Isotopic Resolution of Chromatographically Separated IdeS Subunits Using the X500B QTOF System

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Analysis of protein subunits using high resolution mass spectrometry (HRMS) is commonly performed during the development of biotherapeutics. In comparison to intact mass analysis, subunit level approaches offer greater ability to chromatographically separate subunits and improved sensitivity to detect and quantify a wide range of post translational modifications.

Recently for subunit analysis there has been a drive towards collection of mass spectrometric data which has been isotopically resolved to provide greater confidence in peak identification. The X500B QTOF is specifically designed for generation of high quality data across a number of biopharmaceutical workflows including intact mass, subunit and peptide analysis for a wide range of therapeutic modalities.

Presented in this technical note is the use of the SCIEX X500B QTOF System for confirmation of antibody subunits generated by digestion with IdeS. This molecule is used as an example of the resolution possible on this platform for proteins or their subunits within a similar mass range. Mass spectrometric and data processing parameters to achieve high quality data are presented and discussed.

Key Feature of X500B QTOF Solution

- High resolution mass spectrometer for a wide range of biopharmaceutical applications
- Isotopically resolved subunit analysis by HRMS
- Compact benchtop footprint reduces laboratory space requirements
- Easy to use hardware and software accessible for a wide range of users

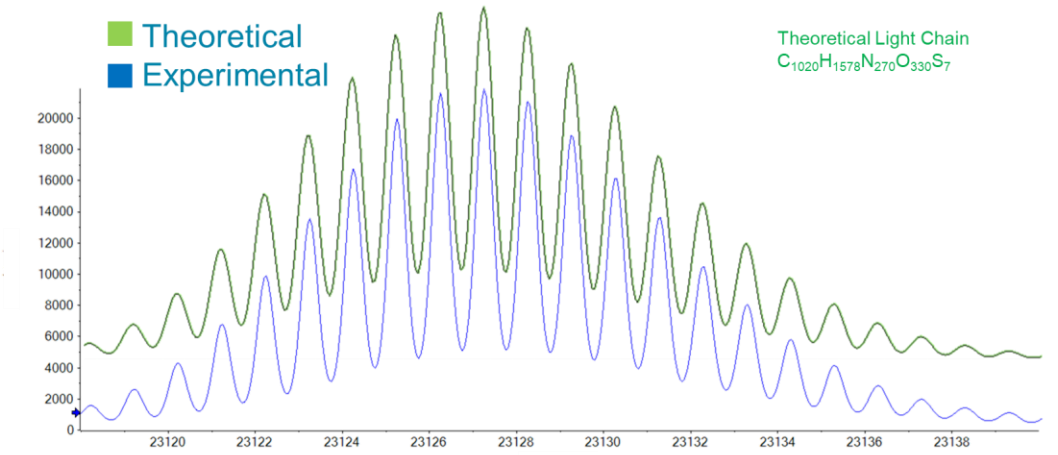

Figure 1. Comparison of Theoretical and Experimental Reconstructed Light Chain Spectra from IdeS Digested NIST Antibody.

Methods

***Sample Preparation**:*

IdeS reduced NISTmAb was first digested with IdeS protease (Cat V7511, Promega) following the protocol from Promega. Briefly, 1 unit of IdeS protease was incubated with 1 µg of IgG at 37°C for 1h in PBS. The resultant sample was further reduced with TCEP using the above protocol. 5 µl samples were subjected to LC-MS analysis.

Chromatography:

Separation was accomplished using a Shimadzu ExionLC™ System fitted with an Agilent PLRP-S column (2.1mm X 50mm, 300Å, 5µm) at 80°C using the gradient shown in Table 1. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile.

Table 1. LC Gradient Conditions

Time (min	%A	%B	Flow Rate ml/min
Initial	*75*	*25*	*0.25*
3.0	*75*	*25*	*0.25*
9.0	*10*	*90*	*0.25*
11.4	*10*	*90*	*0.25*
11.5	*75*	*25*	*0.25*
17.0	*75*	*25*	*0.25*

Mass Spectrometry:

A SCIEX X500B Mass Spectrometer with a Turbo V™ Source was used for data acquisition. Data was acquired using TOF-MS mode with intact protein mode (IPM) turned off. MS instrument conditions are listed in Table 2.

Table 2. MS Parameters.

Parameter	Setting
Scan Mode	*Positive*
GS1	*50*
GS2	*50*
Curtain Gas	*35*
Temperature	*400°C*
Ion Spray Voltage	*5000 V*
Time Bins to Sum	*6*
Accumulation Time (ms)	*0.5 sec*
TOF Start Mas (Da)	*400*
TOF Stop Mas (Da)	*3000*
Declustering Potential	*150.0*
Collision Energy	*10*

Data Processing:

Data were processed using SCIEX OS software 1.4.0.18067 and Bio Tool Kit software 1.0. Reconstruction parameters are shown in Table 3

Table 3. Reconstruction Parameters.

