Detection of Phosphorylated Peptides from Data Dependent MS ^{3} Neutral-Loss Scans using a Linear Ion Trap Mass Spectrometer

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Overview

Unambiguous identification of phosphorylated proteins using tandem mass spectrometry is often hindered by insufficient fragmentation when peptide information is limited to only the data from MS/MS spectra. Benefiting from the increased sensitivity in a linear ion trap mass spectrometer, Data Dependent scanning was used to trigger MS^3 fragmentation after a specific neutral loss from a phosphate group was detected, generating more diagnostic ions for accurate sequence determination as well as identification of the exact site of phosphorylation.

Introduction

Phosphorylation is recognized as one of the most important post-translational modifications of proteins, and is associated with many proteins that have a regulatory function in cells. Phosphoproteins are generally found in low quantities within cells. This low abundance, coupled with the higher acidity of phosphopeptides, increases the complexity of their analysis by mass spectrometry in positive ESI mode. In tandem mass spectrometry, phosphopeptide precursor ions typically exhibit a prominent neutral loss of a phosphate group (98 Da) during fragmentation. However, identification of the phosphoprotein and determination of the exact site of phosphorylation is often limited by inadequate peptide fragmentation and diagnostic sequence ion information.

To overcome the lack of peptide fragmentation information typical in phosphopeptide analysis, a new Data Dependent experiment was created to selectively trigger MS^3 scans on only the MS/MS fragment ions for which a specific, prominent neutral loss ion was detected. This new instrumental method was designed, in part, to take advantage of the superior sensitivity afforded by the design of the new linear ion trap mass spectrometer, the Finnigan LTQ. The increased ion capacity and improved trapping efficiency of the linear ion trap significantly enhances the quality of the MS^2 data, which, in turn, improves the quality and abundance of the MS^3 data which is key to unambiguous identification of phosphorylated proteins.

Results

This neutral loss-driven MS^3 experiment was used to analyze a mixture of alpha and beta caseins, after separation by capillary LC, followed by analysis online with the Finnigan LTQ linear ion trap mass spectrometer. The flow chart of data dependent scan events is shown in Figure 1. An MS survey scan is initially performed, followed by 3 MS/MS scan events. If the MS/MS scan event detected a neutral loss ion of 98, 49 or 32.7 Da (corresponding to +1, +2, and +3 charge states), and this neutral ion intensity was among the top 3 intensities, an MS/MS/MS scan event was triggered. If a neutral loss was not detected, it would repeat with another MS scan and continue the process. Using both neutral loss values ensured that MS^3 scans would be acquired for both singly- and doubly-charged phosphopeptides, and helped increase the overall protein coverage.

Protein identifications were made with BioWorks 3.1 software using a minimum XCorr of 2.5 for the doubly-charged peptides as criteria for acceptance. Because only the MS^3 ions were submitted to Sequest, the database search time was reduced compared to the same experiment in which all of the MS^2 ions were also considered. Figure 2 shows MS/MS and MS^3 spectra of three phosphorylated peptides found. For each, the neutral loss ion had lost an H_3PO_4 moiety, but was still an intact peptide. Further fragmentation of this ion (in MS^3) yielded a classical peptide fragmentation pattern, with a b- and y-ion series searchable by BioWorks 3.1.

