Higher-Energy Collisional Dissociation in the Existing High Pressure Multipole Region of a Stand-Alone Ion Trap Mass Spectrometer

Philip M. Remes[1], Jae C. Schwartz[1], Graeme Mcalister[2], Julie Horner[1], Terry Zhang[1], Reiko Kiyonami[1], August Specht[1], Joshua J. Coon[2]
[1]Thermo Fisher Scientific, San Jose, CA, [2]University of Wisconsin-Madison, Madison, WI

Overview

Purpose: Characterize higher-energy collisional dissociation (HCD) in the existing high pressure multipole (MP00) of a linear ion trap mass spectrometer (ITMS).

Methods: Conditions for efficient transfer of ions through the various optics were studied along with appropriate dissociation parameters. TMT-labeled peptides were used for comparative evaluations of HCD with other common modes of dissociation.

Results: HCD can be performed in < 3 milliseconds with MS/MS efficiency of ~40%. The traditional rCID low-mass cutoff is circumvented, allowing an ITMS to analyze low mass fragment ions.

Introduction

A method for higher-energy collisional dissociation (HCD) in an ion trap mass spectrometer (ITMS) has been developed. Precursor ions are selected in the ITMS and passed to the high pressure RF-only multipole (MP00) for dissociation. Fragment ions are then transferred back to the ITMS for mass analysis. Conventionally, collision induced dissociation in an ITMS is performed by resonantly exciting precursor ions (rCID) in the presence of buffer gas. This method is robust and informative. However, low-mass fragment ions are not captured and generally only primary fragmentation pathways of lower energy are observed. By performing HCD in MP00, both of these limitations are overcome, opening the way for applications such as peptide quantification by isotopic labeling strategies.

Methods

Sample Preparation

A trypic digest of a standard 12-protein equilmolar mix was labeled with Thermo Scientific Pierce Tandem Mass Tag sixplex (TMTsixplex) reagents, cleaned up with Thermo Scientific Stage C18 tips, and diluted to 1 pmol/µL with 50:50 acetonitrile:water, 0.1% formic acid. The reporter ion ratios for 126:127:128:129:130:131 were 10:4:1:1:4:10. The proteins in the mix were transferrin, lysozyme, ß-lactoglobulin, BSA, GAPDH, myoglobin, cytochrome C, a-lactalbumin, ovalbumin, carbonic anhydrase, ß-casein, and a-casein.

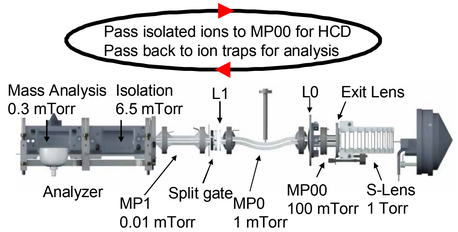
Labeled and non-labeled versions of the 12-protein mixture were used in the collision energy calibration section.

The peptide MRFA and Ultramark 1621 were utilized from the standard LTQ ion trap calibration solution. Angiotensin I (Sigma) was made in 50:50 methanol:water at 1µM.

Mass Spectrometry

Data labeled QqQ were acquired on a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer. All other data were acquired with a Thermo Scientific Velos Pro linear ion trap mass spectrometer.

FIGURE 1. Velos Pro instrument setup. To perform HCD, ions are isolated in the higher-pressure cell of the dual-cell analyzer, transferred to MP00 at high energy (40-100 eV) , and finally transferred back to the analyzer for analysis. The transfer of ions takes approximately 1 ms for each direction.



Results

General Observations

- HCD spectra resemble triple quad CID spectra (Figures 2 and 3).
  - Often produces equal intensity series of y ions
  - TMT fragment ratios are the same for HCD and QqQ
- rCID spectra usually have a few preferred fragmentation channels with high intensity.
- PQD spectra have aspects of both rCID and HCD.

FIGURE 2. MS/MS of a TMT-labeled peptide *m/z* 620 (2+), via various activation methods.

