Methods for In-Depth Characterization of a Biotherapeutic Antibody

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Overview

Full, in-depth characterization of biotherapeutics has become more and more important for reasons of efficacy, immunogenicity, comparability, etc. Improvements in characterization methods have resulted from improvement in techniques, methods, chemistries, software, and instrumentation. We developed a series of methods for the characterization of biotherapeutics via both top-down and bottom-up approaches. A monoclonal antibody was fully characterized at the intact and peptide mapping levels. By utilizing high resolution mass spectrometers, we were able to characterize different isoforms of intact antibodies at 150 kDa. The data demonstrate the ratio of different modifications in the intact therapeutic that were difficult to infer from the peptide map. On the other hand, we developed a long gradient method for antibody peptide mapping. This method gives good separation of peptides and is mass spectrometry friendly. Various modifications such as lysine variation, oxidation, and asparagine deamidation were identified in the peptide map. Complementary results form top-down and bottom-up methods provide a better view on biotherapeutic products.

System Components

Waters® BioSuite™ Intact Protein MS System

Waters® 2796 Bioseparations Module
Waters® Micromass® Q-Tof 2™ Mass Spectrometer

Waters® BioSuite™ Peptide Mapping MS/MS System

Waters® 2796 Bioseparations Module
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

MS/MS Conditions:

-Scan Mode
  -Survey Scan Mode
-Number of Components = 3
-Collision Energy
  -Use Collision Energy Profile
-MS/MS Scan Duration
  -Scan Time (sec) = 1.9
  -Inter Scan Time (sec) = 0.1

HPLC Conditions:

Waters® BioSuite™ PA-A C_{18} 2.1 X 150 mm, 3 µm column
Mobile Phase A: 0.2% formic acid in water
Mobile Phase B: 0.2% formic acid in acetonitrile
Gradient: 0-40% buffer B in 150 mins

Results and Discussions

A monoclonal mouse IgG 1 was characterized via both top-down and bottom-up approaches. For the analysis at the intact level, a mass spectrometer with 10,000 resolution was used for accurate mass measurement. The mass accuracy specification for Q-Tof 2™ type instruments is around 5 ppm. This make it possible for the accurate mass acquisition of intact antibodies and the identification of their post translational modification products.

A denaturing SEC-MS method was used to separate reduced and alkylated IgG 1 samples before MS analysis. Acetonitrile is used as a denaturing reagent to detach IgG light chains and heavy chains before they are separated by SEC. Deconvoluted mass spectra of IgG 1 light chain and heavy chain are shown in Figure 1 and Figure 2, respectively.

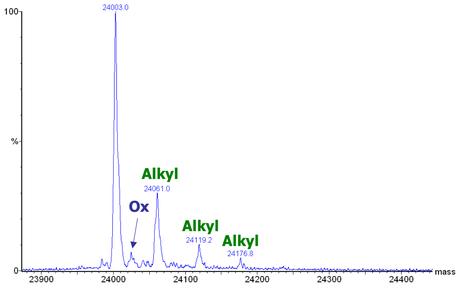

Figure 1: Deconvoluted ESI Mass Spectrum of IgG 1 Light Chain

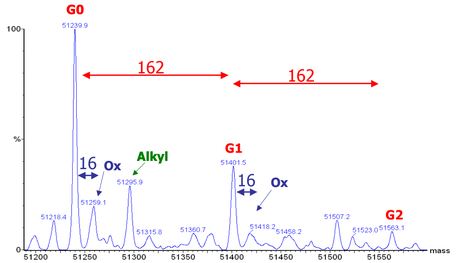

Figure 2: Deconvoluted ESI Mass Spectrum of IgG 1 Heavy Chain

Glycosylation is the only modification that can be identified on intact IgG 1 mass spectrum. By measuring IgG 1 light chain and heavy chain separately, we were able to obtain better resolution and identify more modifications at smaller masses such as alkylation and oxidation.

The amino acid sequence of the IgG 1 that we purchased for this study was unknown. In order to identify post translational modification products, we had to identify the sequence of the antibody first. The strategy for peptide mapping is shown in Figure 3.

