Subunit charge variant characterization in the native state

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Monoclonal antibody (mAb) therapeutics often contain charge variants that arise from a wide array of post-translational modifications (PTM), including sialylation, deamidation, oxidation, glycation, and C-terminal lysine.[1] Comprehensive and dynamic analysis of these variants is critical to ensuring therapeutic safety and efficacy. Currently, multiple strategies are capable of performing charge variant analysis, such as ion exchange chromatography (IEX), capillary isoelectrical focusing (cIEF) and capillary zone electrophoresis (CZE). IEX and cIEF are most popular, but their data interpretation can be challenging, and the results often require validation by orthogonal analytical techniques. In contrast, CZE combines the benefits of native state analysis with the speed and resolution expected from a capillary electrophoresis technique. When coupled with high-resolution mass spectrometry (MS), CZE can easily separate and identify different charge variants. Nevertheless, these strategies are typically carried out on the intact level, which does not provide location information for these PTMs. Since the same modification in different domains of an antibody can result in different biological consequences,[2] here we introduce a subunit approach using IdeS digestion and CZE.

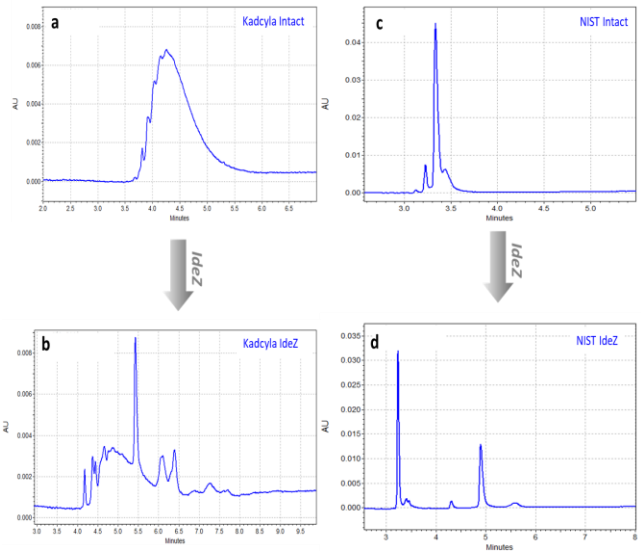

Figure 1. Comparison of intact level and middle up CZE charge variant analysis of antibody drug conjugate (ADC) Kadacyla (a: intact; b: IdeS middle up) and NISTmAb (c: intact; d: IdeS digest)

Figure 2. a) CZE Rapid Charge Variant Analysis Kit (P/N: C44790) and; b) pre-assembled cartridge (P/N A55625)

Key features

- A quick 10 minute analysis time for domain specific charge variants profiling of mAbs, ADCs and new modalities
- Improved resolution compared to intact CZE for challenging molecules
- A methodology results in deeper characterization of the molecule providing domain specific information
- Seamless bridge for peak characterization with ZipChip Native TOF kit
- Ready to use kits and pre-assembled cartridge enables easy and quick training

The Rapid Charge Variant Analysis kit (Figure2) not only separates, quantifies and identifies charge variants, but also provides high-level location information on the modification sites when used to analyze IdeS digested subunit samples

Additionally, this method can profile sample stability. As Indicated by the degradation study where, under elevated pH and temperature conditions, samples showed significant

changes in their charge variant profiles. More importantly peak identification was achieved by analyzing the same sample using the Native TOF kit on the microfluidic ZipChip system coupled to SCIEX time-of-flight (TOF) high-resolution technology[3-5], streamling the charge variant peak characterization process.

Scientists in the biopharma analytical development environment, faced with the challenge of characterization of complex antibody and antibody drug conjugates, can now avoid labor intensive IEX and cIEF methods development and optimization for charge heterogeneity assessment with the use of the CZE_UV kit from SCIEX at both intact and subunit levels and achieve peak characterization with microfluidic ZipChip Native kit.

Methods

Instrumentation

All experiments were performed on the PA 800 Plus Pharmaceutical Analysis System (SCIEX). A Pre-Assembled Cartridge (SCIEX P/N A55625) was used for the separation of all samples.

