Analysis of Intact Proteins Using Liquid Chromatography

Introduction

Accurate mass analysis of intact proteins has evolved significantly over recent years. These developments have enabled the identification of novel protein modifications and the discovery of small post-translational modifications not previously identified. The use of high resolution mass spectrometers for intact protein analysis is now common practice in proteomics.

Accurate Mass Analysis of Intact Ribosomal Proteins

A 21,400 peptide (>4) and/or by a cutoff score (10^-10). As a result 542 proteins were identified. In some cases several different masses of the same protein were identified which correlated with known N- and/or C-terminal processing.

Dipeptide Analysis of Membrane Proteins

Analysis of Membrane Proteins

The experiment was performed in three steps:

1. For each of the three step, a 2-5 mg sample was dissolved in 1:10 of 80% acetonitrile/2% formic acid.
2. The solution was filtered and subjected to a 0.5 mL volume of 0.1% formic acid.
3. The solution was then directly infused into the mass spectrometer.

Chromatographic conditions for analytical separation:

Mobile Phase B: 5/95 water/acetonitrile, 0.1% Formic Acid
Mobile Phase A: 0.09% formic acid in acetonitrile
Sample: 3µl AOHUPO-MPI standard
Column: ProteCol™-C8 HQ1003 3 µm; 1000 Å 150 mm x 300 µm ID
System: TSP4000 pump, Surveyor autosampler, Thermo linear ion trap
Detector: Thermo Xcalibur mass control, Surveyor autosampler, Thermo linear ion trap
Sample Preparation

The ribosomal protein results were obtained from Nicholas Williamson and colleagues. Once obtained, the dried fractions were reconstituted in 100 mM ammonium bicarbonate and digested with trypsin off-line. The dried fractions were analyzed using the mass spectrometer to determine the presence of the ribosomal proteins. All data were acquired and reference mass corrected via a dual-spray electrospray ionisation (ESI) source. Each scan or data point on the Total Ion Chromatogram (TIC) is an average of 10 scans. The average mass of each protein was calculated using the formula described above. The MS data were then searched against the NCBI non-redundant database using MASCOT. The database search was performed with a mass window of ± 10 ppm. The results were then confirmed using a cutoff score of 10^-10.

Conclusions

By combining different phases, with the right buffer chemistry, and the right pre-treatment protocol, it is possible to accurately map the "ribosomal" landscape of membrane proteins. For small proteins one should use a small pore size pre-column for better retention. For large proteins one should use a larger pore size pre-column for better retention.

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