Native Microchip CE-MS for In-Depth Biopharmaceutical Characterization

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Overview

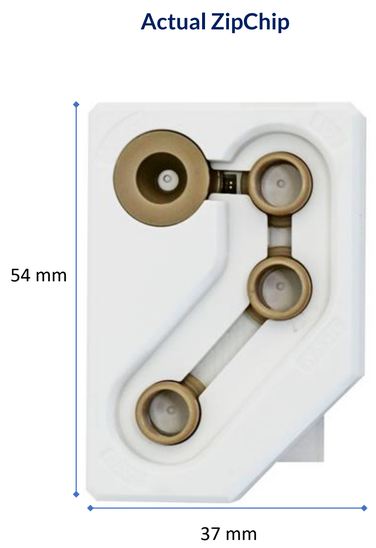
The field of native mass spectrometry has advanced significantly over the past several years. We believe that the integration of a powerful online separation can advance the field of native mass spec even further. Capillary Electrophoresis (CE), coupled via ESI, is a natural fit for native mass spec analysis because it can be performed in native solvent conditions without concern for interactions with a chromatographic stationary phase. Additionally, the slow diffusion of large molecules is a benefit to electrophoretic separation efficiency, often enabling resolution between very minor structural differences between proteoforms. Successfully exploiting CE-ESI-MS for native analysis requires a level of optimization (of surface chemistry, channel geometry, solvent conditions, etc…) that has been beyond the reach of traditional CE-MS platforms. The continued advancement of our fully integrated microfluidic CE-ESI platform has enabled us to start tackling these extremely challenging applications. Here we demonstrate the unique capabilities of this approach by monitoring deamidation of a monoclonal antibody.

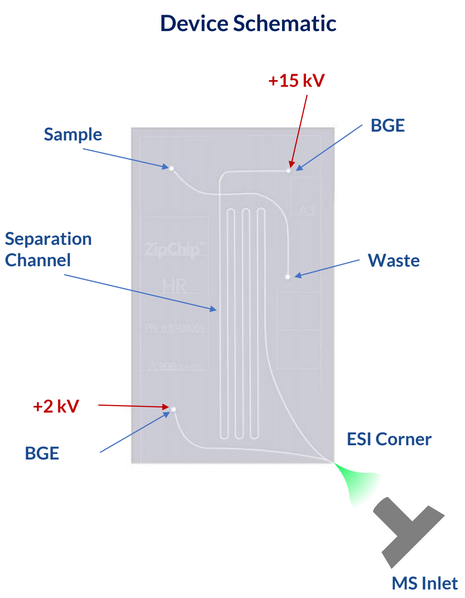
Methods

Sample Preparation and Analysis. The NIST monoclonal antibody reference material was used as a representative sample for this work. The NIST mAb was incubated at 45°C in 50 mM phosphate buffer at pH 8 to accelerate deamidation. Time points were collected at 0, 24, 48, 72, 96, and 120 hours. Samples were buffer exchanged into ZipChip Native Antibody BGE and diluted to a concentration of 0.25 mg/mL for analysis.

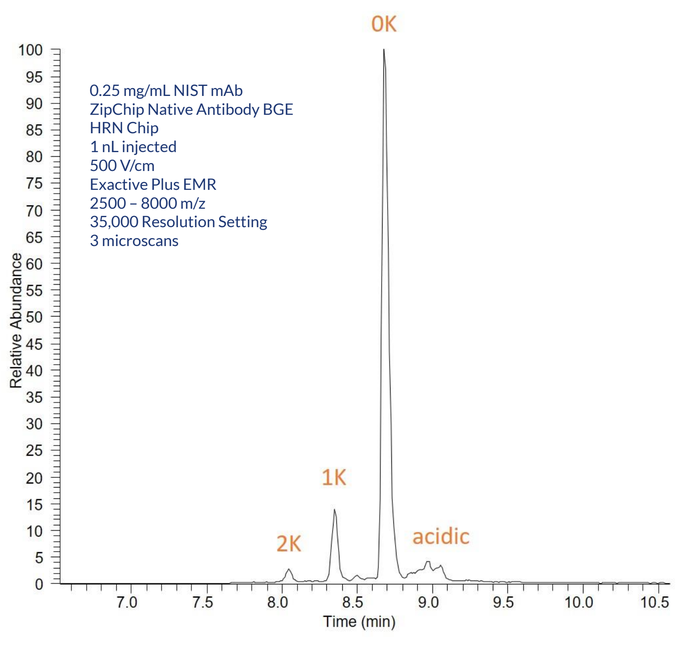
Instrumentation. All work was performed using a commercially available microfluidic CE-ESI system (ZipChip, 908 Devices Inc.). The microfluidic devices utilize a covalently attached, neutral polymer surface coating to prevent analyte interactions and suppress electroosmotic flow. For the work shown here, a "high resolution native" (HRN) chip was used. This chip incorporates a 22 cm long separation channel and uses a new surface coating process to achieve high resolution protein separations under native conditions. Data were collected on a Thermo Exactive Plus EMR mass spectrometer.

