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Application News

No. B74

MALDI-TOF Mass Spectrometry

Detection of High-mass Proteins Using a Benchtop MALDI-TOF Mass Spectrometer

The applicability of MALDI-TOF mass spectrometry to perform protein detection is well recognized in the life science field. In this field, SDS polyacrylamide gel electrophoresis and size exclusion chromatography have been historically used, however, they have drawbacks such as being time-consuming or lacking accuracy in molecular weight determination. Due to its ability to provide more accurate molecular weight information, MALDI-TOF mass spectrometry has become the primary tool for the analysis of protein primary structures. Moreover, in recent years, the analysis of proteins at the femtomole and subfemtomole levels is often required, which is increasing the demand for higher sensitivity measurements with MALDI-TOF mass spectrometry.

The mass range of MALDI-TOF mass spectrometry is potentially unlimited and gives full access to low- to high-mass molecules, such as antibodies. Monoclonal antibodies (see Fig. 1) are utilized for diagnostic and therapeutic purposes. In their development and quality control processes, it is very important to use fast and reliable analytical methods.

Here, we demonstrate the capability of a benchtop MALDI-TOF mass spectrometer (MALDI-8020, Fig. 2)to perform high-throughput protein detection with high sensitivity.

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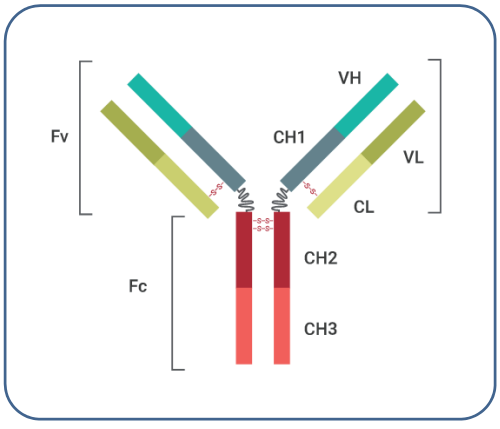

Fig. 1 Generalized Structure of an Antibody

Sample Preparation and Measurement Conditions

The samples - bovine serum albumin (BSA) and immunoglobulin A (IgA) - were purchased from Sigma-Aldrich. These were prepared at a concentration of 500 fmol/µL and 20 pmol/µL, respectively. Fifty shots were accumulated per profile (200 profiles per spectrum). The mass spectra were recorded using the average masses.

Tuning	Linear
Polarity	Positive
Laser rep. rate	200 Hz
Accumulation rate (shots/profile)	50
Profiles	200

Fig. 2 Benchtop MALDI-TOF MS: MALDI-8020

Results

To demonstrate the MALDI-8020 sensitivity, Fig. 3 shows the mass spectrum of BSA. The singly- (approx. 66 kDa), doubly- (approx. 33 kDa) and triply-charged (approx. 22 kDa) ions were observed with good signal-to-noise ratio which compared favourably with the linear mode performance of a conventional MALDI-TOF instrument.

Fig. 4 shows the mass spectrum of IgA. The singly-charged mass expected at approx. 160 kDa was observed along with the doubly-charged ion (approx. 80 kDa). The signal detected at approx. 54 kDa is consistent with the mass of the heavy chain (approx. 55 kDa expected).

