

Application News

No. B78

MALDI-TOF Mass Spectrometry

MALDI-in source decay for the detection of methionine oxidation using the MALDI-8020 benchtop linear MALDI-TOF mass spectrometer

Matrix-assisted laser desorption/ionization (MALDI) is a rapid, simple and sensitive tool for obtaining information on the molecular weight as well as the primary structure of a peptide or protein. Among the different fragmentation methods, in-source decay (ISD) is used in 'top-down' proteomics to obtain the sequence of proteins. It leads to c- and z-fragment ion series via a hydrogen radical-transfer mechanism. One of the advantages of ISD over post-source decay (PSD) is that ISD is theoretically not limited by the sample mass and thus allows the sequencing of large proteins directly, without the need of an enzymatic digestion.

A schematic of the MALDI-ISD workflow is illustrated in Fig. 1.

In the pharmaceutical industry, as part of the quality control (QC) process, it is important to track any changes, either due to product formulation or degradation, as they can affect the therapeutic role, leading to a potential loss of activity or development of toxicity. An example of modifications that can occur with degradation is the oxidation of methionine residues of a peptide or protein as this amino acid is highly susceptible to oxidation.

Here, we demonstrate the capability of the MALDI-8020 benchtop linear TOF mass spectrometer to determine the methionine oxidation (Met-O) of Exendin-4 peptide through accurate intact mass analysis and top-down sequencing. This can be a potentially valuable tool in the QC environments to pinpoint undesired changes during the production process.

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■ Samples and method

Exendin-4 peptide was purchased from Sigma-Aldrich. The oxidation of methionine was carried out as follows: Exendin-4 was incubated with 1 % hydrogen peroxide (H₂O₂, pH neutral), at 37 °C for 15 minutes. Following oxidation, the sample solution was acidified and purified using a ZipTip® C₁₈ microcolumn (Millipore).

For the intact mass analyses, the native and Met-O samples were mixed with alpha-cyano-4-hydroxycinnamic acid (CHCA, 5 mg/mL, 1:1 acetonitrile/0.1 % trifluoroacetic acid) and spotted (1 µL) onto the target. For the ISD-analyses, the native and Met-O samples were mixed with 1,5-Diaminonaphthalene (1,5-DAN, saturated in 1:1 acetonitrile/0.1 % trifluoroacetic acid) and spotted (1 µL) onto the target.

The acquisition parameters of the MALDI analyses are summarised in Fig. 1.

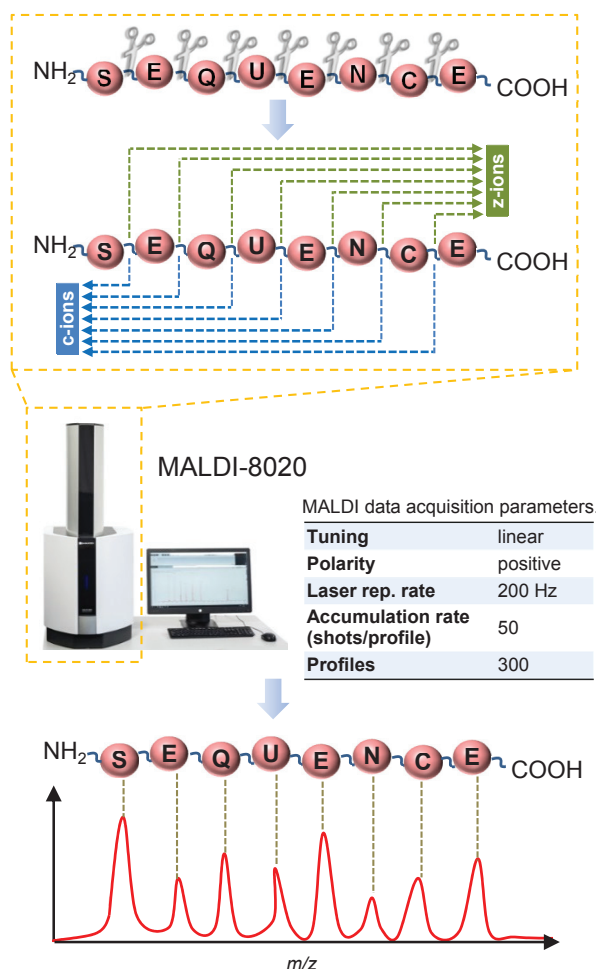


Fig. 1 MALDI-ISD workflow.
Table: MALDI acquisition parameters.

