Application Note

Biopharma and Research

Fast and Simple Protein Molecular Weight Confirmation Using the Agilent InfinityLab LC/MSD XT Mass Selective Detector

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Abstract

Protein characterization is an important element of life science research and biopharmaceutical analysis. This application note describes the analysis of intact protein samples using an economical, robust, and easy-to-use liquid chromatograph (LC) coupled to a mass selective detector (MSD).

An Agilent 1260 Infinity II LC coupled an Agilent InfinityLab LC/MSD XT single quadrupole mass selective detector was used to confirm intact mass. The separation and mass measurement obtained by the InfinityLab LC/MSD XT together with the built-in LC/MSD bioanalysis tools in Agilent OpenLab CDS ChemStation software enables fast and simple confirmation of intact protein mass.

Introduction

Liquid chromatography/mass spectrometry (LC/MS) has been used widely for protein analysis. Quick protein molecular weight confirmation, impurity analysis, as well as identification of post translational modifications (PTMs), can be achieved using LC/MS. Single quadrupole mass spectrometry has commonly been adopted in the QC environment for its simple operation, low cost, and robustness.

For analysis of intact proteins by mass spectrometry, a wide mass range is required. Mass spectrometers measure the mass-to-charge ratio (*m/z*) of protein samples. Therefore, the particular *m/z* required depends on both the molecular weight of the protein of interest, as well as the charge that the protein carries. The lower the charge, the higher the *m/z* that is measured. Further, the mass detector measures a distribution of charge states in the mass spectrum. This charge-state envelope is mathematically transformed to the molecular weight of the compound. This data transformation is called deconvolution.

The InfinityLab LC/MSD XT is ideally suited for protein analysis due to its wide mass range (2 to 3000 *m/z*), robustness, and stability. In this work, LC/MSD XT was used with an electrospray ionization source for protein analysis.

Experimental

Instrumentation

The analysis of two protein samples were carried out on an Agilent 1260 Infinity II LC with the Agilent InfinityLab LC/MSD XT single quadrupole-based system comprising the following modules:

- Agilent 1260 Infinity II quaternary pump (G7111B)
- Agilent 1260 Infinity II vial sampler with sampler cooler and integrated column compartment (G7129A)
- Agilent InfinityLab LC/MSD XT (G6135BA)
- Bioanalysis software (M8363AA)

Mass detection

To confirm protein molecular weights between 15 and 30 kDa of intact proteins, an LC/MS method with a mass range setting of 600 to 2,000 *m/z* was used in this experiment. The full molecular weight range of the InfinityLab LC/MSD XT (3,000 *m/z*) can be used for larger proteins.

Software

Agilent OpenLab CDS ChemStation Edition for LC and LC/MSD XT system with Bioanalysis software package, Rev. C.01.07 SR4

Column

Agilent PLRP-S 1000 Å,
2.1 × 50 mm, 5 µm (p/n PL1912-1502)

Chemicals

LC/MS grade acetonitrile and formic acid were purchased from Sigma-Aldrich (St. Louis, Missouri). Water was obtained from a Milli-Q system (Millipore, Bedford, MA).

Sample preparation

Wild-type ketoisomerase with C-terminal His-tag (wt ketoisomerase) and wild-type superfolding green florescent protein with C-terminal His-tag (wt GFP) were purified on Ni-NTA resin. Two microliters of wt ketoisomerase were injected at 200 µg/mL, and 2 µL of wt GFP at 70 µg/mL in 10 mM PBS buffer (pH 7.4).

Table 1. Chromatographic and MS conditions.

Agilent 1260 Infinity II LC	
Mobile phase A	0.1% Formic acid in water
Mobile phase B	0.1% Formic acid in acetonitrile
Flow rate	0.5 mL/min
Gradient	0 minutes 10% B 0.1 minutes 15% B 4.5 minutes 55% B 5 minutes 10% B
Stop time	6.5 minutes
Column temperature	80 °C
Agilent InfinityLab LC/MSD XT	
Data storage	Full, profile mode
Ion mode	Positive, ESI
Scan range	*m/z* 600 to 2,000
Fragmentor	150 V
Gain EMV	1
Threshold	0
Drying gas temperature	350 °C
Drying gas flow	12.0 L/min
Nebulizer pressure	45 psig
VCap (Positive)	3,500 V

Results and discussion

The LC separation of wt ketoisomerase gives two peaks: a major peak at 4.18 minutes and a smaller peak at 4.51 minutes (Figure 1A). The mass spectrum of the major peak (Figure 1B) is deconvoluted using the built-in deconvolution tool, and gives the deconvoluted spectrum (Figure 1D). The ion sets used to generate the deconvoluted spectrum is also provided by the software (Figure 1C). The deconvoluted mass of wt ketoisomerase is 15,980 Da, and matches the expected molecular weight (MW). The peak at 4.51 minutes is also analyzed, and the deconvoluted mass is 31,957 Da. This second peak appears to be the dimer of the protein. These results show that LC separation followed by mass selective detection provides valuable information about the sample composition. This approach can be used to assess impurities in protein samples as well.

