Using a branched RF-ion trap to combine EAD, ultraviolet-photodissociation (UVPD) and CID fragmentation

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INTRODUCTION

Collision induced dissociation (CID) is the widely used mode of fragmentation on tandem mass spectrometry (MS/MS) systems. As a dissociation technique, CID is efficient, fast and can be applied over a wide range of compounds. In some applications, more selective fragmentation techniques can offer complementary MS/MS information. Electron activation dissociation (EAD) and ultraviolet-photodissociation (UVPD) offer more selective means of fragmentating molecules, thus offering complimentary insight into the structure of gas phase ions. Here, we proposed using a branched radio-frequency ion trap (RF-ion trap) to allow both EAD and UVPD to be performed sequentially. Combining these fragmentation approaches with TOF-based detection offers a flexible system with higher selectivity for quantitation and full identification capability.

MATERIALS AND METHODS

Chemical:
Bosentan and its metabolites were obtained from Toronto Research Chemicals (Toronto, ON). Ubiquitin (UBT) and carbonic anhydrase (CA) were obtained from Sigma-Aldrich (St. Louis, MO).

MS Conditions:
EAD, UVPD and CID were performed on a modified research-grade quadrupole time-of-flight mass spectrometer, based on ZenoTOF 7600 system geometry. The conventional EAD cell has 2 electron filaments on either side of the chimera cell. To enable UVPD, an electron filament was replaced by a mirror to redirect the laser beam in the center of the cell (Figure 1). The mirror was selected to ensure >95% reflectivity at the selected wavelength.

Two different solid-state lasers were enabled on the system covering different wavelengths for different application areas. A 266 nm low energy pulse laser (0.5 μJ) was operated at 30 kHz and used for pharmaceutical compound analysis (Teem Photonics, France). Control of the 266 nm laser was based on activation for a specific duration up to 120 ms after ion trapping. A second laser (213 nm) was operated at high pulse energy (20 μJ) with a maximum repetition rate of 2kHz (Crylas Inc, Fremont, CA). Control of the 213 nm laser was based on the number of pulses (1 ms/pulse) applied expressed as a percentage of the trapping time selected in the software control. Therefore, for a trapping time of 50 ms and 10% UV activation, a total of 5 pulses would be applied.

A research version of SCIEX OS software was used to control the acquisition of data. The software provides the ability to perform EAD and UVPD independently. The UVPD implementation capitalized on the EAD timing control and used the conditions to trap and release precursor and fragment ions. Data processing, was performed using a research version of PeakView software which enabled a-1, x and x-1 fragment ion assignment. The spectra were noise-filtered prior to assessment of sequence coverage.

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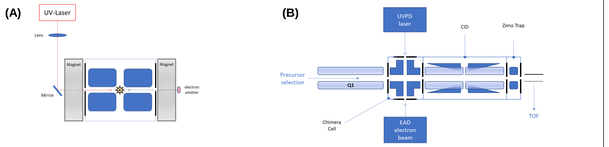
RESULTS

The branched RF-ion trap of the ZenoToF 7600 system is normally fitted with 2 electron emitters, with 1 on either side of the cell [1, 2]. One of these emitters was physically removed and replaced by a 45° angle mirror to redirect the UV laser mounted directly above, outside of the vacuum chamber. Two UVPD windows were used to maintain vacuum and transmit the laser beam (Figure1a). Figure 1a show the collision cell arrangement in which both EAD and UVPD occur in the branched RF-ion trap (also referred to as Chimera cell). CID occurs in the back portion. All 3 fragmentation modes are performed at 8-12 mTorr of nitrogen gas.

Figure 1. Schematic of RF-ion trap. A) Depiction of the front-view cross section of the RF-ion trap with UV-laser introduction from tip of the instrument, and mirror location to focus beam at center of trap. B) Representative top view of part of the ions path of the research grade 7600 depicting the region for UVPD and EAD.



Operating at 266 nm

Desmethyl-bosentan was used to verify system performance and for comparison to previously acquired UVPD (266 nm) data using an hybrid quadrupole linear ion trap (QqLIT) [3]. The unique UVPD fragment observed (m/z 366.2) was used to verify laser alignment. With an irradiation time of 75 ms, a >35% fragmentation efficiency was obtained, which is consistent with result obtained on a QqLIT [3] . Common and unique fragment ions were observed with EAD (10eV) and UVPD (266 nm), when compared to CID (45eV). The fragmentation information obtained with all 3 methods provided a complimentary picture of the positional side chain groups of the molecule. The ability to combine fragmentation information from different excitation techniques can offer additional insight into the structure of the analyte and improve confidence in the proposed analyte identification. One of the drawbacks of the 266 nm laser used in this work is that the energy per pulse is low, which means that longer irradiation time are required to achieve efficient fragmentation for small compounds. This can limit the chemical space that can be explored with this slow energy pulse laser (for example, inefficient beyond tri-peptide).

Figure 2. MS/MS spectra of desmethyl-bosentan using UVPD (top) and EAD (bottom) fragmentation.

