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A Structures for Lossless Ion Manipulations (SLIM) Module for Collision Induced Dissociation

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Abstract

A collision induced dissociation (CID) structures for lossless ion manipulations (SLIM) module is introduced and coupled to a quadrupole time-of-flight (QTOF) mass spectrometer (MS). The SLIM CID module was mounted after an ion mobility (IM) drift tube to enable IM/CID/MS studies. The efficiency of CID was studied by using the model peptide leucine enkephalin. CID efficiencies (62%) compared favorably to other beam-type CID methods. Additionally, the SLIM CID module was used to fragment a mixture of 9 peptides after IM separation. This work also represents the first application of SLIM in the 0.3 to 0.5 Torr pressure regime, an order of magnitude lower in pressure than previously studied.

Graphical Abstract

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Keywords

Collision Induced Dissociation; Ion mobility spectrometry; RF Confinement; ion optics; peptide fragmentation; manipulation; conveyor

Introduction

Collision induced dissociation (CID) is ubiquitous in mass spectrometry (MS). Since the advent of widely used “soft” ionization methods (viz. electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI)[1, 2]), CID has been invaluable for proteomics identification[3–8] and quantitation[9–11]. An important CID figure of merit is the CID efficiency (E_{CID} ; Equation 1)

$$E_{CID} = \frac{(\sum I_{product})(100\%)}{I_0} = (E_{Fragmentation})(E_{Collection})(100\%) \quad (1)$$

where $I_{product}$ is product ion intensity and I_0 is the initial precursor ion intensity. CID efficiency can also be calculated as the product of fragmentation efficiency (Equation 2) and collection efficiency (Equation 3).

$$E_{Fragmentation} = \frac{(\sum I_{product})(100\%)}{\sum I_{products} + I_{precursor}} \quad (2)$$

$$E_{Collection} = \frac{(\sum I_{products} + I_{precursor})(100\%)}{I_0} \quad (3)$$

where $I_{precursor}$ is remaining precursor ion intensity.

Previously, fragmentation techniques, such as photodissociation^[12], surface-induced dissociation[13, 14], and collision-induced dissociation[15–22] have been coupled to IMS for mobility-separated fragmentation of precursor ions. Fragment ions will retain the arrival ions of their respective precursors, provided the fragmentation occurs after the IM separation. In addition, recent reports have also demonstrated IM-selection for action spectroscopy [23, 24]. In this work, we introduce a new structures for lossless ion manipulations (SLIM) CID module for CID/MS. SLIM devices have been previously demonstrated for ion mobility (IM) separations[25–28], mobility-based ion selection[29], and ion trapping[30]. In this study, we demonstrate that SLIM is adaptable (and highly suitable) to applications outside of IM. We also show that SLIM devices are not limited to ~4 Torr and can provide effective ion transmission at lower pressures. The SLIM CID module was used to dissociate ions after an IM stage, providing fragmentation precursor peptides after a mobility separation. In this work we used the well-studied ESI thermometer

ion, leucine enkephalin[31], as well as a mixture of nine peptides, to evaluate the effectiveness of the SLIM CID module.

Experimental Arrangement

Leucine enkephalin was prepared in a 1 μ M solution of 50/50/1 (vol/vol/vol) water/methanol/acetic acid. An equimolar 1 μ M/each solution of 9 peptides (angiotensin I & II, bradykinin, fibrinopeptide A, kemptide, melittin, neurotensin, renin substrate tetradecapeptide, and substance P) was also prepared in 50/50/1 water/methanol/acetic acid. Water was purified by a Barnstead Nanopure set to 18 M Ω resistivity (ThermoScientific, Waltham, MA), peptides were purchased from Sigma-Aldrich (St. Louis, MO), and methanol and acetic acid were purchased from Fisher Scientific (Pittsburgh, PA). Solutions were infused at 300 nl/min from a chemically etched emitter nanoelectrospray source[32] into a home-built IM/MS^[21, 33, 34]. The design of the SLIM module, shown in Figure 1, was based upon previous designs used for ion mobility separations^[25] and other manipulations.^[35, 36] Briefly, ions are confined laterally using DC from ‘guard’ electrodes and vertically by pseudopotentials generated by RF applied in opposite phase to each adjacent electrode. The DC/RF electrodes have a superimposed DC gradient applied across a section of electrode such that ions will experience a constant electric field and traverse the device from high to low DC potentials (left to right in Figure 1). Two SLIM surfaces fabricated from PCBs are then mounted parallel to each other. The DC-only guards are black in Figure 1, and electrodes with both DC and RF are red. There are three independently controllable DC regions on the device. The first two regions (gradient 1 and 2) each span 11 DC-only guard electrodes and the final region contains a planar quadrupole-like geometry (exit region) to focus ions into the center of the device for entrance into the mass spectrometer (Agilent 6538 QTOF MS with a 1.5 m flight tube, Agilent Technologies, Santa Clara, CA). Therefore, higher electric fields suitable for CID can be applied across two regions: between the first two gradients and between the second gradient and quadrupole-like region. The DC fields in the gradients were restricted to a maximum of 15–16 V/cm, so that fragmentation was minimized when CID fields were not applied. The guards in the first two regions were biased 10 V_{DC} higher than the neighboring RF/DC electrodes. All three electrodes in the exit region were biased to the same V_{DC}. For IM/CID/MS, ions were accumulated in the ion funnel trap^[37–39] and released into the drift tube (4 Torr, 16 V/cm constant field) in 488 μ s pulses. The drift tube was followed by a rear ion funnel with a conductance limiting orifice, a short rf-only transmission quad, another conductance limiting orifice, and the SLIM CID module. Therefore, the pressure in the SLIM region could be varied without affecting the drift tube pressure.