Results

Exendin-4 is a naturally occurring peptide present in the saliva of the Gila monster (*Heloderma suspectum*). The synthetic form, Exenatide, is used in the treatment of diabetes mellitus type 2.

The MALDI-MS/MS spectrum of native Exendin-4 is shown in Fig. 2 (top panel). The mass labels shown correspond to the detected monoisotopic peaks.

The camera image shown in the inset in Fig. 2 (top panel) demonstrates the good sample homogeneity obtained for the optimised sample preparation with 1,5-DAN matrix. The expanded regions (green and blue) of the mass spectrum (Fig. 2, middle and bottom panels) show good resolution values of the monoisotopic masses of the fragment ions (m/z 1200-2800 range).

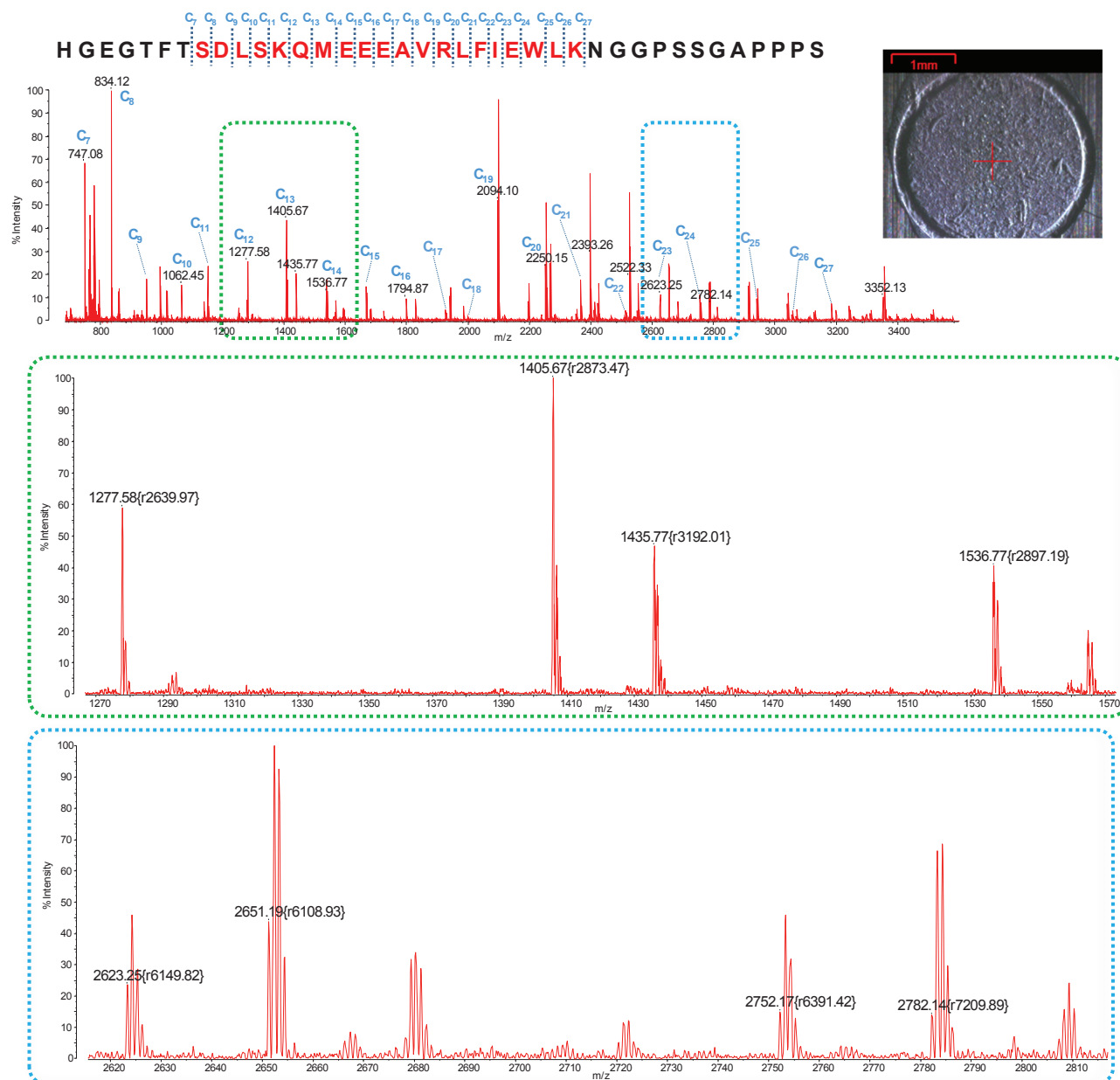


Fig. 2 MALDI-MS/MS spectrum of native Exendin-4 (top panel).

Top panel (inset): camera image of the sample spot prepared using an optimised preparation using 1,5-DAN matrix. Middle (green) and bottom (blue) panels: expansions of the mass spectrum showing the isotopically resolved fragment ions.