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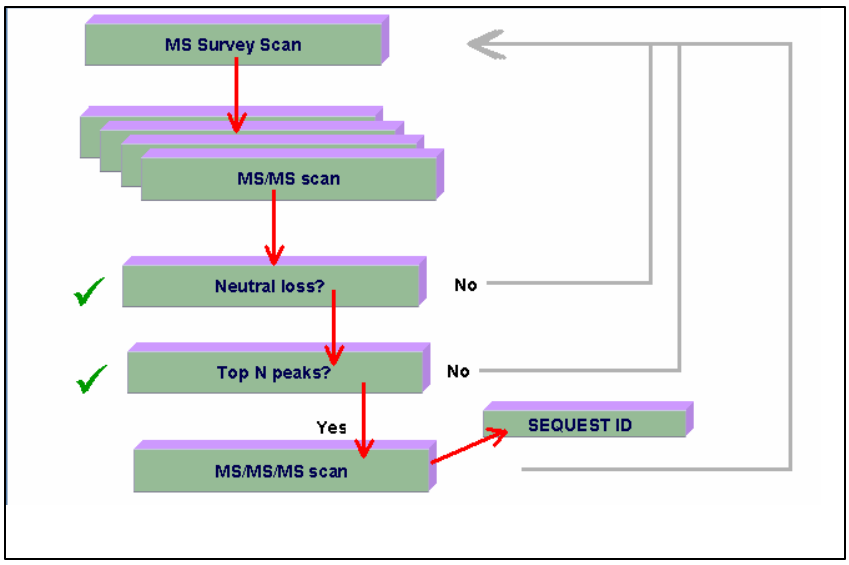

Figure 1: Automated phosphorylation site analysis using Data Dependent MS3 on the LTQ.

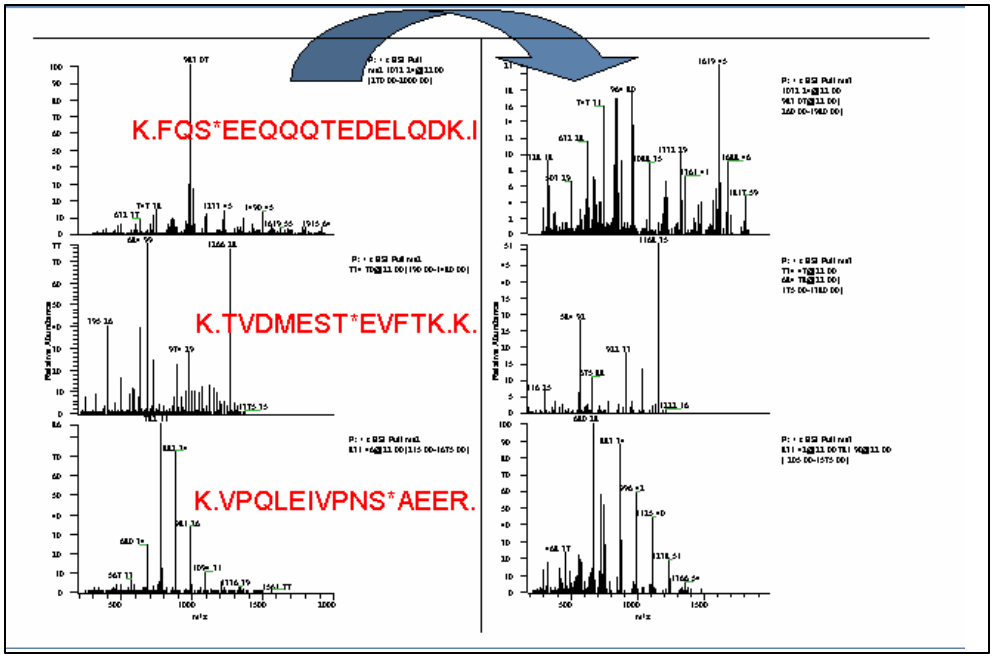

FIGURE 2: MS/MS and MS3 spectra of three phosphorylated peptides found in the casein digest.

Conclusions

The Finnigan LTQ linear ion trap mass spectrometer shows unparalleled sensitivity for the analysis of a digest of alpha and beta caseins, which is evident by the detection of peptides at levels as low as 10 fmole digest on-column. The automated Data Dependent neutral loss scanning algorithm outlined is of great utility in identifying sites of phosphorylation on peptides whose fragmentation using only MS/MS would not normally be sufficient for identification. The MS3 data was searched using TurboSEQUEST within BioWorks and was successful in identifying the exact site of phosphorylation on the peptides.