High Sensitivity Analysis of intact Proteins Using Linear TOF

Product used：Mass spectrometer (MS)

Introduction

The JMS-3000 SpiralTOF has a unique 17m flight path that offers the highest resolution MALDI-TOFMS system currently available. However, ions with a very short lifetime or that undergo spontaneous dissociation during their flight cannot be detected by the SpiralTOF (or a conventional reflection TOF). To address this situation, the SpiralTOF with Linear TOF option can be used for the high sensitivity analysis of intact proteins.

In this work, we demonstrate the measurement of intact proteins by using the Linear TOF option for the JEOL SpiralTOF system.

Experimental

Protein standards were obtained from Sigma-Aldrich.

a. Pepsin (porcine gastric mucosa), P-6887
b. Albumin (bovine serum), A8471
c. Conalbumin (chicken egg white), C0755
d. IgG (bovine serum), I5506

The protein standard samples were dissolved in water at a concentration of 1 pmol/μL. Sinapinic acid (SA) was used as the matrix and was dissolved in 1:1 water / acetonitrile containing 0.1% trifluoroacetic acid. Next, the protein standard solution and SA solution were mixed together 1:1 by volume. Afterwards, 0.5μL of this mixture was placed on the MALDI target plate (250 fmol/spot). Finally, the dried sampl n the JMS-S3000 SpiralTOF MS system.

