Bottom-up characterization of a monoclonal antibody Trastuzumab with CESI-Ms coupled to the Orbitrap mass spectrometers

Jim Thor,¹ María R. Santos,² David K. Bush,¹ Hans Leddaw,¹ Rosa Viné,¹ Alan Biek,¹ Antonius A. M. Heemskerk,² Barry L. Karger¹ and Alexander R. Ivanov²

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Overview

Purpose: To provide a comprehensive characterization of bottom-up tryptic peptides from the Trastuzumab monoclonal antibody using capillary electrophoresis (CE)-HRM mass spectrometry (HMS). We have determined the oligosaccharide composition, glycan profile and the redox state of the cysteines in the antibody.

Methods

Protein Preparation: Trastuzumab was reduced with diithothreitol, alkylated with iodoacetamide and digested with Trypsin (mass spectrometry grade) and desalted with ammonium acetate. The peptide mixture was analysed using Orbitrap at a resolution of 100,000 ppm and at an isolation width of 1 ppm and fragmented using a collision energy of 50 with a range of 100-800 m/z.

Results:

Sequence Coverage

An aspiny (2D gel) plot of the CESI-Ms experiment and the sequencing coverage maps for both the heavy and the light chain were shown in Figure 3. The peptides were fragmented using a collision energy of 50 at a resolution of 100,000 ppm and the sequencing coverage was achieved for the light chain using both SequestHT and 1% FDR and BSMS with 1% FDR.

For the heavy chain, the coverage of 14.4% in Sequel4 and 10.9% in BSMS with the use of the alternative settings. The difficulty in the identification of the peptides in Sequence4 and BSMS was mainly due to the use of primary MS/MS spectra and the fact that the Sequest database was not updated with the latest tryptic peptides

Glycopeptide Analysis:

The results of the MS/MS analysis of both HCD and HCD MS2 spectra demonstrated the presence of glycopeptides containing both 95% high intensity peaks and 4% low intensity peaks, and correct glycan position. The results were also manually annotated, although they showed a general trend across the fragments and direct on glycosylation fragmentation compared to HCD spectra.

Conclusions

The work demonstrates that a combination of CESI, alkylated and highly efficient separation technique with HRM mass spectrometry and advanced bioinformatics analysis allows for comprehensive characterization of Trastuzumab sequence and glycosylation in single fragments. Our CESI-Ms results correlate with several data analysis approaches showed 100% sequencing coverage of the antibody (containing the representative glycopeptide), including the most abundant and low abundant forms as well as low abundant non-modified and modified forms. When MS/MS data was not available, we used extensive software for oligosaccharide of MS/MS peaks to identify other common oligosaccharides. CESI-Ms coupled with a combination of MS/MS high sensitivity detection, HRM mass spectrometry and CE/MSD results and success in identifying glycopeptides and glycan structures.

References

1. Department of Chemistry, University of California, San Diego, La Jolla, CA 92037, USA. 2. Department of Pathology, Leeds University Medical Center, Leeds, The Netherlands. © Centre d’Enzymologie Proteïnes (CEP), Saint-Jean-d’Angély, France. ©Thermo Fisher Scientific, San Jose, California.

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