Introduction

With an increasing number of innovative biopharmaceuticals leaving the market, the development of therapeutic proteins is an ongoing priority. A key step in protein characterization is the analysis of its glycosylation profile, which can have a significant impact on productivity, efficacy and pharmaceutical properties. An antibody-like linkage-specific sialyltransferase (ST) that catalyzes the formation of Galβ(3→4)Manβ(1→3)Manβ(1→4)GlcNAc (Arm3,4) on N-glycans of both fucose-containing and non-fucose-containing protein glycans has been described (1). The Arm3,4 isomer does not appear to be well-sialylated in any of the reactions, which is consistent with previous reports (2). The majority of the Fc-domain N-glycans are fucosylated and sialylated glycans. It is therefore essential that N-glycosylation be targeted and controlled at the optimal level. Once the optimal profile has been established, cell culture conditions are not necessarily maintained in downstream purification. However, modifying cell culture conditions can go too far; other approaches are needed to produce multiple versions of the glycosylation profile, so that differences in N-glycosylation may be evaluated for corresponding effects on drug efficacy, pharmacokinetics of a protein, as well as its immunogenicity.

Materials

Secondary N-Glycan Remodeling of Therapeutic Proteins: α(2-6) Sialyltransferase

GlykoPrep Rapid N-Glycan Sample Preparation with 2-AB (product code 2267) was manufactured using AssayMAP PA50 Cartridges. N-glycan samples were prepared using GlykoPrep Rapid N-Glycan Sample Preparation with 2-AB and ProZyme, Inc., Hayward, CA, USA; 2Roche Diagnostics, Penzberg, Germany. GlykoPrep, Sialidase A, Sialidase S and Signal are trademarks or registered trademarks of Glyko, Inc. We thank Beckman Coulter, Inc. for the use of the BC PA 800 System and the assistance of Marie Anderson, and to ProZyme for method details.

Results & Discussion

Sample preparation and analysis: The GlykoPrep kit coupled with fast UPLC is a powerful method to rapidly acquire, analyze and process data on entire complex glycosylation profiles in a single run. The technique has been used to determine time of harvest in mower biomass, and can also be used to optimize biopharmaceutical manufacturing settings (1).

Sample: Enbrel (a recombinant, soluble, IgG1 protein) is used for the illustration. Enbrel (manufactured using ProZyme) has been used in clinical trials for 12 years, and is currently being studied in 30+ additional trials for a variety of indications. The recombinant IgG exists in at least 2 isoforms, with at least 2 different glycosylation patterns. The IgG includes a variable region (VH and VL) and a constant region (Fc). The humanization of the antibody region reduces its immunogenicity, but can result in a significantly altered glycosylation profile. As the reaction was nearly completed by 2 hours in the UPLC timecourse, we performed a 2 hour reaction with ATPs-labeled Sialidase S for CE analysis. Sialidase S was bound to an AssayMAP PA50 cartridge, and the enzyme was added to a Waters ACQUITY UPLC BEH glycan column (1.7 μm, 2.1 x 100 mm).

Linkage analysis by carbohydrate digestion: After the sialylation reaction, the 2-AB-labeled Enbrel and Rituxan were treated with glycosidase enzymes to generate monosaccharides and free sugars. The Enbrel and Rituxan samples were analyzed by Agilent 1290 UPLC coupled with an in-line evaporative light scattering detector. The Linkage Analysis by Carbohydrate Digestion (LACD) and Fucose Digestion (FD) experiments were performed on the same sample set. The LACD and the FD experiments were performed on the same sample set. The LACD and the FD experiments were performed on the same sample set.

Figure 7 shows the LACD profiles for Enbrel and Rituxan at 2h, 6h, 24h, 48h, and 72h. At 2h, the N-glycan profile consists of 2/3 of those left unsialylated, suggesting a limitation of the ST. As the reaction was nearly completed by 2 hours in the UPLC timecourse, we performed a 2 hour reaction with ATPs-labeled Sialidase S for CE analysis. Sialidase S was bound to an AssayMAP PA50 cartridge, and the enzyme was added to a Waters ACQUITY UPLC BEH glycan column (1.7 μm, 2.1 x 100 mm). After the ST reaction, the 2-AB-labeled Enbrel and Rituxan were treated with glycosidase enzymes to generate monosaccharides and free sugars. The Enbrel and Rituxan samples were analyzed by Agilent 1290 UPLC coupled with an in-line evaporative light scattering detector. The Linkage Analysis by Carbohydrate Digestion (LACD) and Fucose Digestion (FD) experiments were performed on the same sample set. The LACD and the FD experiments were performed on the same sample set.

Conclusions

GlykoPrep sample preparation coupled to fast UPLC-FLR detection and CE-GFP enabled accurate and predictable production of α(2-6) sialyltransferase before and after enzymatic modifications.

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