Accurate Mass Analysis of Intact Ribosomal Proteins

BACKGROUND

Bottom-up proteomic studies rely on the identification of proteins by the accurate mass and fragmentation analysis of peptides generated by proteinase digests of isolated proteins or complex protein mixtures. While this approach ‘identifies’ which proteins are present, the information about the intact protein(s) is lost. Although SDS-PAGE provides some information about the protein’s apparent mass, it is not accurate enough to give an indication of whether the protein is modified or not.

With the recent advances in high resolution MS instruments, accurate mass analysis of intact proteins is now routine.

INTRODUCTION

The ribosome is a cluster of proteins responsible for the biosynthesis of peptides and proteins. It translates the genetic information in the form of messenger RNA into a sequence of amino acids. The ribosome has a small and a large subunit, each containing a number of individual proteins. The total number of proteins in the 80s ribosome is ~49 for the large subunit and ~33 for the small subunit. Recent studies have suggested that ribosomal proteins maybe involved in functions other than protein biosynthesis, such as pathological events or developmental defects. Since ribosomal proteins are relatively small (Mw= 6,000 to 40,000), they can be rapidly identified by accurate LC MS analysis of the intact proteins.

In the present application ribosomal proteins isolated from rat liver were separated on a ProteCol™ C8 HQ1003 column.

SAMPLE PREPARATION

80S ribosomal proteins were isolated from a rat liver microsomal preparation (Williamson et al; 1997, Eur. J. Biochem. 246: 786-793). One optical density unit at 260 nm of 80S ribosomal proteins was mixed with 2 volumes of 6M Guanidine HCl to denature the proteins. 1 % (v/v) formic acid was subsequently added to precipitate the nucleic acids. The mixture was centrifuged for 15 min at 13,000 rpm and the supernatant was collected into a sample vial ready for LCMS analysis.

LCMS CONDITIONS

LC: Agilent 1100 LC system
Detection: Agilent 6220 ESI-TOF LC/MS Mass
Column: SGE ProteCol™ C8 HQ1003, 150 x 2 mm
Solvent A: Aqueous 0.1 % (v/v) Formic Acid
Solvent B: Acetonitrile / 0.1 % (v/v) Formic Acid
Gradient:
- 0 min 5 % B
- 80 min 45 % B
- 81 min 85 % B
- 82 min 85 % B
- 83 min 5 % B
- 88 min 5 % B

DATA ANALYSIS AND RESULTS

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 15,000 transients, producing a spectrum every second. Mass spectra were created by averaging the scans across each peak and background subtracted against the first 10 seconds of the TIC. Acquisition was performed using the Agilent Mass Hunter software version B.02.01 and analysis was performed using Mass Hunter version B.03.01

The resulting base peak chromatogram shows very high peak capacity - 119 discrete protein masses were identified; 46 of which were identified as 80S ribosomal proteins. In some cases several different masses of the same protein were identified which correlated with known N- and/or C-terminal processing.

SUMMARY

The ProteCol™ C8 HQ1003 is the ideal LC column for intact protein analysis due to its intermediate hydrophobicity, but most importantly its wide 1000 Å pore size which enables fast analyte diffusion.

ACKNOWLEDGEMENTS:

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<th>No.</th>
<th>RT [min]</th>
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<td>L37</td>
</tr>
<tr>
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<td>S30</td>
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<td>42.3</td>
<td>18449</td>
<td>L21 Ng to KR</td>
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