Identification of HPLC-Fractionated Tryptic Peptides Using the NanoMate™ 100 System

Ultimate “Peak Parking” by Fractionation

Advion BioSciences, Inc. (Ithaca, NY) has demonstrated use of the NanoMate™ 100 system for protein identification from fractionated tryptic digests of a protein mixture by nanoelectrospray mass spectrometry. The NanoMate 100 is an automated sample-handling system coupled with the ESI Chip™ (a 10 x 10 array of nanoelectrospray nozzles etched in silicon).

Proteomic studies currently use various separation techniques followed by on-line, nanoscale, full-scan LC/MS/MS analysis of tryptic digests. While this approach works well, its drawbacks include relatively long runs and special techniques such as “peak parking” to obtain the best data. Using Advion’s nanoscale technique, capillary LC fractions from tryptic digests of protein mixtures are collected off-line and the fractions analyzed by automated nanoelectrospray ionization mass spectrometry.

Methods

A standard protein mixture of BSA, myoglobin, β-lactoglobulin, and cytochrome c was denatured in 6 M guanidine- HCl and digested by trypsin. The tryptic digests were separated by capillary LC.

HPLC Conditions

Column: PepMap™ C$_{18}$ (300 µm i.d. by 15 cm, 3-µm particles)
HPLC System: UltiMate™ capillary nano HPLC
Gradient: 8 minutes, 0% B
68 minutes, 90% B
Mobile Phase A: 5% CH$_3$CN (v/v) in water with 0.1% TFA
Mobile Phase B: 80% CH$_3$CN (v/v) in water with 0.1% TFA
Flow Rate: 3 µL/minute
Fractions: Collected over 1-minute intervals

Nanoelectrospray MS and Database Search Instruments: NanoMate, ESI Chip, Thermo Finnigan LCQ™ Deca
Spray Voltage: 1.4 kV
Flow Rate: 100 nL/minute
Acquisition Time: 1.5 minutes
Scan Modes: MS survey scan and data-dependent MS/MS scan
Searches: SEQUEST/horse.fasta and bovine.fasta databases
Results
Fractionated samples (3 µL) were collected and analyzed by the NanoMate system over a period of 1.5 minutes. MS/MS analyses of all fractions were complete in 90 minutes. Several peptides were identified within each fraction for each of the proteins digested. An example of the data from the fractions taken from 34 to 40 minutes is shown below.

A representative peptide, identified in the 34 to 35 minute fraction, matched a peptide for cytochrome c (peptide TGQAPGFTYTDANK) as shown in the figure below.

Protein Coverage
100% of the tryptic peptides greater than 500 Da were identified for cytochrome c by this method. In addition, 80% of the tryptic peptides greater than 500 Da were identified for myoglobin.

Summary
The NanoMate 100 System has demonstrated utility as a nanoelectrospray platform for rapid analysis of separated samples via 100% coverage found for cytochrome c. High-quality MS/MS data were obtained for each fraction with 1.5-minute acquisition times containing as many as five tryptic fragments, and yielding unambiguous identification of the target proteins.

Advantages
• Analysis is not time sensitive.
• Short analysis can be used for screening. Long analysis allows for mining of low-abundance proteins.
• Signal intensity does not change with time.
• Sample fractions can be saved and archived.
• MS time is conserved. Analyze only the peak elution window of interest.
• The decoupling of chromatography from MS/MS acquisition allows for greater flexibility and speed of analysis.

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