Reagents

Reagents used to prepare the samples and the separation buffers were provided in the CZE Rapid Charge Variant Analysis Kit (SCIEX P/N C44790). The therapeutic proteins adalimumab and Ado-trastuzumab emtansine® were purchased from Myoderm (Norristown, PA). NIST mAb was purchased from NIST. IdeS was purchased from Promega.

Sample preparation

NIST mAb and Ado-trastuzumab emtansine were diluted to 1 mg/mL in CE Grade water (SCIEX P/N C48034) before it is transferred to sample vial and subject to intact level CZE analysis.

Heat stress treatment: adalimumab samples were incubated in 50 mM Tris buffer (pH8.4) at 40°C for 10 days. 50µg of samples were taken out at different time points. All samples were buffer exchanged into pH 7.4 Tris buffer and 25 µg were digested with IdeS enzyme at 37°C for 2 hours. Unstressed NIST mAb, adalimumab and Ado-trastuzumab emtansine were diluted to 2 mg/mL into pH 7.4 Tris buffer and digested with IdeS enzyme. The digested sample was further diluted with CE grade water to 1 mg/mL before subjecting to CZE analysis.

Instrumentation

The capillary electrophoresis instrument used was a PA 800 Plus equipped with UV detection and a 214 nm bandpass filter (SCIEX P/N 144437).

Separation and analysis

Separations were performed with the CZE Rapid Charge Variant Analysis Kit. The kit provides necessary acid wash, separation buffer and CE grade water. Specifically, separations were performed at 500 V/cm (15kV for 30 cm pre-assembled cartridge) to match the typical conditions used on ZipChip HRN chip for protein analysis and sample was injected by pressure (0.5 psi for 10 s). Data acquisition and analysis were done using 32 Karat™ Software version 10.

Results and discussion

mAb Analysis

High-resolution separation of commercial mAbs can be achieved in less than 10 minutes for both intact and middle up approach. Both analysis can be run in a single sequence using the same cartridge, and method (Figure 1c, Figure 1d).

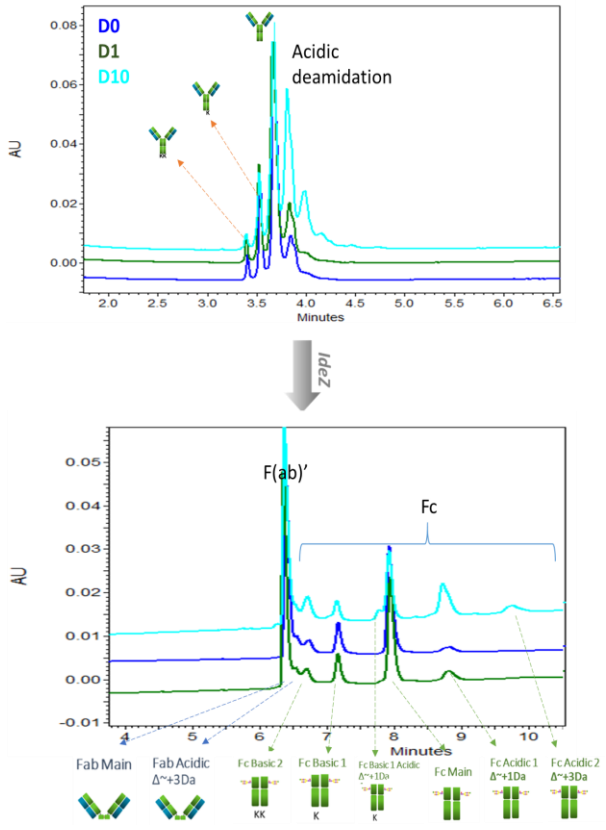

Figure 3. Native state CZE electropheragram overlay of intact (top) and IdeS digested (bottom); adalimumab under stressed (Day1 (D0), Day10 (D10)) and unstressed (Day0 (D0)). Peak identification was achieved using ZipChip® coupling SCIEX TripleTOF® 6600 System with Native CZE application.

