

## Introduction

The development of bioanalytical techniques for rapid identification of proteins is important in biopharmaceutical industry. Tandem mass spectrometry (MS/MS) has become a valuable tool for characterization of biomolecules owing to its reliability, speed and sensitivity. The ease of interfacing the MS with liquid chromatography (LC) make mass spectrometry a powerful technique for applications. This study demonstrates a systematic approach for obtaining extensive sequence information over the whole length of a protein in a single series of experiments using high-resolution and high mass accuracy tandem mass spectrometry.

Ovalbumin, a major protein in avian egg-white which belongs to the serpin super-family, was used for the analysis. Ovalbumin comprises of a single polypeptide chain of 385 amino acid residues which has a single carbohydrate chain linked covalently with Asn292.

## Objective

To develop a rapid technique for the determination of amino acid sequence information and possible post-translational modifications (PTMs) using a high resolution LC/MS/MS method. The approach involves enzymatic cleavage of the protein to a collection of peptides which are then separated by LC and analyzed directly by MS/MS.

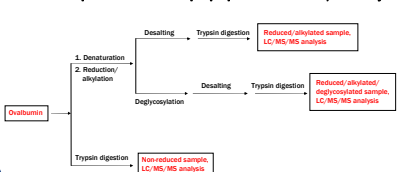


Figure 1: Thermo Scientific LTQ XL™ Orbitrap instrument (LC/MS/MS) used for the analysis

## Experimental

The analysis was carried out on high resolution LTQ XL™ Orbitrap (Thermo Electron Corp., Waltham, MA) hybrid mass spectrometer in conjunction with an Accela™ high-speed chromatography system. Mass spectral data was acquired using high mass accuracy full scan and normal accuracy data dependent product ion spectra. All the data was analyzed using Bioworks™ software to produce amino acid sequence information.

### Schematic representation of sample preparation for LC-MS/MS analysis



### Non-reduced ovalbumin peptide map

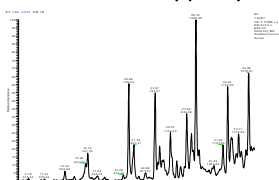


Figure 2: Mass spectral profile for the tryptic digest of non-reduced ovalbumin sample.

### Reduced/alkylated/deglycosylated ovalbumin peptide map

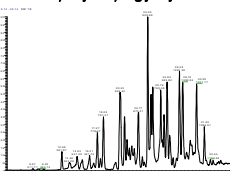


Figure 3: Mass spectral profile for the tryptic digest of reduced/alkylated/deglycosylated ovalbumin sample. The retention time and mass/charge values are shown.

### MS spectrum of the peptide ISQAVHAAHAEINEAGR

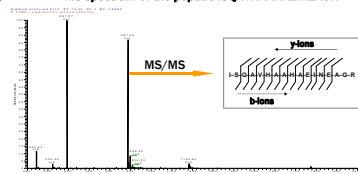


Figure 4: MS/MS spectrum of the peptide ISQAVHAAHAEINEAGR. The sequence with residues identified as b-ions (in blue) and y-ions (black). Residues highlighted in red were not found. Representative sequence for b<sub>2</sub>, b<sub>3</sub>, y<sub>2</sub> and y<sub>3</sub> fragment ions is shown.

### MS spectrum of the peptide LIEWTSSNVMEER

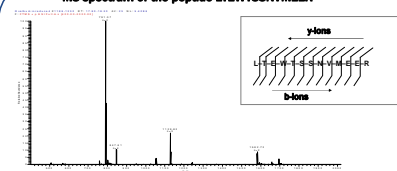


Figure 5: MS/MS spectrum of the peptide LIEWTSSNVMEER. The sequence with residues identified as b-ions (in blue) and y-ions (black). Residues highlighted in red were not found. Representative sequence for b<sub>2</sub>, b<sub>3</sub>, y<sub>2</sub> and y<sub>3</sub> fragment ions is shown.

### MS/MS spectrum of the peptide LIEWTSSNVMEER

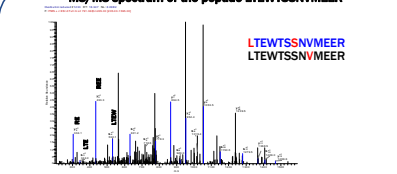


Figure 6: MS/MS spectrum of the peptide LIEWTSSNVMEER. The sequence with residues identified as b-ions (in blue) and y-ions (black). Residues highlighted in red were not found. Representative sequence for b<sub>2</sub>, b<sub>3</sub>, y<sub>2</sub> and y<sub>3</sub> fragment ions is shown.

### Protein Sequencing

GSIGASMEF CFVFKELV KHANENFYC PIAMISALAM VYLGAQDSTR TQNNKVRFD  
KLPQGDSE AQGTSVNH SSRLDNLQ TPNQVYSFS LASRYAEER YPLPEYLC  
VKELYRGLE PINQTAAQ ARELINSWE SQTGURRV LQPSVDSQT AMVLYNAVF  
KGLVETKFD EDQAMPFRV TEQESKVPQM MYQGLFRVA SMASEXMKL ELPASQTMG  
MLVLLPDEVS GLEQLSIN FENLWTSV NVMEERKRV YLPRMMEER YLTVSLMAM  
GTDVFSKHA NLSQSSAES LKISQAVHAA HAEINEAGRE VVQSAEGDV ASVSEEPRA  
DHFFLCRH LMTNVLFG RGVSP

Ovalbumin sequence Sequence Coverage for tryptic digested ovalbumin (residues in red) : 97.2% coverage

### MS/MS spectrum of the peptide ISQAVHAAHAEINEAGR

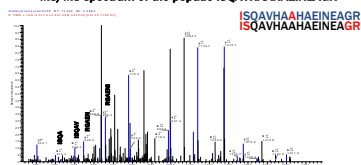


Figure 6: MS/MS spectrum of the peptide ISQAVHAAHAEINEAGR. The sequence with residues identified as b-ions (in blue) and y-ions (black). Residues highlighted in red were not found. Representative sequence for b<sub>2</sub>, b<sub>3</sub>, y<sub>2</sub> and y<sub>3</sub> fragment ions is shown.

## Results and Conclusions

Confirmation of the sequence was accomplished by LC-MS/MS analysis combined with data processing and sequence similarity database searching tools. Extensive sequence coverage was achieved with the reduced/alkylated/deglycosylated sample (96%) compared with the non-reduced sample (97%). The method demonstrates the advantage of fast chromatography combined with high resolution, high mass accuracy mass spectrometry to confirm sequence identity of proteins. The method is simple, rapid and useful approach for the characterization of complex proteins. The method can be extended for characterization of unknown proteins sharing identical peptides with related database sequences.

## References:

- Seibald-Barnes C.A. and Lim A. Applications of mass spectrometry for the structural characterization of recombinant protein pharmaceuticals. Mass Spectrometry reviews 26 (2007) 370-388.
- Huntington J.A. and Stein P.E. Structure and properties of ovalbumin. J. Chromatogr. B 756 (2001) 189-198.