Characterization of glycans derived from monoclonal antibody by capillary electrophoresis–mass spectrometry

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Glycosylation of monoclonal antibodies (mAb) can have effects on its biological activity and immunogenicity. Due to the importance of mAbs as therapeutic agents, there is a growing demand for monitoring the carbohydrate structures attached to mAb. For enhanced detection sensitivity and higher resolution, shorter run times, novel sample/reagent consumption and flexibility, capillary electrophoresis (CE) has an immense potential for the analysis of biopharmaceuticals. Further, there is growing interest in exploring CE coupled to mass spectrometry (MS) for the higher sensitivity and better compound identification with high mass accuracy. Improvements in CE technology have made CE a widely used tool for protein characterization. In the present work, coupling of an Agilent 7100 CE system to an Agilent 6520 Q-TOF MS was achieved with a coaxial sheath liquid interface. The CE-MS setup equipped with electrospray and the orthogonal sprayer reduces the risk of contamination and improves the MS source cleanliness, while glycans were released by deglycosylation (PNGase F digestion) and released glycans were labeled with 8-amino-1-2,3,4-triolsulfonate (APTS). APTS-labeled glycans were purified and analyzed by CE-TOF MS. The CE-MS platform, combined with the powerful data processing capabilities of Agilent MassHunter and BioConfirm software packages, enabled biopharmaceuticals. Further, there is growing interest in characterization. In the present work, coupling of an Agilent CE-MS method is demonstrated for mAb glycan profiling.

In this work, the utility of CE-MS method is demonstrated for mAb glycan profiling. These results clearly indicate CE-MS as an alternative robust analytical tool gaining advantages in separating the microheterogeneously modified species from CE and the MS analysis provides molecular weight information. The powerful data processing capabilities of Agilent MassHunter and BioConfirm suite further enhance the robustness and detailed identification of the glycan modifications of mAb. CE-MS extends the level of molecule identification for well-established glycan analysis by CE-MS and can complement LC-MS data.

Results and Discussion

The released glycans from mAb were labeled with APTS followed by CE-MS analysis. Using PNGase F crated, all the glycans were migrated within 20 min (Figure 2). The successfully identified the uncharged N-linked glycan species G0, G1, G2, G3, G2F and G3F+1NANA in replicate runs. All the peak assignments were based on accurate mass measurements from CE-TOF MS (Table 1). The mass spectra for individually resolved glycans is depicted in Figure 3. The relative percentage ratio analysis of individual glycan moieties reveal the presence of both the major and minor forms of glycan modifications (Table 1).

Conclusions

1. In this work, the utility of CE-MS method is demonstrated for mAb glycan profiling.
2. These results clearly indicate CE-MS as an alternative robust analytical tool gaining advantages in separating the microheterogeneously modified species from CE and the MS analysis provides molecular weight information.
3. The powerful data processing capabilities of Agilent MassHunter and BioConfirm suite further enhance the robustness and detailed identification of the glycan modifications of mAb.
4. CE-MS extends the level of molecule identification for well-established glycan analysis by CE-MS and can complement LC-MS data.

References