Characterization of Intact and reduced therapeutic monoclonal antibodies using microflow size exclusion chromatography coupled with mass spectrometry

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ABSTRACT

Monoclonal antibodies (mAbs) are a major part of protein therapeutics. Although mAbs are relatively stable molecules, post-translational modifications (PTM), sequence variations, and degradation products, can lead to clinical complications and potency loss. Alongside mass spectrometry, microLC has emerged as a superior method for characterization of these proteins. The analytical advantages of high throughput, higher MS sensitivity and smaller amounts of sample required are clearly beneficial. Size exclusion chromatography (SEC) is a well-accepted technique for the detection of intact proteins and accurate quantification of protein aggregates in biological drug products. In this presentation, we report the rapid microLC/MS using microLC SEC characterization of low-microgram levels of an intact mAb and its heterogeneity using a microLC system coupled with a Qq-TOF mass spectrometer (TOF)

INTRODUCTION

Monoclonal antibodies (mAb) are becoming major target oriented bio therapeutics to treat an array of human diseases. Current therapeutic monoclonal antibodies are immunoglobulin G (IgG)1 derivatives. These antibodies have typically been produced by mammalian cell culture using Chinese hamster ovary (CHO) cells, mouse NSO, or SP2/0 plasma cell lines1. In recent years, mass spectrometry has emerged as a superior method for the characterization of the heterogeneity of these proteins due to post-translational modifications (PTMs), sequence variations generated from proteolysis or transcriptional
translational errors, and degradation products which are formed during processing or final product storage Pharmaceutical companies typically go through a four level characterization procedure using different mass spectrometric methods. Characterization Level 1 analyzes the intact protein by determining the accurate molecular weight and to some degree the heterogeneity of the recombinant mAb. The analysis of intact antibodies is also important after formulation and storage of the therapeutic drug. Characterization Level 2 starts after reduction of the disulfide bonds in the antibody with or without alkylation, including chromatographic separation (SEC, RP-HPLC) of the heavy chain (50 kDa) and light chain (25 kDa). Exact molecular weights of the heavy and light chains and the degree of heterogeneity can then be determined.

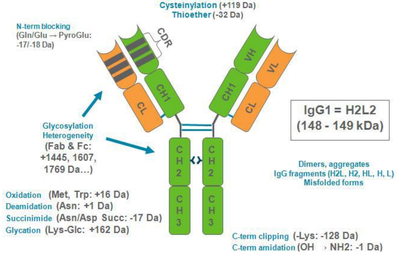

Figure 1. Reported IgG1 Structure Heterogeneity. Therapeutic antibodies produced using recombinant DNA technologies are generally complex, heterogeneous, and subject to a variety of enzymatic or chemical modifications during expression, purification, and long-term storage.

MATERIALS AND METHODS

Experimental Conditions

LC

Column:	SEC Super SW 3000 1.0 × 300 mm (TOSOH)
Flow Rate:	8 µL/min isocratic flow
Mobile Phase:	For UV: 150 mM Na-Phosphate, pH 6.5 For MS: 25mM Ammonium formate
Column temperature:	30 °C
Sample loop:	10 µL
Detection:	210 nm and 280 nm, 5 mm 90 nL flow cell

MS

Interface:
AB SCIEX TurboIonSpray® ion source with 50 µm hybrid electrode
Micro flow LC/MS analysis was performed on the TripleTOF™ 4600 System using the Turbo V™ Source (AB SCIEX).

Sample

Mouse monoclonal IgG (approx. 150 kDa, Waters) in buffer. Sample was used for UV and mass spectrometry work. For heavy and light chain LCMS analysis, the intact protein was reduced with DTT at 60ºC for 35 minutes.

ekspert™ nanoLC 400 system

AB SCIEX TripleTOF® 4600 LC/MS/MS System

Results

Intact Protein Analysis of Therapeutic with UV

Size exclusion chromatography (SEC) is the most common technique used to analysis monoclonal antibody (mAb).

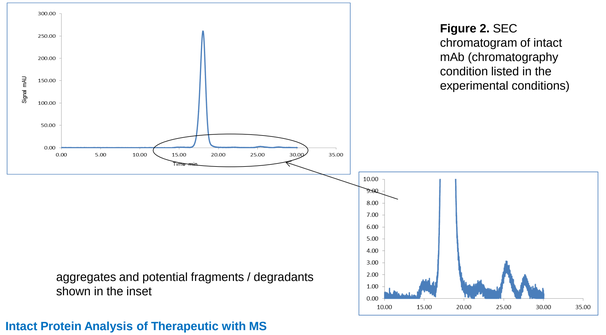

Figure 2. SEC chromatogram of intact mAb (chromatography condition listed in the experimental conditions)

Intact Protein Analysis of Therapeutic with MS

The analysis of larger proteins (~150 kDa) is most suited to a time-of-flight (TOF) mass analyzer by electrospray ionization, because of its superior resolution and high mass range. Multiply-charged ion envelopes resulting from the electrospray ionization of intact antibodies easily fit within the mass range of this system. LC/MS on the TripleTOF™ 4600 system was used to determine the intact molecular weight of monoclonal anti-actin

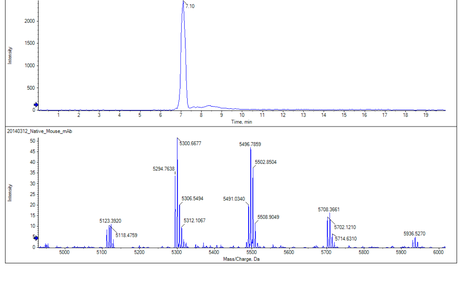

Figure 3. Intact mAb Characterization by size exclusion LCMS. The upper panel shows the total ion chromatogram of the intact mAb. The lower pane shows the TOF MS spectrum of the Intact Antibody.

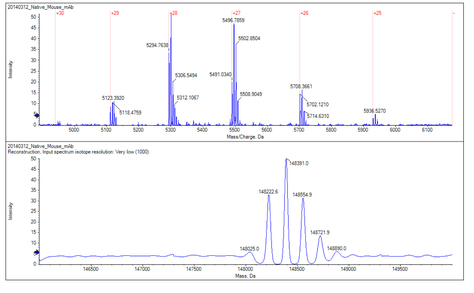

Figure 5. The bottom pane shows the mass reconstruction of the intact mAb. Differences between each peak correspond to a difference of one Hex.

CONCLUSIONS

This study demonstrates the use of SEC micro columns in non-denaturing intact mAb analysis. SEC micro columns allow the use of very low flow and therefore higher sensitivity in the mass spectrometer. Additionally, there is a benefit in that proteins that cannot handle denaturing conditions can be run in native mode using size exclusion chromatography. This technology is particularly useful for the analysis of cysteine-linked antibody-drug conjugates. Native-mode intact antibody analysis can be particularly challenging as native conditions severely restrict ionization of the intact protein. Scientists facing sensitivity challenges in native mode should consider microflow SEC for the added sensitivity benefits.

REFERENCES

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TRADEMARKS/LICENSING