Influence of Mass Resolution on Intact Protein Analysis by Mass Spectrometry

Da Ren, Himanshu S. Gadgil , Paul Rainville, Reb J. Russell, Tom Wheat and Jeff R. Mazzeo
Waters Corporation, Chemistry Applied Technologies, Milford, MA, USA

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Overview

With the recent development of new ionization techniques, especially MALDI and ESI, mass spectrometry has become more and more popular in the world of protein analysis. Various kinds of mass spectrometers have found utility in the biopharmaceutical industry. Among all the factors that differentiate these mass spectrometers, resolution is one of the major factors that affects instrument performance. In this research, we take an in-depth look at instrument resolution from both theoretical and experimental aspects. Enolase and monoclonal IgG 1 from mouse are used to measure the performance of different mass spectrometers from quadrupole to time of flight instruments. Results from different instruments show different capabilities in identifying isoforms and modifications from intact proteins. Combining the calculated data with experimental results gives a guideline in choosing mass spectrometers for different purposes and different processes in biotherapeutic production.

System Components

Waters® BioSuite™ Intact Protein MS System

Waters® Micromass® ZQ™ Mass Spectrometer

Waters® Micromass® Q-Tof micro™ Mass Spectrometer

Experimental

MS Conditions:

-Source = ESI(+)
-Capillary (kV) = 3.3
-Cone (V) = 35
-Temperature (°C)
  -Source = 150
  -Desolvation = 400
-Gas Flow (L/Hr)
  -Cone = 50
  -Desolvation = 500
-Scan Mode
  -MS Mode
-Collision Energy (V) = 6

Software:

-Waters® MassLynx™ 4.0 with MaxEnt 1

Background

The isotopic pattern in a small molecule mass spectrum is relatively simple. But for intact protein analysis, mass spectra become much more complicated due to isotopic contributions from large number of protein molecules. It makes more sense to measure the average mass instead of measuring the monoisotopic peak which has extremely low intensity for proteins of the size of Enolase. The molecular peak for large proteins will be presented as a Gaussian shape peak that is composed of several intensive isotope distributions. The intensity of each peak can be calculated by the following equation:

$$A = \frac{n!}{(a)!(b)!(c)!\ldots}(x_1)^a(x_2)^b(x_3)^c\ldots$$

where A stands for the intensity; n stands for the total number of atoms from one element; a, b, and c stand for the number of atoms of each isotope; r_1, r_2, and r_3 stand for the intensities of different isotopes. The peak width of the intact protein can be acquired by measuring the half height of the Gaussian distribution. If P is the width of isotopic envelope and I is the instrument peak width, the overall peak width of intact protein W is:

$$W = \sqrt{I^2 + P^2}$$

With P being constant for a given protein, the final measured peak width of a protein is in inverse proportion to the resolution of a mass spectrometer.

Results

The ESI mass spectra of Enolase were obtained on a Waters ZQ™ (Figure 1) and a Waters Q-Tof micro™ mass spectrometer (Figure 2). Enolase samples were loaded to a Symmetry300™ Sentry C_4 guard column before being eluted out by high organic solvent.

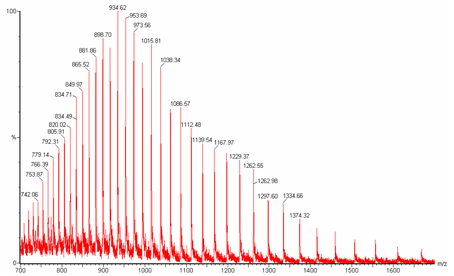

Figure 1. Enolase Mass Spectrum from Waters ZQ™ Mass Spectrometer

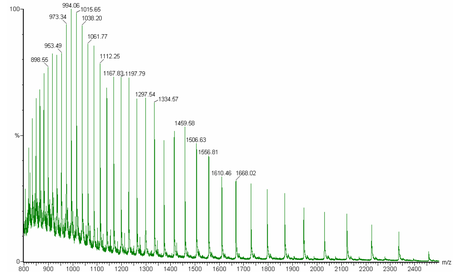

Figure 2. Enolase Mass Spectrum from Q-Tof micro™ Mass Spectrometer

Figure 3 shows the m/z 1198 peaks from both instruments. The theoretical peak width of Enolase is about 13 Da and the charge state of that peak is 39. Without considering instrument peak width, the Enolase 39+ ion should be as wide as 13/39 = 0.33 Da. The peak width we got from the single quadruple instrument is about 1.15 Da, and about 0.48 Da from the Q-Tof instrument.