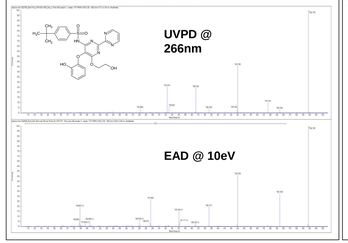
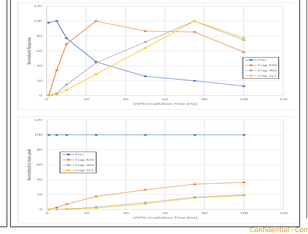


Figure 3. Fragmentation efficiency for desmethyl-Bosentan as a function of UVPD irradiation time with 266 nm laser



Operating at 213 nm

With this wavelength, it is possible to move to wider class of chemicals, including peptides and intact protein as previously demonstrated [4-5]. To evaluate the fragmentation efficiency of this laser, the depletion of the precursor ion of the $[M+12H]^{+12}$ and the $[M+7H]^{+7}$ of ubiquitin was determined as a function of the number of laser pulse applied. Figure 3 shows that >90% depletion was achieved within 20 pulses for both charge states. This represents <20 ms of irradiation time to generate the maximum fragmentation of the precursor. As previously reported, UVPD of protein generates a-1 and x-1 fragment ions that are unique to this type of fragmentation [5]. Figure 5 shows the normalized response for the $(a\text{-}1)_{38}{}^{+5}$ at m/z 795.5 and $(a\text{-}1)_{27}{}^{+5}$ at m/z 815 as a function of the number of laser pulses applied for the $[M+12H]^{+12}$ precursor ion. The peak response at approximately 20 pulses suggests that applying more pulses can lead to secondary fragmentation and consequently complicate the MS/MS interpretation. Figure 6 shows that 96% sequence coverage is obtained for the UVPD of the $[M+7H]^{+7}$ ion of ubiquitin using 10 pulses in 10ms. These condition were used to minimize secondary fragmentation that leads to internal fragments.

Figure 4. Depletion of the ubiquitin $[M+12H]^{+12}$ and $[M+7H]^{+7}$ as a function of the number of laser pulses

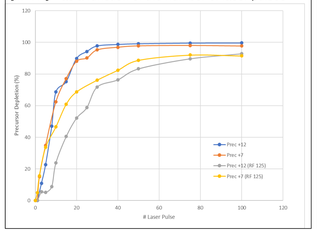


Figure 5. Formation of the $(a\text{-}1)^{+35}$ ion as a function of the number of laser pulses for ubiquitin $[M+12H]^{+12}$

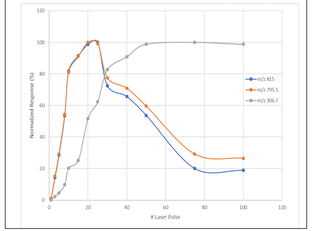


Figure 6. Sequence coverage for the UVPD of+7 ion with 10 laser pulses (10ms Irradiation)

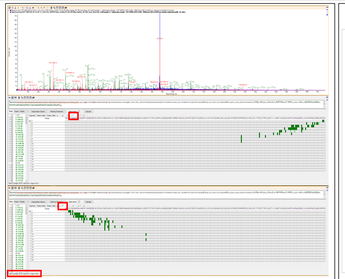


Figure 7. UVPD-MSMS and sequence coverage for carbonic anhydrase $[M+31H]^{+31}$. Three laser pulse were used. Only a, x, y ions were considered.

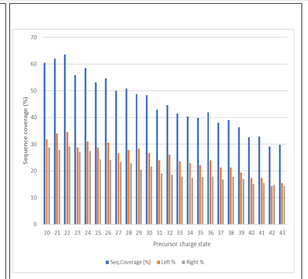


Figure 8. Sequence coverage obtained for carbonic anhydrase as a function of charge state. All data were acquired with 3 laser pulses.

Similar to ubiquitin, using a high number of laser pulses (>8) for carbonic anhydrase left little-to-no residual precursor ions, regardless of the charge state. Therefore, to avoid spectral complication resulting from internal fragmentation, UVPD-MS/MS was performed for all visible charge state s (20 to 413) using 3 laser pulses. Sequence coverage was calculated based on single bond cleavage (no internal fragmentation) and considering only a, x and y ions as fragment type. All spectra were denoised prior to peak assignment and matching. Figure 8 shows coverage as a function of charge states. Lower charge states clearly shows improved coverage, likely due to the reduced spectral complexity in the low mass region as the precursor mass increases. The coverages reported here are consistent with previously reported values [4,5]

CONCLUSIONS

UVPD was implemented in a branched RF-ion trap by replacing an electron filament. This offers the ability to perform UVPD and EAD MS/MS analyses independantly on a single platform. UVPD can generate unique fragments that can complement CID and EAD fragmentation and increase confidence in sequence assignment. The use of low power and high repetition rate laser can yield efficient fragmentation at the pressure regime used (8 mTorr) as long as the irradiation time is a few tens of milliseconds (50-100) making it still attractive for small molecule applications. However, using higher energy pulse laser (tens of uJ) in the same pressure regime can reduce the fragmentation time significantly (<10 ms) even for large molecules. Given the short fragmentation times, and the selectivity provided by UVPD fragmentation, combining with LC and SWATH DIA will be the focus of our future work.

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