

# Mass Spectrometric Analysis of Surfactant Proteins

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## Introduction

The major role of pulmonary surfactant is to reduce surface tension at the air-liquid interface, thereby facilitating alveolar expansion during inspiration and preventing their collapse at the end of expiration (1). Lack or malfunction of surfactant at birth results in the development of the respiratory distress syndrome (2). Bovine lipid extract surfactant (BLES) is the major therapeutic surfactant currently used to treat the above pathology in Canada. Surfactant is composed of phospholipids and surfactant apoproteins. Surfactant protein B (SP-B) and surfactant protein C (SP-C) are essential for surfactant function. In this study, SP-B and SP-C have been analyzed by nano-electrospray mass spectrometry.

Discrepancies between the observed mass and the theoretical mass of the reported SP-B and also for SP-C were observed, indicating that either post-translational modifications or sequence alteration occurred.

Using mass spectrometry, the correct amino acid sequence of SP-B was determined. SP-C was also analyzed and its correct sequence were determined.

## Experimental

### Material:

Bovine lipid extract surfactant (BLES) was a gift of BLES Biochemicals, Inc. (London, Ontario, Canada) and it was obtained from lung lavage from cows. SP-B, SPC and phospholipids were extracted from BLES with organic solvent, then separated on an LH-60 column (3). The fractions corresponding to SP-B and SP-C were pooled, respectively, and analyzed by MS.

### ESI-MS and LC/MS/MS:

Purified SPB and SPC were analysed using a Micromass Q-TOF instrument (Micromass, Manchester, UK) equipped a nano ESI source. The samples (10  $\mu$ L) were loaded into gold coated borosilicate glass capillaries (Proxeon Biosystem, Odense, Denmark) and the capillaries were opened manually to give a orifice of 20  $\mu$ m. The mass spectrometer parameters were as follows: capillary voltage, 1.2-1.5 kV, cone voltage 40 V; collision energy 10 for MS scan; for CID of the tryptic peptides of SPB or SPC, collision energy of 30-50 were used according to the  $m/z$  of the precursor ions. The reduction of 30  $\mu$ g of SPB was carried out at room temperature for 1 hr using tris(2-carboxyethyl)phosphine hydrochloride (TCEP) at 10 mM.

Ten  $\mu$ g of purified SPB was reduced with TCEP, alkylated with iodoacetamide, and digested with 0.2  $\mu$ g trypsin (Promega, Madison, WI) in 50 mM ammonium hydrogen carbonate (pH 7.8) at 37 C for four hours. The resulting digest solution corresponding to 1  $\mu$ g SPB was injected for LC/MS/MS analysis on a Waters CapLC coupled to a QTOF Global mass spectrometer (Micromass, Manchester, UK). The LC system consisted of a reversed phase C18 precolumn (300  $\mu$ m X1 cm, 5  $\mu$ m C18) and an analytical column (75  $\mu$ m X 15 cm, 5  $\mu$ m C18) (LC Packings, Amsterdam, Netherlands). The total flow rate was 2.4  $\mu$ L/min while the flow rate through the analytical column was about 300 nL/min after splitting. Solvents used for elution of peptides were A: 0.2% formic acid in water, and B: 0.2% formic acid in acetonitrile. A gradient started from 5% B to 95% B in 50 min was used for elution of peptides. A data dependent analysis (DDA) function (Micromass, Manchester, UK) was used to detect multiply charged peptide ions eluted from the analytical column in a mass range 300 to 1900  $m/z$  and sequentially perform MS/MS on the multiple charged ions that passed a selection criteria. The obtained MS/MS spectra were processed to search the SwissProt database using Mascot, together with manual verification of the unmatched spectra.

## Results

### ESI MS of SPB

MW of SPB dimer determined to be 17397.4 Da (Figure 1b). Reduction of SPB dimer produced a SPB monomer with MW 8705.6 Da (Figure 1c) indicating 7 disulfide bonds were formed upon dimerization.

