O24

Intramolecular Disulfide and Charge Variant Separation and Characterization of Various Antibody Subunits with CE-MS/MS

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Summary

The analysis of antibodies is an ever-evolving field of research. Antibodies can vary, for example, in their glycosylation or amino acid sequence based on the host cell, the purification process, and the storage conditions. The characterization on subunit level enables a detailed mass spectrometric characterization of posttranslational modifications (PTMs) of monoclonal antibodies (mAbs). For the subunit analysis, various mAbs were enzymatically digested and further reduced. However, the implemented reduction often leaves the intramolecular disulfide bridges intact, sometimes without realizing it. Here, we present a capillary electrophoretic (CE) method based on a neutral-coated capillary for the separation of Immunoglobulin G-degrading enzyme of *Streptococcus pyogenes* (IdeS) digested and reduced mAb subunits followed by mass spectrometry (MS) and MS/MS. Our CE approach enables the separation of (i) different subunit moieties, (ii) various reduction states, and (iii) positional isomers of these partly-reduced subunit moieties. The location of the remaining disulfide bridges can be determined by middle-down Electron transfer higher energy collisional dissociation (EThcD) experiments. Applying the presented CE-MS/MS method, reduction parameters such as the use of chaotropic salts were studied. For the antibodies investigated, urea significantly improved the subunit reduction compared to the approach without a chaotropic salt. Using urea in the reduction process, subunit proteoforms like lysin or glycosylation variants could be separated and identified as well as several other low abundant charge and size variants. The presented CE-MS method is a powerful tool for the variant characterization of mAbs on the subunit level. It not only enables understanding of disulfide bridge reduction processes in antibodies, but also the characterization of variants.

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