Data Processing. Data were visualized using Thermo Xcalibur QualBrowser. Data files were processed with an in-house software program to accurately identify and assign relative quantitative abundance values to all of the observed species. All of the samples were run 3x to assign a standard deviation to each of the measurements.

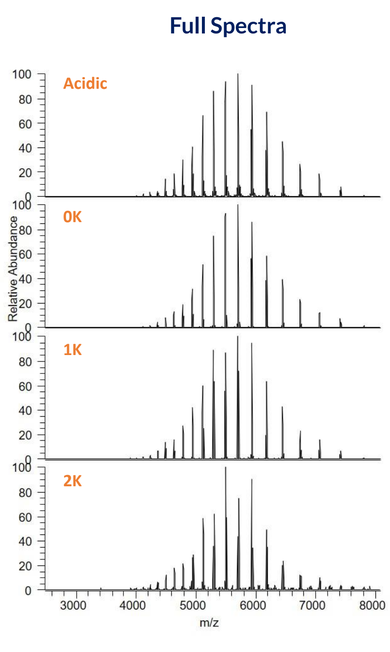




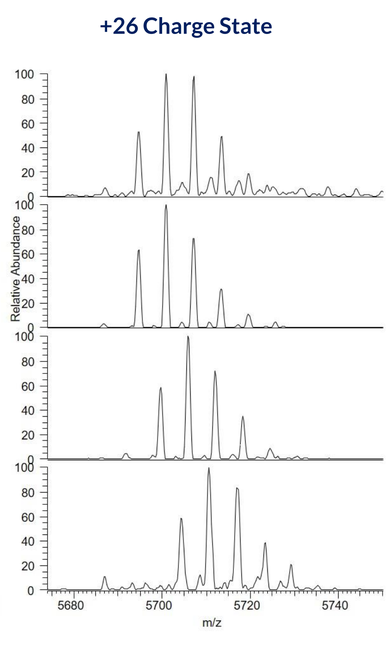
Native Antibody Analysis with ZipChip CE-ESI-MS



