Protein Identification and Quantification using an Ion Trap with Enhanced Detection in the Low Mass Range

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Overview

Purpose: To overcome low mass limits on ion traps, a new activation method has been developed, called pulsed-Q dissociation, PQD.

Methods: Peptides from a protein digest were analyzed using NanoLC-MS/MS using conventional CID as well as the new technique, PQD.

Results: Low mass ions, such as y_1 and b_1, were observed within the MS/MS spectrum of many precursor ions. Low mass and isobaric labels were also used for relative quantification in the MS/MS mode.

Introduction

Protein identification with ion trap mass spectrometers relies on the detection of sequence ions in the MS/MS fragmentation spectra. The b- and y- ion series provide complementary information based on fragmentation of the peptide backbone in opposite directions. Traditionally, ion traps miss the y_1 and b_1 fragment ions because of the low mass cut-off. Isobaric tags used for peptide quantification are also often absent in the MS/MS spectrum because they have an *m/z* range from 114 to 117 and typically derive from precursor ions with an *m/z* greater than 400.

A newly developed dissociation technique, called pulsed-Q dissociation (PQD), provides a solution to the low mass cut-off issue with ion traps. By varying the Q values during fragmentation, PQD allows good precursor fragmentation for the detection of all low mass ions without the low mass cut-off limitation. PQD has been effective at detecting y_1 and b_1 ions, as well as reporter ions for peptides labeled with isobaric tags.

Protein identification with the PQD method will be demonstrated with digests of simple protein mixtures and digests of complex cell lysates. Detecting y_1 and b_1 sequence ions improves the confidence of matching an MS/MS spectrum to a peptide sequence in the database. The ability of the PQD method to efficiently fragment and detect the isobaric tags will be demonstrated with protein digests of simple mixtures. The resulting ratio of labels will be compared to the theoretical ratios to determine the overall variance in the method.

Methods

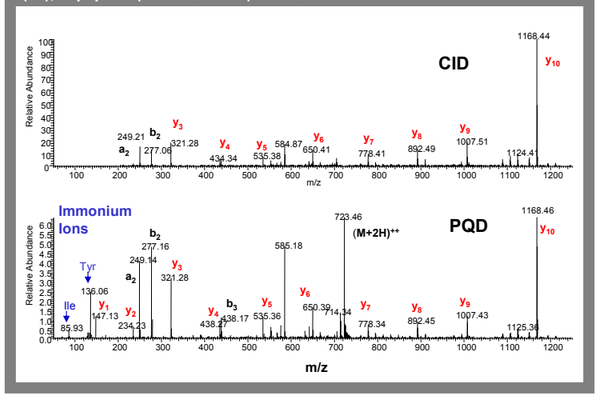
LC/MS

HPLC System:	Surveyor™ MS Pump plus MicroAS autosampler
Columns:	Agilent® C18 trap column PicoFrit® 75 µm x 15 µm tip x 10 cm C18 column
Gradient:	0-50% B, 90 min. (A: 0.1%FA H_2O, B: 0.1%FA ACN) Nanoflow-ESI, with post-split flow rate of 200nL/min
Sample Load:	5 µl (100fmol/ µl) injection using Surveyor MicroAS
Mass Spectrometer:	Finnigan™ LTQ™ linear ion trap using Data Dependent MS/MS on top 4 ions with PQD or CID.
Spray voltage:	2.0kV
Capillary temp:	160°C
Capillary Voltage:	46.0V
Tube Lens (V):	160V
MS^n Target:	$4x10^4$, 2µscans, 200ms
Scan types:	Full MS: 400-1200 *m/z*
Collision Energy:	45%
Dynamic exclusion:	Off

Sample Preparation: Bovine Serum Albumin, BSA, was enzymatically digested and re-constituted to 1pmol/µl with water containing 0.1% formic acid. Four identical mixtures containing 50 pmol of each of digested protein were prepared and dried, and re-constituted with 30 µl dissolution buffer provided by the iTRAQ™ kit. The four tubes were labeled individually with all four iTRAQ reagents: 114, 115, 116 and 117, respectively. The contents of each iTRAQ-labeled tube were combined and purified on a cation exchange cartridge. The resulting mixture was eluted from the exchange cartridge with 500 µl elute buffer provided in the iTRAQ kit. The prepared sample mixture (100 fmol/µl with iTRAQ reagents labeled at 1:1:1:1 ratio) was used for LC-MS/MS analysis.

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), two or more peptides

FIGURE 1. Comparison of CID and PQD scans for 20fmol: YICDNQDTISSK (+2), a peptide (scans 286-297) from BSA .



