

Using AP MALDI for Phosphopeptide Mining on an Ion Trap Mass Spectrometer

Scott M. Peterman and Joseph J. Mulholland

Thermo Electron, Somerset, NJ 08873

Introduction: Phosphorylation is one of the most important post-translational modifications due to its important role in a number of biological activities. Limitations to phosphopeptide mining by LC/MS include low relative abundance, increased possibility of poor retention on standard HPLC columns, and reduced ionization efficiencies compared to unmodified tryptic peptides. Our approach for phosphopeptide mining utilizes atmospheric pressure matrix-assisted laser desorption ionization (AP MALDI) coupled to an ion trap. AP MALDI/ITMS has shown routine detection limits below 50 femtomoles for full-scan MS spectra and sub-femtomole detection for MS/MS (1). In addition, the use of Data Dependent/Dynamic Exclusion™ software allows for direct analysis of a protein mixture of bovine casein digest without fraction collection. The casein mixture was chosen due to it being well characterized (2-3).

Experimental: Mass spectra were obtained on a Finnigan™ LCQ Deca XP Plus (Thermo Electron) with an AP MALDI source (Mass Technologies, Burtonsville, MD) with pulse energy of 300 μJ at 337 nm. Collision induced dissociation (CID) parameters were altered from the “standard” parameters of normalized collision energy of 35 %, a *q*-value of 0.25, and a 30-msec activation time compared to “low *q*” values of 19-25%, 0.2, and 200-msec, respectively (2).

All unmodified peptides, the casein proteins, and the MALDI matrix alpha-cyano-4-hydroxycinnamic acid were purchased from Sigma (St. Louis, MO) and all phosphopeptide standards were purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). Comparison and confirmation of peptide assignments were done using 20-minute gradient on a C8 column (50 x 1.0 mm, Thermo Electron).

Results: **Figure 1** shows comparative full scan MS/MS on three different phosphopeptide standards using “low *q*” and “standard” CID parameters. Utilization of “low *q*” CID parameters yielded slightly greater TIC for the standard **DHTGFLpTEpYVATR** and over a two-fold increase in the total product ion intensity for the remaining two phosphopeptide standards. In addition, the resulting product ion spectrum shows product ions resulting from amide backbone cleavage are greatly attenuated forming almost exclusively the phosphate and water loss product ions.

AP MALDI/ITMS analysis of a complex mixture of bovine caseins digest spiked with phosphopeptide standards utilizing “low *q*” activation parameters was also performed. **Figure 2a** shows a portion of the resulting full scan mass spectra showing the complex full scan mass spectra generated by spotting the entire digest mixture. The ions marked in red represent identified phosphopeptides as indicated by the characteristic [M-98]⁺ product ion. **Figures 2b** and **2c** show the full scan MS/MS spectra for the phosphopeptide standard P-318 (m/z 1863) acquired in three successive MS/MS scans. Note the intensity of the precursor ion which illustrates the instrumental advantage of precursor ion concentration/isolation afforded with an ion trap. Clearly, the resulting MS/MS spectra acquired using “low *q*” CID parameters yields an intense product ion consisting of the characteristic neutral loss compared to results shown in **Figure 2c**.

Reference: 1. Laiko, V. V., Moyer, S. C., Cotter, R. J., *Anal. Chem.* 2000, **72**, 5239-5243
2. Peterman, S. M., Winnik, W., Paul, G., Kagan, M. Mulholland, J. J. Maximizing the Phosphate Loss Transition using Standard LC/MS Techniques by Ion Traps and Triple Quadrupole Mass Spectrometers. Presented at the 2003 Pittsburgh Conference, March 2003.
3. Bateman, R. H., Carruthers, R., Hoyes, J. B., Jones, C., Langridge, J. I., Millar, Vissers, J. P. C. *J. Am. Soc. Mass Spectrom.* 2002, **13**, 792-803

