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Proteomic Coverage Comparison of On- and Off-line LC-IMS-MS with Traditional LC-MS Platforms

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Overview

- An ESI-IMS-TOF MS was designed that utilizes high-pressure electrodynamic ion funnels on both sides of the drift cell. These ion funnels allow the pressure in the drift cell to be increased from 4 Torr to 12 Torr, enabling higher IMS resolving power.
- *Salmonella typhimurium* was analyzed with LC-MS and NanoMate™-MS. The LC and NanoMate™ sample introduction methods were compared to determine which method results in more features.
- *Shewanella oneidensis* was studied with LC-IMS-MS and LC-MS (LTQ-FT) using the same LC system, enabling direct comparison of IMS and MS.
- IMS-MS fragmentation measurements were acquired to determine if they aid in increasing the quality of IMS-MS peptide/protein identifications.

Introduction

The ability of ion mobility spectrometry coupled with mass spectrometry (IMS-MS) to separate ions by conformation (Figure 1) has shown great potential for analyzing complex samples. However, challenges posed by the extreme complexity of many biological samples have demonstrated the need for IMS-MS measurements with higher resolution and broader dynamic range.

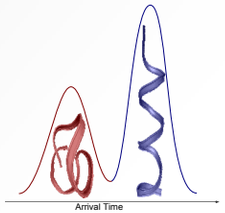

Figure 1: IMS separates ions of the same *m/z* by shape.

While higher resolution can be obtained by either increasing the length of the drift cell or the drift gas pressure, a wider dynamic range is achieved either by on-line coupling of a LC system to the IMS instrument or off-line sample fractionation and sample introduction using a NanoMate™.

Since previous LC-IMS-MS experiments have resolved peptide isomers indistinguishable by LC-MS alone, we provide a comparison of LC-IMS-MS, LC-MS, NanoMate™-IMS-MS and NanoMate™-MS.

Methods

Instrumentation

A new ESI-IMS-TOF MS was recently completed that incorporates electrodynamic ion funnels before and after the drift cell allowing the drift cell pressure to be increased from 4 to 12 Torr, providing increased IMS resolution. A schematic of the ESI-IMS-TOF MS equipped with two electrodynamic ion funnels to maximize ion transmission is shown in **Figure 2**. This instrument is comprised of six primary components:

1) ESI source
2) high-pressure hourglass ion funnel to focus and trap ions prior to ion injection
3) ion gate
4) ion mobility drift cell
5) secondary high-pressure ion funnel to focus the diffuse ion beam post-IMS separation
6) orthogonal TOF MS to measure mass to charge ratios (*m/z*) of the ions after IMS separation. The drift cell used the same modular design concept with each modular unit floated at different voltages as previously reported in [1].

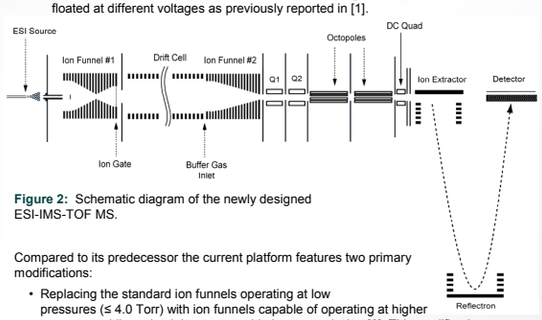

Figure 2: Schematic diagram of the newly designed ESI-IMS-TOF MS.

Compared to its predecessor the current platform features two primary modifications:

- Replacing the standard ion funnels operating at low pressures (≤ 4.0 Torr) with ion funnels capable of operating at higher pressures while maintaining comparable ion transmission [2]. This modification was crucial since increasing pressure allows higher electric fields to be attained and consequently higher IMS resolving power.
- Coupling an orthogonal TOF MS (Agilent Technologies, Santa Clara, CA) by adding two differentially pumped short quadrupole chambers at the IMS and TOF MS interface (Q1 and Q2 in **Figure 2**) to facilitate the high pressure IMS operation.

Software

To identify peptides and proteins observed in the IMS experiments, each spectrum was deisotoped using the THRASH [3] algorithm implemented in Decon2LS (http://ncrr.pnl.gov), and features were delineated based on drift and elution time profiles.

Currently, programs are being designed to align IMS data to an existing accurate mass and time (AMT) tag database and then identify peptides by appropriate mass and elution time tolerances.

Because IMS allows the drift time of each ion to be determined, in the future this dimension will be added to the AMT tag database. The proposed AMT tag database generation with peptide mass, elusion time, and IMS drift time is shown in **Figure 3**.

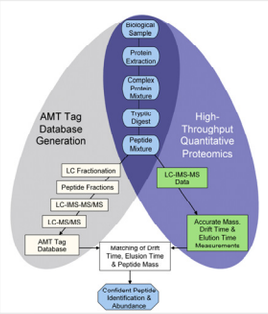

Figure 3: Approach for peptide identification using IMS

Results

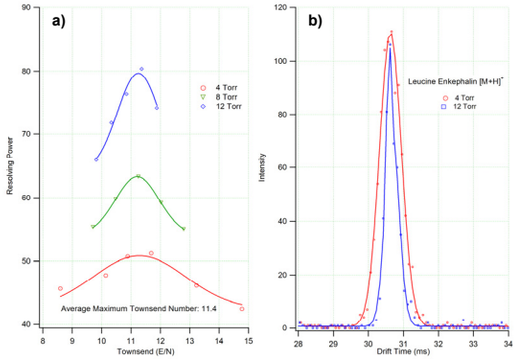

Figure 4: **a)** A plot of resolving power (Rp) versus Townsend (E/N) for the +1 charge state of leucine enkephalin at 4, 8 and 12 Torr. For all pressures, the maximum Rp occurred at ~11.4 Td. **b)** Arrival time distributions (ATD) of (leucine enkephalin)$^+$ for E/N=11.4 Td at 4 and 12 Torr.

