Multi-dimensional Capillary LC/MS/MS for Peptide Separation and Protein Identification

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Objective

To develop a method using a fully automated multi-dimensional capillary LC/MS/MS system to analyze complex peptide mixtures from digests of whole proteins and identify those proteins as an alternative to gel electrophoresis based methods.

Introduction

While 2-D gel electrophoresis is currently the method of choice for proteome analysis, this approach does have some disadvantages namely in the analysis of membrane proteins, low copy number proteins, and proteins with high or low isoelectric points. To overcome some of these disadvantages, multi-dimensional HPLC methods have been explored as possible alternatives.

In this study we present results utilizing a fully automated multidimensional capillary HPLC system to separate complex peptide mixtures from protein digests where the eluent is directly coupled to a flow-thru nanospray source of a quadrupole-TOF hybrid (QTOF) instrument. The data from multi-dimensional analysis are processed and searched against NCBI databases.

General Experimental Conditions

LC/MS instrument: Micromass CapLC™ system and Q-TOF2™ system

Strong cation exchange column (SCX): 320 µm ID x 5 mm L, PolySULFOETHYL Aspartamide™ SCX, 5 µm, 300 Å (PolyLC Inc, Columbia, MD)

Analytical reversed-phase column (RP): 75 µm ID x 100 mm L, Symmetry® PicoFrit™ or IntegraFrit™ C18, 3.5 µm, 100 Å

Reversed-phase desalt/trap column: 320 µm ID x 5 mm L, Symmetry® C18, 5 µm, 300 Å

Mobile phase A: 0.1% formic acid in 98% water, 2% acetonitrile

Mobile phase B: 0.1% formic acid in 98% acetonitrile, 2% water

Loading solvent: 0.1% formic acid in 98% water, 2% acetonitrile

Step elution solvent: a. 0, 25, 50, 75, 100, 125, 150, 200, 225, 250 mM KCl in 5% ACN; b. 0, 50, 100, 200, 300, 400 mM ammonium formate in 5% ACN. Elution solvents were injected through vials in the autosampler tray using a 5 µl sample loop.

Flow rate: 200 nl/min for reversed-phase column gradient

Gradient: 5-50%B for 30 min, 50%-80%B for 2 min and hold at 80% for 10 min.

Sample information: tryptic digest of bovine serum albumin (BSA), apomyoglobin (MYO), bovine carbonic anhydrase (BCA), human transferrin (HTRF) and bovine cytochrome c (CYTC).

Mass Spectrometric Conditions

Voltages: capillary 2.25 kV, cone 30 V, extractor 0 V, RF lens 0.9 V

Source Temperature: 80 °C

Cone Gas Flow: 50 L/hr

Nanoflow (nebulizer) Gas Pressure: 2 psi

Collision Gas: Argon

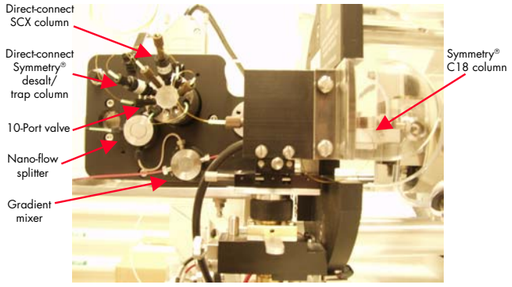
(Collision) Gas Cell Pressure: 15 psi

Resolution and Ion Energy: LR 5, HR 5; Ion energy 1.8 V

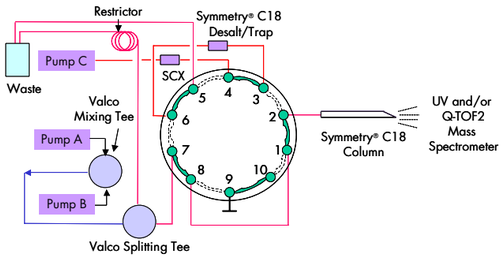
Data-Directed Analysis: Survey scan of 1 sec (0.1 sec interscan delay) was utilized with a collision energy profile for each precursor ion based on mass and charge state. Precursor ions with charge states 2-4 were selected for MS/MS fragmentation.

CapLC™ and Q-TOF2™

Stream Select Module and Nano-Spray Source



Multi-dimensional CapLC™ Stream Select Module - SCX, Desalt/Trap and Analytical RP C18 Columns



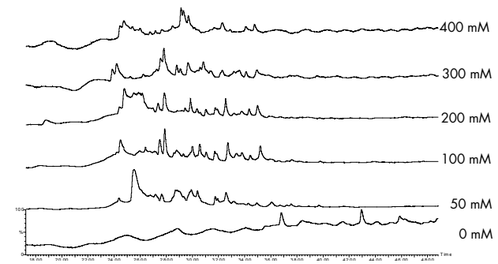
Valve position 1: Sample eluted from SCX and trapped on desalt/trap C18 column
Valve position 2: Sample eluted from desalt/trap C18 column and analytical C18 column

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.

