Characterization of a Novel Ion Mobility-Tandem Mass Spectrometry Approach

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Overview

• Collision induced dissociation (CID) efficiency of a segmented quadrupole was evaluated.
• CID efficiency inside the quadrupole was higher than that at the quadrupole terminal.
• An IMS-MS/MS approach was applied to a tryptic digest of BSA.
• We confidently identified 20 peptides within ±15 ppm for parents and fragments.
• FDR was found to be 0.6% using a decoy database approach.

Introduction

Ion mobility is increasingly being utilized for the separation of peptides and proteins in tandem fashion with liquid chromatography and mass spectrometry (LC-MS/MS). Integrating an ion dissociation step for ion mobility separated ions enriches the information content of the data by providing the fragmentation pattern of precursor ions. Traditionally, CID was chosen to induce the ion fragmentation for drift time-specific precursor ions. CID is usually implemented by increasing the potential difference between two elements at the end of, or following, the drift tube to deposit enough energy into the ions causing ion fragmentation. Here, we compare the efficiency of two CID approaches utilized in a quadrupole downstream of the drift tube.

Methods

• FWHM = 18.5

Results

Collection, fragmentation and CID efficiencies

Example of “RHPEYAVSVLLR” IMS-MS/MS

Conclusions

• We evaluated the CID efficiency of IMS-MS/MS instrument using a segmented quadrupole.
• CID efficiency of nearly 100% was observed for neutral PI ions inside the quadrupole compared to 25% at the quadrupole terminal.
• CID efficiencies of different peptide showed similar trends.
• Rich information content was observed in the MS/MS spectrum along with good MMA (<20 ppm).
• The IMS-MS/MS approach was validated using tryptic digest of BSA.
• Using MMA of ± 15 ppm for the precursors and the fragments, 13 fragments per parent and IMS information, we identified 20 peptides of the BSA tryptic digest.
• An average of 14 fragments were identified per peptide.
• FDR was evaluated using a decoy tryptic digest database of glyoxal phosphorylated (PYG).
• Only one peptide was identified for PYG.
• Incorporating IMS-MS/MS information decreased the FDR from 35.3% to 0.6%.

Acknowledgements

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References


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Instrument Setup

• The instrument setup: ion funnel trap, 86 cm drift tube, segmented quadrupole and an Agilent oa-TOF
• Pressure in:
  – drift tube: 4 Torr (nitrogen)
  – segmented quadrupole: 200 mTorr
• Segmented quadrupole RF: f = 880 MHz, Vpp=200 V
• The collision energy was varied by the DC gradient between the fourth and the IR segments.

False Discovery Rate (FDR)

• An average of 14 fragments were identified per peptide
• Most of the peptides can be identified using only three collision energies

CAS 100-51-6 M+ P1 : Remaining parent intensity (Dissociation)

Full Potential Distribution

( sum of the effective and DC potentials)

No CID

CID at the terminal of the segmented quadrupole

CID inside the segmented quadrupole – Higher collection efficiency

Mass measurement accuracy (MMA)

Example of “RHPEYAVSVLLR” IMS-MS/MS

Precursor ions

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Instrument Setup: Quadrupole and a drift Tube (with Gas).