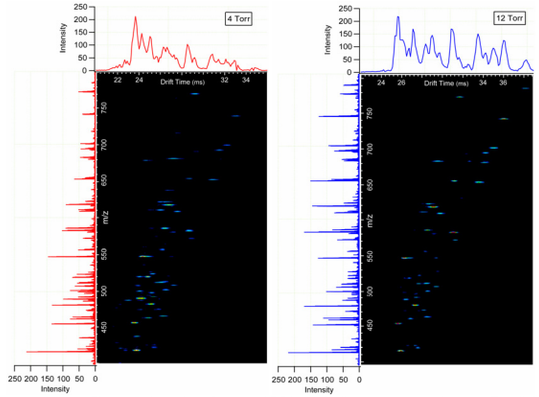

Figure 5: A section of a nested BSA tryptic digest spectrum taken at 4 Torr (left) and 12 Torr (right) where drift time is displayed on the x-axis and *m/z* on the y-axis. The smaller spot sizes of the 12 Torr nested spectrum indicate the increase in resolving power from 4 Torr.

ESI-IMS-TOF MS performance evaluation

To evaluate the performance of the ESI-IMS-TOF MS instrument, its resolving power (Rp) and sensitivity were determined. **Figure 4** illustrates the Rp curves for (leucine enkephalin)$^+$ in nitrogen at 4, 8 and 12 Torr. The highest observed Rp at 4 Torr was 51 increasing to 80 at 12 Torr (Figure 4a). An arrival time distribution (ATD) of (leucine enkephalin)$^+$ clearly illustrates the decrease in peak width with increasing Rp (Figure 4b).

Figure 5 illustrates the nested spectra for a tryptic digest of 0.01 mg/mL BSA at 4 and 12 Torr. Similar intensities in the total ion chromatograms (TICs) and mass spectra were observed for each pressure and the higher resolving power at 12 Torr nitrogen is highlighted by the increased definition in contoured features. While higher Rp at increased pressures has been reported previously [4-5], gains in this arena have come at the cost of sensitivity. However, the addition of the high pressure electrodynamic ion funnel to the ESI-IMS-TOF MS has allowed sensitivity to be retained while increasing resolving power.

Comparison of LC-IMS-MS and LC-MS

A sample of *Salmonella typhimurium* was evaluated to compare the features observed for LC-MS, NanoMate™-MS and NanoMate™-IMS-MS and the number for each are denoted in **Table 1**. NanoMate™-IMS-MS had the most observed features, so efforts are being placed on developing bioinformatics programs that match the complex IMS data to the AMT tag database for peptide/protein quantification.

A comparison of LC-MS and LC-IMS-MS is provided in **Table 2**. Again more features are observed for the IMS data compared to the MS data (taken on an LTQ-FT) using the same LC platform.

Table 1: Features detected for LC-MS, NanoMate™-MS, and LC-IMS-MS when analyzing *Salmonella typhimurium*

	Features Detected
LC-MS	7021
NanoMate™-MS	7305
NanoMate™-IMS-MS	7509

Table 2: Features detected for LC-MS and LC-IMS-MS when analyzing *Shewanella oneidensis* MR-1

	Features Detected
LC-MS	12364
LC-IMS-MS	19575

Fragmentation after the drift cell

There are two advantages of IMS-MS fragmentation compared to MS/MS: 1) ions have already been separated in the drift cell, so fragment ions will have the same drift time as the parent ions, and 2) small ions need less energy to fragment than larger ions. Utilizing this fact and the ability of IMS to separate ions, the fragmentation voltage can be ramped linearly as the drift time of the experiment increases, allowing optimum fragmentation of each ion rather than using a fixed voltage, which is optimum for an intermediate ion, but over-fragments small ions and under-fragments large ions. The parent and fragment spectra for a 4 peptide mixture with multiple charge states are shown in **Figure 6**.

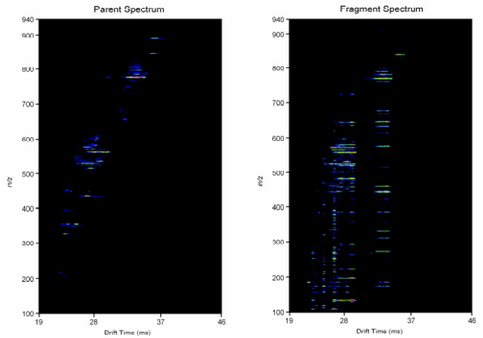

Figure 6: Parent and fragment nested spectra for a 4 peptide mix with different charge states of fibrinopeptide A, neurotensin, angiotensin I and bradykinin.

Conclusions

- Modifications to our previous ion funnel design allowed the ESI-IMS-TOF drift cell pressure to be increased from 4 to 12 Torr without substantial loss of sensitivity. An additional benefit of increasing the drift gas pressure was increasing the resolving power from 51 at 4 Torr to 80 at 12 Torr.
- A larger number of features were detected using the off-line fractionation of NanoMate™ compared to on-line fractionation of LC, likely because the solvent mixture could be varied for more efficient electrospray ionization.
- More features were found in the IMS-MS measurements than by just MS alone. Currently, software is being developed to compare the IMS-MS data to the AMT tag database so that the number of peptides and proteins observed can be quantified.

Acknowledgements

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