Characterization of a Monoclonal Antibody using a High-Resolution and Accurate-Measurement Mass Spectrometer with Multiple Fragmentation Techniques

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Overview

Purpose: Characterization of a monoclonal antibody using a high-resolution and accurate-measurement mass spectrometer equipped with multiple fragmentation techniques.

Methods: The monoclonal antibody, with or without enzymatic treatment, was analyzed by liquid chromatography-mass spectrometry.

Results: The intact antibody and light chain were characterized; Over 98% of the heavy and light chain sequence was covered by the bottom-up approach.

Introduction

Monoclonal antibodies (mAbs) have become a promising type of therapeutics for biopharmaceutical industry. Thorough characterization of mAbs is needed for research and production. Mass spectrometry (MS) with high resolution and accurate mass has become an essential analytical tool for mAb structural determination. A monoclonal IgG1 kappa antibody, composed of two heavy chains and two light chains for a total of 1328 amino acids, is characterized in this study.

Methods

The mAb was enzymatically treated before liquid chromatography (LC)-MS analyses. Both intact proteins and peptides eluted from the column were analyzed using an Orbitrap™ Elite mass spectrometer. The intact protein was directly injected onto a ProSwift™ RP-10R column (1mm x 5cm) and further evaluated by the Orbitrap™. Aliquot of mAb was deglycosylated and reduced with dithiothreitol before the LC-MS analysis. For bottom-up approach, the mAb was denatured, reduced, alkylated and then digested using trypsin. Multiple fragmentation techniques were applied towards the amino acid sequencing. LC-MS spectra of intact or reduced mAb were analyzed using a piece of protein deconvolution software (Protein Deconvolution 2.0). The bottom-up data was interpreted with Proteome Discoverer 1.3, providing an easy and fast workflow for investigating amino acid sequence.

Results

Intact mAb Analysis by LC-MS

Intact mAb was directly injected onto a ProSwift™ RP-10R column (1mm x 5cm) and gradient eluted into the Orbitrap™ Elite. The solvents include aqueous solvent as 0.1% FA in water and organic as 0.1% FA in acetonitrile. The full MS spectrum below was acquired at 15,000 resolution, with mass range from 1500 to 3500 m/z. As shown in Figure 1, the spectrum shows a clean distribution of the whole charge envelope, in which each peak represents the protein with a different amount of charges on it. The peaks are well resolved from each other in this scan extracted from a LC-MS run.

FIGURE 1. Full MS Spectrum of the intact mAb with a well resolved charge envelope.

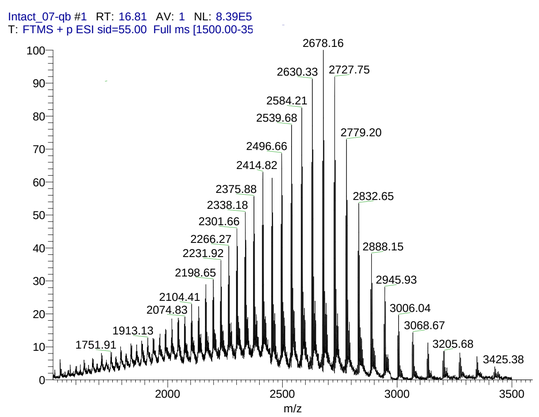


FIGURE 2. Expanded view of the full MS Spectrum of the intact mAb.

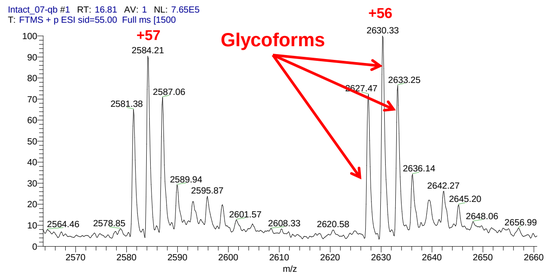


Figure 2 presents peaks at charge states +56 and +57. For each charge state, the spectrum revealed a clean distribution of 4 major glycoforms and a few minor forms.