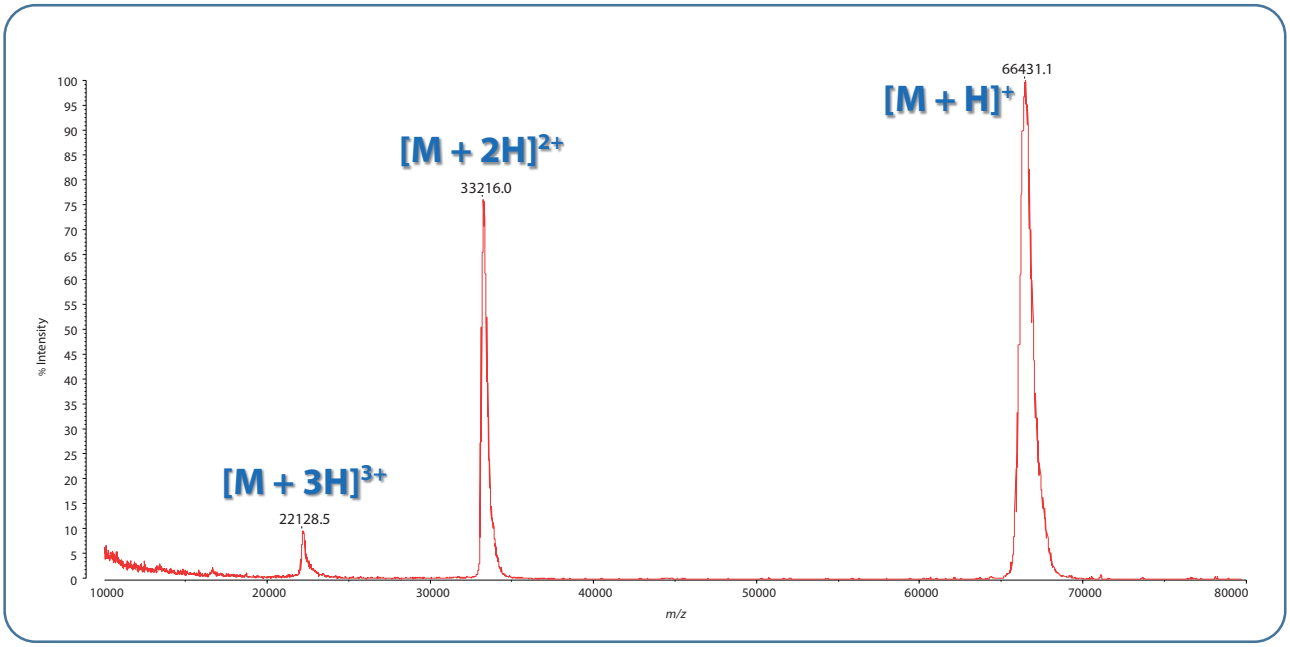

Fig. 3 Mass Spectrum of BSA (500 fmol/µL; 250 fmol on-target)

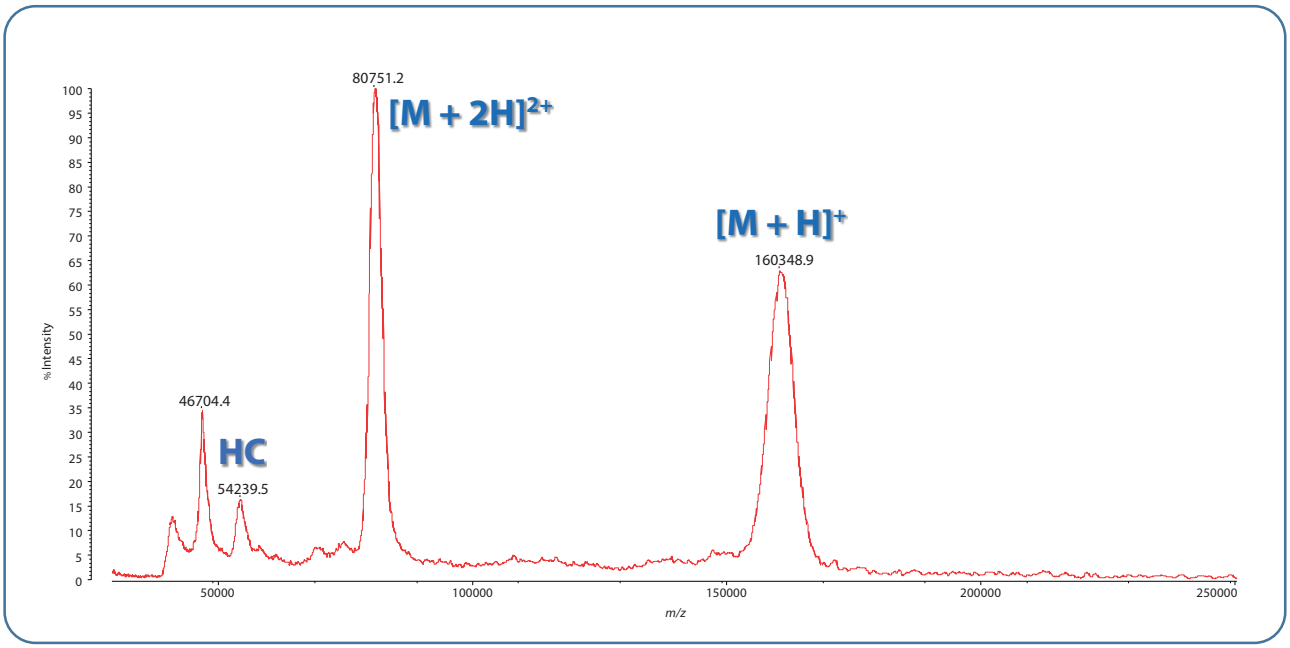

Fig. 4 Mass Spectrum of IgA (20 pmol/µL; 10 pmol on-target). HC = heavy chain

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