Charge Separation for Protein Applications on a QqTOF Mass Spectrometer

Igor V. Chernushevich\(^1\), Lorne M. Fall\(^1\), Nic Bloomfield\(^1\), Alexander V. Loboda\(^1\) and Andrey A. Shevchenko\(^2\)

\(^1\)MDS SCIEX, Concord, Canada; \(^2\)Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany

Overview

Novel charge separation method is applied in a quadrupole – TOF mass spectrometer (QStar) to improve detection limits of peptides and proteins when analyzed from complex mixtures.

Introduction

In many protein applications performed with nonspecific, chemical noise (predominantly singly charged) may mask or distort the signal and require a high signal to noise ratio to be attained. These conditions can be achieved, for example, by performing high-sensitivity experiments, but require tuning and are associated with long run times. The same applies to the scan recording “trapping” of the precursor ions, which detect multiply charged ions only. SRMMS or secondary electron ionization (SEI) MS\(^2\) can also detect multiply charged species in the presence of singly charged ions, but does not work well in low charge state ions (2+). Finally, software charge separation methods are limited to cases when chemical noise is either highly repetitive (Fourier transform ion-chromatograms) or too diverse to be distinguished on the basis of mass and intensity alone.

In the present work we describe results obtained with a novel “multiple charge separation” (MCS) technique, which allows efficient identification of uncharged singly charged ions when analyzing highly complicated mixtures. The method is described in detail elsewhere [3].

Figure 1. Tunable charge separation spectra of a protein digested with trypsin. The quadrupole (Q1) was set to transmit ions with the charge state corresponding to an ion of m/z 668. The QIC-MS/MS spectrometer is tuned to sequentially transmit ions with a charge state of 1+ through 4+. The middle segment shows fragment ions above the m/z of the precursor ion. The trapping duty cycle enhancement [6] was tuned for approximately 1.5 times of the precursor m/z. The use of Q2 pulsing is illustrated in Figure 3. Upper panel: an example of MS/MS with Q2 trapping OFF (Top) and ON (Bottom). Lower panel: two expanded segments are shown, together with MS/MS spectra of two identified peptide ions.

Table 1. Sequence TAG information from protein mixture: 1 fM/L Lactic Dehydrogenase (left) and 3 fM/L apo-Transferrin (right).

<table>
<thead>
<tr>
<th>m/z</th>
<th>Charge State</th>
<th>Sequence</th>
<th>Protein Identified</th>
<th>Peptide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>513.97</td>
<td>2</td>
<td>LV</td>
<td>TRFE_BOVIN</td>
<td>SEROTRANSFERRIN</td>
</tr>
<tr>
<td>520.27</td>
<td>2</td>
<td>NV</td>
<td>TRFE_BOVIN</td>
<td>SEROTRANSFERRIN</td>
</tr>
<tr>
<td>573.31</td>
<td>2</td>
<td>GF</td>
<td>OCPHOR_1</td>
<td>RABBIT MRNA FOR MUSCLE</td>
</tr>
<tr>
<td>668.89</td>
<td>2</td>
<td>PGSEL</td>
<td>TRFE_BOVIN</td>
<td>SEROTRANSFERRIN</td>
</tr>
<tr>
<td>673.67</td>
<td>3</td>
<td>NGDT</td>
<td>TRFE_BOVIN</td>
<td>SEROTRANSFERRIN</td>
</tr>
<tr>
<td>678.36</td>
<td>2</td>
<td>LL</td>
<td>TRFE_BOVIN</td>
<td>SEROTRANSFERRIN</td>
</tr>
</tbody>
</table>

Results

Multiple Charge Separation (MCS) mode is very similar to TOF MS, in that it records a full electrospray spectrum without scanning, but allows varying degrees of singly charged ions to be detected. This variability is tunable by the user as depicted in Fig. 1. Fig. 1A shows a standard TOF MS spectrum of BSA digest at 4 fM/L; very few if any multiply charged ions can be detected above background (see also Figure 2). Fig. 1B was recorded with MCS “on” and shows a significant suppression of background. Suppression comes at some cost: about 50% of the multiply charged ions survive. Fig. 1C depicts a complete suppression of the majority of ions being charged to singly charged state. This high degree of separation comes at a higher cost, only about 10% to 20% of the original multiply charged ions remain. However, the gains of signal to noise and the ability to detect these ions are quite apparent. The benefits of charge separation are particularly apparent at low femtomolar concentration levels, where a regular TOF MS spectrum is difficult to interpret. MCS immediately identifies those multiply-charged signals that are known to be non-specific and eliminates them from the charge toward the lower mass ions, thereby making the experiment the electrospray equivalent of conventional MALDI peptide maps.

CONCLUSIONS

The novel charge separation method is applicable to partial or almost complete elimination of singly charged background ions from the spectrum of multiply charged ions of interest. Typically, singly charged background ions are not detected in the linear ion trap experiments, thereby resulting in significantly lower detection limits of peptides or proteins under investigation. The suppression of chemical noise reduces the need for chromatography and potentially can make this method the electrospray equivalent of conventional MALDI peptide maps. Alternatively, the precursor ions found through the charge separation method can be subjected to MS/MS and a sequence tag can be formed for database search. Combination of the Q2 trapping/ion-multiplication technique with the absence of chemical noise allows the multiplex of the ion-multiplication to be accomplished on a single fragment concentration.

REFERENCES


Acknowledgments

The authors would like to acknowledge John Hogl for guiding in the idea and developing the scan type on lower ion trap instruments, and to thank John Lobbins for creative thinking.

Trademark/Licensing

API QSTARTM Pulsar Hybrid LC/ESI/MS system, AnalystTM Software and BioAnalystTM Software are trademarks of Applied Biosystems/SCIEX. Bovine Serum Albumin (BSA) and Lactate Dehydrogenase (LDH) are obtained from the American Type Culture Collection. MDS SCIEX is a trademark of MDS Inc.