Results

A strong y-ion series is seen with CID in the MS/MS spectrum for the BSA peptide (Figure 1, top spectrum), but the y_1 and y_2 ions are missing. However, both these y-ions are observed with PQD activation in the bottom MS/MS spectrum, along with the immonium ions from tyrosine and isoleucine. The CID and PQD spectra were obtained in the same run by alternating scans that were a fraction of a second apart. The PQD is not quite as efficient as CID in precursor fragmentation because some residual precursor ion is observed in the PQD, but not in the CID spectrum. However, the CID and PQD spectra are otherwise quite similar, and all the major ions observed in the CID spectrum were also present in the PQD spectrum.

The PQD technique also detects low mass tags such as the iTRAQ label. The iTRAQ label attaches to the N-terminal amino group of peptides and to the epsilon amino group of lysine. It fragments from the peptide to produce the iTRAQ signature ion (*m/z* 145) in the MS/MS spectrum. The signature ion further fragments into iTRAQ reporter ions (114 to 117 *m/z*, depending on the tag) plus a balance group which varies with the tag to produce just one iTRAQ signature ion (*m/z* 145). The balance group makes the iTRAQ label isobaric.

Strong y-ion and b-ion series are observed in the PQD spectrum, shown in Figure 2, for an iTRAQ-labeled peptide from a BSA digest. The four iTRAQ reporter ions are present at nearly the same intensity in this spectrum. The lysine y_1 ion is seen at *m/z* 291 instead of *m/z* 147 because the iTRAQ label attached to the epsilon amino group of lysine at the C-terminus of the peptide. This labeled lysine ion is often seen in CID spectra, and when followed by MS^3 on the *m/z* 291 ion, the iTRAQ reporter ions can also be quantified in the MS^3 scan.[1]

FIGURE 2. PQD scans for QTALVELLK in bovine serum albumin digest derivatized with the iTRAQ reagent.

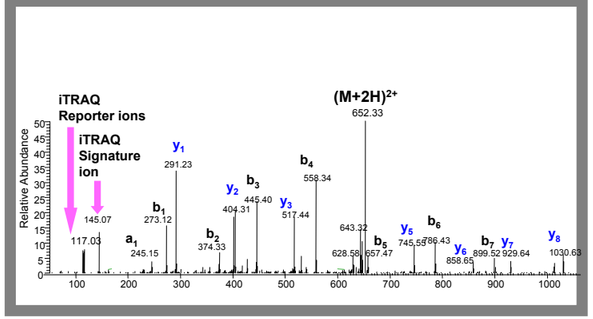
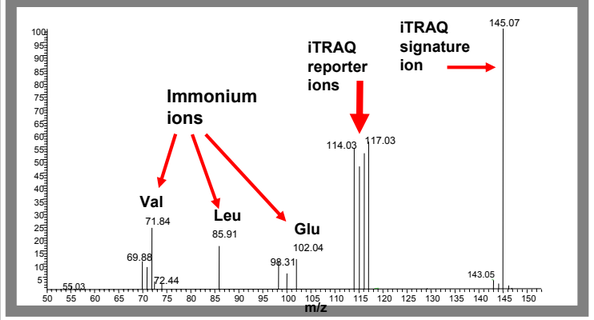


FIGURE 3. PQD scans for QTALVELLK in bovine serum albumin digest derivatized with the iTRAQ reagent, low mass range only.



Expanding the low range for the PQD scans of the doubly-charged precursor ion for QTALVELLK (scan 612) in Figure 3 reveals the iTRAQ signature ion, the four expected iTRAQ reporter ions and three prominent immonium ions. The immonium ions are consistent with the peptide sequence.

The four iTRAQ reporter ions have roughly the same intensity, with a relative standard deviation of 8.7% for the raw intensities, which is comparable, if not better than previously reported data.[2] When the 115, 116 and 117 iTRAQ reporter ions are compared to the 114 ion, the ratios vary from 0.87 to 1.05. This is in good agreement with the theoretical ratios of 1:1:1:1.

The MS/MS spectrum with PQD activation in Figure 4 shows a good y-ion series and clearly detects the iTRAQ signature ion and all four iTRAQ tags. The intensities of the iTRAQ reporter ions are more clearly shown in the pink color insert along with absolute and relative intensities of the ions. The relative standard deviation of the raw intensities is surprisingly low, at 4.1%. The ratios relative to the 114 iTRAQ reporter ion vary from 0.92 to 1.01, which is in excellent agreement with the theoretical ratios.

The results for all twenty iTRAQ-labeled peptides are shown in Table 1. The peptides here represent about 36% of the BSA sequence. The average relative standard deviation for the raw intensities of the four iTRAQ labels from all twenty BSA peptides was 10.5%. This compares well with previously reported data.[3]

FIGURE 4. PQD scans for QNCDQFEK in bovine serum albumin digest derivatized with the iTRAQ reagent.

