ASMS 2002

Automated Multi-Dimensional Capillary LC/MS/MS for Protein Identification

Jeffrey W. Finch, WenSheng Xu, Scott J. Berger, and Steven A. Cohen
Waters Corporation, Life Sciences R&D, Milford, MA, USA

Overview

- Results are presented for a fully automated multi-dimensional capillary LC/MS/MS system for complex protein digest 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 34 out of 46 possible 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 with 2-D gel electrophoresis. Online multi-dimensional LC, utilizing a strong cation exchange (SCX) column coupled to a nanoscale reversed-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 1and 2). The 10-port valve was configured with a Waters OPTI-PAK™ SCX column (350 µm x 5 mm, 5 µ, 300 Å), Waters OPTI-PAK™ Symmetry® C18 trapping/desalting column (350 µm x 5 mm, 5 µ, 300 Å), and a Waters Symmetry® PicoFrit™ C18 nanoLC column (75 µm x 100 mm, 3.5 µ, 100 Å), as previously 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 µL/min), followed by step elution of peptide fractions (0, 25, 100, 150, 200, 300, 400, and 500 mM KCl in 5% ACN/5 mM K_2HPO_4 (pH 3.0), 5 µL injections from vials in the autosampler tray) onto the trapping/desalting column. After desalting (3 min wash of loading 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: 98% H2O/2% ACN w/ 0.1% formic acid, Pump B: 98% ACN/2% H2O w/ 0.1% formic acid, gradient: 5-75%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/hr, source temp: 80 °C, nebulizer gas: 2 psi, collision gas: argon, 15 psi. Data Directed Analysis: 1 sec TOFMS 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.

Figure1: Stream Select Module and nanospray source

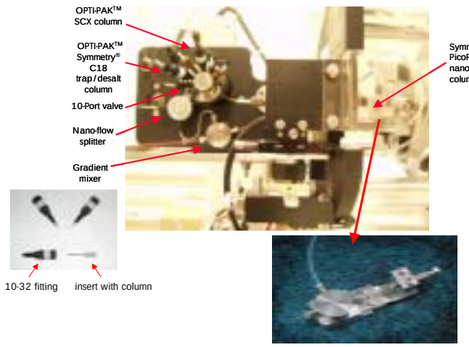
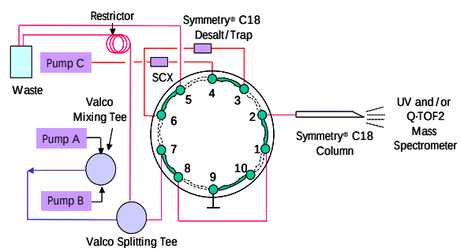


Figure 2: Configuration and flow diagram of multi-dimensional CapLC™ with Stream Select Module



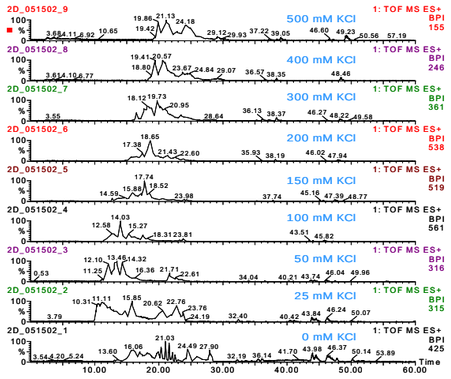
Valve position 1: Sample eluted from SCX and trapped on trap/desalt C18 column

Valve position 2: Sample eluted from trap/desalt C18 column and analytical C18 column

This configuration permits online desalting of peptides eluted from the SCX column without introducing salt into the MS interface. Thus, the peptides desorbed from the SCX column are captured by the trapping column, and the salt is flushed to waste prior to switching the valve to run the reversed-phased separation for LC/MS/MS analysis.

Results and Discussion

Figure 3: BPI chromatograms of nine elution steps with KCl for 2-D nanoLC separation of enriched ribosomal subunit protein digest



The entire 2-D LC/MS/MS analysis can be run unattended under full MassLynx™ software control in ~12.5 hours (including load, wash, and nanoLC 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 ≥ 11, which was the criteria used to consider a positive identification (Figure 5).