Parameter	Setting
Start Mass	*Fc 25000 Da* *LC 22600 Da* *Fd 25200 Da*
Stop Mass	*Fc 26000 Da* *LC 23600 Da* *Fd 26200 Da*
Step Mass	*0.05 Da*
Input Spectrum Isotope Resolution	*30000*

Results and Discussion

IdeS digestion and reduction of a therapeutic monoclonal antibody results in three roughly equivalent mass fragments which correspond to the Fc/2, light chain, and Fd fragments of the monoclonal antibody. This digestion strategy has gained significant attention since it was first reported for use on therapeutic monoclonal antibodies.[1] In particular, IdeS digestion serves to reduce overall sample complexity compared to intact mass measurement and reduces the complexity of sample preparation and data interpretation frequently associated with peptide mapping experiments.

Decreasing sample using and IdeS digest enables greater interrogation of the protein subunit when compared to intact mass analysis. As each fragment generated is around 25 kDa it is possible to generate isotopically resolved spectral data for each component. As shown in Figures 2-4, under the conditions described, isotopically resolved spectra for each subunit were obtained with major relevant features observed compared to previous reports[2]

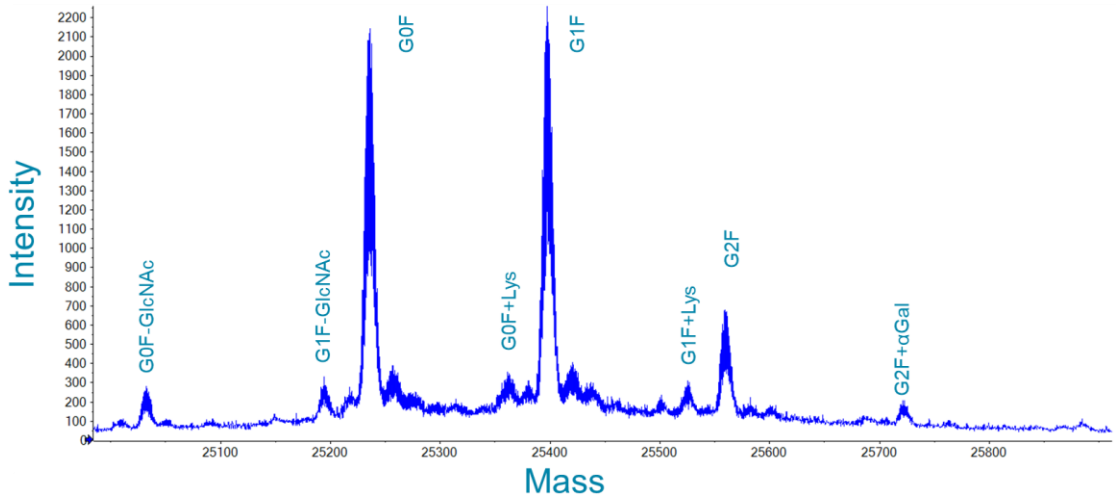

Figure 2. Reconstructed Spectrum for Fc/2 Fragment of NISTmAb Standard Digested with IdeS.

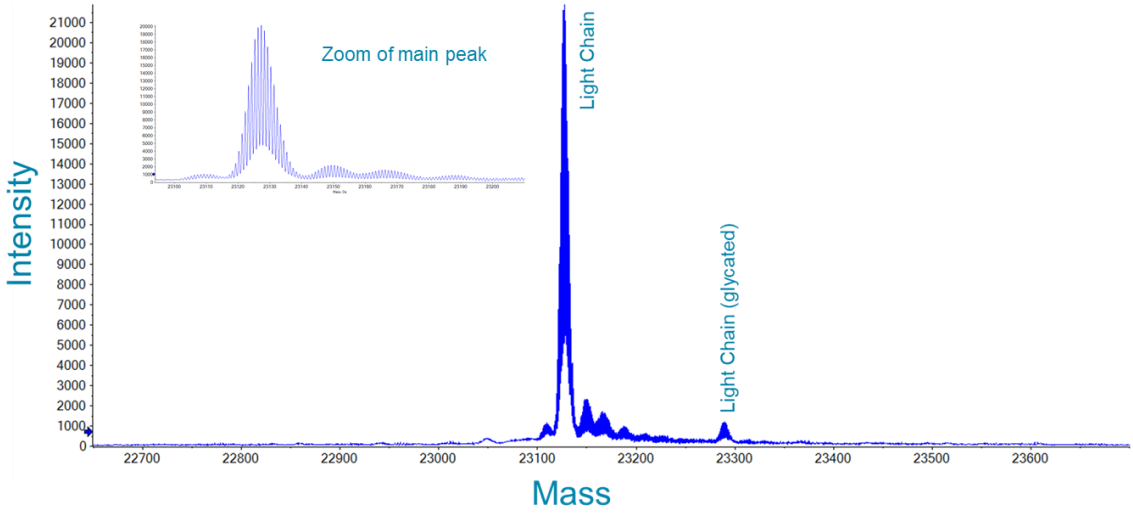

Figure 3. Reconstructed Spectrum for Light Chain of NISTmAb Standard Digested with IdeS and Reduction.

