Overview

- Results are presented for a fully automated multi-dimensional capillary LC/MS/MS system for complex protein digestion analysis, utilizing novel SCX and trapping desalting column formats that connect directly to a multi-port switching valve.
- Analysis of a global tryptic digest of an enriched preparation of yeast large ribosomal subunit proteins resulted in identification of 32 of 46 out of potential 46 proteins.

Introduction

There is an increasing need for new technology developments with capabilities of analyzing proteins in complex mixtures, including low abundance proteins, hydrophobic proteins, and highly acidic and basic proteins which are difficult to analyze by 2-D gel electrophoresis. Online multidimensional LC, utilizing a strong cation exchange (SCX) column coupled to a nanoscale reverse phase column, has proven to be a powerful alternative technique in order to fractionate peptide mixtures prior to MS/MS analysis for efficient protein identification [1-3]. In this study, we present results utilizing a fully automated multi-dimensional capillary LC/MS/MS system to analyze a global tryptic digest of an enriched preparation of yeast large ribosomal subunit proteins, as an alternative to traditional gel-based analysis.

Experimental

A Micromass CapLC™ and QTOF2™ system was utilized with a 10-port Stream Select Module (Figures 1 and 2). The 10-port valve was configured with a Waters OMEGA™ SCX column (350 μm x 5 mm x 5 μL, 300 Å), and a Waters OMEGA™ Symmetry C18 trapping/desalting column (350 μm x 5 mm x 5 μL). A Waters Symmetry™ PicoFrit C18 nanoflow column (75 μm x 100 mm x 3.5 μL, 100 Å) was pre-crucially described [4]. A global tryptic digest of an enriched preparation of yeast large ribosomal subunit proteins (1 μg in 2 μL) was loaded onto the SCX column (loading solvent: pump C: 0.1% formic acid/5% ACN, 15 min at 10% B, followed by step elution of peptides 0, 25, 100, 150, 200, 300, 400, and 500 μM KCl in 5% ACN/5 mM K2HPO4 (pH 3.0), 5 μL injections from six samples into the autosampler tray) onto the trapping/desalting column. After desalting (3 min wash of trapping solvent at 10 μL/min), the peptide fractions from each step were separated on the analytical column connected to the nanoLC interface (flow rate ~230 nL/min). Pump A: H2O/2% ACN/0.1% formic acid, pump B: 98% ACN/2% H2O/0.1% formic acid, gradient: 7-57% B over 45 min, 75-80% B over 1 min and hold at 80% for 6 min. NanoLC MS/MS conditions: capillary voltage: 2.35 kV, cone: 35 V, cone gas: 50 L/h, source temp: 80 °C, nebulizer gas: 2 psi, collision gas argon, 15 psi. Data Directed Analysis 1 sec TDMS survey scan, collision energy profile based on mass and charge state, with precursor ions of +2,+3 selected for MS/MS from the six most intense precursor ions in a single function cycle. ProteinLynx™ Global Server 1.1 was utilized to search MS/MS spectra against a yeast protein database.

Results and Discussion

The entire 2-D LC/MS/MS analysis can be run unattended under full Masflex™ software control in ~2.5 hours (including load, wash, and nanoflow column equilibration times). The large number of MS/MS data acquired for each elution step is demonstrated in Figure 4, where ~100 MS/MS spectra were acquired for the 25 mM KCl step. From these spectra, there were 39 hits in the database, with 23 yielding a score of >31, which was the criteria used to consider a positive identification (Figure 5).

The database search of MS/MS data acquired for each KCl elution step identified 34 out of 46 known proteins. A total of 34 out of 46 known proteins were identified (74%). Interestingly, the 0 mM KCl (initial loading) step contained almost exclusively singly-charged species of low molecular weight, with only a few +2 species which yielded interpretable MS/MS spectra that matched proteins in the database. As expected, there is a general trend indicating greater numbers of matching MS/MS spectra for proteins that are higher in molecular weight (~12,444 Da), as these proteins likely yield a greater number of unique tryptic peptides. The lower molecular weight, highly basic proteins typical of ribosomes would not be expected to yield as many MS/MS spectra in the database matches, due to higher probability of missed cleavages and higher proportion of low molecular weight peptides that do not yield an interpretable fragmentation pattern, or which are not unique.

Conclusions

- A fully automated 2-D LC/MS/MS method at the nanoflow scale has been demonstrated for a complex digest of yeast large ribosomal subunit proteins.
- A total of 34 out of 46 (74%) possible proteins were identified.
- The total analysis time of the 2-D method was ~12.5 hours.
- Direct connection of OMEGA™ capillary format allows easy configuration and replacement of the SCX and trapping/desalting columns with a 10-port valve for either a 1 or 2-D method.
- The Symmetry™ C18 OMEGA™ trap column is robust and can effectively remove K+/H+ buffer and high concentrations of KCl through many injection cycles without the need to replace columns.
- The three-column configuration improves robustness, avoiding introduction of salt/buffers into the analytical nanoflow column and MS source.

References