ZipChip

ZipChip Analysis of Intact Antibodies for Rapid Characterization of mAb-based Biotherapeutics

INTRODUCTION

In this work, we describe a method for monoclonal antibody (mAb) analysis using the ZipChip™ method for monoclonal antibody (mAb) analysis that couples capillary zone electrophoresis (CZE) separation of intact charge variants with direct electrospray ionization mass spectrometry (ESI-MS) analysis. Primary benefits of the method are:

- No sample preparation beyond dilution is required
- Separation of charge variants with on-line MS identification
- Fast intact antibody analysis in gentle, near native conditions
- High quality MS data
- Single method for charge heterogeneity, mass information, and glycoform characterization

ZipChip technology seamlessly integrates sample injection, electrophoretic separation, and ESI onto a simple glass chip. The electrophoretic separation and ESI are executed by applying precisely-controlled voltages and pressures to the chip within the ZipChip Interface (ZCI) unit. The ZCI attaches directly to the standard source mounts of mass spectrometers and provides optimal alignment of the ESI plume for sensitive detection of the intact mAb structural variants

The ZipChip intact mAb method requires minimal sample preparation, yields high-quality mass spectrometry data, and results in near-baseline resolution of many mAb variants. Mass spectra are generated for each variant in the ZipChip separation; and the deconvoluted spectra are used to help identify the variants. A NIST mAb reference material was used for this demonstration. The NIST mAb is an $IgG1_K$ designed to mimic the lifespan from cell culture production to drug formulation of a biotherapeutic.1 Thus, this sample is a good representation of a typical IgG-based drug substance. The method presented here is applicable to a wide range of mAb molecules and formulations, providing high-quality mass spectra and exceptional resolution of subtle structural variants.

METHODS

Sample preparation. The NIST mAb reference material (RM 8671, NIST) was used for the work described here. For analysis, the mAb stock (10 mg/mL mAb in formulation buffer) was diluted to 0.5 mg/mL with LC/MS grade water.

ZipChip MZE ESI-MS analysis conditions. The ZipChip parameters used for analysis are provided in Table 1.

Chip Type	ZipChip HR
BGE	ZipChip Intact Antibody Kit
Field Strength	500 V/cm
ESI potential	3.5 kV (set automatically)
Injection Volume	0.75 nL
Pressure Assist	Enabled

Table 1. ZipChip Analysis Parameters

MS parameters. Data were acquired using a Thermo Exactive Plus EMR. Table 2 lists the MS parameters used for analysis. The instrument was operated in EMR mode to maximize the trapping efficiency for large ions, which increases the sensitivity for intact mAbs. Additionally, applying both In-source CID and HCD Collision Energy significantly improve the quality of the mass spectra as has been shown previously.[2] Note that Collision Energy may not be necessary when operating in Normal mode or using a different model of Thermo Orbitrap MS.

Ion Transfer Mode		
EMR MS mode	On	
Trapping gas pressure	6	
Spectrum data type	Profile	
C-trap charge detector	Off	
Scan Parameters		
Scan Range	2500-6000 m/z	variable
Fragmentation:		
In-source CID	80	
CE	60	
Microscans	1	
AGC target	1.00E+06	variable
Max Ion Inject Time	50 ms	
Resolution	35,000	variable
ESI Source		
Sheath gas flow rate	2	
Aux gas flow rate	0	
Sweep gas flow rate	0	
Spray voltage	0	Determined by ZCI
Spray current	na	
Capillary Temperature	200	
S-lens RF level	100	

Table 2. Exactive Plus EMR MS Parameters

Data processing. Mass spectra were deconvoluted using Thermo Scientific BioPharma Finder 1.0 SP1.

RESULTS AND DISCUSSION

Figure 1 shows the base peak electropherogram for the intact NIST mAb standard. The analysis was complete in less than 3.50 minutes. While a 10 µL aliquot of sample was loaded in the sample well for analysis, an on-chip injection of less than 1.0 nL was performed, which results in the injection of approximately 325 pg of protein. Three main mAb variants (peaks 1-3 in Figure 1, inset a) are separated with an average resolution of 1.45. With this method, the more charged, or more basic, variants of the mAb have a faster migration time in the electropherogram. A decrease in migration time indicates a decrease in net charge of the variant. Thus, it can be inferred from the ZipChip separation that peak 1 has a higher net charge than the main variant, peak 3.