None of these MWs matches the bovine sequence reported in SwissProt (P15781, MW 8549.6 Da), nor the number of reported cysteines.

### LC-MS/MS of SPB

Six unique tryptic peptides observed and were sequenced. Two of MS/MS spectra are shown in Figure 3. The whole sequence assembled as shown in Figure 2. MW of SPB dimer and monomer match this sequence. This sequence is identical to sheep SPB but one amino acid.

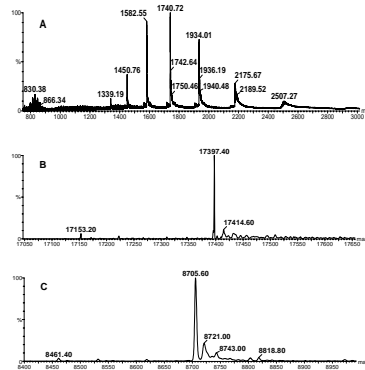


Figure 1. Mass spectra of SPB. A) raw ESI mass spectrum of SPB dimer. B) deconvoluted spectrum of SPB dimer. C) deconvoluted spectrum of reduced SPB monomer.

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FPIPLPYCWLCRALIKRIQAMI PKGALAVAQAQV RVVPLVAGGICQCLA ERYSVILLDTLLGRMLPQLVCR LVLRCSSM Human
LPIPLPYCWL CRTLIKRIQAVI PKGVLAVTVGQVCHVVPVLPVGGICQCLA ERYVI LLNMLLDR LPLQVCGVLRCSH Dog
FPIPLPFCWLCR TLIKRIQAVI PKGVLAVTVGQVCHVVPVLPVGGICQCLA ERYVI LLNMLLDR LPLQVCGVLRCSH Pig
FPIPLPFCWLCR TLIKRIQAVI PKGVLAMTVQAQVCHVVPVLLVGGICQCLVERYS VI LLDTLLGRMLPQLVCGVLRCSH Sheep
FPIPLPYCWL CRTLIKRIQAVI PKGVLAMTVQAQVCHVVPVLLVGGICQCLVERYS VI LLDTLLGRMLPQLVCGVLRCSH Bovine
FPIPLPYCWL LRTLIKRIQAVI PKGVLAMTVQAQVCHVVPVLLVGGI IQQLVI EYS VILXTDLLGR LPNLVCGVLRCSG SwissPort
Bovine
    
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Figure 2. Alignment of bovine SPB sequence determined and other species SPB sequences. The assignments of L and I in Bovine SPB are made using sheep SPB as a template. Altered positions between bovine and other species are marked as red.

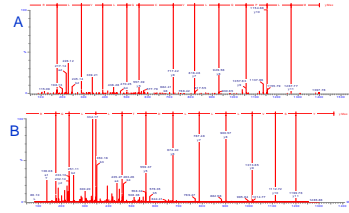


Figure 3. MS/MS spectra of two tryptic peptides of SPB. A) MS/MS spectrum of peptide at 699.9  $m/z$ . B) MS/MS of peptide at 681.9  $m/z$ .

### ESI MS and MS/MS of SPC

MW of SPC determined to be 4041.8 Da (Data not shown), 14 Da less than that from SwissPort. Amino acid sequence determined (Figure 3) shows an L to V substitution at position 22.

Bovine SPC LIPCCPNNIKRLILVVVVVLLVVVIVGALLMGL

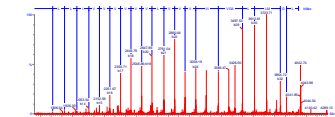


Figure 4. MS/MS of triply charged SPC at 1348.3  $m/z$ . Series of b ion confirmed the sequence from position 12 to 34.

## Conclusion

Bovine SPB and SPC sequences were determined by LC/MS/MS. The discrepancy between MW and the sequence in the database, and the number of cysteines has been resolved using the present approach.

## Reference

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- 3) Curstedt, T., Jornvall, H., Robertson, B., Bergman, T., and Berggren, P. (1987) *Eur J Biochem* 168, 255-62.