

Analysis of Low Mass Ions in Peptide Fragmentation Spectra With a Linear Ion Trap

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Overview

Purpose: Demonstrate a technique to remove the “Low Mass Cutoff” for ion trap mass spectrometers and assess the quantitative capabilities of such a technique.

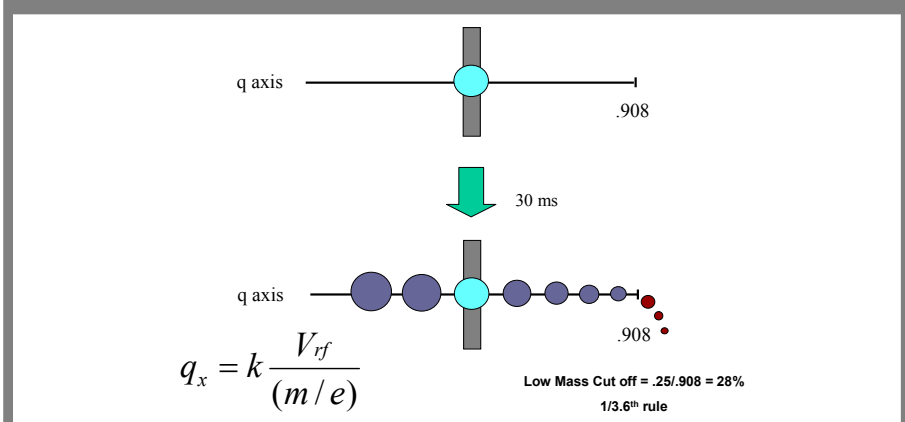
Methods: A novel fragmentation mechanism, pulsed-Q dissociation (PQD), was used to create, capture, and analyze low mass fragment ions on a linear ion trap mass spectrometer. This system was used to analyze peptides, protein digests and iTRAQ™ labeled protein digests.

Results: PQD fragmentation of peptides resulted in robust fragment production and the ability to routinely scan product ions down to m/z 50. Many interesting low mass ions were seen in these spectra, including y_1 and b_1 sequence ions, immonium ions and iTRAQ reporter ions. Ability to easily quantify relative protein levels in iTRAQ labeled samples was demonstrated.

Introduction

Fragmentation of ions in ion trap mass spectrometers has traditionally been done using collisionally induced dissociation (CID). CID results in highly efficient precursor ion fragmentation subject to a “low mass cutoff”, or the so-called “1/3 Rule”. The mechanism for CID, shown in Figure 1, does not allow for trapping of fragment masses below 28% of the precursor mass. Consequently, ion trap CID spectra are missing certain classes of potentially interesting and useful ions, such as immonium ions and y_1 and b_1 sequence ions.

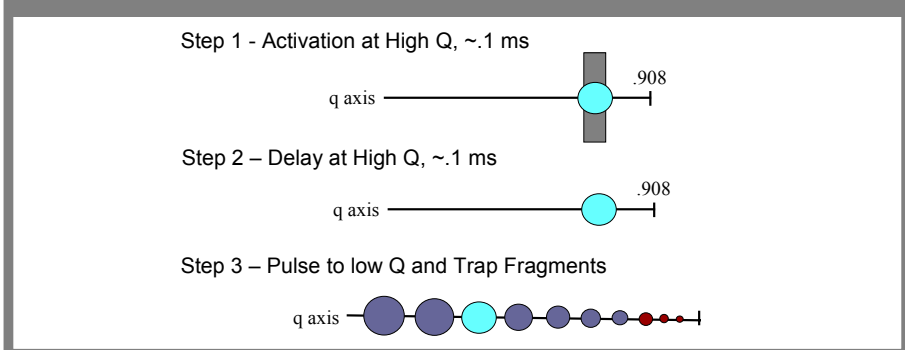
FIGURE 1. Mechanism for ion trap CID. Product ions are subject to the “1/3 Rule”.



Recently, a variety of stable isotope reagents have been developed for relative quantification in proteomics, including ICAT®, SILAC, ^{18}O , AQUA™, and iTRAQ (1,2,3). Most methods enable quantification in the full MS scan, and peptide identification based on subsequent fragmentation (MS/MS) of precursor ions, with the exception of iTRAQ, where both the identification and quantification are performed in the MS/MS scan. Fragmentation of iTRAQ labeled peptides results in conversion of the iTRAQ tag into 4 reporter ions at m/z 114, 115, 116, or 117. These low m/z reporter ions fall below 28% of the mass of all but the smallest labeled peptides. As such, this restriction was thought to make the iTRAQ reagent incompatible with ion traps.