Only one peak at 3.59 minutes is observed in the wt GFP sample (Figure 2A). The apex mass spectrum of the peak and the deconvoluted spectrum are shown in Figures 2B and 2C. The deconvoluted mass matches the expected MW (27,597 Da). A common loss of the first methionine, cyclization and oxidation can occur during expression and fluorophore core formation, which leads to a loss of 151 Da, and results in the expected molecular weight.

These data demonstrate that the GFP sample is of uniform composition.

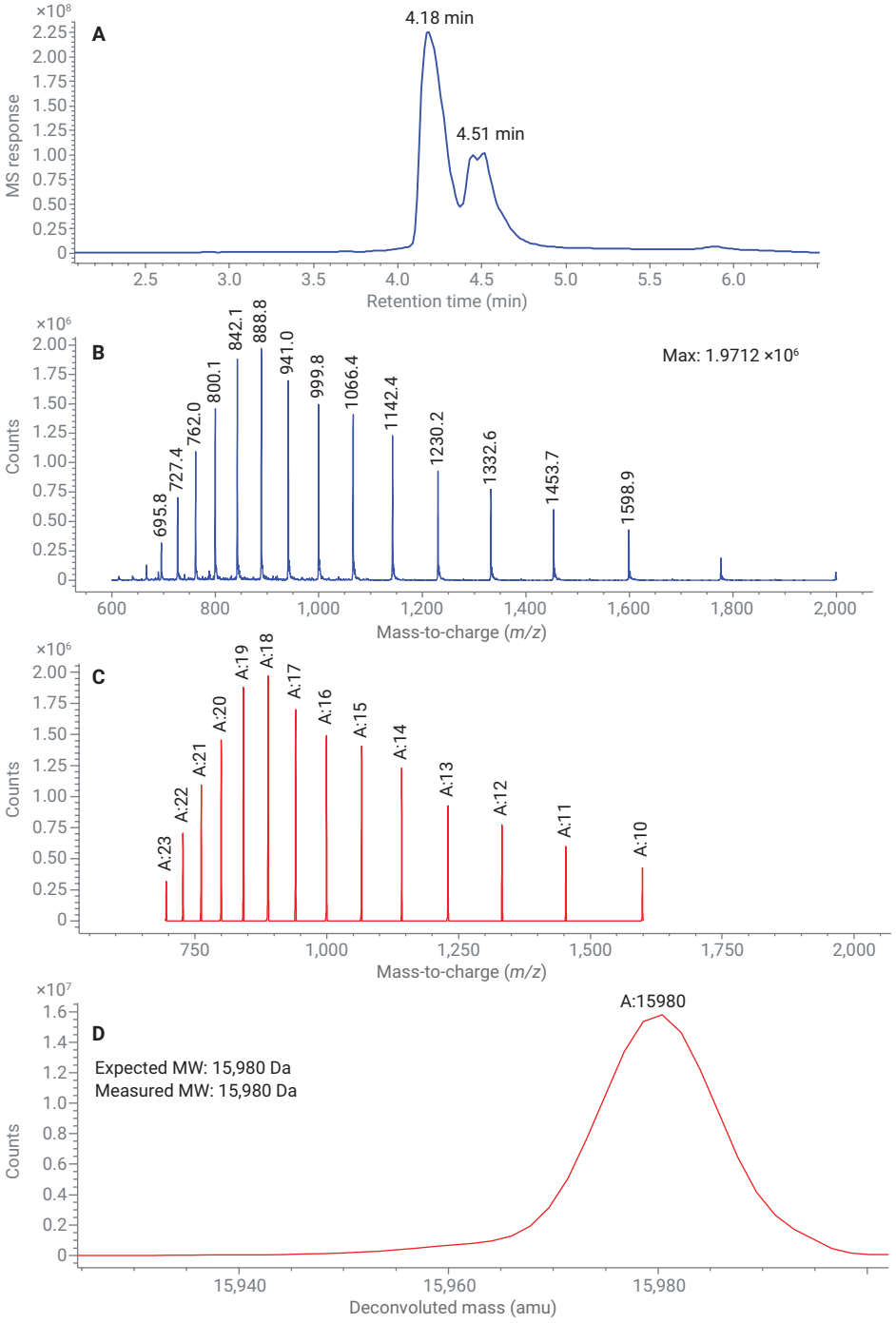

Figure 1. LC/MS mass analysis of wt ketoisomerase. A) LC separation of wt ketoisomerase showing two peaks. B) Mass spectrum of major peak (4.18 minutes). C) Ion sets used to generate the deconvoluted mass spectrum. D) Deconvoluted mass spectrum measuring 15,980 Da ketoisomerase matches with the expected mass.

