

SpiralTOF-TOF

Analysis of Bovine Serum Albumin

Introduction

The JMS-S3000 “SpiralTOF™” is a MALDI-TOFMS that uses an innovative SpiralTOF ion optics system to achieve the highest resolution currently available for a MALDI instrument (Fig. 1 and 2). Additionally, this system can be equipped with a TOF-TOF option that can acquire high-energy collision-induced dissociation (CID) product ion spectra for monoisotopically selected precursor ions. The distance to the ion gate is 15 m, more than one order of magnitude longer than that of conventional MALDI TOF-TOF instruments, thus allowing the monoisotopic selection of the precursor ion. The second TOFMS incorporates a re-acceleration mechanism and an offset parabolic reflectron, another innovative ion optical system developed by JEOL. This unique design

enables the seamless observation of product ions ranging from very low m/z up to that of the precursor ion.

In a previous application note¹, we reported the analysis of bovine serum albumin (BSA) by Spiral and Linear modes. In this work, we show the analytical result for BSA by using the JMS-S3000 “SpiralTOF™” with the TOF-TOF option.

Results and Discussion

As a starting point, the tryptic digest of BSA (tBSA) was analyzed using just the Spiral mode. For the calibration step, a peptide mixture was used as an external mass calibrant, which resulted in an RMS error of 4 ppm. Subsequently, the mass spectrum peak list for this sample was submitted to the MASCOT peptide mass fingerprint

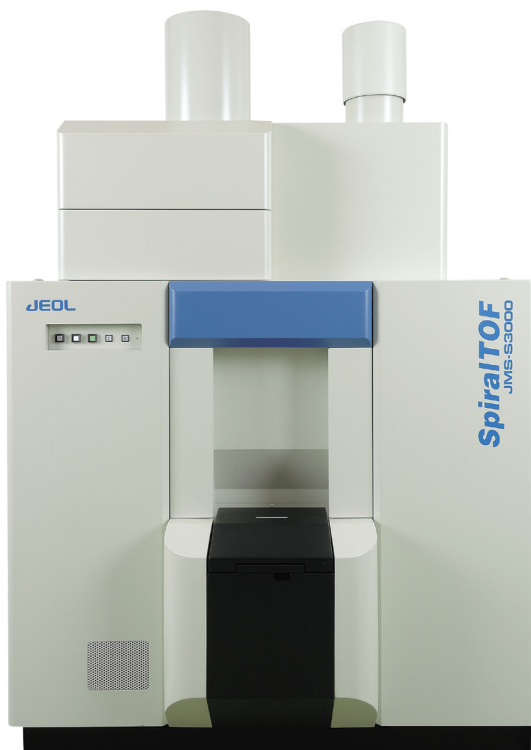


Figure 1. JMS-S3000 SpiralTOF with TOF-TOF attachment.

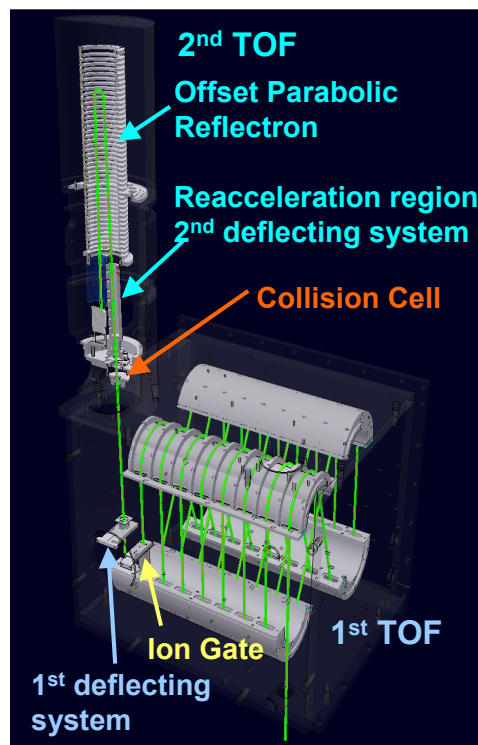


Figure 2. Ion trajectory of SpiralTOF and TOF-TOF attachment.

