Identification of Phosphorylation Sites in α-Casein

Advion BioSciences
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Using Chip-based Nanoelectrospray with Automated Data-Dependent Neutral Loss Mass Spectrometry

Advion BioSciences, Inc. (Ithaca, NY) has demonstrated the use of the NanoMate®100, combined with an ion trap mass spectrometer, to identify phosphorylation sites of α-casein using data-dependant scanning followed by automatically triggered neutral loss mapping. The NanoMate 100 is an automated chip-based nanoelectrospray ionization system (nanoESI) that provides extended analysis times to detect and to characterize phosphopeptides.

Advantages

- Extended acquisition time for MS/MS analysis with automated neutral loss mapping in a single analysis for increased information content
- Excellent spray stability for maximum sensitivity
- Automated chip-based nanoelectrospray enables one-time spray optimization for analysis of 96 samples
- Microfabrication enables reproducible spray from laboratory to laboratory
- Soft ionization technique
- Ability to save unused sample

Reversible protein phosphorylation plays a pivotal role in many regulatory mechanisms such as metabolism, cell division, cell growth and cell differentiation. Knowledge of the residues being phosphorylated in a protein can provide insight into the mechanisms of regulation. This work serves to demonstrate the the NanoMate and LTQ™ as a sensitive, reproducible and stable method to identify phosphorylation sites of bovine α-casein.

Method

α-casein (Sigma, St. Louis, MO) was dissolved in 50 mM ammonium bicarbonate pH 7.8 at 1 mg/mL. Trypsin, in a 1 µg/µL stock solution, was then added to the solution at 1:60 (w/w). Digestions were performed at 37 °C for 16 hours and stopped by the addition of 0.1% (v/v) acetic acid. The digests were stored at -70 °C.
Calf intestinal phosphatase (CIP) (Biolabs, Boston, MA) was dialyzed against 20 mM ammonium biocarbonate pH 7.8 overnight at 4° C using Slide-A-Lyzer Mini Units from Pierce (Rockford, IL). Four units of dialyzed CIP (8 units/mL) were added to 10 mL of the above digests and incubated at 37° C for 2 hours. The CIP treated digests were stored at 70° C until use.

Chip-based nanoESI/MS Analytical Conditions

Analyte:	Trypsin digest of bovine α-casein Calf intestinal phosphatase (CIP) was used for treatment of the α-casein digest
Solvent:	50% Methanol with 0.1% acetic acid in water
Sample Concentration:	50 and 100 fmol/µL α-casein
Sample Volume:	5µL

NanoMate 100 Conditions

Approximate Flow Rate:	100 nL/ minute
Spray Voltage:	1.5 kV positive ion mode
Spray Pressure:	0.25 psi

Finnigan LTQ™ Conditions

Acquisition Time:	5 minutes
Capillary temperature:	150 °C
Normalized collision energy:	25 %
Maximum ion collection time:	50 ms
Number of microscans:	2-3

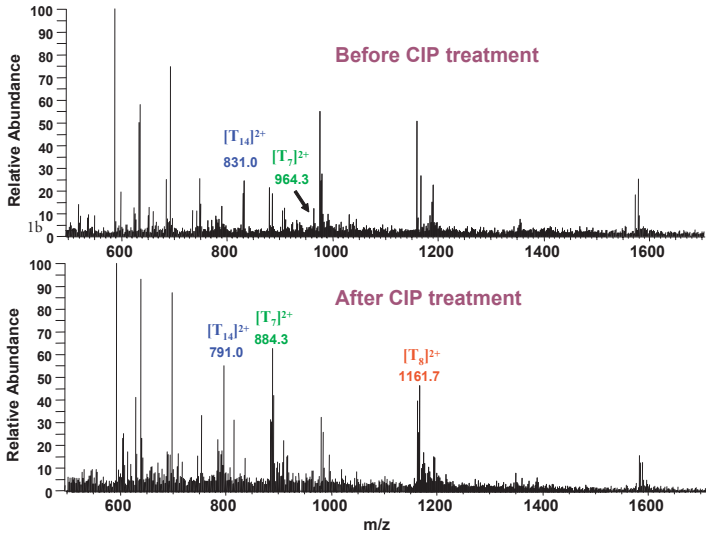

Figure 1 - MS spectra of α-casein tryptic digests before and after CIP treatment 1 pmol/µL

CIP treatment was used for the α-casein digests to identify the T_8 phosphopeptide containing five phosphogroups that failed to be detected in the full scan positive ion mass spectrum.