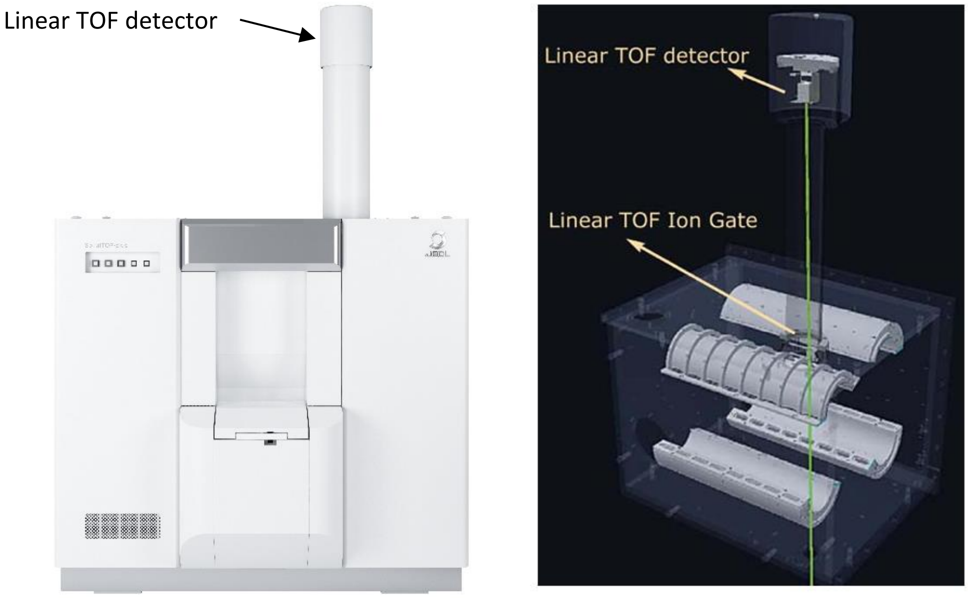

Figure 1. SpiralTOF with Linear TOF option

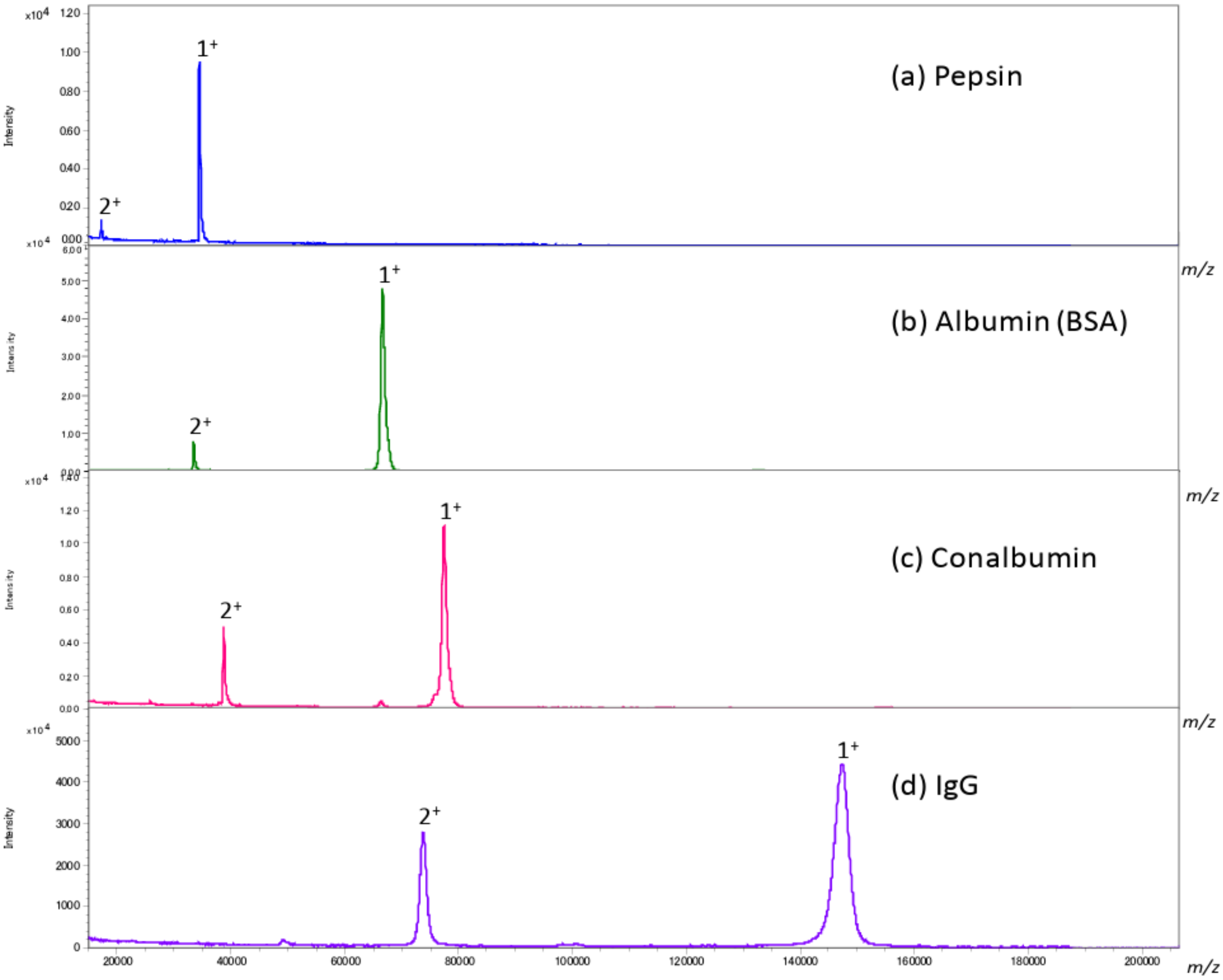

Figure 2. MALDI mass spectrum of protein standards, (a) Pepsin (250 fmol/spot),
(b) Albumin (250 fmol/spot) (c) Conalbumin (250 fmol/spot), (d) IgG (250 fmol/spot).

Results & discussion

The MALDI mass spectra are shown in Figure 2 for each protein sample. Peaks corresponding to single- and double-charge protonated molecules were observed at the expected *m/z* values for the primary structure of these proteins.

The SpiralTOF with Linear TOF option provided
・Good peak shape in the high *m/z* region
・Excellent signal-to-noise ratio for these sample at this concentration (250 fmol)
High-quality mass spectra were achieved for intact proteins by using the JEOL JMS-3000 system. Also worth noting, the Linear TOF can measure intact molecules up to *m/z* 500,000 (data not shown in these examples).