Results

Leucine enkephalin was chosen as a model peptide for fragmentation as the fragmentation patterns are widely documented and understood^[31]. Figure 2 shows representative spectra of protonated leucine enkephalin. The DC voltage in Figure 2a between the end of gradient 2 and the exit region (V_{CID}) was 0 V. Applying a V_{CID} of 30 V (Figure 2b) results in extensive fragmentation, including cleavage of all peptide bonds (y₄, b₂, b₃, and b₄). The CID efficiency was 62%, comparing favorably to efficiencies in a triple-quadrupole and for 200

mTorr in a segmented quadrupole CID (36% in both cases)[21], and dipolar resonant excitation CID of methionine enkephalin at 80 mTorr (44%)[33].

Next, the CID efficiencies from applying V_{CID} between gradient 1/gradient 2 and gradient 2/exit region were measured (Supplemental Figure 1). V_{DC} was increased for each case until the fragmentation efficiency remained roughly constant (Equation 2). The maximum CID efficiency from gradient 2/exit region CID was 62% (30 V_{CID}). The maximum efficiency when V_{CID} was applied between gradient 1 and 2 was 50% (20 V_{CID}). The fragmentation efficiency for V_{CID} between gradient 2 and the exit region was 80% and between gradient 1 and gradient 2 was 84%. Therefore, the increase in CID efficiency for application of V_{CID} in between gradient 2 and the exit region was due to increased collection efficiency (77% versus 60%). Although both methods are equally efficient with the application of 20 V_{CID} , the collection efficiency was 60% between gradient 1 and 2 and 81% between gradient 2 and the exit region. Changes in collection efficiency are likely due to stronger ion focusing of product ions in the quadrupolar region than the RF/DC region, where ions move in closer proximity to surfaces^[26, 27].

Figure 3 shows two nested IM/MS spectra of a 9 peptide mix. Figure 3A was taken with 0 V_{CID} , and Figure 3B was taken with a 45 V_{CID} potential between gradient 2 and the exit region. After the application of CID, characteristic dissociation “ladder” patterns appear in the nested spectra. The product ions in the nested spectra appear vertically aligned with the arrival time of the precursor. Extensive dissociation was observed, showing the utility of IM/SLIM CID for ‘all-ion’ fragmentation.

Conclusions

We have introduced a CID-capable SLIM module including two CID regions. The most efficient CID was observed when the V_{CID} was applied between the second voltage gradient and the exit region. SLIM CID resulted in extensive fragmentation of the thermometer peptide ion protonated leucine enkephalin. SLIM CID coupled to an IM separation was exemplified with all-ion fragmentation of a mixture of peptides. In the future, SLIM CID will be coupled to high resolution SLIM IM separations to give higher peak capacities and direct connectivity of precursor ions to product ions without requiring mass selections for data-independent analysis experiments. Additionally, this study showed the ability of SLIM devices to transmit ions at lower pressures than pressures used in previous studies (i.e. 4 Torr). Present work is ongoing to optimize SLIM CID for higher pressures for more direct coupling to IM measurements without losses of resolving power or sensitivity due to changes in pressure. Once SLIM CID is integrated into existing SLIM modules, slower heating trapping/longer activation time experiments can be performed which will allow for higher CID efficiencies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Layout of electrodes on one of the two planar SLIM surfaces. Black (guard) electrodes are DC-only, and red electrodes are RF/DC. The DC was divided into two separate gradients of equal length, followed by an independently controlled DC for the planar pseudo-quadrupole region at the exit, where RF on the guard electrodes is the same phase and the RF on the central electrode is 180 out of phase with respect to the guards. Ions traverse the SLIM module from left to right.

Figure 2.

Representative spectra of protonated leucine enkephalin at 265 mTorr, 750 kHz, 200 V_{p-p} RF. (a) $V_{CID} = 0$. (b) $V_{CID} = 30$, CID efficiency = 62%.

Figure 3. Nested IM/MS spectra of a mix of 9 peptides at 365 mTorr, 750 kHz, 200 V_{p-p} RF. (a) 0 V_{CID} (b) 45 V_{CID}.