Application of Data Independent Acquisition for Top-Down Characterization of IgG Light Chain

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Introduction

Within the past few years, monoclonal human immunoglobulin gamma (IgG) antibodies have become popular biotherapeutic candidates. Mass spectrometry, a powerful technique, emerged to meet the need of high-throughput analysis for structural characterization of monoclonal antibodies (mAbs). Most commonly, the characterization of mAbs is performed by a "bottom-up" approach, in which the mAb is digested by protease and analyzed at the peptide level. However, due to the known limitations with this approach, a more favorite "top-down" approach can be used to analyze the intact or the large domain of the mAbs. Here, we report a method using data-independent strategy with the TripleTOF® LC-MS/MS instrument to characterize light chain of IgG by top-down approach. Intact molecular weight of both light chain and heavy chain was determined in TOF MS and matched the value determined by primary sequence with known modifications. The high resolution MS/MS spectra were acquired by a data independent acquisition method, in which all charge species (+22 to +32) of light chain within mass range of m/z 755 to 1076 were isolated, one per isolation window, and fragmented by CID. The combined fragment information of the given precursors provided thorough information and improved sequence coverage for top-down protein sequencing. Data showed that such improvement was mainly affected by identification of precursor charge-dependent and precursor charge-favored fragment ions. This valuable information can be missed during a traditional top-down method, in which only the most abundant precursors are selected for fragmentation.

Experimental

Sample Preparation: 50 µg of Intact mAb (IgG1) was subjected to dithieothreitol (DTT) at 60°C for 45 min to reduce the antibody to the light and heavy chains.

Chromatography: Chromatographic separation of reduced light chain and heavy chain was performed on a high flow UHPLC system. Approximately 1 µg of reduced sample was loaded onto a Poroshell 300 SB-C8 column. Light and heavy chain were separated by a linear gradient of 20%-55% of B in 15 min, using 100% H_2O, 0.1% FA (solvent A) and 100% ACN, 0.1% FA (solvent B).

SWATH® Acquisition using TripleTOF® 6600 LC-MS/MS system: The MS analysis was performed on a TripleTOF 6600 system using SWATH acquisition. A variable precursor isolation window was applied. The Q1 isolation window width was determined by the precursor charge state of light chain to maintain one charge species per window. High resolution MS/MS spectra were acquired for each Q1 window, with windows covering all charge species (+22 to +32) of light chain within mass range of m/z 755 to 1076 and fragmented by CID. Data was processed using PeakView® Software.

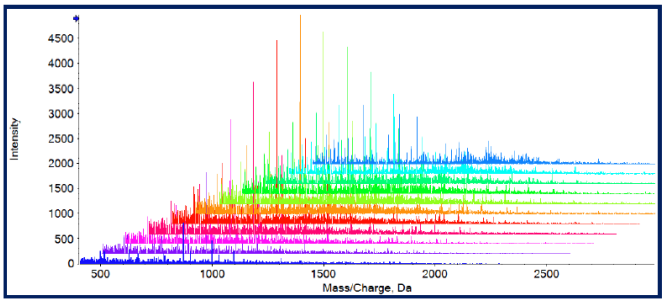

Figure 1. MS/MS spectrum from SWATH acquisition: In this SWATH acquisition analysis, the instrument stepped through m/z of 755 to 1076 and generated high resolution MS/MS spectrum (represented by each color) for this mass range with a cycle time of 2.4 sec. A variable Q1 window was applied to maintain fragmentation of one charge state per mass window.

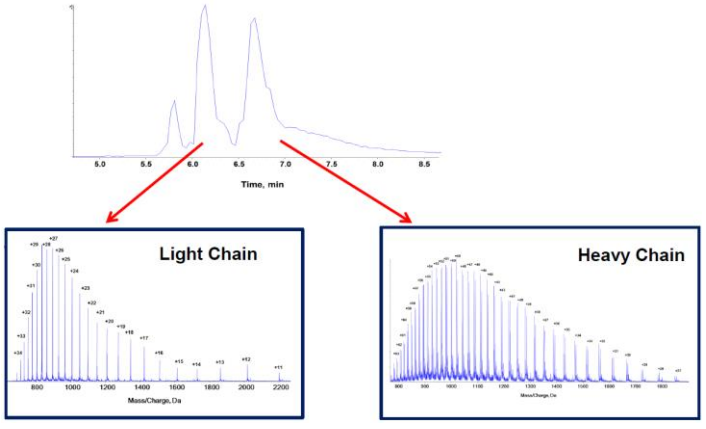

Figure 2. LC-MS chromatogram. Shown are the peaks from 1 µg reduced antibody sample. The light and heavy chains elutes at 6.2 min and 6.8 min respectively. Also shown below is the charge state envelope for each chain used for mass reconstruction, confirming the mass of the 2 IgG chains.