A new fragmentation mechanism, pulsed-Q dissociation (PQD), eliminates the “1/3 Rule” for ion traps. Developed for the Finnigan LTQ™, PQD (shown in Figure 2) involves precursor activation at high Q, a time delay to allow the precursor to fragment, then a rapid pulse to low Q where all fragment ions are trapped. The product ions can then be scanned out of the ion trap and detected. In principle, PQD should now allow analysis of useful low mass ions such as peptide immonium ions and iTRAQ reporter ions with the Finnigan LTQ linear ion trap mass spectrometer.

FIGURE 2. Mechanism for linear ion trap PQD. No low mass cutoff.



Methods

- Samples:**
- 1) BSA digest (Michrom Bioresources, Auburn, CA)
 - 2) Human transferrin (Sigma, St. Louis, MO), reduced, alkylated and proteolyzed.
 - 3) 6 protein mixture created from digested horse myoglobin, horse cytochrome-C, BSA, bovine beta-lactoglobulin, bovine carboxypeptidase A and bovine carbonic anhydrase (all from Michrom Bioresources, Auburn, CA).

Basic PQD performance was assessed with BSA and transferrin digests. For iTRAQ analysis, an equimolar mixture of 6 proteins (listed above) was prepared. 4 identical aliquots were labeled with 114, 115, 116, and 117 iTRAQ reagents as per product instructions and then combined to give a sample which should yield a 1:1:1:1 ratio for each protein.

LC/MS

HPLC System: Surveyor™ MS Pump plus MicroAS autosampler
 Columns: C18 trap column
 Picofrit 75 μm x 15 μm tip x 10 cm C-18 column
 Gradient: 0-50% B, 90 minute (MA: 0.1%FA H₂O, MB: 0.1%FA MeCN)
 Nanoflow-ESI, with post-split flow rate of 200nL/min
 Sample Load: 5 μl (100fmol/ul) injection with Micro AS

Mass Spectrometer: Finnigan LTQ
 Spray voltage: 2.0kV
 Capillary temperature: 160 °C
 Capillary Voltage: 46.0
 Tube Lens (V): 160
 MSⁿ Target: 4x10⁴, 2 μs cans, 200ms
 Scan types: Full MS: 400-1200 m/z
 Data-Dependent™ MS/MS on top 4 ions with PQD (Collision Energy:45%)
 Data-Dependent MS/MS on top 4 ions with CID (Collision Energy:45%)
 Dynamic Exclusion™: Off

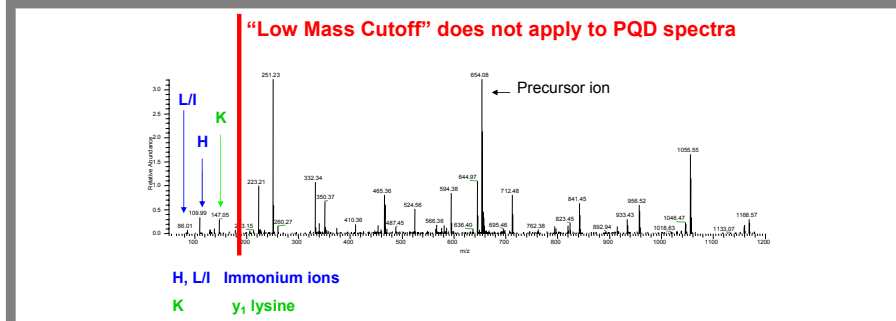
Database Searching:

BioWorks™ 3.2 with SEQUEST®
 Basis for peptide ID: Xcorr vs. Charge State filter (+1, Xcorr 1.5; +2, Xcorr 2.0; +3, Xcorr 2.5)
 2 or more peptides

Results

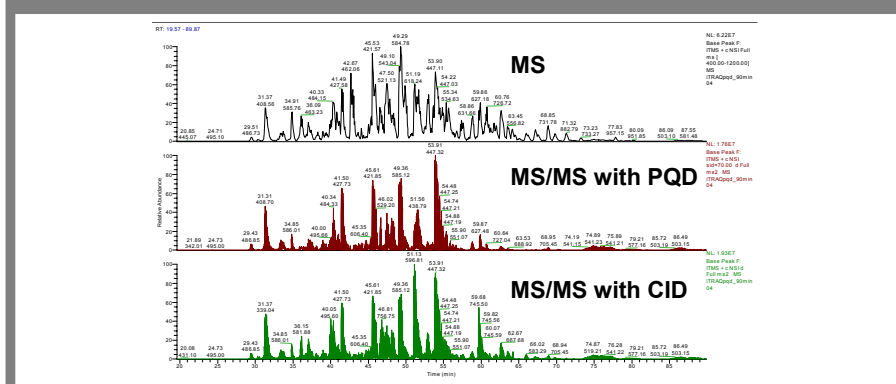
PQD can be used like any other scan mode on the Finnigan LTQ. In these experiments, four Data Dependent PQD scans were followed immediately by four Data Dependent CID scans with Dynamic Exclusion disabled so that both scan modes would be used to analyze the same peptides. Note that PQD scans require approximately the same time to execute as CID spectra, so there is no time penalty to perform these scans and no loss of protein coverage. Figure 3 shows a PQD fragmentation spectrum of a BSA peptide HLVDPEQNLIK. The precursor ion was at m/z 653. If the “low mass cutoff” applied, we would not expect to see any ions below m/z 653 x 0.28 = m/z 183. This spectrum shows the clear presence of immonium ions for L/I and H plus the y_1 sequence ion for K.

