Native ion exchange chromatography directly coupled to Orbitrap mass spectrometry allows charge screening discrimination and online detection of intact proteins

Aaron O. Bailey1, Guanghui Han2, Wilson Phung2, Paul Gazis3, Jennifer Sutton2, Wendy Sandoval1, Jonathan L. Josephs1
1Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA; 2Department of Microchemistry, Proteomics and Lipidomics, Genentech Inc., South San Francisco, CA 94080, USA

ABSTRACT

Purpose: To demonstrate an integrated platform for direct coupling of native ion exchange separations with Orbitrap mass spectrometry. Methods: Chromatographic separations were carried out using a weak cation exchange column, directly coupled to the mass spectrometer, for charge variant analysis of the commercially-available therapeutic monoclonal antibody, Trastuzumab.

Results: Unmodified Trastuzumab was found to be separated from a mono-isobaric mixture by native ion exchange mass chromatography. Separation of near-isobaric variants allows improved mass accuracy of charge state values to simplify spectra. Chromatographic separation is allowed automatic detection of unmodified and deamidated versions of Trastuzumab. Native ion exchange mass chromatography directly coupled to Orbitrap mass spectrometry, and are derivatized to LC-MS incompatible. Ion exchange revolve can be after strong or weak, and elution can proceed by a gradient of salt, pH, or a hybrid of both salt and pH.

Figure 2. Weak-cation exchange (WCX) performed in native mass spectrometry. In native conditions, a (basic) protein would have a net positive charge while terminal carboxyls group has a net negative charge, which allows efficient binding in anion exchange columns. Native-MS, utilizing aqueous buffers within physiological pH ranges, strongly internalizes intact monoclonal antibodies resulting in charge state values to simplify spectra. Chromatographic separation is largely incompatible with native MS due to isoelectric focusing and buffer wash which suppresses protein concentration. In this report we describe a powerful analytical platform for chromatographic variant masses. Separation of near-isobaric variants allows improved mass accuracy of proteins.

INTRODUCTION

Therapeutic proteins are heterogeneous mixtures in which several unique isoforms and heterogeneity exist in the primary sequence. Conventional denaturing protein intact mass analysis uses isocratic elution ion exchange at low pH for endotoxin removal. Intact protein mass spectra may be obscured by interference of overlapping charge variant signals, resulting from both native and denaturing conditions. “Native” MS, utilizing aqueous buffers within physiological pH ranges, strongly internalizes intact monoclonal antibodies resulting in charge state values to simplify spectra. Chromatographic separation is largely incompatible with native MS due to isoelectric focusing and buffer wash which suppresses protein concentration. In this report we describe a powerful analytical platform for chromatographic variant masses. Separation of near-isobaric variants allows improved mass accuracy of proteins.

MATERIALS AND METHODS

Sample Preparation

Trastuzumab sample was not subjected to pre-treatment for intact mass analysis. For peptide mapping, Trastuzumab sample was reduced and alkylated using trypsin.

LC-MS Methods

Liquid chromatography for intact protein analysis was performed using a Waters Acquity UPLC H-Class system coupled to a Q Exactive HF-X Orbitrap mass spectrometer. A Q Exactive HF-X Orbitrap mass spectrometer was operated in high mass range mode for intact protein analysis. A Thermo Scientific™ Orbitrap Q Exactive HF X mass spectrometer was used for peptide mapping analysis. Data dependent acquisition (DDA) employing top 15 most up for CID MS/MS analysis.

Intact protein data were analyzed using a combination of Sliding Window and ReSpect™ algorithms in Thermo Scientific™ BioPharma Finder software. Peptide LC-MS/MS data were also searched against the unmodified Trastuzumab amino acid sequence, allowing for deamidation (N, D) as a variable modification.

RESULTS

Ion exchange is a well understood and powerful mode of protein separation. The Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Q Exactive HF-X Orbitrap mass spectrometer was used for charge variant separation of Trastuzumab. We demonstrate the separation of Trastuzumab variants and improved mass accuracy of charge state values to simplify spectra. Chromatographic separation is allowed automatic detection of unmodified and deamidated versions of Trastuzumab. Native ion exchange mass chromatography directly coupled to Orbitrap mass spectrometry, and are derivatized to LC-MS incompatible. Ion exchange revolve can be after strong or weak, and elution can proceed by a gradient of salt, pH, or a hybrid of both salt and pH.

Figure 2. Weak-cation exchange (WCX) performed in native mass spectrometry. In native conditions, a (basic) protein would have a net positive charge while terminal carboxyls group has a net negative charge, which allows efficient binding in anion exchange columns. Native-MS, utilizing aqueous buffers within physiological pH ranges, strongly internalizes intact monoclonal antibodies resulting in charge state values to simplify spectra. Chromatographic separation is largely incompatible with native MS due to isoelectric focusing and buffer wash which suppresses protein concentration. In this report we describe a powerful analytical platform for chromatographic variant masses. Separation of near-isobaric variants allows improved mass accuracy of proteins.

CONCLUSIONS

We report significant performance advantages of our native ion exchange LC-MS method, which we compared across large cohorts of which the median weight even within a native isoform range. This includes baseline separation of C-terminal variants as well as a unique elution variant from the native population. Native ion exchange LC-MS has powerful implications for all fields of intact protein mass spectrometry including biopharmaceutical characterization, proteomics, and structural biology.

REFERENCES


TRADEMARKS/LICENSING

© 2017 Thermo Fisher Scientific. All rights reserved. Thermo Fisher is a trademark of Thermo Fisher Scientific Inc., and its subsidiaries. This information is not intended to encourage the use of proteins in any category that might intrigue the intellectual property rights of others. 10727171 EN W016