Membrane Stabilizing and Lipid Trafficking Domains of Surfactant Peptide B_{1-25}

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Introduction

In this work, we investigate the lipid trafficking mechanism of surfactant peptide B (SP-B_{1-25}) in mammalian pulmonary surfactant (PS) model lipid systems. We utilize deuterium (^{2}H) and phosphorus (^{31}P) NMR to observe effects of SP-B_{1-25} on lipid acyl chain and head group dynamics. It has been shown that this conserved region (SP-B_{1-25}) of SP-B promotes surface tension reduction at alveolar air-water interfaces in-vitro by specifically enriching the alveolar surface with DPPC lipids. Our previous NMR studies have shown cubic lipid morphologies in 4:1 DPPC/POPG liposomes containing therapeutic levels of SP-B_{1-25} at physiologic temperature (37 °C). We suggest SP-B_{1-25} induces this non lamellar lipid phase, possessing highly curved membrane architecture, that allows specific and rapid lipid transit between lamellae to the alveolar air-water interface.

Experimental

^{2}H and ^{31}P NMR spectra were collected for hydrated lipid assemblies containing 0 and 5 mol% peptide. Deuterium spectra were collected on a 500 MHz Bruker Avance III spectrometer and Bruker Broad Band Observe (BBO) probe utilizing a standard quad echo pulse sequence (90-t-90-t-acq).

Results and Discussion

Our results elucidate distinct effects of the N-terminus (SP-B_{1-12}) and C-terminus (SP-B_{12-25}) SP-B_{1-25} domains on lipid dynamics and organization. We show isotropic lipid head group phase behavior upon addition of 5 mol% SP-B_{1-12} in 4:1 DPPC/POPG liposomes, evidenced by the sharp isotropic resonance at 0 ppm **(Figure 1, top)**. This was not observed for SP-B_{12-25} and suggests a unique role for the N-terminal peptide fragment in modifying lipid head group organization, and is likely early induction of membrane curvature. ^{2}H spectra indicate lipid acyl chains remain in an ordered lamellar phase in samples containing SP-B_{1-12} and SP-B_{12-25}, suggesting a complementary role for both peptide fragments in formation of cubic lipid morphology **(Figure 1, bottom)**.

Conclusions

This study aimed to characterize functional motifs of SP-B_{1-25} and their potential roles in lipid trafficking. We hypothesize the proline rich N-terminal (SP-B_{1-12}) fragment is highly dynamic and deeply embedded in the lipid bilayer, while the amphipathic C-terminal (SP-B_{12-25}) fragment serves to stabilize the peptide by partitioning at the membrane surface. These insights warrant further investigation of peptide/lipid interactions and motivates our efforts in characterizing a high resolution NMR structure of SP-B_{1-25} in a lipid environment.

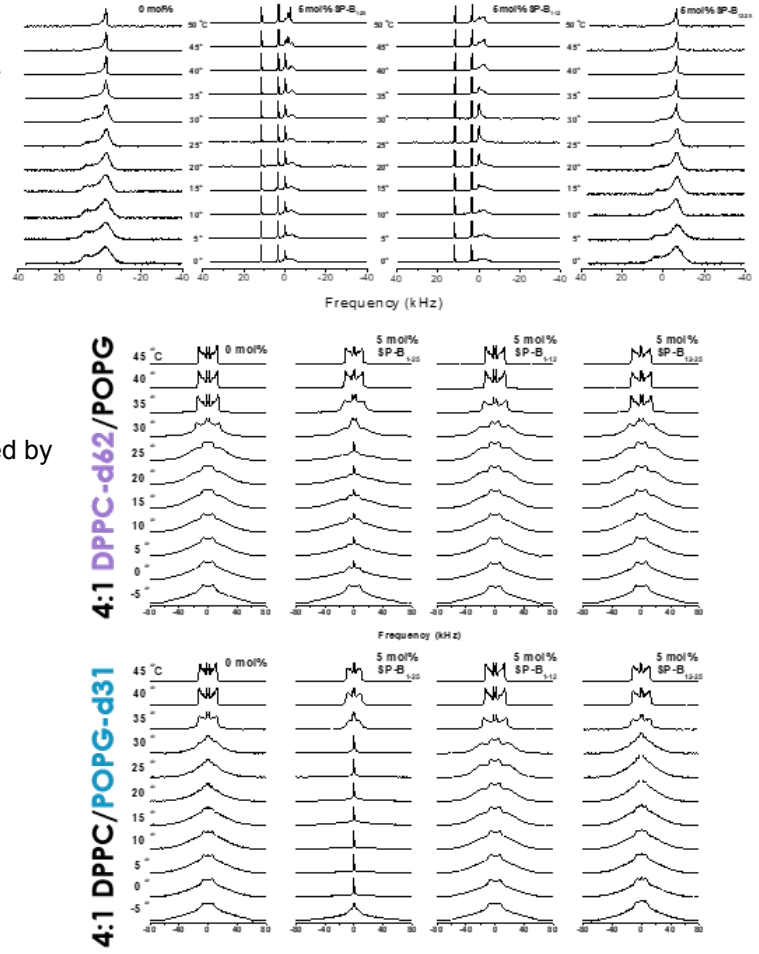

Fig.1 (Top) ^{31}P and (bottom) ^{2}H NMR spectra of 4:1 DPPC-d_{62}/POPG containing 0 and 5 mol% peptide.

Acknowledgements

The National High Magnetic Field Laboratory is supported by the National Science Foundation through NSF/DMR-1157490/1644779 and the State of Florida.