To perform a Mascot-ISD search, a custom version of the Swiss-Prot database was created by adding the sequence of Exendin-4. The ISD search was then performed using the integrated Mascot search tool in the MALDI Solutions software and the results are shown in Fig. 3. A significant score (Fig. 3 a) and sequence coverage (72 % ; Fig. 3 b) were obtained.

Following oxidation, an intact mass analysis was performed to check the success of the oxidation reaction. The spectra in Fig. 4 show the (isotopically resolved) intact mass of native (red trace) and Met-O (blue trace) Exendin-4, obtained with CHCA matrix, with good mass accuracy and resolution. As expected, a mass shift of +16 Da, corresponding to the chemical modification of the single methionine in Exendin-4 to its sulfoxide form, was detected.

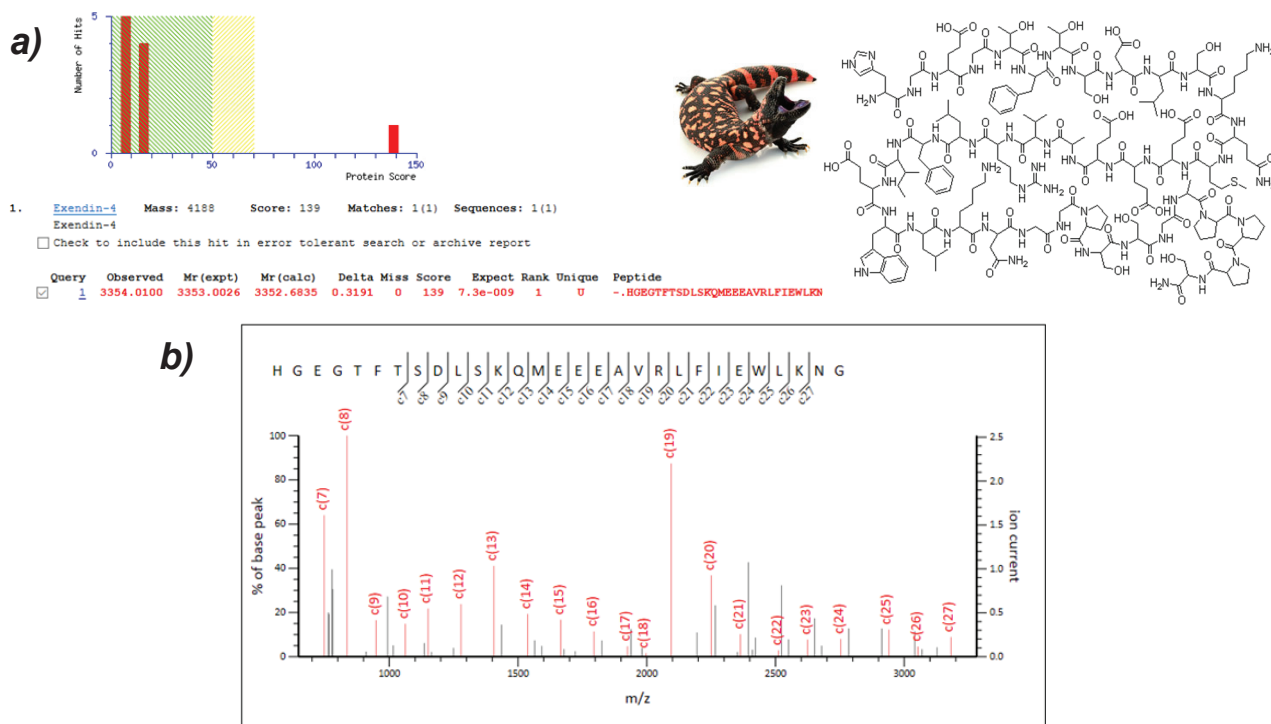


Fig. 3 a) Mascot-ISD score; b) Mascot matched fragment ions spectrum graphic.

