Analysis of Phosphopeptides Using Nano LC/MS/MS

INTRODUCTION

Phosphorylation is recognized as one of the most important post-translational modifications of proteins. Phosphorylated proteins play a key role in mediating cellular signal transduction pathways. Various techniques are available for detection and identification of phosphorylated proteins, including gel electrophoresis, HPLC and Edman degradation. One of the current technologies for identification of proteins involves the separation of proteolytic digests of proteins with Capillary LC and mass spectrometric detection.

RESULTS AND DISCUSSION

In this application an UltiMate™ Nano LC system, a Switchos™ II advanced micro column switching unit, a FAMOS™ micro autosampler and a ThermoFinnigan LCQ Deca ion trap mass spectrometer were used for the analysis of phosphopeptides. Synthetic peptides corresponding to the non-, mono- and diphosphorylated tryptic fragment 485–496 (ALGADDSYYTAR) of human protein tyrosine kinase ZAP-70 were used to evaluate the LC-MS system.

A sample of 10 µL containing 75 fmol of each peptide was injected onto a C18 precolumn (300 µm x 1 mm). Peptides were preconcentrated and desalted with 0.1% formic acid at a flow rate of 50 µL/min for 2 min. The separation of the peptides was performed on a 75 µm I.D. PepMap™ column with a gradient from 2–36% acetonitril containing 0.1% formic acid at a flow rate of 0.2 µL/min. A Thermo Finnigan nano spray ion source and a coated spray needle with 10 µm I.D. tip were used to interface the LC and MS system.

The base peak chromatogram and the extracted MS ion current of the di-phosphorylated peptide are shown in Figures 2A and 2B, respectively. Peak widths are

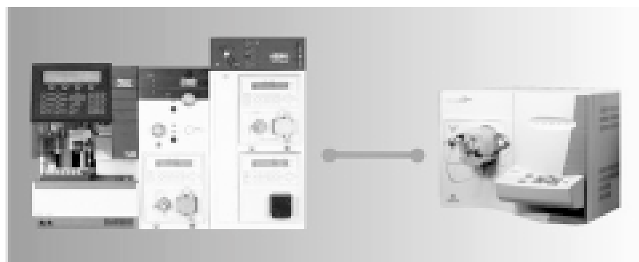

Figure 1. UltiMate NanoLC system.

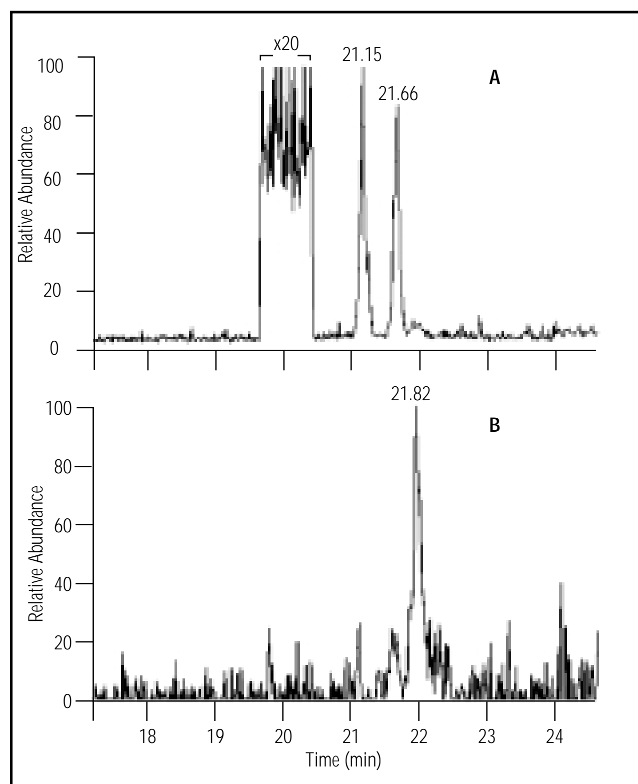

Figure 2. Base peak chromatogram (A) and the extracted MS ion current of the di-phosphorylated peptide (B).

approximately 0.2 min at the base of the peaks. The peak shape is excellent with the use of formic acid as ion-pairing agent in a concentration of 0.1%. The use of 0.1% formic acid resulted in a 10-fold increase in MS sensitivity compared to mobile phases containing 0.05% TFA.