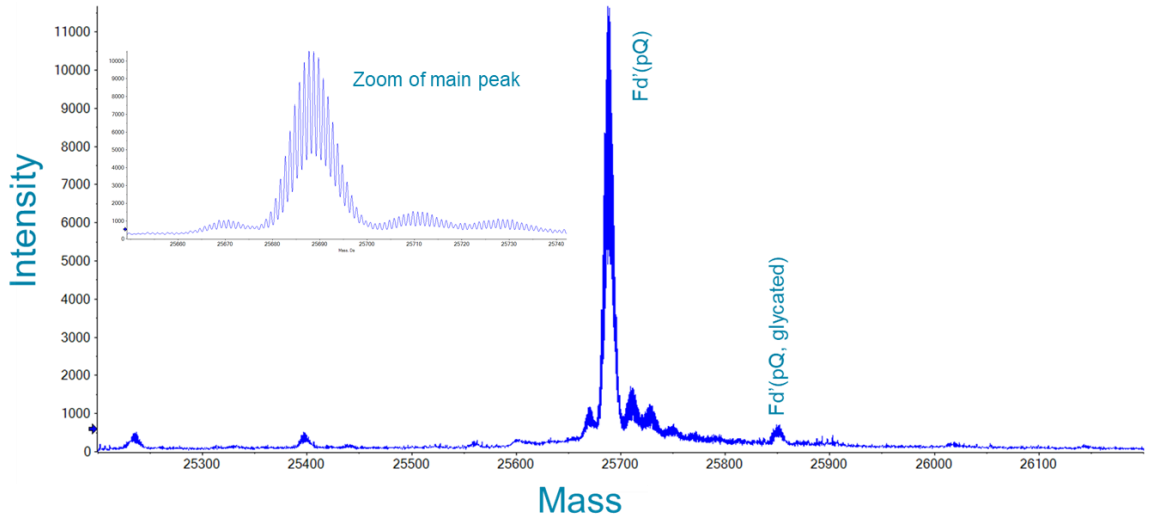

Figure 4. Reconstructed Spectrum for Fd of NISTmAb Standard Digested with IdeS and Reduction.

To verify the quality of our data, we generated a theoretical spectrum based on the elemental composition of the light chain of the NISTmAb standard. The theoretical spectrum was modeled to generate a continuous spectrum with the same resolution used for reconstruction of the experimental data, ca. 30,000. As shown in Figure 1, excellent agreement of experimental (blue trace) and theoretical (green trace) was achieved.

Finally, consistent with previous reports[2], the monoisotopic mass for any species observed is not clearly evident as its abundance is too low in intensity to accurately define. In this case, we verified the mass accuracy of our measurement by comparison of theoretical isotopic masses to those observed experimentally for the light chain. Shown in Table 4 are the theoretical and observed reconstructed masses for each isotope clearly visible in the data. The average error across the light chain was 3.46 PPM highlighting the high mass accuracy with which subunit data can be characterized with the X500B QTOF system.

Conclusions

- Isotopically resolved IdeS subunit analysis on the X500B QTOF system provides accurate assessment of post translational modification with high mass accuracy.
- Strong agreement of theoretical and experimental spectral data for subunits is achieved.
- Presented data is highly consistent with previous reports.

Table 4. Theoretical and Observed masses for light chain with corresponding PPM values.

Theoretical	Observed	PPM
23118.318	*23118.222*	*-4.13*
23119.320	*23119.222*	*-4.25*
23120.323	*23120.226*	*-4.19*
23121.325	*23121.237*	*-3.82*
23122.328	*23122.238*	*-3.89*
23123.330	*23123.25*	*-3.48*
23124.333	*23124.256*	*-3.33*
23125.335	*23125.257*	*-3.39*
23126.338	*23126.255*	*-3.58*
23127.340	*23127.257*	*-3.60*
23128.343	*23128.264*	*-3.40*
23129.345	*23129.267*	*-3.37*
23130.347	*23130.273*	*-3.22*
23131.350	*23131.277*	*-3.14*
23132.352	*23132.281*	*-3.07*
23133.354	*23133.292*	*-2.70*
23134.357	*23134.291*	*-2.84*
23135.359	*23135.294*	*-2.81*
	AVERAGE	***3.46***

References

1. An, Y., Zhang, Y., Mueller, H.-M., Shameem, M., Chen, X., A New Tool for Monoclonal Antibody Analysis. mAbs. 2014;6(4) :879-893. doi: 10.4161/mabs.28762.

2. John E. Schiel, Darryl L. Davis, Oleg V. Borisov (Eds.). Vol 1201, 2015 State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization Volume2. Biopharmaceutical Characterization: The NISTmAb Case Study. Vol 1201, American Chemical Society.

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