

SpiralTOF™

MALDI-ISD Measurements Using Both the SpiralTOF Mode and the LinearTOF Mode

Introduction

Matrix assisted laser desorption/ionization (MALDI) combined with in-source decay (ISD) is a useful tool for doing top-down sequencing of intact proteins. In this work, we measured and compared the ISD fragment ions generated for several proteins by using both the high resolution MALDI-Spiral mode and the high sensitivity MALDI-Linear mode available on the JEOL SpiralTOF MALDI-MS system.

Experimental

Myoglobin and Bovine serum albumin (BSA) protein samples were separately dissolved into 0.1% trifluoroacetic acid aqueous with the concentration fixed at 10 pmol/ μ L. 1,5-diaminonaphthalene (DAN), which can provide good S/N for ISD fragment ions [1], was used as the MALDI matrix. The DAN matrix was dissolved to 0.1% trifluoroacetic acid aqueous/ 50% acetonitrile with the matrix concentration fixed at 10 mg/mL. Subsequently, the matrix and the sample solutions were mixed 1/1 (v/v), and then 1 μ L of each solu-

tion was deposited and dried on the MALDI target plate. Afterwards, each sample was analyzed in triplicate on the JEOL JMS-3000 SpiralTOF by using both the SpiralTOF mode and the LinearTOF mode.

Results

The Myoglobin and BSA ISD mass spectra for both the LinearTOF and SpiralTOF measurements are shown in Figures 1 and 2, respectively. Both sets of ISD spectra were dominated by the c-ion series. The Linear mode mass spectra showed higher sensitivity overall, especially for ions over m/z 5,000 (shown in the m/z 4000-7000 zoomed regions). However, as expected, the Linear TOF did not provide full isotopic separation of these c-ions (see insets for the myoglobin c35 and BSA c33 ions). Conversely, the Spiral mode measurements fully resolved these ions and their associated isotopes, as shown in the Figures 1b and 2b insets, respectively, but had less sensitivity at higher m/z values.

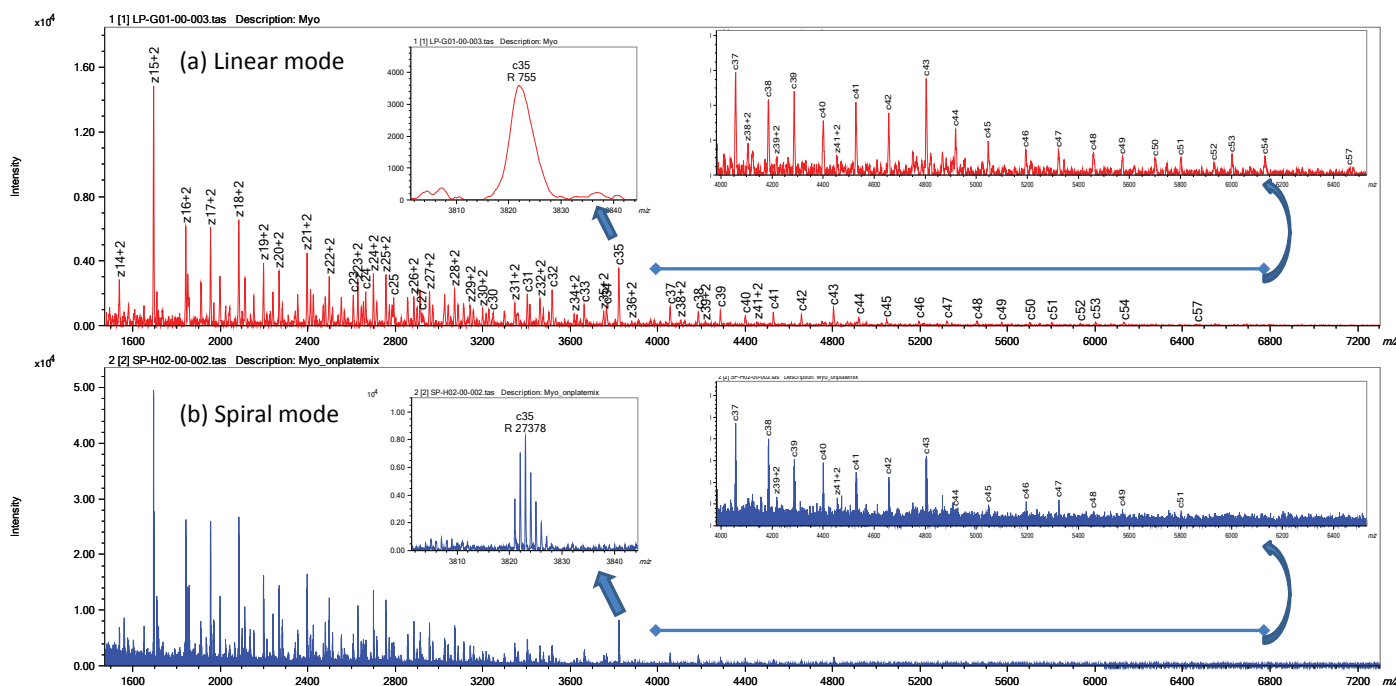


Figure 1. ISD Spectra of myoglobin (MW: 16,952Da) using (a) LinearTOF mode, (b) SpiralTOF mode.