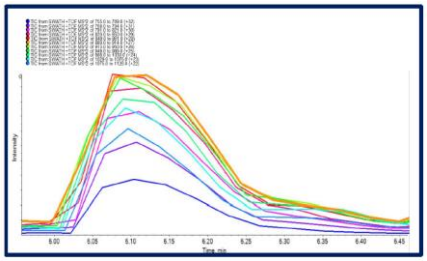

Figure 3 SWATH acquisition of IgG Light Chain. Shown is the overlap of the TIC of SWATH acquisition for the charge states ranging from +22 to +32. Variable mass window is chosen for each charge state to encompass the major peaks.

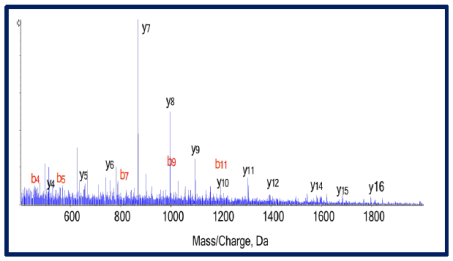

Figure 4 MS/MS analysis for +30 charge state. Shown is the MS/MS spectra of the m/z window of 791-821 which corresponds to the +30 charge state of the light chain. The abundant fragment ions observed are +1 charge of the N & C-term ion ladder. The peaks at lower signal, overlapping with noise, are highly multi-charged > +20.

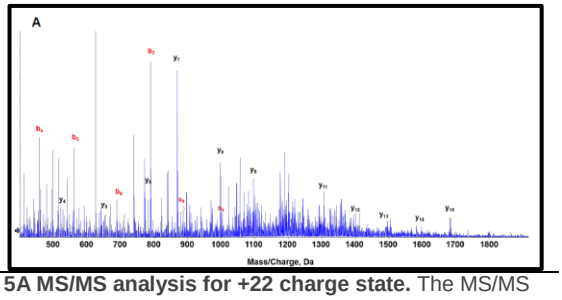

Figure 5A MS/MS analysis for +22 charge state. The MS/MS spectra of the m/z window of 1075-1126 corresponds to the +22 charge state. In addition to the terminal ion ladder, other fragment ions of higher charge states are also observed above the noise level. Figures 5B-D show the zoomed in regions from m/z 850-1500 to take a closer look at the fragment ions identified.

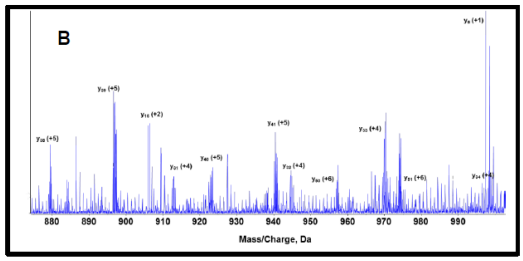

Figure 5B shows the zoomed in MS/MS m/z region of 850-1000 with various multiply charged y ion MS/MS fragments.

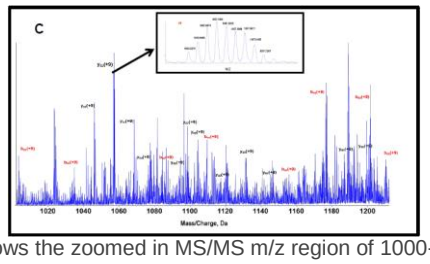

Figure 5C shows the zoomed in MS/MS m/z region of 1000-1250 with the +9 charged MS/MS fragments from the b 82-99 and y 81-96 ions. The inset shows the zoomed in area for the y82 ion confirming the +9 charge state.

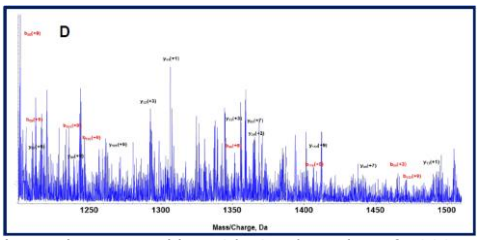

Figure 5D shows the zoomed in MS/MS m/z region of 1200 -1500 identifying +9 charged MS/MS y97-114 and b99-122 fragments of the light chain.

DVLMTQTPLSLPVSLGDQASISCRSSQYIVHSNGNTYL
EWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDF
TLKISRVEAEDLGVYYCFQGSHVPLTFGAGTKLEIKRAD
AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKW
KIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDE
YERHNSYTCEATHKTSTSPIVKSFNRNEC

Table 1 IgG1 Light Chain Sequence Coverage. Highlighted in blue and green are the regions of the light chain that was identified from the various charged b and y-ion fragments, respectively. As can be observed a more complete coverage can be obtained via MS/MS spectra of the various charged state precursor masses of the IgG1 light chain.

Conclusions

- Shown in this study is a top down approach using data independent analysis to obtain better sequence coverage compared to the traditional data dependent approach.
- Variable Q1 mass window was used in SWATH® acquisition analysis to maintain one charge species per mass window provides for selectively generating MS/MS of specific precursor charge states.
- MS/MS spectra from different charge states provide different fragment ion information. Cumulative fragment ion identification from the various charge states can provide a more complete sequence coverage.
- Further investigation will be performed using this top down SWATH acquisition for sequencing of larger domains generated by enzymatically cleaved IgG heavy chain.

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