Analysis of Monoclonal Antibodies in Human Serum as a Model for Clinical Monoclonal Gammopathy by Use of 21 Tesla FT-ICR Top-Down and Middle-Down MS/MS

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Introduction

With the rapid growth of therapeutic monoclonal antibodies (mAbs), stringent quality control is needed to ensure clinical safety and efficacy. Monoclonal antibody primary sequence and post-translational modifications (PTM) are conventionally analyzed with labor-intensive, bottom-up tandem mass spectrometry (MS/ MS), which is limited by incomplete peptide sequence coverage and introduction of artifacts during the lengthy analysis procedure.

Experimental

Here, we describe top-down and middle-down approaches with the advantages of fast sample preparation with minimal artifacts, ultrahigh mass accuracy, and extensive residue cleavages by use of NHMFL's 21 tesla FT-ICR MS/MS. The ultrahigh mass accuracy yields an RMS error of 0.2– 0.4 ppm for antibody light chain, heavy chain Fc/2, and heavy chain Fd subunits. The corresponding sequence coverages are 81%, 72%, and 65% with MS/MS RMS error ~4 ppm (**Fig. 1**).

Results and Discussion

Extension to a monoclonal antibody in human serum as a monoclonal gammopathy model yielded 53% sequence coverage from two nano-LC MS/MS runs. A blind analysis of five therapeutic monoclonal antibodies at clinically relevant concentrations in human serum resulted in correct identification of all five antibodies. NHMFL's nano-LC 21 T FT-ICR MS/ MS provides nonpareil mass resolution, mass accuracy, and sequence coverage for mAbs, and sets a benchmark for MS/MS analysis of multiple mAbs in serum, without the need for invasive biopsy. This is the first time that extensive cleavages for both variable and constant regions have been achieved for mAbs in a human serum background.

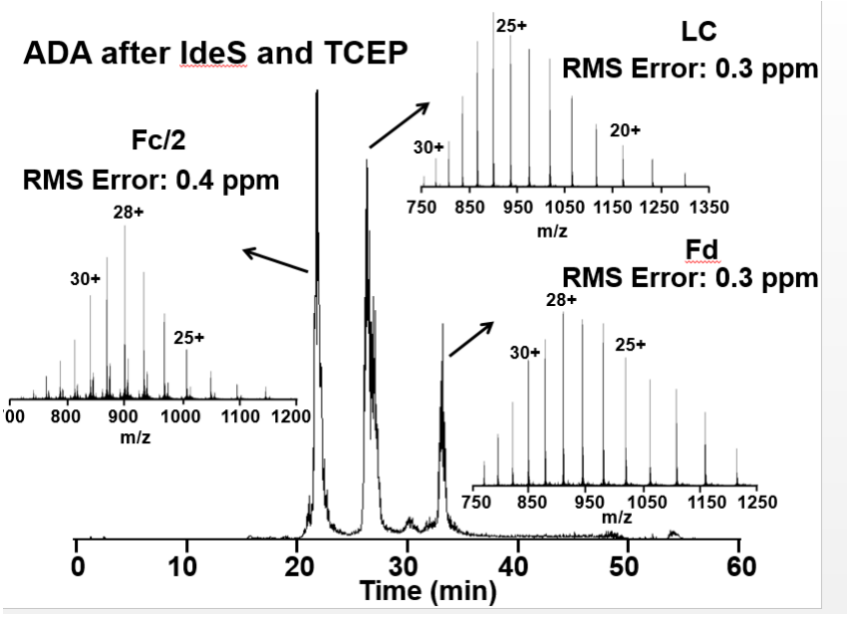

Fig. 1. Total ion current chromatogram for adalimumab subunits after IdeS digestion and TCEP reduction. Broadband (+) ESI 21 T FT-ICR mass spectra of adalimumab Fc/2 (58 spectra averaged, 1 µS/spectrum), light chain (98 spectra averaged, 1 µS/spectrum), and Fd (39 spectra averaged, 1 µS/spectrum) charge state distributions are shown in insets. 0.25 pmol of digested and reduced adalimumab was loaded onto the nano-LC column. 119 peaks from the 7 most abundant charge states were assigned with an rms error of 0.4 ppm, 0.3 ppm, and 0.3 ppm for Fc/2, light chain, and Fd.

Acknowledgements

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Reference

[1] He, L., *et al.*, J. Amer. Soc. Mass Spectrometry, **28**, 827-838 (2017).