FIGURE 3. PQD spectrum of BSA peptide HLVDPEQNLIK. Note the presence of low m/z immonium and y_1 sequence ions below the “low mass cutoff”. This cutoff does not apply to PQD spectra.



The iTRAQ labeled 6 protein mixture was analyzed using PQD. Figure 4 shows the base peak chromatograms from the MS, CID, and PQD scans. They are qualitatively very similar. Note that the base peak intensity for CID and PQD are nearly identical, indicating strong ion formation by PQD.

FIGURE 4. MS and MS/MS elution profiles of iTRAQ peptides.



The spectrum from a representative labeled peptide is displayed in Figure 5. It shows the strong presence of iTRAQ reporter ions at m/z 114-117. The inset shows that the relative intensities of these ions is very nearly the 1:1:1:1 theoretical ratio. It is important to note that the iTRAQ reporter ions did not interfere with identification of the protein during database searching with SEQUEST. Searching was done with a static modification of 144 Da to peptide N-termini and a differential modification of 144 Da to K residues.

FIGURE 5. Ratio calculation for identified peptide QNCDQFEK* by PQD MS/MS. Inset shows iTRAQ reporter ions and calculated ratios of relative abundance.

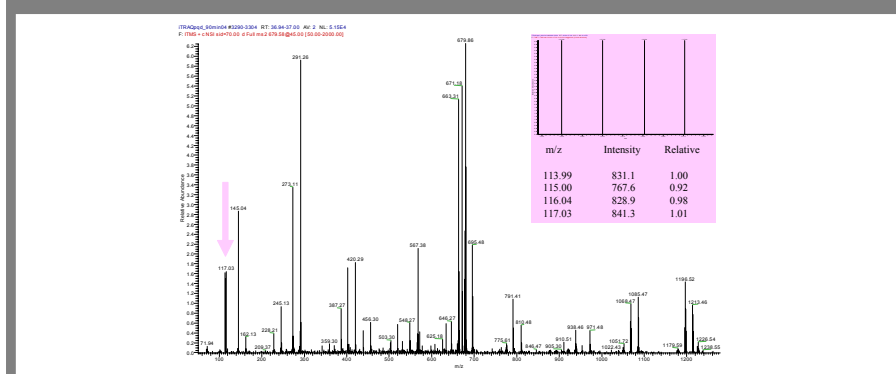


Table 1 summarizes results from iTRAQ analysis of the 6 protein mixture. Many peptides were identified for each of the proteins in the mixture. Protein sequence coverage was generally excellent. Relative quantitation of the proteins in the 4 samples showed good agreement with the expected ratios of 1:1:1:1. iTRAQ peak ratios were consistent for both intense and weak peptide ions.

TABLE 1. Results of iTRAQ analysis of the 6 protein mixture

Protein	# Peptides	Coverage %	114/114	114/115	114/116	114/117
BSA	23	38	1.00	0.95	0.91	0.93
Carboxypeptidase	7	19	1.00	0.89	0.81	0.86
beta-Lactoglobulin	6	51	1.00	0.98	0.93	0.99
Cytochrome-C	6	47	1.00	1.04	1.05	1.10
Myoglobin	4	26	1.00	0.98	0.90	0.98
Carbonic Anhydrase	3	15	1.00	1.23	1.26	1.22

Standard deviations of iTRAQ peak ratios for all 49 peptides used in this analysis were 16% or less which is in good agreement with other recently published studies¹.

Conclusions

PQD eliminates the “low mass cutoff” for fragment ions on the Finnigan LTQ. This scanning method is easily incorporated into general Xcalibur™ software methods and can be used in conjunction with CID, MSⁿ, and other scan functions. This study shows the use of PQD on the Finnigan LTQ to create and analyze:

- 1) Immonium ions
- 2) y_1 and b_1 sequence ions
- 3) iTRAQ reporter ions: good experimental agreement with theoretical values and standard deviations in line with other recently published studies.

References

1. DeSouza et al., Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and ICAT. J Proteome Rsch. 2005 Mar-Apr; 4(2)

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