ONLINE IEX-MS CHARACTERIZATION AND MONITORING OF MAB CHARGE HETEROGENEITY USING AN OPTIMIZED CATION EXCHANGE RESIN AND COMPACT TOF MS MASS SPECTROMETER

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INTRODUCTION

Ion exchange chromatography (IEX) is a method of choice for the analysis of charge variants of recombinant proteins, encountered with biopharmaceutical drug candidates. Traditionally, IEX separations require high concentrations of salts that are not compatible with mass spectrometry (MS) analysis, which has led to a gap in the characterization of charge variants. It has been shown that direct, MS-based characterization of these charge variants is possible if suitable salts are employed. In this study, MS-compatible IEX separations are combined with a new instrument and applied to a case study on identifying the charge variants formed upon formaldehyde degradation.

METHODS

Forced Degradation of Trastuzumab

A sample of Trastuzumab (50 μl, 2 mg/mL) was buffer exchanged and concentrated to 200 μg/mL with a BioRad Spin®-12 filtration column (25mM NaCl, 25mM Tris, 1mM EDTA, pH 8.0). It was then incubated in the presence of 20μg/mL FabRICATOR® (Qi Wang, 2008) in 25mM NaCl, 25mM Tris, 1mM EDTA, pH 8.0 for 1 week at 1°C, followed by 1 week at 4°C, and finally 1 week at 1°C. The samples were then diluted to 2 mg/mL in 100mM sodium phosphate, pH 8.0 and was injected for IEX analysis.

ionX-MS

Separation of charge variants by IEX was performed on the new BioRad® Spin®-12 column (25mM NaCl, 25mM Tris, 1mM EDTA, pH 8.0) using a Biichi Biorad® 1100 LC instrument, which consists of an ACQUITY IEX 2000 pump, an ACQUITY Bioisolation column (250 x 2.1 mm), an ACQUITY 250 μA MS detector, and an ACQUITY single quadrupole MS. Charge variants were separated and monitored by monitoring base peak (m/z 400) at a resolution of 60000. Each peak was deconvoluted by a MaxEnt1 algorithm. The panel on the left shows combined raw spectra for each peak and the right displays the corresponding MaxEnt1 deconvoluted masses for each peak.

RESULTS

The results are presented in Tables 1 and 2. Table 1 shows digestion IEX-MS results and possible assignments for each peak.

DISCUSSION

Until recently, the investigation of charge variants required tedious labor-intensive chromatography followed by MS analysis. The development of IEX methods that do not necessitate buffer exchanges or the use of high concentrations of salts has been a major challenge. Traditional IEX methods, however, have both chromographic separation of charge variants and mass spectrometry detection, which can lead to misidentifications due to the use of a single ion chromatogram and possible misinterpretation of charge variants. New IEX methods, such as those developed by Leblanc, Fussl et al. (2017) and Fussl, F et al. (2017), are able to provide high-resolution separations of charge variants while also achieving mass spectrometry detection. Furthermore, the use of a chromatographic method has a distinct advantage over other charge-based approaches, such as capillary electrophoresis or mass spectrometry, in that it can simultaneously separate and detect multiple charge variants. As a result, it is possible to confidently assign these variants to other peaks, which in the case of Trastuzumab, is essential, especially in the process of identifying and optimizing the major conformational variant.

CONCLUSION

A new IEX method was developed with water-MS compatibility, as shown in Figures 1 and 2. The TOF MS separations were performed on a BioRad Spin®-12 column packed with a new chromatographic resin, which allows for high-resolution separations of charge variants without the need for buffer exchanges. Furthermore, the use of a chromatographic method has a distinct advantage over other charge-based approaches, such as capillary electrophoresis or mass spectrometry, in that it can simultaneously separate and detect multiple charge variants. As a result, it is possible to confidently assign these variants to other peaks, which in the case of Trastuzumab, is essential, especially in the process of identifying and optimizing the major conformational variant.

REFERENCES


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