Using the ZipChip interface, the charge variant separation is directly integrated with on-line ESI-MS. Therefore, a mass spectrum can be generated for each peak in the separation by averaging the MS scans across

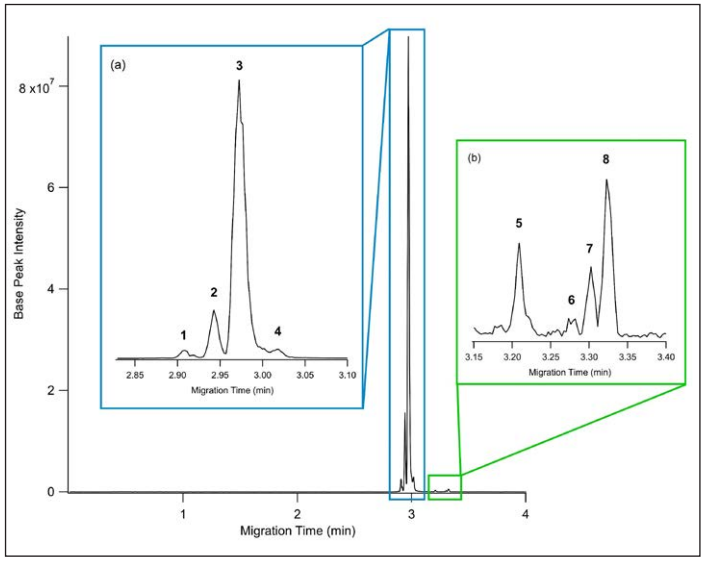

Figure 1. Electropherogram showing the ZipChip separation of the intact NIST mAb variants. The separation was performed with a field strength of 500 V/cm and completed in less than 3.5 minutes. Inset (a) shows three main mAb charge variants are separated (peaks 1-3) with an additional low abundance acidic variant (peak 4). Inset (b) shows the smaller protein species that migrate after the mAb variants (peaks 5-8). These species are thought to be host cell protein contaminants.

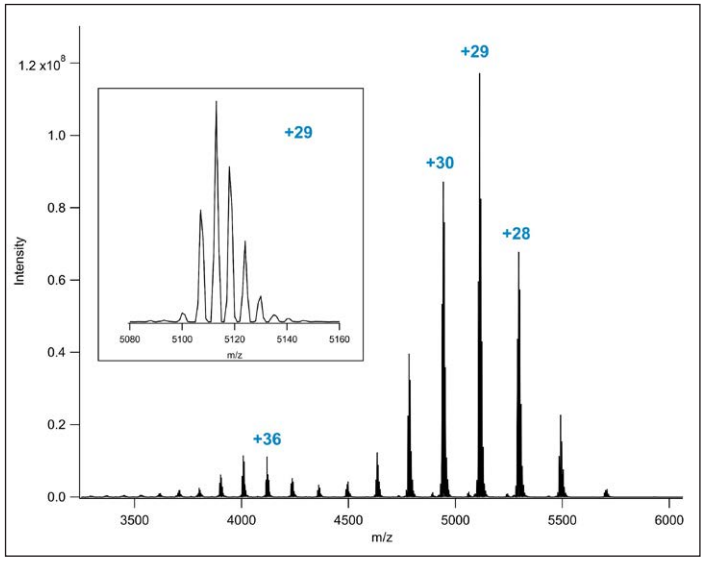

Figure 2. The raw mass spectrum for peak 3 generated by averaging the MS scans across the width of the peak. The most abundant charge state, +29, is enhanced in the inset to show the resolution of glycoforms. A second distribution of charge states centering around +36 and +37 is also detected.

the width of the peak. The mass spectrum shown in Figure 2 was generated by averaging the scans across the width of the main variant, Peak 3. As labeled in the figure, the most abundant charge states detected are +31 to +28, with a second population of charge states centered around +36. The background electrolyte (BGE) used for this analysis contains less organic modifier and acid than typical denaturing conditions and causes less unfolding of the mAb in solution. This results in charge states that are closer to that achieved with native spray MS.

Mass spectra were generated for each variant by averaging MS scans across the width of the peak and were then deconvoluted using BioPharmaFinder. Figure 3 provides deconvoluted spectra for peaks 1-3 with some identified glycoforms labeled. A summary of the peaks detected in the separation, and the deconvoluted masses are listed in Table 3. With this approach, a mass shift of ~127 Da is easily detected between the three charge variants. This mass shift is characteristic of a known modification to mAbs that also induces a change in net charge: C-terminal lysine truncation. Comparison of the migration times of peaks 1-3 to the deconvoluted masses in Table 3 reveals that the decrease in mass of 127 Da is indeed accompanied by a decrease in migration time that indicates a reduction in net charge. Taken together, these two qualities confirm that the most abundant variants result from C-terminal lysine truncations of the heavy chain.

There is also a low abundance acidic variant detected in the electropherogram. The similarity in mass of the peak 3 and 4 variants (~1 Da) implies that deamidation could be the modification causing the reduction in net charge. For proteins of this size a ~1.0 Da mass shift as is seen with deamidation would result in an m/z difference of only 0.03 for a +29 charge state. This small difference is difficult to resolve in the mass domain and accurately deconvolute, complicating identification of deamidation at the intact level. However, the separation in the MZE domain provided by ZipChip reveals the presence of this acidic variant.