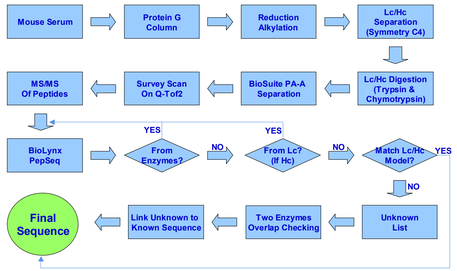

Figure 3: Peptide Mapping Strategy for IgG 1

DVLMTQTPRSLQVSLGDQASISRSSQNIVHSNGNP
YLDWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSG
SGTDFTLKISRVFARSLGVSKTGFPGSSVPWTFGG
GTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCF
LNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKD
STYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPI
VKSFNRNEC

Figure 4: IgG 1 Light Chain Sequence

IgG 1 light chain sequence (Figure 4) is acquired according to the strategy demonstrated in Figure 3. Two enzymes (trypsin and chymotrypsin) were used for IgG 1 digestion. Figure 5 shows the LC/MS spectrum of IgG 1 tryptic digestion

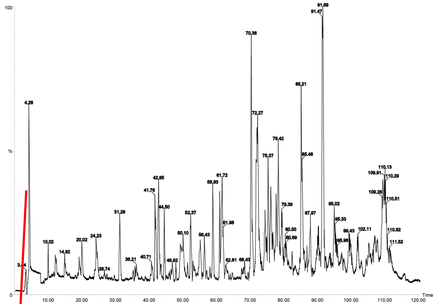

Figure 5: IgG 1 Tryptic Peptide Mapping LC/MS Spectrum

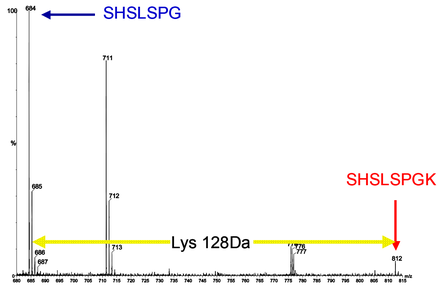

Figure 6: IgG 1 LC/MS Peak (4.28 min) Showing Lysine Variation

LC/MS spectrum of peptide mapping peak at 4.28 min is shown in Figure 6. Peptides with 128 Da difference were found in this peak. MS/MS data confirmed that their sequences are SHSLSPG and SHSLSPGK, respectively, which indicates lysine variations.

Deamidation is another post translational modification found in IgG 1 peptide map. DLNVK and DLDVK are the two peptides identified by their MS/MS spectra. For confirmation, synthesized peptide samples DLNVK, DLDVK, and DLisoDVK were spiked into IgG 1 digestion and the LC separation with spiked samples is shown in Figure 7.

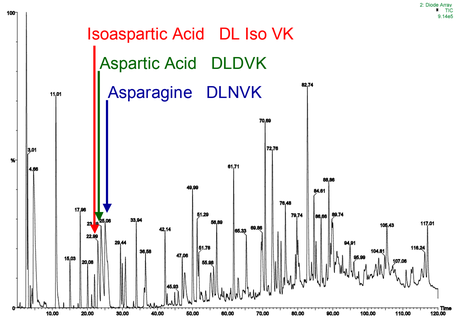

Figure 7: IgG 1 Peptide Mapping of IgG 1 Spiked Sample

The existence of DLNVK and DLDVK in IgG 1 is confirmed by retention time overlap with known samples in the peptide map. Asparagine and aspartic acid are different by one mass unit which can be picked up by MS, but the identification of aspartic acid and iso aspartic acid is only feasible by LC separation.

Conclusions

- A top-down method for biotherapeutic antibody analysis can be used for intact mass measurement as while as the identification of some isoforms
- A bottom-up method reveals the sequencing information of antibody. Most of the post translational modifications including deamidation can be identified at this level
- Antibody characterizations at intact and peptide mapping level are complementary to each other