Increased Peptide and Protein Identification Rate for Proteomics Samples by Controlling Peptide Charge States Using CaptiveSpray nanoBooster

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ASMS 2013, MP24, #486

Introduction
The CaptiveSpray ionization source provides nanospray sensitivity with robust operation. The CaptiveSpray source utilizes a tapered, etched capillary emitter tip attached to the inlet of the MS, which draws in gas around the emitter, desolvating and focusing the ions into the MS. We describe here the usage of modified CaptiveSpray ionization source equipped with a device (nanoBooster) allowing the modification and vapour enrichment of the gas which flows around the emitter. Depending on the solvent vapour introduced, a modification of the charge state of peptides during the ionization process can be achieved. In the presented study we optimized peptide and protein identification rates by testing acetonitrile enriched gas streams guided into the CaptiveSpray ionization source.

Methods

Tryptic peptides are separated on a nanoLC system. For mass spectrometric detection using nanoflow rates at 300 nL/min a modified CaptiveSpray source is coupled to an Ultrahigh Resolution (UHR) Q-TOF system (impact). The modification of the ion source comprises usage of the nanoBooster, which allows the addition of vapor from organic solvents to the inlet gas of the CaptiveSpray ionization source (Figure 1).

Acetonitrile enriched nitrogen is used to determine the effect of the enrichment of organic solvents to the inlet gas on peptide charge distribution, signal intensities, signal to noise behavior and overall identification rates.

A tryptic digest of the human HeLa cell line was used as model system. Effect of the nanoBooster was determined on different sample amounts (100ng and 1μg) and different gradient lengths (60, 90, 240 min).

Results

We describe here the effects of the nanoBooster using acetonitrile as dopant leading to a higher average charge state compared to the standard lab air setup (Figure 2 a). Solvents leading to charge striping, as for example methanol, can also be applied with the nanoBooster, but are not in scope of the presented study. Initial experiments analyzing tryptic peptides of BSA reveal an increase of the average charge state from 2.32± to 2.66± when using acetonitrile enriched gas supply. For a HeLa digest, representing a very complex peptide mixture, the average charge state increased from 2.16± to 2.34±. Peptides, which are detected as singly charged species using standard setup, were predominantly detected as doubly charged ions using acetonitrile enriched nitrogen (Figure 2 b).

Base peak signal intensities increase up to 5 times for 100 ng of HeLa digest (Figure 3) due to charge state enhancement. The effect on signal intensity using 1μg sample amounts (e.g. > 1 μg) is less distinct but still detectable. The resulting increase in signal intensity but also the charge state enhancement directly results in increased protein identification rates on the Q-TOF system (Figure 4). As singly charged peptides are typically not considered for the fragmentation process and thus do not contribute to identification, an increase in identification rate was expected using the charge enhancement with the addition of acetonitrile vapor to the inlet gas. The effect on the increase of identification rates is more significant (+25%) on lower amounts, e.g. 100 ng HeLa, compared to high amounts, e.g. 1 μg HeLa. Still an increase in identification rates of 5% can be observed if high amounts are separated using a relatively short gradient of 90 min.

Summary

We have demonstrated that the CaptiveSpray ionization source equipped with the new nanoBooster enables an increase in the overall charge state if acetonitrile is used as dopant. This effect is combined with an increase in signal intensity resulting in increased protein identification rates on the Q-TOF system. The effect on protein identification rates strongly depends on sample amounts loaded on column and is much more significant for low amounts, but is not limited to those kinds of samples.

The enhanced charging described here clearly shows the capability of in-spray charging of peptides without the need of addition of supercharging agents to the LC solvent and thus without influencing the chromatographic performance. Thus the CSI source equipped with the nanoBooster is also advantageous for ETO measurements requiring higher charge states (+3).

Conclusions

• The CaptiveSpray ionization source equipped with the nanoBooster allows the addition of vapor from organic solvents to the inlet of the gas.

• Depending on the solvent, charge enrichment and charge stripping for peptides and proteins is enabled via in-spray charging.

• Acetonitrile enriched N2 vapor in the CaptiveSpray ionization source significantly improved the intensity across all peptides. Moreover, the protein identification rates increased significantly.