Conclusion

Protein analysis using a 1260 Infinity II LC coupled to an InfinityLab LC/MSD XT was successfully demonstrated. Two intact protein samples were analyzed, and the deconvoluted masses matched the expected MWs. Mass-selective detection provides an economical, robust, and easy-to-use approach for protein molecular weight confirmation.

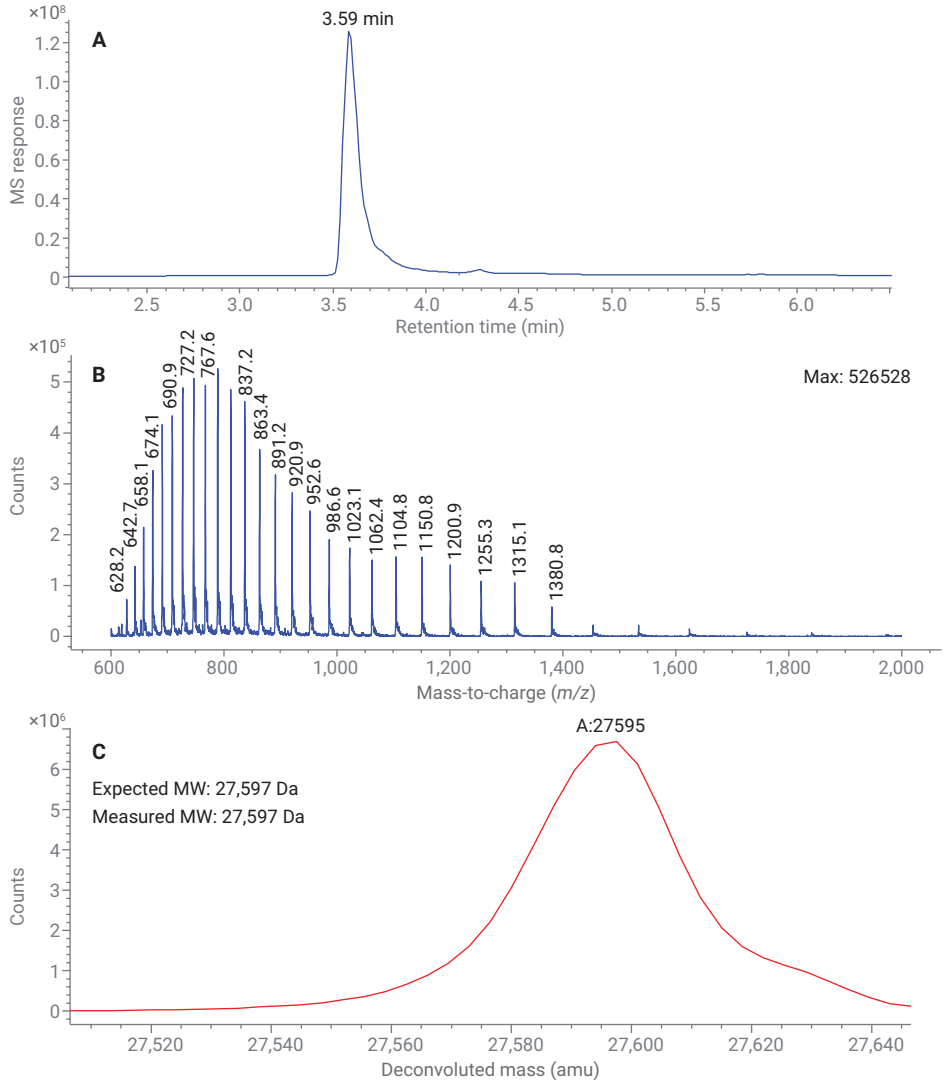

Figure 2. LC/MS mass analysis of wt green fluorescent protein. A) LC separation of wt GFP ketoisomerase showing one peaks. B) Mass spectrum of GFP. C) Deconvoluted mass spectrum measuring 27,597 Da GFP protein matches with the expected mass.

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