Table 1. Percentage corrected area of each identified species in middle up CZE_UP profiles

	% Fab_Main	%Fab_Acidic	%Fc_Basic	%Fc_Basic1	%Fc_Basic1_Acidic	%Fc_Main	%Fc_Acidic1	%Fc_Acidic2
Humira D0	90.9	9.1	10.9	17.0	_	65.8	6.3	_
Humira D1	92.4	7.6	11.2	16.0	_	62.6	10.4	_
Humira D10	79.8	20.2	3.9	12.2	3.9	35.6	27.9	8.1

High pH and heat stressed adalimumab was analyzed with both intact and middle up approaches. The results are shown in Figure 3. All peaks were identified using a ZipChip® system coupled to the SCIEX TripleTOF® 6600 System[5]. The data revealed that both workflows are sample stability indicating. After 10 days of stress, acidic variants increased significantly compared to the control. With IdeS digestion below the hinge region, this middle up approach highlighted deamidation mainly happened on the Fc domain, dominantly with a single deamidation, while the Fab domain stayed relatively stable.

Additionally, IdeS digest removed all covelent link between the two Fc/2 chains. During the CZE analysis, Fc rather than Fc/2 chains was observed, which further demonstrates that the CZE Rapid Charge Variant Analysis Kit provides native like conditions and maintains all non-covalent interactions during analysis.

ADC analysis

Compared to mAbs, ADCs typically have more charge heterogeneity due to additional manufacturing steps. Ado-trastuzumab emtansine is an antibody-drug conjugate consisting of the humanized monoclonal antibody trastuzumab covalently linked, on lysine redidues, with the neutral cytotoxic agent DM1. Both the payload and the manufacturing process can induce changes in the ADC surface charge, resulting in great heterogeneity. The charge variant analysis for Ado-trastuzumab emtansine is typically carried out using the more time consuming high resolution cIEF,, as the faster and easier CZE method does not have enough resolution at the intact level. However, scientists face great challenges in method characterization, especially when the cIEF profile deviates from the standard.

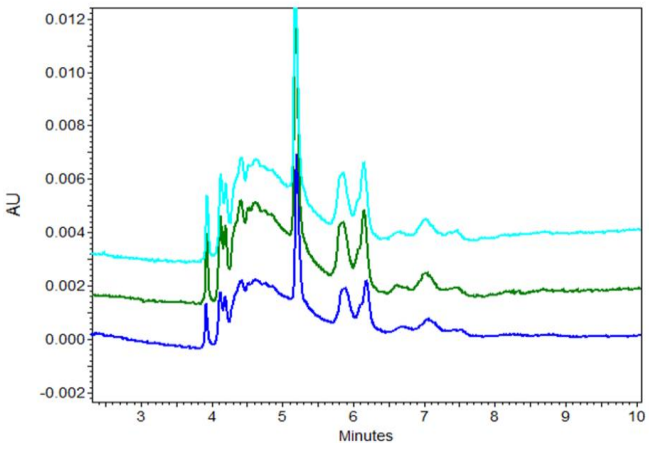

Figure 4. Native state CZE electropheragram overlay of triplicate injections of IdeZ digested Ado-trastuzumab emtansine.

With the help of IdeS, we can achieve domain specific high resolution charge variant analysis of Ado-trastuzumab emtansine in less than 10 min with a CZE method (Figure 1, Figure 4) with good reproducibility The IdeS treated sample can also be analyzed on ZipChip without any addional method development enabling peak identifications.

Conclusions

PTM domain information: A subunit approach for charge variant analysis provides an opportunity to easily identify domain information for charge related PTMs.

Platform capable: Ready to use kits and the pre-assembled cartridge enable easy and quick training. Additionally, the same method and reagents can be used for both intact and IdeS digested subunits, allowing batch analysis.

Easy peak characterization: Good profile correlation between native state CZE_UV and ZipChip Native TOF was demonstrated.

Opportunity for method development and optimization: Resolution can easily be optimized by using a longer bare-fused silica capillary, adjusting capillary temperature or separation voltage.

References

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