Reversed-Phase Chromatograms of Peptide Mixtures After Step Elution with Ammonium Formate from SCX Column

System: CapLC™ with nano valve
Column: 75 µm ID, 12 cm L, C18
Detection: 0.145 AU at 214 nm

Sample: tryptic digests of 5 proteins
Injection: 2 µl, 1 pmol/µl
Loading solvent: 0.1% formic acid/2% ACN

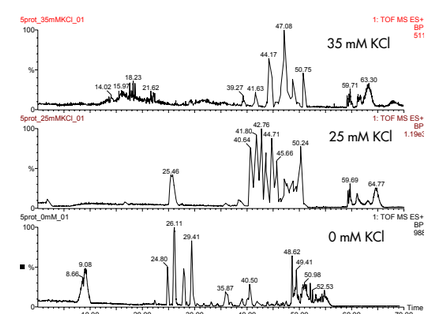


Most peptides eluted from the SCX column between 50 mM and 400 mM

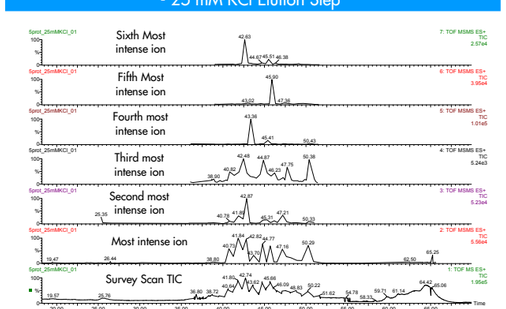
Chromatograms of Three Step Elutions with KCl

System: CapLC™ - QTOF™
Column: 75 µm ID 10 cm L, C18
Flow: 200 nl/min

Sample: tryptic digests of 5 proteins
Injection: 200 fmol/ul, 5 µl
Loading solvent: 0.1% formic acid/2% ACN



Data-Directed Analysis Chromatograms of Six Most Intense Precursor Ions - 25 mM KCl Elution Step



Up to six precursor ions above a set threshold are selected in each survey scan in a single function cycle

Example of Database Search Result

```
User            : wxu
Email           : wensheng_xu@waters.com
Search title    : 25 mM KCL
MS data file    : F:\Temp home of pkl files\5prot_25mMKCl_01.pkl
Database        : NCBInr 20020220 (883557 sequences; 276611002 residues)
Timestamp       : 7 Mar 2002 at 20:26:13 GMT
Significant hits: gi|2190337      (X58989) serum albumin [Bos taurus]
                  gi|4557871      (NM_001063) transferrin precursor; PRO1557 protein [Homo s
                  gi|4139756      Chain F, Igg1 Fab Fragment (Of E8 Antibody) Complexed Wit
                  gi|115460       Carbonic anhydrase II (Carbonate dehydratase II) (CA-II)
                  gi|113582       SERUM ALBUMIN PRECURSOR
                  gi|3913386      CYTOCHROME C
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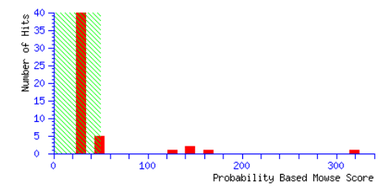
Probability Based Mowse Score

Score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 49 indicate identity or extensive homology (p<0.05).



List of Peptides from Database Search at 25 mM KCl Elution Step

Protein*	Peptides matched	Mr (expt)	Mr (calc)	Peptide
BSA	9	608.30	608.28	AFDEK
		732.43	732.41	VLTSSAR
		788.47	788.46	LVTDLTK
		817.45	817.42	ATEEQLK
		885.28	885.41	DDSPDLPK
		1138.45	1139.46	CCTESLVNR
		1162.62	1162.62	LVNELTEFAK
		1928.73	1929.74	CCAADDKEACFAVEGPK
		1952.96	1954.95	DAIPENLPPLTADFAEDK
HTRF	5	697.30	697.29	CQSFR
		734.41	734.40	GDVAFVK
		1167.40	1165.50	HQTVPQNTGGK
		1247.54	1248.60	SASDLTWDNLK
		1279.43	1280.53	CDEWSVNSVGK
CYTC	4	677.39	677.37	YITGPK
		1467.69	1469.68	TGQAPGFTYTDANK
		1469.68	1469.68	TGQAPGFTYTDANK
		1492.71	1494.69	EETLMEYLENPK
BCA	3	1011.55	1011.53	VGDANPALQK
		1018.49	1018.47	DFPIADGER
		2002.01	2001.96	DFPIADGERQSPVDIDTK

*Peptides from myoglobin were found at 35 mM KCl elution step

Summary

- Multi-dimensional capillary HPLC coupled with mass spectrometry can be used to fractionate complex peptide mixtures for peptide sequencing and protein identification.
- Elution buffers (ammonium formate, KCl) proved to be effective for eluting peptides from the SCX column.
- The Symmetry® C18 desalt/trap column is effective for removing high concentrations of salts/buffers and trapping the peptides.
- The multi-dimensional system using the ten-port valve allows automated on-line desalting and trapping of peptides for LC/MS/MS analysis.
- The multi-dimensional capillary LC system is flexible and can be easily configured for one-dimensional or two-dimensional separations using Waters direct-connect format for SCX and C18 trapping columns.
- Waters direct-connect format for SCX and C18 trapping columns also minimizes void volume.