Characterization of intact monoclonal antibody with microfluidic chip electrophoresis mass spectrometry

ABSTRACT

Heterogeneity of monoclonal antibodies (mAbs) including glycosylation variants, carboxyl terminal truncation, and deamination products is an important analytical problem in the biopharmaceutical industry. High resolution mass spectrometry is a powerful tool for the characterization of mAbs; however some proteoforms might not be observed due to the sample complexity. In this study, a microfluidic chip electrophoresis system (ZipChip from 908 Devices) coupled to a high resolution Thermo Scientific Biopharma Q-Exactive mass spectrometer was used to separate and characterize the NIST mAb (RM 8671), capturing distinct lysine variants and respective proteoforms. Preliminary data provides identification of most proteoforms listed in the NIST mAb Referenced Material 8671.

INTRODUCTION

The main goal of this work was to develop a method to characterize heterogeneous mAb on an intact protein level. In order to resolve the complexity of mAb, a powerful combination of an efficient separation system, coupled to high-performance mass spectrometer (MS), and data analysis software is necessary. The separation of charge variants is still a challenging task, especially on an intact protein level. Capillary electrophoresis (CE) provides an ideal separation technique for charge variants of mAb. Microfluidic CE provides advantages in characterizing mAb given its good separation efficiency and short migration time based on high field strength and short separation channel. High-performance MS including high mass accuracy and resolution is also important for antibody characterization. Orbitrap mass spectrometer is a good option for this application, and the Q-Exactive BioPharma MS is suitable for the analysis of very large biomolecules. The challenges of protein MS data analysis usually increases with its molecular weight. The software BioPharma Finder is specifically designed to characterize the complex protein via delicate deconvolution of mass spectra for individual time windows in combination with a thorough comparison to a reference database with all variants. In this study, a powerful combination of microfluidic CE separation, MS detection, and data analysis is presented to solve one of the most challenging workflows in the Biopharma industry.
MATERIALS AND METHODS

Reference material from the NIST mAb standard (8671) with the known sequence and post translational modifications (PTMs) was used in this study. The NIST mAb is a recombinant humanized IgG1κ antibody, and process-related impurities have been removed through various purification steps. The heavy chain of this mAb is known to have high abundance of PTMs such as N-terminal pyroglutamation, c-terminal lysine clipping, and glycosylation. 10μg/μL of raw sample was diluted with deionized water into 0.5μg/μL for the experiments. No desalting procedure is necessary for this series of measurement. The chip was primed with background electrolyte solution (BGE) consisting of 0.2% acetic acid and 10% IPA (pH=3.17). Once a stable electrospray was observed, 10μL of 0.5μg/μL mAb was pipetted into the sample reservoir. Pressure injection with 2psi for 4s was used for sample loading, introducing 0.4μL with 0.2ng of mAb was introduced to the separation channel. High voltage of ~20kV was applied for CE separation within 22 cm-length of channel, and ~2kV was used for nano-ESI. Each run was finished in 4 min.

Q-Exactive mass spectrometer was set to the optimized condition for ultra-high mass biomolecule, and maintain the scan rate at 6 scans/sec. In-source CID (100 e.v.) was applied to assist desolvation. m/z range (2500–6000) was selected for this mild-denatured condition. BioPharma Finder 2.0 was used in this study for data analysis. The sequence and PTM information of the NIST mAb was first set up in protein sequence manager. The identification of different variants can be accomplished after the mass spectra were deconvoluted to the intact mass with ReSpect.

Work Flow

Intact mAb  ZipChip + QE Biopharma  BioPharma Finder

Figure 1. Characterization of mAb is accomplished by separation with the ZipChip device, on-line analysis with high resolution Q-Exact BioPharma mass spectrometer, and identification with BioPharma Finder software.