In addition to the main intact mAb variants of the NIST material, smaller protein species are detected (Fig. 1, inset b) These species elute as well-defined peaks that are well resolved from the mAb variants, eluting between 3.20 and 3.40 minutes. The deconvoluted masses of these protein species are ~47 kDa which is not consistent with the theoretical mass of the light or heavy chain of the NIST mAb. Thus, it is theorized that

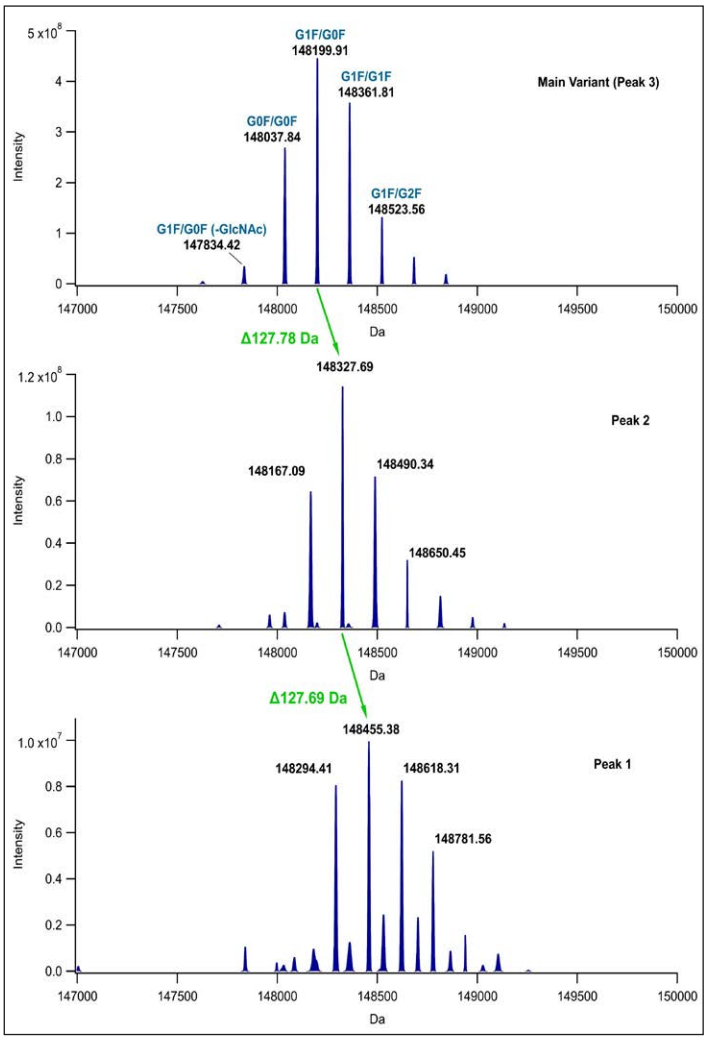

Figure 3. Deconvoluted mass spectra for MZE peaks 1-3 in the electropherogram. The mass shift between the variants is approximately 127 Da. The masses can be used to assess the different glycoforms of the mAb and some common glycoforms are labeled in the Main Variant (peak 3) spectrum

Peak #	Migration Time	Mass*	ID
Peak 1	2.91 min	148455.38 Da	2x C-terminal K
Peak 2	2.94 min	148327.69 Da	1x C-terminal K
Peak 3	2.97 min	148199.91 Da	0x C-terminal K
Peak 4	3.02 min	148201.60 Da	Deamidation
Peak 5	3.21 min	47626 Da	Host cell protein
Peak 6	3.27 min	47488 Da	Host cell protein
Peak 7	3.30 min	47259 Da	Host cell protein
Peak 8	3.32 min	47145 Da	Host cell protein

Table 3. Intact mAb Charge Variants and Masses Detected during Analysis

*For mAb variants the (G0F/G1F) glycoform mass is listed

these species are residual host cell proteins.

CONCLUSIONS

The ZipChip method for intact mAb analysis enables separation of intact variants with direct ESI-MS analysis. This method typically requires no sample preparation beyond dilution of the sample to an appropriate concentration. The BGE conditions used in this method result in a CZE separation resolution of ±1 change in net charge and near-native charge states generated in the ESI. Sufficient resolution is obtained in the MS domain to easily identify glycoforms of the intact mAb variants. Mass information from the deconvoluted spectra can be used in conjunction with mobility information from the CZE separation to more confidently identify the charge variants. Several variants of the mAb were identified using this method, along with masses for minor basic and acidic variants. By integrating high speed CZE separation, on-chip ESI ionization, and MS analysis into an automated workflow, the method enables extremely fast, simple, and robust mAb analysis.

References:
1. *Report of Investigation for Reference Material* 8671; National Institute of Standards and Technology: Gaithersburg, MS, 2016.)
2. Samonig, M.; Huber, C.; Sheffler, K. LC/MS *Analysis of the Monoclonal Antibody Rituximab Using the Q Exactive Benchtop Orbitrap Mass Spectrometer*, Application Note 591, Thermo Scientific, 2013.)

908devices

www.908devices.com