Rapid Sample Preparation of Biologics to Support High-throughput and High-resolution Glycan Analysis by Capillary Electrophoresis

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Abstract

Structural characterization of the glycan moieties of biologics, especially recombinant monoclonal antibody therapeutics (mAb), is critical during clone selection, cell culture optimization and product characterization. High-throughput screening methods are required in order to return results promptly to allow multiple iterations for selection of optimal candidates. This poster gives an overview of a state-of-the-art screening protocol with rapid sample preparation coupled with capillary electrophoresis (CE)-based glycan analysis that includes: automatic sample preparation with optional purification modules to allow direct screening of cell-culture samples; glycan labeling for laser-induced fluorescent (LIF) detection; cleanup to reduce excess reagent peaks and desalting followed by oligosaccharide CE profiling and/or carbohydrate sequencing; and glucose unit (GU)-value-based structural prediction.

Introduction

Glycans released from subnanomolar amounts of mAbs are quickly dependent upon the number of hydrophilic groups presented.6 As a consequence, the GU shifts of the glycans is also presented.

Methods/Discussion

Figure 2 shows an electropherogram for known standards as a control. Exoglycosidase array digestions were performed on rMAb A to identify the peaks present in the UPLC-fluorescence and CE-LIF IgG glycan profiles. Subsequent profiling of the digestion products was conducted using both separate desalting and desialylation, the resulting CE-LIF profiles exhibited similar selectivity to UPLC-fluorescence (data not shown), which facilitated structural annotation. Fluorescent glycoforms were accompanied by the experimentally annotated oligosaccharide digestion pathway for the IgG glycan pool. Each electropherogram was aligned using the lower and upper bracketing standards; however, for better clarity, only the migration window between ten and fifteen minutes is displayed. The relative abundance of the peaks corresponding to APTS-labeled asialo IgGs was consistent with that generated using UPLC-fluorescence.2,4 For these APTS-labeled asialo N-glycans, structural annotation was performed using a bottom up approach, considering that the peaks present in the lowermost trace corresponded to the Biantennary N-glycan and its analogue carrying a bisecting GlcNAc residue.

The GU shifts following the consecutive removal of terminal sugar units when using UPLC-fluorescence were in agreement to those previously reported, i.e., removal of a 4,4’-linked galactose residue caused a shift of 0.86 GU and removal of an 8’-linked fucose or a β-linked GlcNAc residue resulted in a shift of 0.4 GU. A noteworthy observation in CE-LIF was that the GU shifts following digestion showed deviations based upon the presence of other structural components due to the resulting differences in the hydrodynamic volume. The removal of an 8’-linked fucose from the core fucosylated biantennary asialo-N-glycan FA2G2, resulted in a GU shift of 1.06, whereas the removal from its structural analogue containing a bisecting GlcNAc residue resulted in a GU shift of 0.86. Previous in silico-based studies on the molecular dynamics introduction of bisecting GlcNAc in silico, in vitro and in vivo. European Journal of Biochemistry 2004, 271 (3), 118-134.

Conclusion

1. The optimized separation parameters resulted in full separation of all selected rMAb N-glycans in less than 7 minutes at 25°C.
2. At 35°C, the separation time was decreased to 5 minutes, still featuring full resolution of the sample IgG N-glycans.
3. With the use of highly stable capillary coatings, faster separations are expected at higher separation temperatures. The optimal separation conditions at 25°C were applied to demonstrate high-throughput glycosylation profiling of two mAbs in a 96-well format for automated overnight operation.

4. With the help of a liquid handling robot, the entire glycan release, derivatization, sample cleanup and separation process can be fully automated.

References