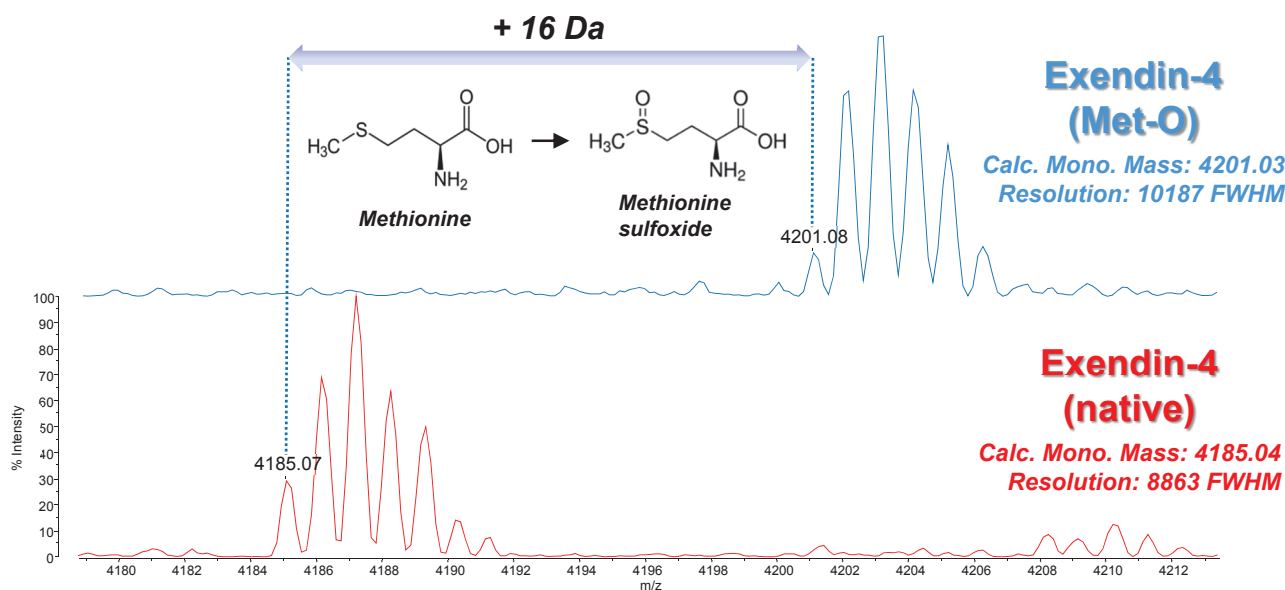


Fig. 4 MALDI-MS mass spectra of intact native (red trace) and Met-O (blue trace) Exendin-4 obtained with CHCA matrix.

The MALDI-MS/MS spectrum of Exendin-4 (Met-O) is shown in Fig. 5 a). The mass labels shown correspond to the detected average peaks. From the N-terminus, c-ion series (C_9 - C_{13}) and c-ions shifted by 16 Da (C_{14} - C_{29}) were detected. The C_{14} ion (highlighted in green) corresponds to the site of oxidation involving the methionine residue.

Fig. 5 b) illustrates a portion of the mass spectrum (m/z 1400-1650) showing the monoisotopically resolved fragment ions of native (red trace) and

Met-O (blue trace) Exendin-4. The C_{14} ion (HGEFTFTSDLSKQM) exhibited a mass shift of 16 Da corresponding to the oxidation of methionine, as expected.

The Mascot-MS/MS search was conducted on the MS/MS spectrum of Exendin-4 (Met-O) using the custom database and specifying the oxidation (M) as fixed modification during the search. The Mascot-MS/MS results are shown in Fig. 5 c), and highlight a significant score.