Figure 4: Data Directed Analysis chromatograms of the six most intense precursor ions (charge states +2, +3) for the 25 mM KCl elution step

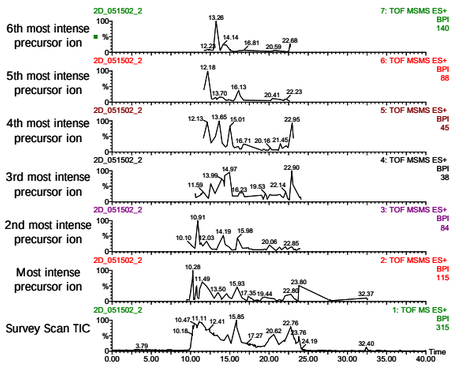
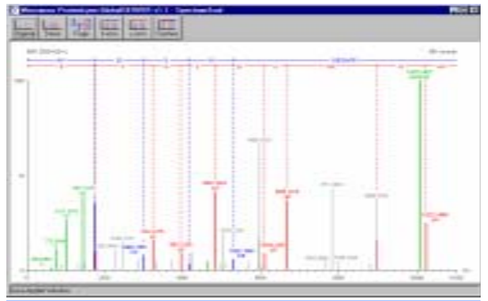


Figure 5: MS/MS spectrum of ribosomal tryptic peptide from 25 mM KCl elution step showing fragment ions which match the predicted sequence of the L13 ribosomal protein found in the yeast database



Peptide Sequence: AVQDNGESAFR; Protein ID: L13a
m/z 597.292, charge: 2, mw: 1192.547; delta: 0.021; score: 14.3

Table 1 contains a summary of the ribosomal proteins in the yeast database which matched MS/MS spectra acquired from the various salt elution steps. 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 (~11-44 kDa), 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 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.

Figure 6: Example display of ProteinLynx™ Global Server database search results for the 150 mM KCl elution step.

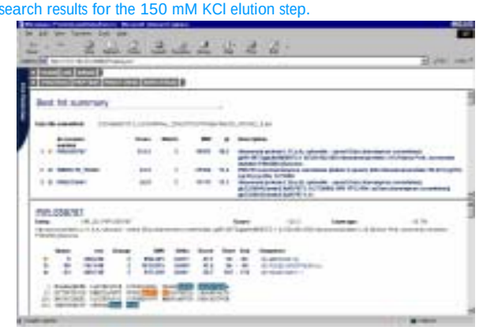


Table 1: Summary of yeast ribosomal subunit protein identification from database search of MS/MS data acquired for each KCl elution step

Subunit	MW (kD)	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8	Step 9
L41	3.3									
L40	6.0									
L39	6.2									
L29	6.5									
L38	8.7									
L37	9.7									
L43	9.9									
P1	10.5									
P2	10.7									
L36	11.0									
L30	11.3									
L33	12.0									
L42	12.2									
L31	12.8									
L34	13.5									
L22	13.6									
L35	13.8									
L26	14.1									
L23	14.3									
L32	14.7									
L14	15.0									
L27	15.4									
L25	15.6									
L12	15.8									
L28	16.6									
L24	17.6									
L21	18.1									
L11	19.6									
L6	19.9									
L17	20.4									
L18	20.5									
L20	20.7									
L9	21.6									
L19	21.6									
L16	22.1									
L13	22.4									
L15	24.3									
L1	24.4									
L10	25.2									
L2	27.3									
L7	27.5									
L8	28.0									
P0	33.6									
L5	33.6									
L4	39.0									
L3	43.7									

*Color scheme	Scores
Red	31+
Orange	21-30
Yellow	11-20
White	0-10 (No ID)

Conclusions

- A fully automated 2-D LC/MS/MS method at the nanoLC 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-connect OPTI-PAK™ column format allows easy configuration and replacement of the SCX and trap/desalt columns with a 10-port valve for either a 1-D or 2-D method
- The Symmetry® C18 OPTI-PAK™ trap column is robust and can effectively remove KH_2PO_4 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 salts/buffers into the analytical nanoLC column and MS source

References

1. Link, A.J., Eng. J., Schietz, D.M., Carmack E., Mize, G.J., Morris, D.R., Garvik, B.M., and Yates J.R.III, *Nat. Biotechnol.* 1999, 17, 676-682.
2. Washburn, M.P., Wolers, D., and Yates, J.R. III, *Nat. Biotechnol.* 2001, 19, 242-247.
3. Wolters, D.A., Washbrun, M.P. and Yates, J.R. III, *Anal. Chem.* 2001, 73, 5683-5690.
4. Xu, W., Finch, J.W., Plumb, R.S., Stumpf, C.F., and Cohen, S.A., "Mulit-Dimensional Capillary LC/MS/MS for Peptide Separation and Protein Identification", P129-T, ABRF 2002, Austin, TX, March 9-12, 2002, p. 91.