Full Spectra



+26 Charge State

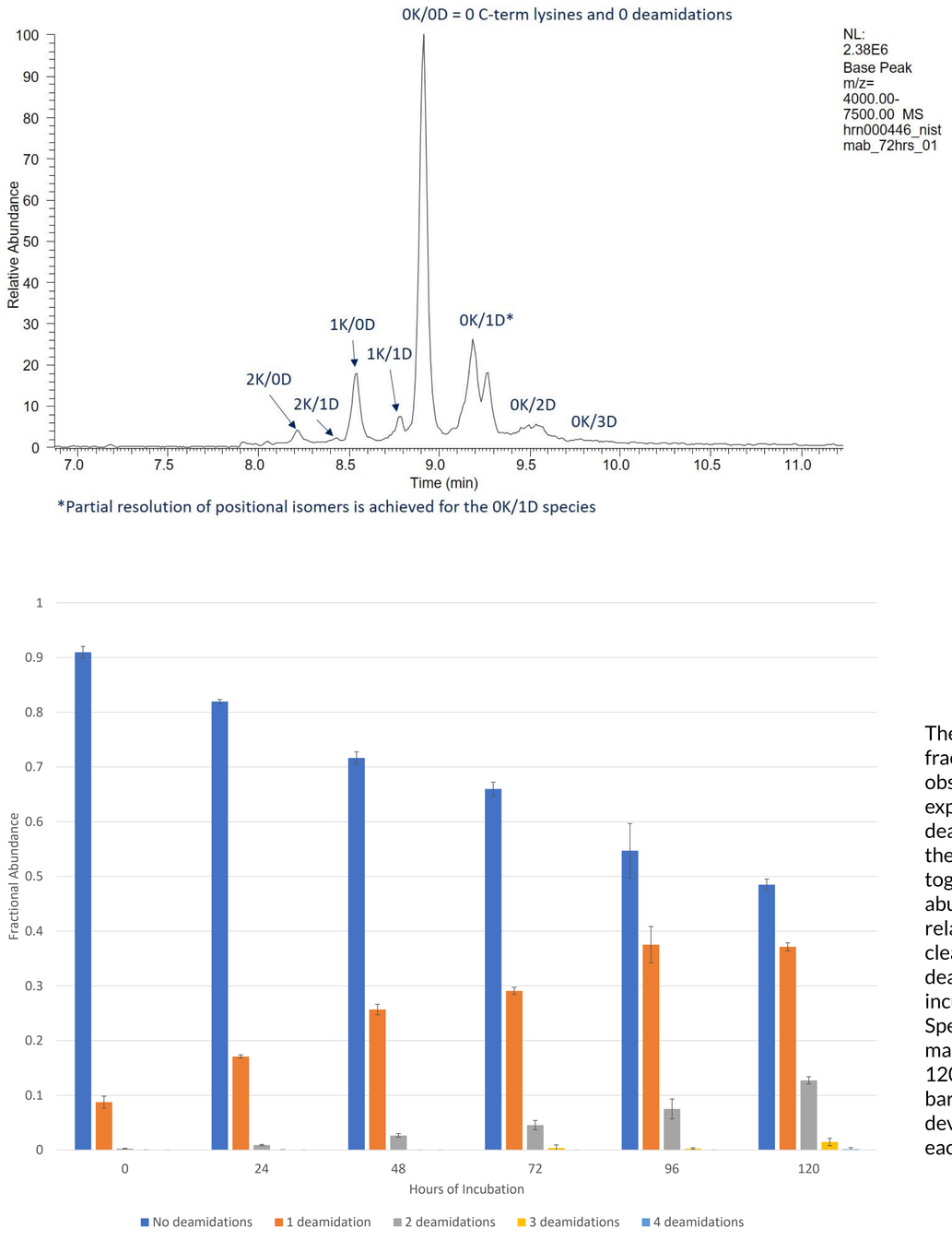


Proteoforms Identified

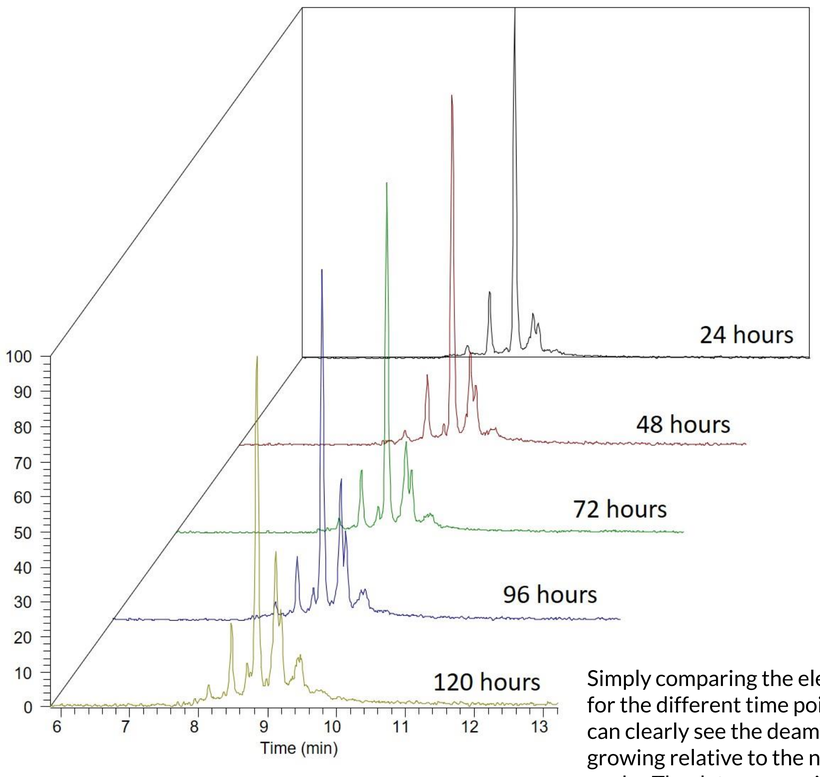
C-term K	Deamidation	Glycoform	Theoretical Mass	Error (ppm)	Relative Abundance
0	0	G0F/G0F	148037.1	5.0	62.8
		G0F/G1F	148199.3	4.9	100.0
		G1F/G1F	148361.2	4.5	87.4
		G1F/G2F	148523.5	0.3	34.4
		G2F/G2F	148685.7	6.1	15.1
	1	G0F/G0F	148038.1	0.4	4.5
		G0F/G1F	148200.3	2.7	8.3
		G1F/G1F	148362.2	3.0	9.2
		G1F/G2F	148524.5	1.6	3.9
		G2F/G2F	148686.7	4.5	1.1
	2	G0F/G0F	148039.1	4.6	0.2
		G0F/G1F	148201.3	9.6	0.6
		G1F/G2F	148525.5	0.3	0.2
1	0	G0F/G0F	148165.3	0.1	6.7
		G0F/G1F	148327.4	3.4	12.9
		G1F/G1F	148489.4	0.4	9.4
		G1F/G2F	148651.7	11.0	4.6
		G2F/G2F	148813.9	11.6	1.5
	1	G0F/G0F	148166.3	1.7	0.8
		G0F/G1F	148328.4	0.8	1.1
		G1F/G1F	148490.4	16.1	0.9
		G1F/G2F	148652.7	2.9	0.7
2	0	G0F/G0F	148293.5	13.2	1.1
		G0F/G1F	148455.6	5.8	1.7
		G1F/G1F	148617.5	19.6	1.3
		G1F/G2F	148779.9	10.1	0.6
		G2F/G2F	148942.0	20.0	0.2
	1	G0F/G0F	148294.5	16.9	0.1
		G1F/G1F	148618.5	22.4	0.2

Keeping the antibody in its native state during the analysis enables the high resolution separation of charge variants and the ability to assign accurate masses to each of the variant peaks. Zooming in on the spectra we see the excellent resolution between glycoform peaks achieved by running the orbitrap detection at a resolution setting of 35,000. The basic variants are clearly identified as C-terminal lysine variants. The small mass shift and slower mobility of the acidic variant indicates a deamidation. Such a small mass shift would not be detected without separating the species prior to MS analysis. The high efficiency of the Native ZipChip separation achieves this result with no additional sample processing required.

Forced Deamidation Time Point Analysis