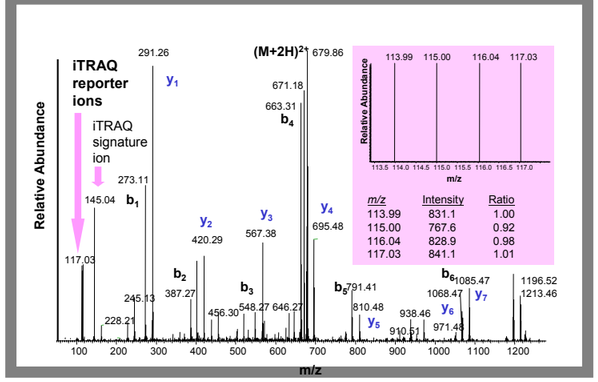


TABLE 1. iTRAQ-labeled peptides from BSA in a 6-protein digest.

Albumin [Bos taurus]	114 Intensities	Std. Dev.	iTRAQ ion ratios 115/114	116/114	117/114
Averages		10.5%	0.95	0.91	0.93
K.T]CVADESHAGCEK*.S	228	17.6%	0.75	0.69	0.73
K.Q]NCDQFEK*.L	817	4.1%	0.92	0.98	1.01
K.S]HCIAEVEK*.D	2507	25.6%	0.55	0.77	0.98
K.V]TK*CCTESLVNR.R	803	14.4%	0.89	0.71	0.81
K.Y]ICDNQDTISSK*.L	382	9.1%	0.92	0.82	0.85
R.L]CVLHEK*.T	4886	5.2%	1.02	0.97	1.10
K.A]EFVEVTK*.L	32224	4.7%	0.87	1.06	1.00
K.L]VTDLTK*.V	68952	3.3%	1.00	0.93	0.97
K.C]CAADDK*EACFAVEGPK*.L	2319	10.5%	0.96	0.82	0.82
K.H]LVDEPQNLIK*.Q	1636	14.9%	0.89	0.75	0.74
R.R]HPEYAVSVLLR.L	1584	8.5%	1.22	1.17	1.10
K.Y]LYEIAR.R	14923	5.2%	0.95	0.88	0.96
R.R]PCFSALTPDETYVPK*.A	2794	13.3%	1.20	0.89	1.15
K.SLHTLFGDELCK*.V	3362	9.6%	1.15	0.98	1.18
K.Q]TALVELLK*.H	9451	7.7%	0.87	0.96	1.05
K.L]VNELTEFAK*.T	11336	7.6%	0.95	0.83	0.91
K.L]GEYGFQNALIVR.Y	4410	14.5%	1.03	0.97	0.73
K.L]FTFHADICTLPDTEK*.Q	102	9.0%	0.81	0.98	0.92
K.T]VMENFVAFVDK*.C	1090	18.8%	0.85	1.00	0.65
K.D]AFLGSFLYEYSR.R	101	10.1%	1.07	0.88	0.87

The average of the iTRAQ ratios for all the peptides from bovine serum albumin were within 10% of the theoretical value of 1:1:1:1. The ratios ranges from a low of 0.55 to a high of 1.22. However, the average ratio for each tag ranged from 0.93 to 0.95. This demonstrates the value of averaging the ratios of all peptides from a given protein to determine an overall level for a given protein.

Conclusions

The new PQD fragmentation technique generates low mass ions below 150 *m/z* which are detectable and quantifiable:

- **Immonium ions are detectable with PQD**
- **y_1 and b_1 ions are detectable with PQD**
- **iTRAQ reporter ions are detectable with PQD**
- **iTRAQ reporter ions are quantifiable with PQD**

References

1. *Quantitative App. Note 353: Identification and Quantification of iTRAQ Labeled Peptides on the Finnigan LTQ using MS/MS and MS3*, R. Kiyonami, T. Schlabach and K. Miller, Thermo Electron Corporation, 2005
2. *Quantitative Proteomic Analysis Using Isobaric Protein Tags Enables Rapid Comparison of Changes in Transcript and Protein Levels in Transformed Cells*, R. D. Unwin, A. Pierce, R. B. Watson, D. W. Sternberg, and A. D. Whetton, Mol. Cell. Proteomics, Jul 2005; 4: 924 - 935.
3. *Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with multidimensional liquid chromatography and tandem mass spectrometry* DeSouza L, Diehl G, Rodrigues MJ, Guo J, Romaschin AD, Colgan TJ, Siu KW. J Proteome Res. 2005 Mar-Apr;4(2):377-86.

Acknowledgements

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