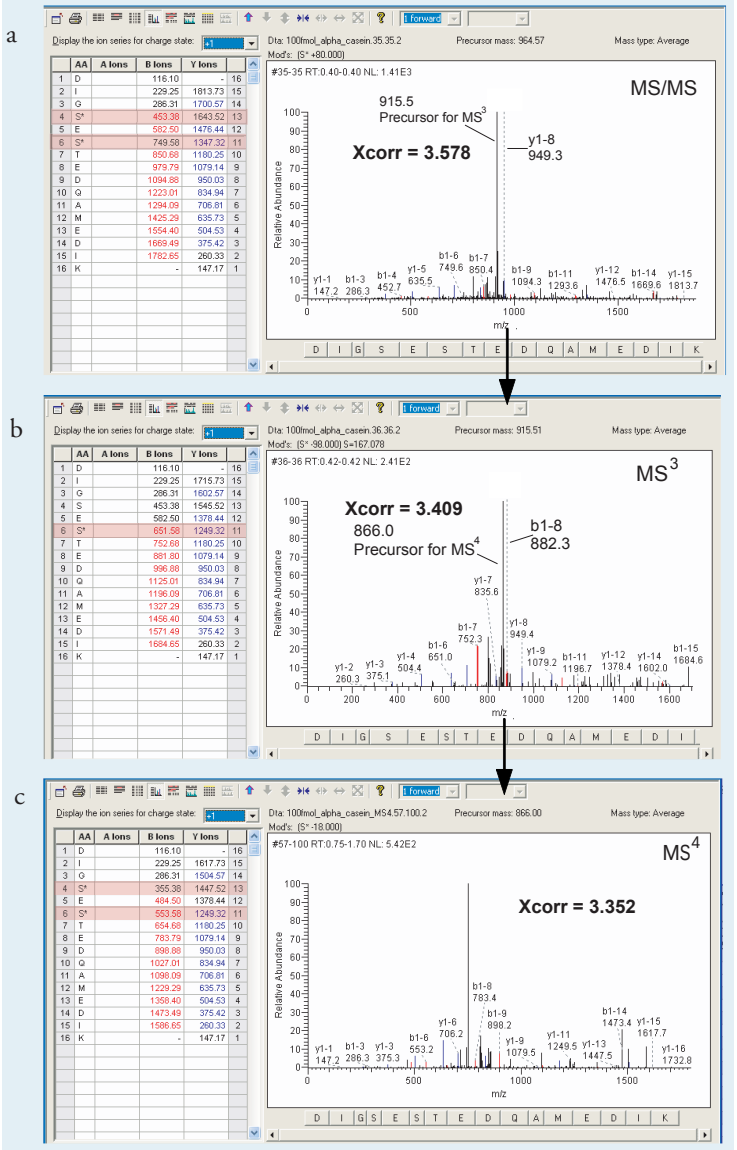

Figure 2 - Identification of phosphorylation sites by automated data-dependant scanning and neutral loss mapping before CIP treatment

(a) - MS/MS spectrum of the precursor ion (T_7) *m/z* 964 for 100 fmol/µL α-casein digest.

(b) - MS^3 spectrum of the ion formed from the neutral loss at *m/z* 915 for 100 fmol/µL α-casein digest, see Figure 2a.

(c) - MS^4 spectrum of *m/z* 866 formed from the second loss of phosphate for 100 fmol/µL α-casein digest, see Figure 2b.