Instrumentation
MATERIALS AND METHODS


2. Results

Figure 2. A) Schematic of high-resolution (HR) microfluidic CE chip with 22 cm channel and integrated nano-ESI emitter. The nano-ESI spray can be monitored with the integrated green laser and camera. B) Schematic of Q-Exactive mass spectrometer including the high-performance quadrupole precursor selection and high-resolution, accurate-mass orbitrap detection. The BioPharma Finder software package is optimized for 3 protein workflows: high-mass-range (HMR) mode for intact analysis, intact protein mode for subunit analysis, and normal mode for peptide mapping.

Sample

NISTmAb, recombinant humanized IgG1κ
Number of amino acids: 1326
Disulfide bonds: 12 (inter-chain), 4 (intra-chain)
High-abundance PTM: N-terminal pyroglutamination, C-terminal lysine clipping, glycosylation

Figure 3. Information of NISTmAb 8671 including amino-acid sequence, disulfide-bond linkage, and post-translational modification.
Results

Figure 4. A) Electropherograms of NIST mAb in a 4 min run on a HR microfluidic chip hyphenated to Q-Exactive BioPharma mass spectrometer. Three different forms of lysine variants were separated under a field strength of 770 V/cm. B) Three mass spectra were extracted from the three peaks on the electropherogram. Lysine variant (128 Da) can be identified after the comparison of deconvoluted mass spectra. Higher number of lysine on the C-terminal of heavy chain provides higher mobility due to the additional positive charges from lysine.
INTRODUCTION

Most biotherapeutics are characterized by high-resolution mass spectrometry (HRMS) Devices)

BioPharma biotherapeutics are usually produced through recombinant expression and purification processes. These processes are complex, leading to the introduction of post-translational modifications (PTMs) in the protein structure. PTMs can affect the biological activity, stability, and efficacy of the protein, making their characterization and identification crucial.

Traditionally, PTM analysis is performed by mass spectrometry (MS) coupled with liquid chromatography (LC). However, these methods can be challenging due to the complexity and heterogeneity of the sample.

Microfluidic chip electrophoresis mass spectrometry (MEC-MS) is a powerful technique for the analysis of intact monoclonal antibodies (mAbs). This technique allows for the separation of protein variants and the identification of PTMs. The use of MEC-MS for the characterization of intact mAbs is presented in this study.

EXPERIMENTAL

The study was conducted using Thermo Fisher Scientific Exactive (RM) mass spectrometer. mAbs were analyzed using a microfluidic chip electrophoresis (MEC) system. The separation was achieved by applying a voltage across the chip, allowing for the discrimination of protein variants based on their migration rates.

RESULTS

Three deconvoluted mass spectra from different migration time interval which are corresponding to different lysine variants. A) mAb with 2 lysine adduct including 5 glycosylated variant was detected B) mAb with 1 lysine adduct including 5 glycosylated variant was detected C) mAb without lysine adduct including 5 glycosylated variant was detected.

Figure 5.

Figure 6. Migration time was used as a parameter to verify the heterogeneous mAb in an example of specific glycosylation form G0F/G0F. A) Total Ion Chromatography (TIC) in a 4 min run of NIST mAb. B) TIC from the specific form of G0F/G0F. C) TIC from the specific form of G0F/G0F+Lys. D) TIC from the specific form of G0F/G0F+2Lys.
CONCLUSIONS

- Clear mAb signal was detected within 4 minutes coupling the 908 ZipChip with Thermo Q-Exactive BioPharma MS. No sample preparation and desalting was necessary.
- Lysine variants of NIST mAb can be separated on a microfluidic chip electrophoresis device. The mobility was increased with additional positive charges added to the c-terminal of heavy chain from lysine.
- 5 glycosylated variants including G0F/G0F, G0F/G1F, G1F/G1F, G1F/G2F, and G2F/G2F were observed from 2-lys, 1-lys, and 0-lys migration time frames.
- Peaks with different lysine variants can be distinguished from the electropherogram.
- 3 orders of dynamic range for detection was achieved.
- Most of the proteoforms listed in the NIST mAb Reference Material can be identified in this study.

REFERENCES


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