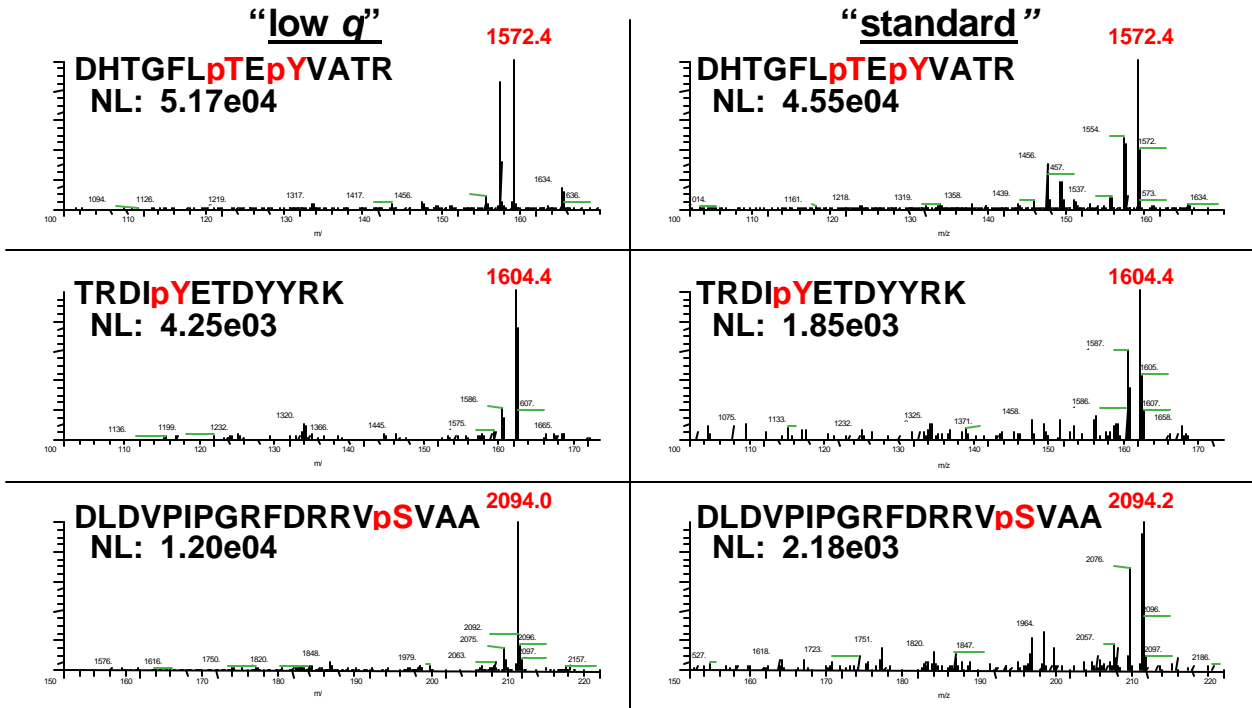


Figure 1. Comparative full scan MS/MS spectra for three different phosphopeptide standards using “low q ” and “standard” CID parameters. Each peptide was spotted at a concentration of 50 femtomoles and the resulting MS/MS spectra was signal averaged using 5 consecutive scans.

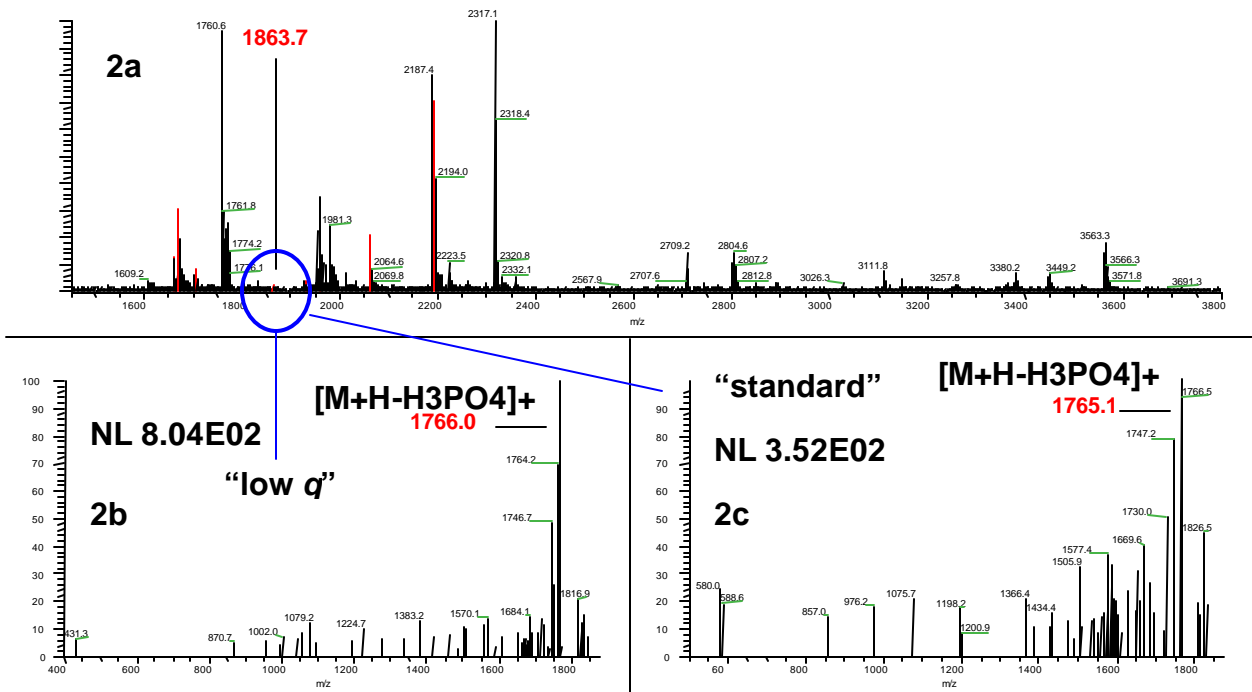


Figure 2. Full scan mass spectra (2a) of bovine casein tryptic mixture spiked with phosphopeptide standards. The region of the full scan mass spectra circled represents the phosphopeptide P-318 isolated and analyzed by a MS/MS utilizing “low q ” (2b) and “standard” (2c) CID parameters.