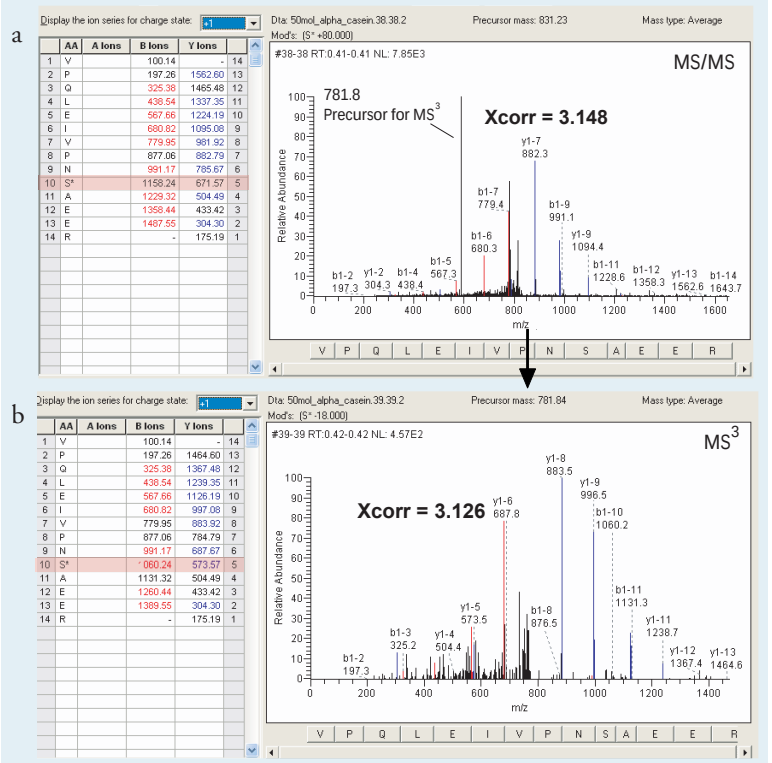

Figure 3 - Identification of phosphorylation sites by automated data-dependant scanning and neutral loss mapping before CIP treatment

(a) - MS/MS spectrum of a precursor ion (T_{14}) at *m/z* 831 for 50 fmol/µL α-casein digest.

(b) - MS^3 spectrum of the ion formed from the neutral loss at *m/z* 781 for 50 fmol/µL α-casein digest.

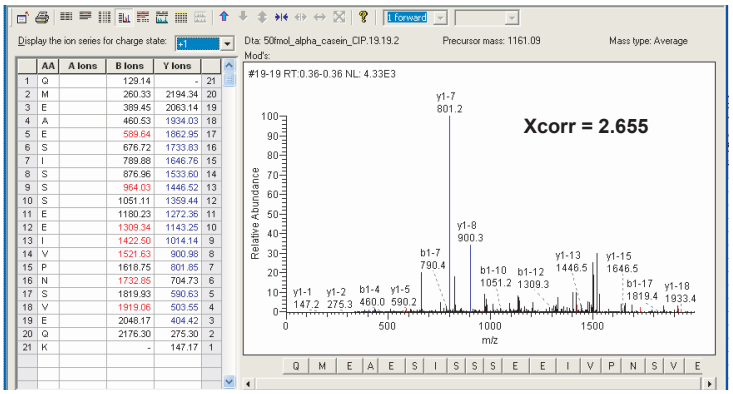

Figure 4 - MS/MS spectrum of *m/z* 1161 (from T_8 phosphopeptide) for 50 fmol/µL CIP treated α-casein digest.

Summary

The NanoMate 100 system combined with the Finnigan LTQ is an effective tool to rapidly and accurately identify peptides containing multiple phosphorylation sites. The system provides increased time and sensitivity needed to complete MS^3 analysis using minimum sample quantities.

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Thermo ELECTRON CORPORATION

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