The MS signal intensity is considerably higher for the non-phosphorylated peptide than for the diphosphorylated peptide. The lower ESI-MS signals for the phosphorylated peptides are due to reduced ionization efficiencies. The detection limit for the non- and mono-phosphorylated peptides is in the low fmol range as can be seen in Figure 2A. In order to perform protein identification, automated data dependent MS/MS spectra were produced for each of the peptides. Differences of 80 m/z could be observed in the MS/MS spectra of the three peptides as a result of the phosphate groups. The MS/MS spectrum of the di-phosphorylated peptide is shown in Figure 3. Using this MS/MS spectrum, TurboSequest identified the human protein tyrosine kinase ZAP-70 from a protein database. The TurboSequest result file is shown in Figure 4.

```
database=C:\Xcalibur\database\human.fasta, accession=gi|346421|pir||A44266
peptide(s)=ALGADDSYYTAR

Analyzing ...

>gi|346421|pir||A44266 protein-tyrosine kinase (EC 2.7.1.112) ZAP-70 -
human [MASS=69876]
MPDPAAHLPF FYGSISRAEA EEHLKLAGMA DGLFLLRQCL RSLGGYVLSL VHDVRFHHFP
IERQLNGTYA IAGGKAHCGP AELCEFYSRD PDGLPCNLRK PCNRPSGLEP QPGVFDCLRD
AMVRDYVRQT WKLEGEALEQ AIISQAPQVE KLIATTAHER MPWYHSSLTR EEAERKLYSG
AQTDGKFLLR PRKEQGTYAL SLIYGKTVYH YLISQDKAGK YCIPEGTKFD TLWQLVEYLK
LKADGLIYCL KEACPNSSAS NASGAAAPTL PAHPSTLTHP QRRIDTLNSD GYTPEPARIT
SPDKPRPMPM DTSVYESPYS DPEELKDKKL FLKRDNLLIA DIELGCGNFG SVRQGVYRMR
KKQIDVAIKV LKQGTEKADT EEMMREAQIM HQLDNPYIVR LIGVCQAEAL MLVMEMAGGG
PLHKFLVGKR EEIPVSNVAE LLHQVSMGMK YLEEKNFVHR DLAARNVLLV NRHYAKISDF
GLSKALGADD SYYTARSAGK WPLKWYAPEC INFRKFSSRS DVWSYGCTMW EALSYGQKPY
KKMKGPEVMA FIEQGKRMEC PPECPPELYA LMSDCWIYKW EDRPDFLTVE
QRMRACYYSLASKVEGPPGS TQKAEAACA
>average mass = 69858

 position  sequence (NCBI BLAST link)
 --------  --------
 485- 496  ALGADDSYYTAR

Protein Coverage: 12/619 = 1.9% by amino acid count, 1284/69858 = 1.8% by mass

Search SWISS-PROT with gi|346421|pir||A44266 via accession, descr./ID, or full text field.

Done.
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Figure 4. TurboSequest result of MS/MS spectrum. Courtesy: Prof Dr. R. Bischoff, RUG, Groningen, and L. Maljers, Thermo Finnigan, Breda, The Netherlands.

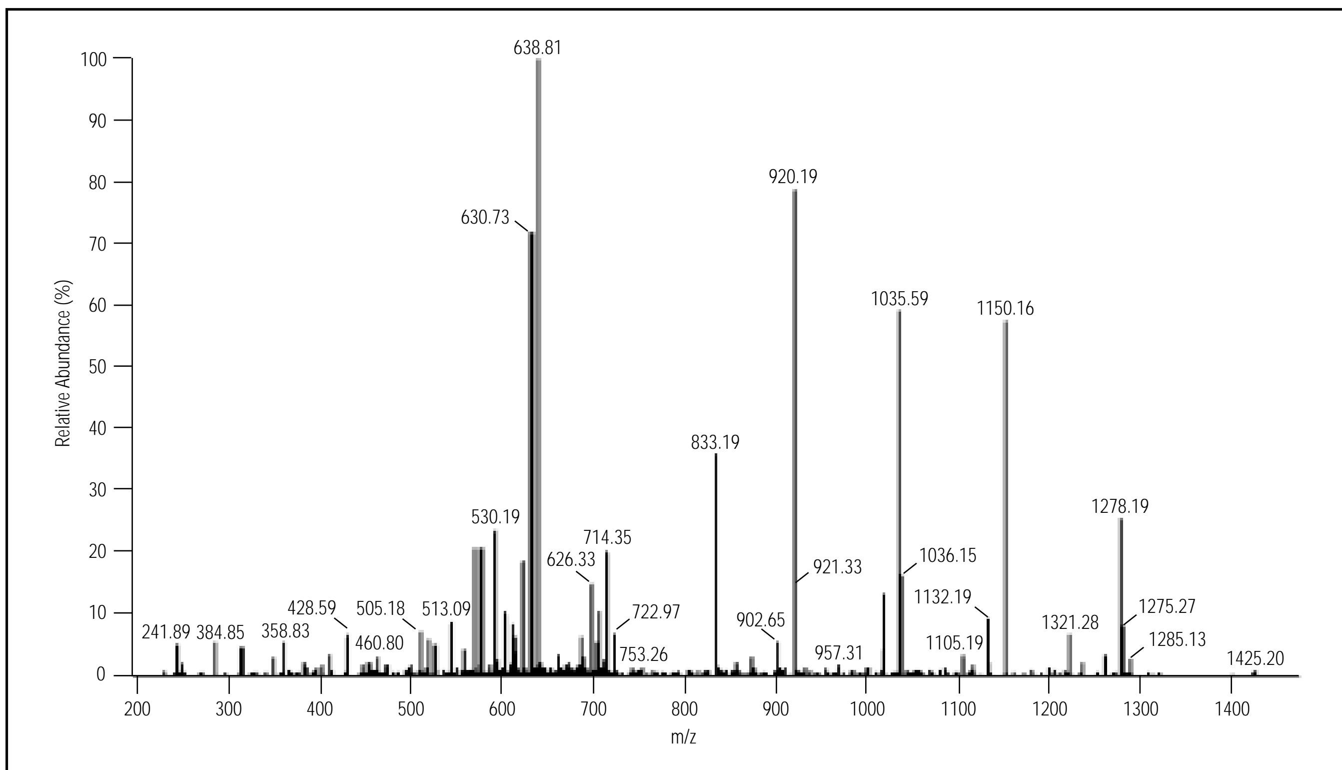

Figure 3. MS/MS spectrum of the diphosphorylated peptide.

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