Using standard sample preparation protocols, monosaccharide analyses were performed on CARPAP, APTS labeled and separated by CE. In order to identify and help illustrate better the co-migrating glycan species associated with the MAbs, the N-linked oligosaccharide maps were further processed by 2D mapping. This work suggests the potential for CE to be used to successfully separate and quantify N-linked oligosaccharide populations associated with MAbs. Additional experimentation will focus on the optimization of chemistry and CE methods to enable separation of these species in a reproducible assay environment.

The goal of this study was to achieve separation of major glycan species associated with monoclonal antibodies (MAbs). The glycan population includes oligosaccharides with and without core fucosylation, fucosylated, sialylated and complex antennary oligosaccharides can be differentiated from one another. Optimization of chemistry and CE methods enabled separation of these species in a simple, reproducible assay environment.

Quantitative analysis of glycans can be performed utilizing a polymeric separation matrix consisting of 0.4% polyethylene oxide (PEO). Beckman Coulter has developed and commercialized technology (Beckman Coulter Carbohydrate Labeling & Analysis Kit). Employing standard protocols (involving the Carbohydrate Labeling & Analysis Kit) for instrument configuration, sample preparation, and separation conditions, we easily obtained baseline resolution between G0, G1 positional isoforms and G1F and G2 oligosaccharide species (Figure 2). A systematic approach was devised in which closely migrating co-migrating glycan species can be resolved (Figure 4). In order to test this separation method on an antibody, we obtained a therapeutic MAb and analyzed its associated glycans (Figure 5). Spiking with oligosaccharide standards to a bisecting GlcNAc have been associated with changes in ADCC activity and thus an impact on therapeutic efficacy. This work suggests that CE can be used to successfully separate and quantify N-linked oligosaccharide populations associated with MAbs. Additional experimentation will focus on the optimization of chemistry and CE methods to enable separation of these species in a simple, reproducible assay environment.

Results and Discussion

The high resolution capillary electrophoresis separations based on mobility and hydrodynamic volume have been developed for quantitative analysis of glycans. Using published protocols and methods, we have been able to successfully separate oligosaccharides differing in terminal galactose. We also showed that by combining the standard PEO separation gel buffer with a LPA gel buffer, we were able to separate fucosylated from afucosylated N-linked oligosaccharides, high mannose structures, and numerous other glycan moieties. This work suggests that CE can be used to successfully separate and quantify N-linked oligosaccharide populations associated with MAbs. Additional experimentation will focus on the optimization of chemistry and CE methods to enable separation of these species in a simple, reproducible assay environment.

Methods and Materials

All separations were performed using the PA 802 Plus Pharmaceutical Analysis System configured with a 480 mm x 75 mm white capillary labeled with amino pyrene tri-sulfonic acid (APTS). The complexity of glycans associated with many molecules calls for high resolution separation in order to assess heterogeneity among carbohydrate isoforms and core fucosylation. Since there is currently no established technology for automated and quantitative analysis of N-linked oligosaccharides, we set out to develop methodology by which isolated, scissored, sialylated, and complex antennary oligosaccharides can be differentiated from one another. Optimization of chemistry and CE methods enabled separation of these species in a simple, reproducible assay environment.

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