FIGURE 3. Deconvoluted Spectrum by Protein Deconvolution 2.0.

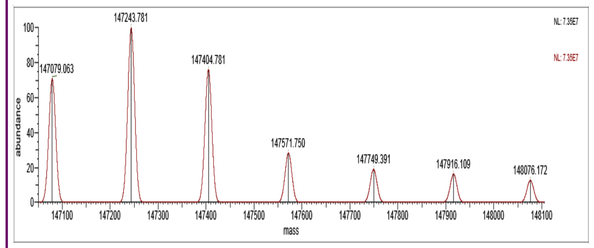


FIGURE 4. Deconvoluted Masses by Protein Deconvolution 2.0.

No.	Average Mass	Sum Intensity	Delta Mass	Relative Abundance
1	147243.781	73,497,330.09	0.00	100.0000
2	147404.781	56,049,679.02	161.00	76.2608
3	147079.063	52,041,818.85	-164.72	70.8078
4	147571.750	20,931,206.56	327.97	28.4789
5	147749.391	13,965,441.12	505.61	19.0013
6	147916.109	11,943,868.05	672.33	16.2508
7	148076.172	9,363,481.54	832.39	12.7399

As shown in Figure 3 and 4, the spectrum was deconvoluted using protein deconvolution 2.0 software, revealed 4 major glycoforms and a few minor forms.

Reduced Intact mAb Analysis

The mAb was deglycosylated with PNGase F and then reduced with dithiothreitol. The protein mixture was injected onto a ProSwift™ RP-10R column (1mm x 5cm) and gradient eluted into the Orbitrap™ Elite. With a gradient of 5-60% in 6 minutes, the heavy chain and light were co-eluting into mass spectrometer for detection, resulting a mixed spectrum of light and heavy chain as shown in Figure 5. Spectrum in Figure 5 was acquired at 15,000, the lowest resolution, on Orbitrap™ Elite.

For achieving isotopically resolved spectrum, high resolution was applied on the light chain at either 60,000 resolution or 240,000 resolution. In Figure 6, the upper panel shows the light at 60,000 resolution (*m/z* 400) and bottom panel at 240,000 resolution (*m/z* 400). The peak at charge state +19, measured at 240,000 resolution is well resolved to the baseline.

FIGURE 5. A mixed spectrum of reduced light and heavy chain.

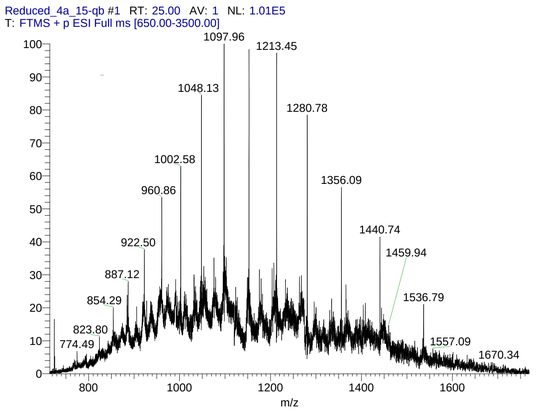
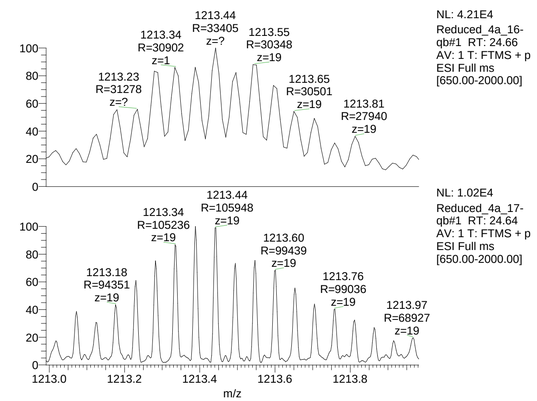


FIGURE 6. Light chain (+19) measured at 60,000 (upper panel) and 240,000 (bottom panel) resolutions.