search, and the protein was identified as BSA. These results are in very good agreement with the results obtained previously for Spiral mode.1
 Next, the 10 highest intensity ions in the tBSA mass spectrum were selected as the precursor ions for automatic measurement using the TOF-TOF option. The product ion spectra for m/z 927.5, 1439.8, and 1567.7 shown in Fig.3 had an immonium ion along with the a-ion, d-ion, and w-ion series. These spectra along with the other 7 product ion spectra were then submitted to the MASCOT MS/MS Ion Search, and the protein was identified as BSA (see Fig.4).

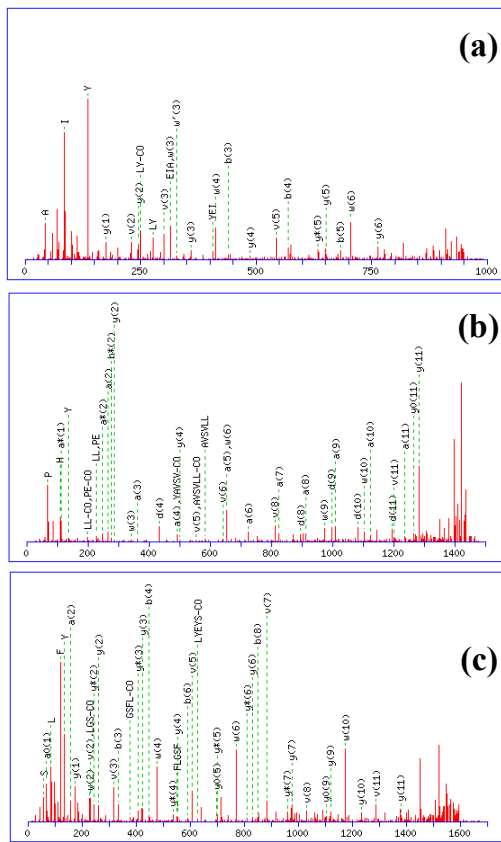


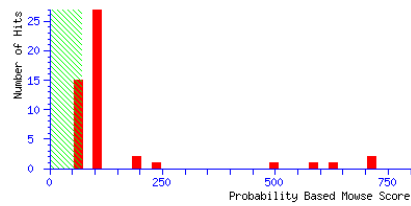
Figure 3. Product ion spectra for m/z (a) 927.5, (b) 1439.8, and (c) 1567.7.

Conclusions

The high resolution tBSA mass spectrum measured using Spiral mode was easily identified using the MASCOT peptide mass fingerprint method, even with a peptide mass tolerance set as narrow as 10 ppm (not shown in this note). Additionally, the high energy CID of the 10 highest intensity monoisotopically selected peaks measured using the SpiralTOF-TOF mode produced product ion spectra that readily identified BSA as the protein through the MASCOT MS/MS Ion Search.

Reference

1)www.jeolusa.com/DesktopModules/Bring2mind/DMX/Download.aspx?EntryId=833&PortalId=2&DownloadMethod=attachment



Protein Summary Report

Format As: Protein Summary (deprecated) [Help](#)

Significance threshold $p < 0.05$ Max. number of hits: AUTO

Standard scoring: MudPIT scoring Ions score or expect cut-off: 0 Show sv

Show pop-ups: Suppress pop-ups Sort unassigned: Decreasing Score Require

Re-Search All Search Unmatched

Index

Accession	Mass	Score	Description
1. gi130794280	71309	714	albumin [Bos taurus]
2. gi11351907	71279	696	Serum albumin precursor (Allergen Bos d 6) (BSA)
3. gi174267962	71221	614	ALB protein [Bos taurus]
4. gi1229552	68118	576	albumin
5. gi176445989	55514	510	serum albumin [Bos indicus]

Figure 4. MASCOT MS/MS Ion search result of tBSA by TOF-TOF mode.