and have prompted the synthesis of additional mutant peptides aimed at delineating functionally significant residues using 2H and 31P NMR.

**Acknowledgements**

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**References**

[1] R. Suzanne Farver. Investigations of Lipid Dynamics and Polymorphisms in Lung Surfactant. Ph.D Dissertation, 2011

**Figure 1.** Deuterium spectra as a function of temperature for A) 4:1 DPPC-d62/POPG with 5% SP-B1-25 (WT), B) CLSE/DPPC-d62 with 5% SP-B1-25 (WT), C) CLSESyn/DPPC-d62 with 5% SP-B1-25 (WT), D) 4:1 DPPC-d62/POPG with 5% SP-B1-25 (C8S, C11S, M21I), E) CLSE/DPPC-d62 with 5% SP-B1-25 (C8S, C11S, M21I), F) CLSESyn /DPPC-d62 with 5% SP-B1-25 (C8S, C11S, M21I)