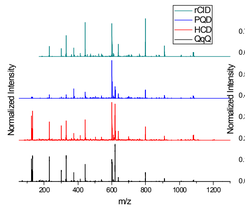
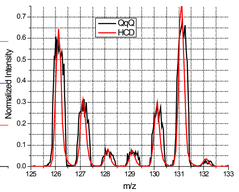


FIGURE 3. Zoom of QqQ and HCD spectra from Figure 2. Reporter ions were prepared with the ratio 10:4:1:1:4:10.



Performance Metrics

- MS/MS efficiency is a common metric for evaluating the performance of an activation method. It quantifies the capacity to both produce fragment ions and collect them.

$$MS/MS Efficiency = \frac{\sum_i fragment_i}{InitialParent}$$

- For HCD, the precursor ion spectrum was acquired without any transfer to MP00.
- rCID is the most sensitive method (Figure 4), and has the broadest optimum in the normalized collision energy scale (NCE)[1].
- HCD and PQD have similar efficiencies.
- The efficiency of the transfer process itself was measured by observing the decrease in signal as an ion is passed back and forth successive times from the high-pressure ion trap cell to MP00 before acquisition (Figure 5).
  - The slope of the data gives the average of the forward and backward transfers.

FIGURE 4. Comparison of the efficiency of ITMS activation methods for various TMT-labeled tryptic peptides.

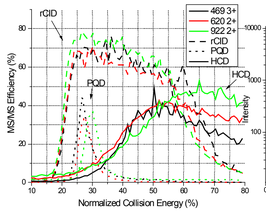
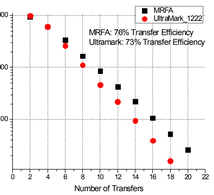


FIGURE 5. Log of intensity vs. number of transfers for MRFA (*m/z* 524) and Ultramark (*m/z* 1522). The slope gives the average transfer efficiency.

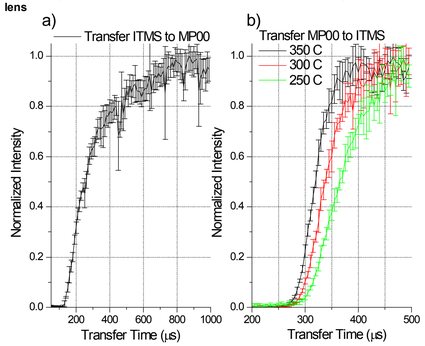


Transfer Time

- Transfer from ITMS to MP00 takes ~1000 µs (Figure 6a)
  - Center section of ion trap is longer (~36 mm) than MP00 (~25 mm), increasing extraction time
- Transfer from MP00 to ITMS takes ~500µs (Figure 6b)
  - Depends on temperature of capillary, which in turn affects the MP00 pressure
  - Capillary conductance (C) is related to temperature through gas viscosity(?)[2]

$$C \propto \frac{diam^4}{\eta \cdot length} \cdot pressure_{avg} \qquad \eta = \frac{Temp^{3/2}}{Temp + const}$$

FIGURE 6. Intensity of MRFA (*m/z* 524) vs. time. a) Ions were released from the ITMS and gated with L0. b) Ions were released from MP00 and gated with split lens



Reporter Ion Ratio Variation

- Relative quantification of isotopic mass tag labeled proteins relies on reliable measurement of reporter ion intensity ratios
- Reproducibility of ratios is fundamentally limited by Poisson statistics:
  - Ion number distribution has mean (?) = variance (s^2)

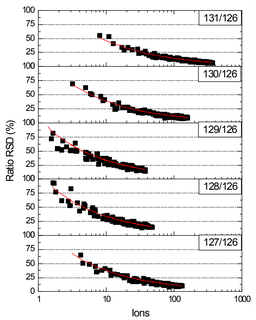
$$RSD = \frac{\sigma}{\lambda}$$

- For the ratio of two peaks with mean number of ions $?_1$ and $?_2$, the ratio RSD is limited by:

$$ratio RSD = \sqrt{\frac{1}{\lambda_1} + \frac{1}{\lambda_2}}$$

- The ratio of TMT reporter ions was measured vs.. number of ions in numerator
- Number of ions was calibrated using method of [3].
- The ratio RSD follows the theoretical limit very closely

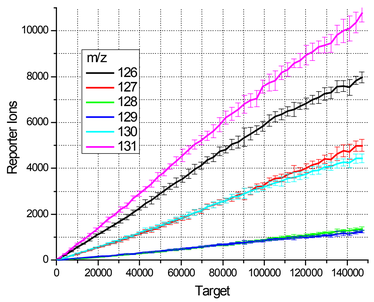
FIGURE 7. RSD of reporter ion ratios for TMT labeled peptide *m/z* 620 (2+) as number of ions is varied. Black dots are measurements, red line is theoretical limit. X axis refers to numerator.



