STRUCTURAL CHARACTERIZATION OF A MONOCLONAL ANTIBODY BY ELECTROSPRAY ION-MOBILITY TIME-OF-FLIGHT MASS SPECTROMETRY

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OVERVIEW

- A newly developed electrospray ion-mobility time-of-flight mass spectrometer (Waters Synapt™ HDMS™) is used to perform structural characterization on a monoclonal antibody.
- The glycosylation profile of the intact antibody is rapidly characterized by simply injecting samples directly onto a reverse-phase system (Waters nanoACQUITY UPLC™) that is in-line with the high-resolution mass spectrometer.
- The detailed characterization on the glycans and the glycopeptides are obtained by two-step sequential fragmentations using the unique two-collision-cell design of the instrument.
- Synapt's ion-mobility function enables the separation of low-mass fragments from other species after the fragmentations of intact light chain subunit, thus yielding partial amino acid sequence of the light chain.

INTRODUCTION

Structural characterizations of intact MAb molecules are challenging due to their high molecular mass, hydrophobic nature, and presence of sugar moieties. Conversely, the analysis of intact protein is also advantageous because it reduces dramatically the time for sample preparation and data interpretation, compared with peptide mapping and sequencing. Furthermore, it also minimizes the chance of introducing putative modifications, which are often observed during peptide mapping.

The aim of this study is to perform structural characterization on a monoclonal antibody (IgG1, κ) by a newly developed electrospray ion-mobility time-of-flight mass spectrometer (Synapt HDMS). The presented study covers the results in the following areas:

- ♦ analyzing intact antibodies to map the glycosylation profile of the antibody
- ♦ obtaining the glycan sequence and the peptide sequence by fragmenting glycopeptides in a sequential two-step fragmentation process using Synapt
- ♦ separating the light and heavy chains of the reduced antibody by ion mobility to yield further characterizations
- ♦ fragmenting the intact light chain subunit to obtain partial amino acid sequence of the light chain

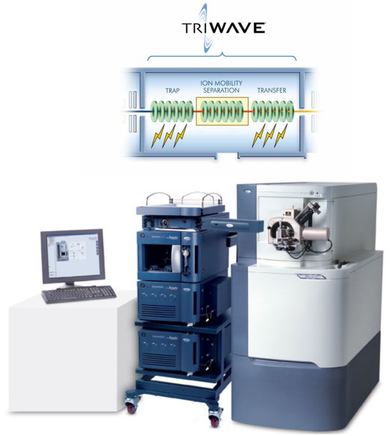
EXPERIMENTAL METHODS

1.Sample Preparations

- Preparation of intact monoclonal antibody
  A mouse monoclonal antibody (IgG1, κ) was received from VICAM group. The intact IgG1 was stored in 0.1M $NaHCO_3$/0.5M NaCl at a concentration of 11.3 mg/mL, and was diluted to 1.0 mg/mL with 50mM of NH_4HCO_3 before analyzed by Synapt.
- Reduction of antibody
  The IgG1 sample was diluted to 2.26mg/mL in a pH 7.5 buffer containing 7.5M Guanidine hydrochloride, 0.1M Tris-HCl, 1 mM EDTA. The protein was reduced with 10mM DTT at 37°C for 35 min.
- Preparation of glycopeptides
  The IgG1 sample was prepared in 0.05% RapiGest SF, 0.1M $NaHCO_3$/0.5M NaCl, pH 8.3. The sample was first heated at 60°C for 30 minutes, and then was reduced with 10mM DTT at 60°C for 30 min and alkylated with 13 mM of IAA. Trypsin digestion was performed at 37°C for overnight. The digest was diluted to 0.015 mg/mL with 0.1% formic acid to get ready for injection.

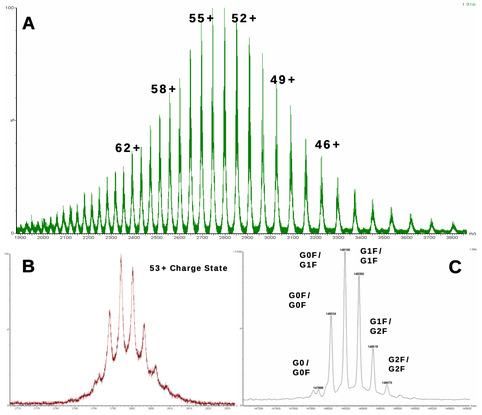
2. Mass Spectrometry and LC System

Mass spectrometric analyses were performed on Waters Synapt HDMS mass spectrometer coupled with Waters nanoACQUITY UPLC system. For the analysis of intact and reduced IgG1, a prototype 300µmx50mm on-line RP column was used for automated on-line desalting.