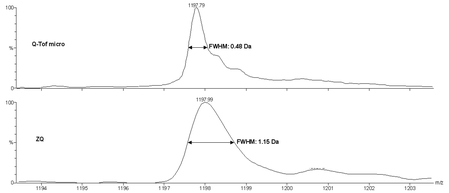

Figure 3. Measured Peak Widths of Enolase 39+ Ion

Waters ZQ™ has a resolution of 1000 and Q-Tof micro has a resolution of 5000. When using a ZQ™ to measure intact protein around 50 kDa, it is possible to differentiate a mass difference of 1.15 X 39 = 45 Da between two adjacent peaks. On the other hand, Q-Tof micro™ can detect modifications with 19 Da or more. The resolution of a mass spectrometer can be changed in a certain range by tuning, higher resolution is attainable by sacrificing sensitivity.

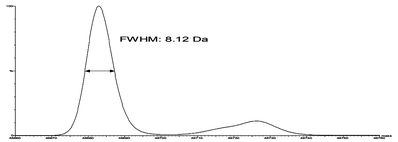

Figure 4. MaxEnt 1 Enhanced Enolase Molecular Peak from ZQ™

MaxEnt 1 is a Waters software that is used for deconvoluting ESI mass spectra composed of complicated charge envelopes. As shown in Figure 4, the peak width of Enolase from ZQ™ after MaxEnt 1 deconvolution is 8.12 Da compared to 45 Da from original mass spectrum. Data from Q-Tof micro™ (Figure 5) also shows the peak width change from 19 Da to 1.38 Da. MaxEnt 1 deconvolution does not only increase the signal-to-noise ratio, but also enhances peak resolution which makes the detection of modifications easier.

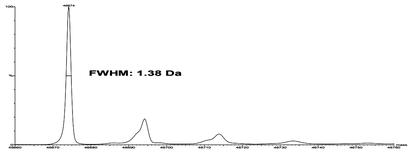

Figure 5. MaxEnt 1 Enhanced Enolase Molecular Peak from Q-Tof micro™

Identification of post translational modification products from IgG 1 is a very important. Figure 6 and Figure 7 are the ESI mass spectra measured from ZQ™ and Q-Tof micro™.

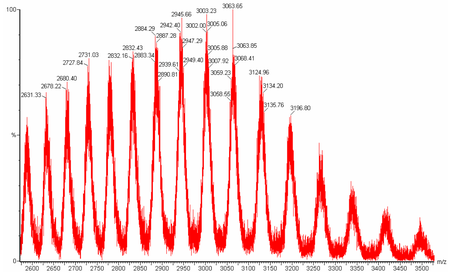

Figure 6. IgG 1 Mass Spectrum from Waters ZQ™ Mass Spectrometer

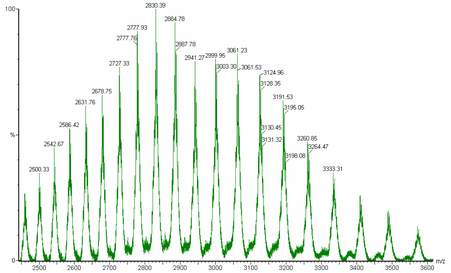

Figure 7. IgG 1 Mass Spectrum from Q-Tof micro™ Mass Spectrometer

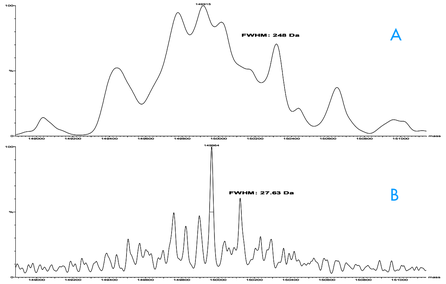

Figure 8. MaxEnt 1 Deconvolution of IgG 1 Mass Spectra
A: ZQ™ data; B: Q-Tof micro™ data

The molecular weight of monoclonal IgG 1 from mouse is about 150 kDa. A Q-Tof micro instrument can easily identify loss of galactoses (162 Da) at this mass range.

Conclusions

- In a mass spectrum, peak width of an intact protein is determined by its theoretical peak width and instrument peak width
- Theoretical peak width is in proportion to molecular weight
- Single quadrupole mass spectrometers are suitable for quick intact protein molecular weight measurement and some PTM identification
- Q-Tof type mass spectrometers can not only measure intact protein mass, but can also identify different post translational modifications with smaller mass shifts
- MaxEnt1 software increases both resolution and signal-to-noise ratio in intact protein ESI mass spectra

References

Yergey, J. A. A general approach to calculating isotopic distributions for mass spectrometry *Int. J. Mass Spectrom. Ion Phys.* 1983, 52 337-349.
Dilip, K. R.; Green, B. N., etc. Accurate Mass Measurement by Electrospray Ionization Quadrupole Mass Spectrometry: Detection of Variants Differing by <6 Da from Normal in Human Hemoglobin Heterozygotes *Anal. Chem.* 2003, 75, 1978-1982.