The base peak electropherogram for one of the forced deamidation time point samples (72 hours) shows the electrophoretic separation of both C-terminal lysine variants and deamidation variants. Deamidated variants can be seen for each of the three different lysine variants, and multiple deamidations can clearly be seen on the most intense, 0K variant. In addition, the single deamidation of the 0K variant reveals partial resolution of multiple peaks. These are likely explained by positional variants with the deamidation occurring at different amino acid sites.



Simply comparing the electropherograms for the different time point samples, we can clearly see the deamidated peaks growing relative to the non-deamidated peaks. The data processing software can use separation in both time and m/z to see details that are not visible in this view.

The data were processed to assign fractional abundance to the observed species. Since this experiment was focused on deamidation, we've grouped all of the glycoforms and lysine variants together to indicate the total abundance of deamidated species relative to non-deamidated. A clear trend of increasing deamidation can be seen versus incubation time at pH 8 and 45°C. Species were detected with as many as four deamidations after 120 hours of incubation. The error bars indicate +/- 1 standard deviation for the 3 replicates of each sample run in this experiment.

Conclusions

We have demonstrated here a fast, efficient method for detection and analysis of antibody degradation (deamidation). This native analysis of the intact antibody does not rely on additional sample prep, such as peptide mapping, which can itself induce further degradation in the antibody and complicate results. Additionally, the method is extremely fast as no enzymatic digests are required, and the separation of the native species is very fast; it is also extremely easy to use as no complicated LC separations are required.

We believe that the continued advancement of microchip CE separation methods and native mass spec performance have now produced a method that can achieve unprecedented molecular characterization of intact monoclonal antibodies as well as other large, complex proteins.