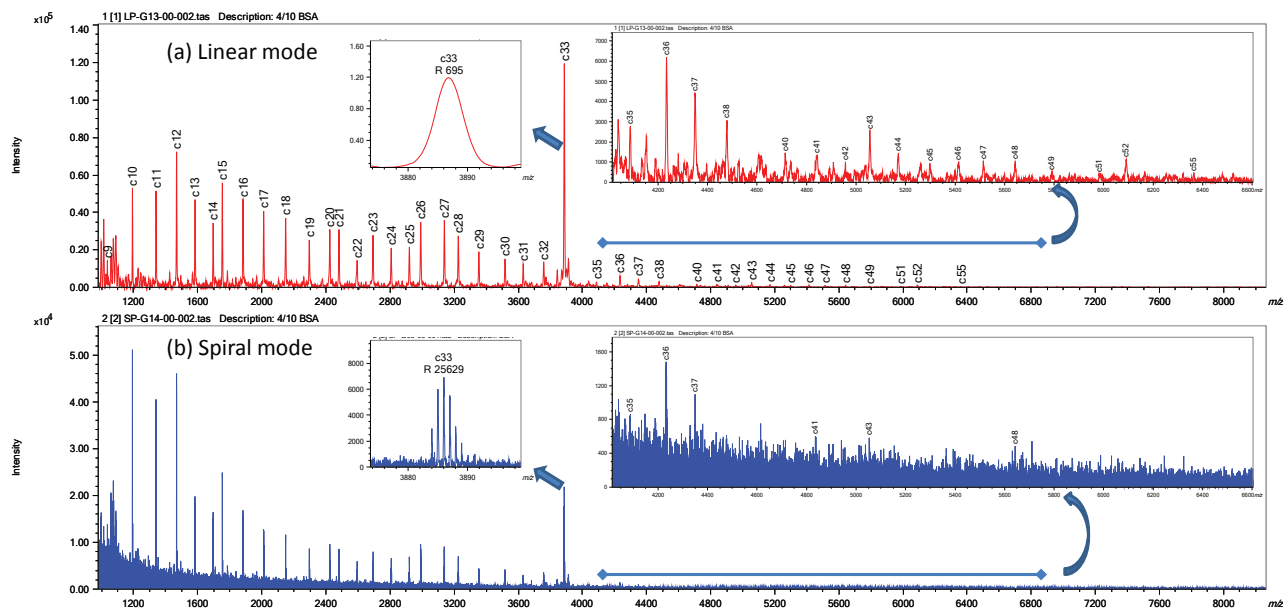


Figure 2. ISD spectra of BSA (MW: 66,430Da) using (a) LinearTOF mode, (b) SpiralTOF mode.

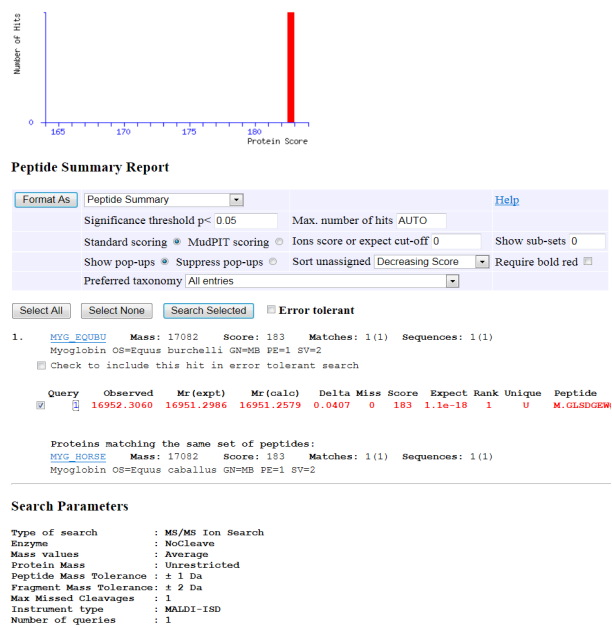


Figure 3. Mascot search result for the myoglobin sample.

A Mascot MS/MS Database Search using the LinearTOF data identified each sample as myoglobin and BSA, respectively. As an example, Figure 3 shows the Mascot database search result for the myoglobin sample. These results showed that, despite the fact that the ISD Linear data does not contain exact masses and does not provide isotopic separation of the ions, the data can still be used with a database search function like Mascot to identify proteins.

Conclusion

In this work, we showed a brief study in which the ISD measurements for standard proteins were measured by using the SpiralTOF mode and the LinearTOF mode. The Spiral-

TOF mode provides high mass accuracy and fully separated isotopic ions while the LinearTOF mode provides higher sensitivity, particularly for ions in the higher m/z region. Additionally, the LinearTOF data can be used in conjunction with a database search analysis to identify proteins.

Reference

[1] Issey Osaka, Mami Sakai, Mitsuo Takayama, 5-Amino-1-naphthol, a novel 1,5-naphthalene derivative matrix suitable for matrix-assisted laser desorption/ionization in-source decay of phosphorylated peptides, Rapid Communications in Mass Spectrometry, Volume 27, Issue 1, pages 103–108, 15 January 2013.