Solution NMR Structural Studies of a Surfactant Peptide, SP-B_{1-25}

A.Smith, J.R. Long (University of Florida)

Introduction

Lung surfactant (LS) is a complex lipoprotein mixture that is found near the air/water interface in the alveoli of the lungs. Its primary purposes are to reduce surface tension and facilitate lipid transfer at or near the interface. There are four protein components of LS, surfactant protein-A, B, C, and D (SP-A, B, C, and D). It has been shown *in-vivo* that only SP-B is required for survival [1]. To date the mechanism of SP-B is unknown. Infants born prematurely have an increased chance of suffering from respiratory distress syndrome (RDS). The pathophysiology of RDS is characterized by a lack of functioning LS. Therefore, it is of interest to understand the mechanism of SP-B so that a synthetic replacement can be found. SP-B is an extremely hydrophobic protein that remains membrane associated and has yet to be heterologoulsy expressed and purified. However, it has been shown that only the first 25 residues of SP-B are required to recapture much of the activity of full length SP-B *in-vivo* [2]. Therefore, we have focused our studies on SP-B_{1-25}. We have previously used solid-state ^{2}H NMR to investigate the effect SP-B_{1-25} has on lamellar lipid systems and have shown that the peptide causes an increase in lipid dynamics. In an effort to understand this mechanism of action we have decided to utilize solution NMR to solve the structure of SP-B_{1-25} in both methanol and DPC micelles.

Results and Discussion

Other groups have shown, via solution NMR, that SP-B_{11-25} is mostly alpha helical, however this construct does not posses the activity of full length SP-B or SP-B_{1-25} [3]. We have also demonstrated that SP-B_{1-25} and SP-B_{1-25} C8S C11S M21I is mostly alpha helical using the low-resolution technique CD spectroscopy. ^{1}H-^{1}H TOCSY and NOESY experiments were completed on SP-B_{1-25}, SP-B_{1-25} C8S C11S M21I, and SP-B_{1-12} in both methanol and DPC micelles. The experiments were conducted on a 600 MHz Bruker Avance II Spectrometer utilizing a cryo-probe. Figure 1 shows the fingerprint region of the ^{1}H-^{1}H TOCSY of SP-B_{1-25} C8S C11S M21I in methanol. Both residue type and sequence specficd assignment have been made for 14 of the 25 residues. Ten of the assignments are shown in Fig. 1, the remaining four assignments are of proline residues in the C_{α} region of the spectrum. However, the spectra of SP-B_{1-25} in DPC micelles will most likely require ^{15}N isotopic labeling to complete the assignments.

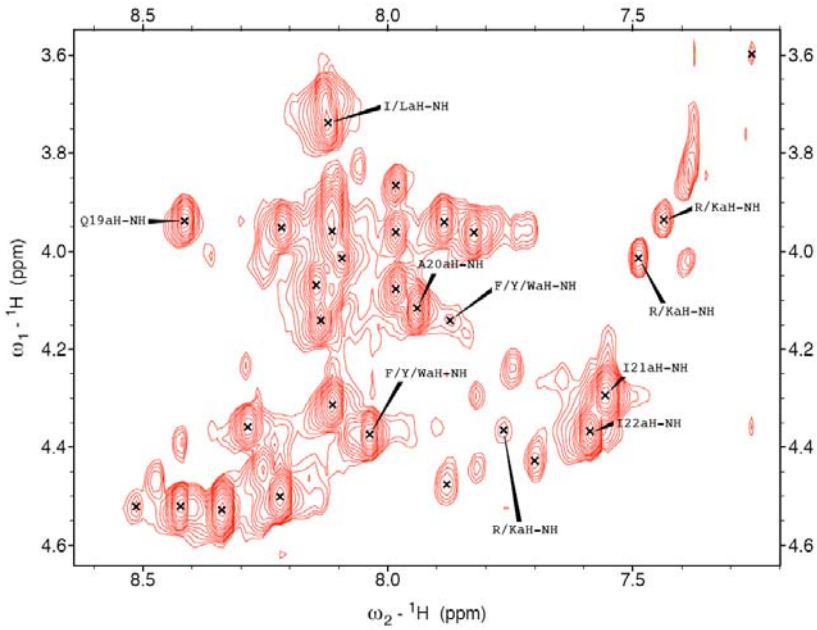

Figure 1: Fingerprint region of a ^{1}H-^{1}H TOCSY spectrum of SP-B_{1-25} C8S C11S M21I in methanol.

References

[1] Lipp M.M., et al., *Science* **273**:1196-1199 (1996).
[2] Gupta M, et al., *Thorax* **56**:871-876 (2001).