RESULTS

Figure 1. (**A**) Positive ion ESI mass spectrum of an intact monoclonal antibody acquired in V mode with resolution 10,000. (**B**) Positive ion ESI mass spectrum for 53+ charged ion of the intact monoclonal antibody. Peaks represent various glycoforms. (**C**) Deconvoluted ESI mass spectra of the intact monoclonal antibody IgG1 containing multiple peaks due to the sugar heterogeneity.



RESULTS

Figure 2. (**A**) Ion mobility DriftScope showing the separation of heavy chains and light chains from the reduced IgG1. (**B**) The summed ESI mass spectrum of heavy chain (**C**) The summed ESI mass spectrum of light chain.

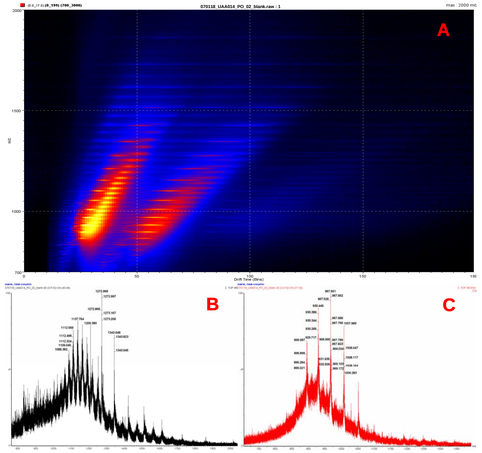
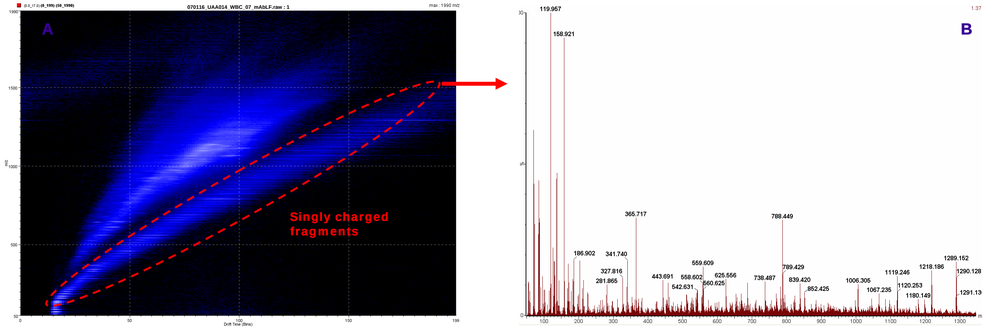
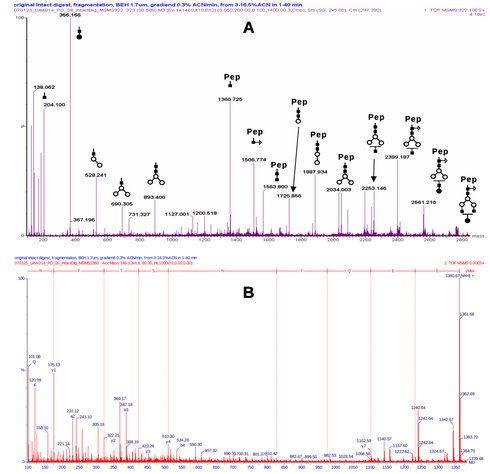


Figure 3. (**A**) Ion mobility DriftScope of the fragment ions from fragmentations of the intact light chain. Singly charged species are clearly separated from the other multiply charged fragments by ion mobility. These fragments contain partial amino acid sequence of the light chain (**B**) The summed mass spectrum of all the singly charged ions generated from the intact light chain fragmentation process.



RESULTS

Figure 4. (**A**) ESI-IMS-Tof-MS/MS with CID of a tryptic glycopeptide from the IgG1. The fragmentation took place in the trap cell when the peptide was eluted from a 75µmx100mm BEH column. The precursor ion of the $[M+3H]^{3+}$ species at *m/z* 922.1 was selected for the fragmentation. (**B**) All the fragment ions were subjected to a second fragmentation in the transfer cell after separated in the IMS cell. The spectrum for ions at *m/z* 1360.7 was summed and yielded the peptide sequence and glycosylation site.



CONCLUSIONS

- ♦Synapt HDMS (ESI-IMS-TOF MS) has been successfully used for the structural characterization of a mouse monoclonal antibody.
- ♦The system combines high-efficiency, ion-mobility based measurements and separations with high-performance quadrupole, time-of-flight mass spectrometry to deliver enhanced specificity and sample definition beyond that achievable by conventional mass spectrometers.
- ♦All of the results demonstrate that Synapt HDMS mass spectrometer is a superior tool to characterize MAb and other complex protein pharmaceuticals.

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