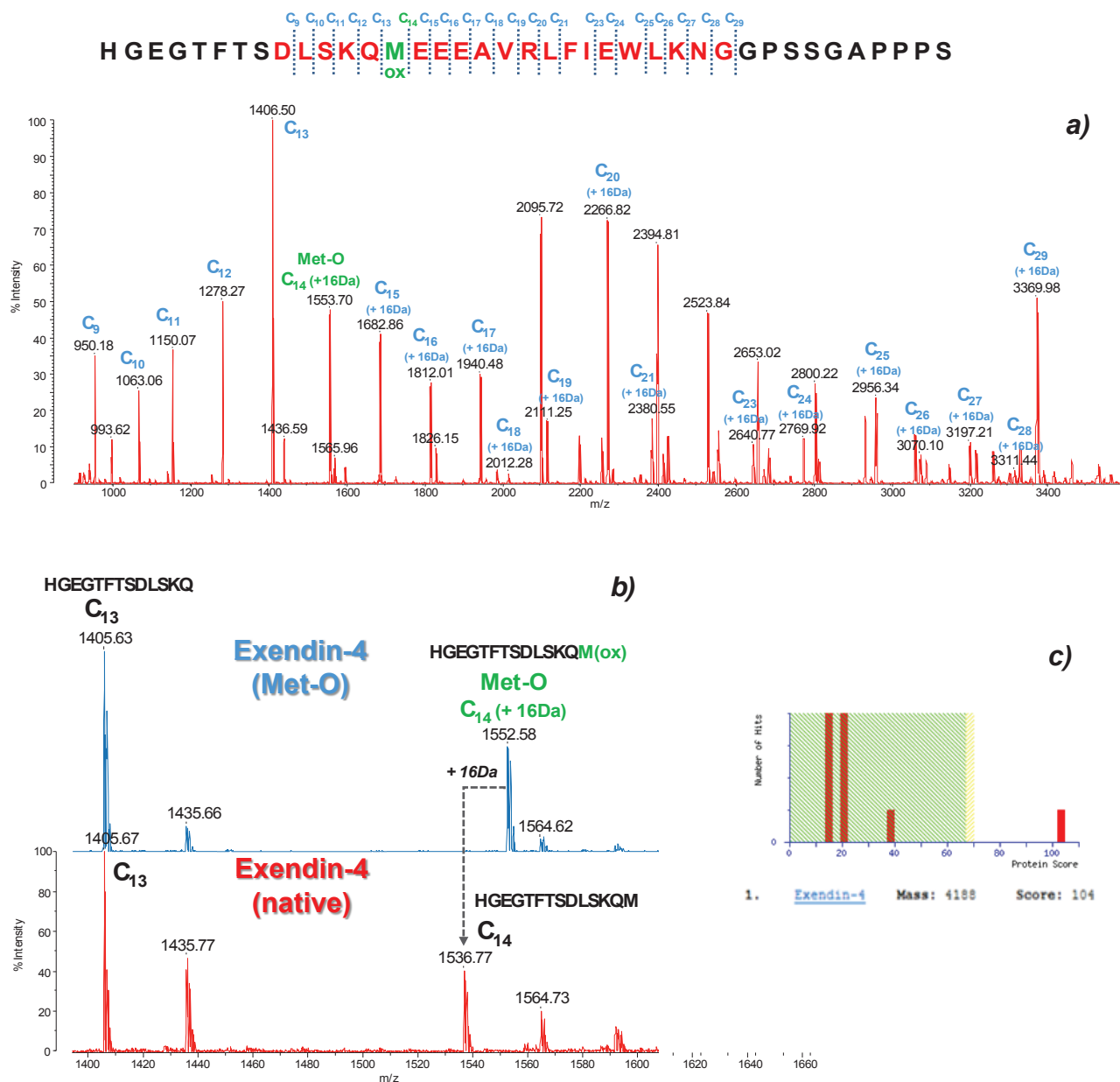


Fig. 5 a) MALDI-MS/MS spectrum of Met-O Exendin-4; b) Expansion of the mass spectrum showing the C_{14} fragment ions and the +16 Da mass shift of the methionine oxidation modified species (Met-O); c) Mascot-MS/MS score.

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First Edition: Mar. 2018



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