Linearity of Reporter Ion Intensity

- Linearity of reporter ion response is maintained for large numbers of precursor ions.
  - LTQ typically isolates 100k ions with > 50% efficiency at 1 Da isolation width (data not shown). Figure 8 shows minimal flattening of response.
- In conjunction with Figure 7, the number of reporter ions vs. ion target setting in Figure 8 reveals the optimum target settings for this 2+ tryptic peptide (*m/z* 620).
  - More than 50 ions/reporter channel is achieved at a target of 10k, assuming the maximum injection time isn't reached, i.e. peptide concentration is high enough
  - 50 ions per channel → 20% RSD for 1:1 ratio

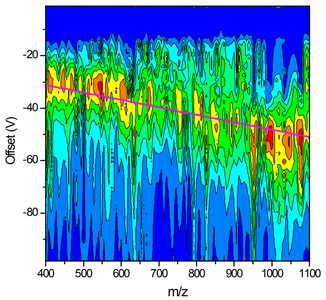
FIGURE 8. Number of reporter ions vs. instrument target setting for *m/z* 620 (2+). Reporter ions have ratio 10:4:1:1:4:10



Collision Energy Calibration

- A normalized collision energy scheme (NCE) [1] was developed for HCD to programmatically determine the optimum MP00 offset for a given mass-to- charge.
- Offset was varied from -10 to -100 in a LC/MS study of thousands of peptides, both labeled and unlabeled, from the aforementioned 12-protein mixture, and also digested bovine fetuin, bovine serum albumin, and equine myoglobin (Figure 9).
- Results could be related to compounds in the standard LTQ calibration solution to implement a charge state dependent NCE scheme.

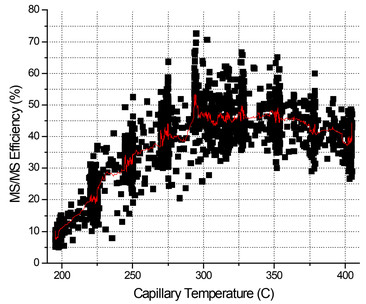
FIGURE 9. Fragment intensity vs. MP00 offset and mass-to-charge for 2+ ions of non-labeled trypic digest of standard 12-protein mix



Effect of Capillary Temperature

- The capillary temperature has an effect on MS/MS efficiency (Figure 10).
- The fragmentation process itself is somewhat dependent on the pressure and temperature of the gas in MP00.
- In addition, scattering losses can increase in MP00 as the pressure is increased from the lower temperature, and ions also take longer to diffuse to the exit.
- Typically temperatures of 250 °C and above are used.

FIGURE 10. MS/MS efficiency as a function of capillary temperature for the peptide angiotensin I, *m/z* 648 (2+).



Conclusion

HCD performed in the existing high pressure multipole (MP00) produces triple-quad-like fragmentation spectra in a linear ion trap MS

- Mass range is now defined by multipole optics i.e. conventional rCID low mass cutoff is circumvented
- MS/MS efficiency is ~40% for peptides.
- Sum of transfer processes is < 3 ms
- Good TMT reporter ion statistics (>50 ions/channel) are achieved with reasonable instrument targets (10-40k)

References

1. Schwartz, J.C., Taylor, D.M. U.S. Patent 6,124,591, 1999.
2. Moore, J.H., Davis C.C, Coplan M.A, *Building Scientific Apparatus, 3rd ed.; Westview* Press: Boulder, **2002**.
3. Schwartz, J.C. U.S. Patent 7,109,474 B2, **2006**.

Acknowledgements

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