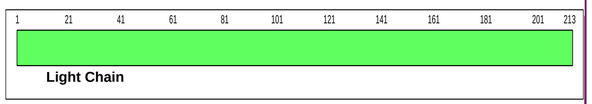


Protein Sequencing by Bottom-up Approach

All tryptic peptides were loaded onto a Halo C18 column, 2.7µm, 20cm followed by 300nL/min gradient. HCD spectra were detected by Orbitrap™; CID and ETD spectra were analyzed using the ion trap part. The sample is not optimized for ETD spectra since trypsin digest could yield majorly +2 ions, which is less favorable for ETD application.

FIGURE 7. Summed sequence coverage of light and heavy chain by complementary fragmentation methods (HCD, CID, ETD).

Description	ΣCoverage	MW [kDa]
light chain chimeric	100.00 %	23.0
heavy chain chimeric	98.23 %	49.2



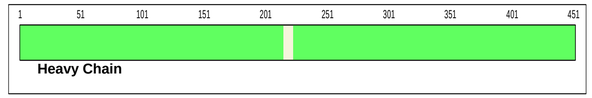


FIGURE 8. Sequence coverage of light and heavy chain by HCD.

Description	Coverage	# Unique Peptides	MW [kDa]
light chain chimeric	97.18 %	29	23.0
heavy chain chimeric	84.26 %	50	49.2

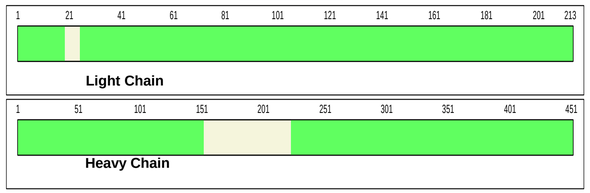


FIGURE 8. Sequence coverage of light and heavy chain by ETD.

Description	Coverage	# Unique Peptides	MW [kDa]
light chain chimeric	100.00 %	33	23.0
heavy chain chimeric	91.57 %	42	49.2

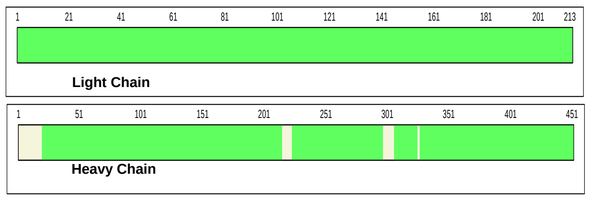
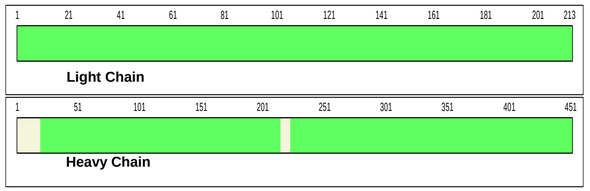


FIGURE 9. Sequence coverage of light and heavy chain by CID.

Description	Coverage	# Unique Peptides	MW [kDa]
light chain chimeric	100.00 %	39	23.0
heavy chain chimeric	94.01 %	55	49.2



Conclusion

- Intact mAb was characterized by LC-MS. The full MS spectrum from Orbitrap™ Elite demonstrates a clean distribution of the whole charge envelope. For each charge state, four major glycoforms and a few minor forms were detected.
- Deglycosylated and reduced mAb was surveyed with a 5-60% gradient online to Orbitrap™ Elite. A mixed spectrum of light and heavy chain was acquired. The light chain was effortlessly resolved to baseline at 240,000 resolution.
- Multiple fragmentation methods were applied for sequencing tryptic mAb. Over 98% of heavy chain and 100% of light chain was sequenced.