Comparison of GlycanMap® analysis, a rapid, high-throughput MALDI-TOF MS glycan analysis method, to fluorescent (2-AA) HPLC for recombinant glycoprotein characterization

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ABSTRACT

Detailed characterization of the glycans present on recombinant glycoproteins remains an important challenge in both the development and production of these clinically important biopharmaceuticals. In order to test the fidelity of a novel, high-throughput analytics system, recombinant glycoproteins representing the major therapeutic platforms were analyzed by a new high-throughput method (GlycanMap® analysis) and the results were compared to a traditional HPLC fluorescent detection method run in a second laboratory. The new GlycanMap® method relies upon a novel chemoselective bead separation technology, on-bead chemical derivatization, quantitative MALDI-TOF mass spectrometry and a custom Bioinformatics platform – with the key assays steps integrated into an automated, 96-well robot-assisted analysis system. The comparison method involves standard HPLC using fluorescence detection, following release of the glycans with PNGase F and reductive amination of the glycans with 2-aminobenzolic acid (2-AA). Overall, results from the two laboratories and two assay methodologies compared well with GlycanMap® showing some advantages over HPLC with respect to detecting glycans on a complex glycoprotein. The GlycanMap® method afforded the ability to simultaneously measure neutral and acidic sugars (e.g. N-acetyl) and absolute glycan levels. It was also more adapted to measure O-linked glycans.

METHODS

GlycanMap® analysis (Figure 1) was performed according to Ezoza Sciences, proprietary methodology, which is based on the methods previously reported by Nishimura, Furukawa, and Miura [1-3]. Briefly, internal standard glycan (70 ppt) was added to duplicate 2-μl aliquots of each sample to aid in quantification. The samples were denatured and then digested with trypsin, followed by heat inactivation. The mixture was then treated with PNGase F. After enzymatic release of glycan, aliquots were subjected to solid-phase processing using BioSpect® beads. Following capture onto the beads, the acid and reducing sugars were released. The glycan was then simultaneously reloaded and labeled, and then spotted onto a MALDI target plate. Steps from initial dilution to spotting onto the MALDI plate were performed using the fully automated Saweedee® technology. For the analysis of O-linked glycans, the oligosaccharides were released chemically in the form of reducing sugars. The released oligosaccharides were subjected to GlycanMap® analysis.

For glycan mapping by HPLC, 100 μg of each glycoprotein (in duplicate) were buffer exchanged and deglycosylated overnight at 37 °C, following PNGase F. Glycans were deglycosylated under non-reducing conditions while Fl Fusion Proteins and Complex glycoprotein were deglycosylated under reducing conditions after overnight treatment with reductive amination with 2-aminobenzolic acid (2-AA). After SPE clean-up on QureClean® Q2 SPE Column (Interscience, Suer, France), glycans were analyzed by HPLC and the GlycanMap® RapiGest® and complex glycan standard solutions (Trask Biosciences, Sunny, CA) were run simultaneously with the samples in the same run, with fluorescence detection at 350/450 nm.

RESULTS

Detected glycans and their proposed structures are shown in Table 1. Glycan structures are depicted using the symbolic nomenclature recommended by the Consortium for Functional Glycomics.

Glycan structures are expressed as a four digit code which represents the number of hexoses (galactose, mannose, or glucose), N-acetylglucosamines (GlcNAc or GalNAc), fucose (Fuc), and N-acetylgalactosamines (NeuAc).

CONCLUSIONS

The GlycanMap® method was also adapted to measure O-linked glycans. As expected, no O-linked glycans were detected in the monoclonal antibodies. The Fl fusion protein and complex glycoproteins both contained complex N-glycans, which are typical for the host cells.

REFERENCES