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Identification of N-Glycans Using an Accurate Mass and Retention Time Database Yield Oligosaccharides Variations in Individual Serum

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Overview

Glycosylation is a common and important post-translational modification. The most common are the N-glycans attached to asparagine (Asn) residues via an N-glycosidic bond. Many properties of glycoproteins are mediated by N-glycans including their conformation, solubility, and antigenicity.

We introduce a means of rapidly identifying released N-glycan structures using their accurate mass and retention time in a glycan database. The method allows us to readily profile N-glycans in biological fluids with deep structural analysis. We have assembled a database of 178 N-glycans. Users can easily update information in the database and search it against LC/TOF or Q-TOF data.

Experimental

N-Glycans were released using standard PNGase F methods and then purified by solid phase extraction (SPE) using graphitized carbon cartridges (GCC). Purified N-glycans were reduced by 1 M $NaBH_4$ in a water bath at 65 °C for 2 h. Reduced N-glycans were desalted and enriched again with GCC-SPE. To create a serum N-glycan library, the glycans were separated into fractions using an off-line HPLC. Structures were systematically elucidated using exoglycosidases to determine the linkages and terminal monosaccharides' positions of each N-glycan.

Each compound was characterized by tandem MS and LC retention time using a microfluidic ChipCube nano-LC system coupled to an Agilent 6520 Q-TOF. N-glycan structures, formulas, and retention times were determined and imported into a MS library software.

Conclusion

To conclude, we developed a comprehensive workflow which allows the rapid identification of released N-glycans by searching against a database using accurate masses and LC retention times. It was further used to identify N-glycans in nine individual sera and the serum oligosaccharides variations among individuals were compared.

Results and Discussion

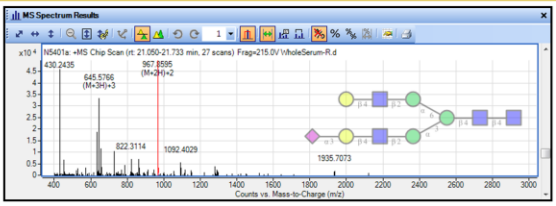

Figure 2: Spectrum identified using the accurate mass and retention time database annotated with glycan structure and a mass fragment peak labeled with its formula.

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Results and Discussion

Creation of Accurate Mass and Retention Time Database

A database of 178 released N-glycans was created using MassHunter PCDL Manager B.07.00 software. The database contains compound names, formulas, accurate masses, retention times and the structures in CFG notation (Figure 1). An Excel spreadsheet with columns containing the key information was first imported into PCDL Manager. Structures were drawn using GlycoWorkbench Version 2.1 and added to each entry in glycoct_condensed format.

Searching MS data for N-Glycans

The database is searchable against TOF or Q-TOF accurate mass data to identify released N-glycans using MassHunter Qualitative Analysis B.07.00. Spectra are annotated with the glycan structure and major mass peaks are labeled with fragment formulas (Figure 2). Retention times can be used as part of the search either optionally or as a required match for a hit.

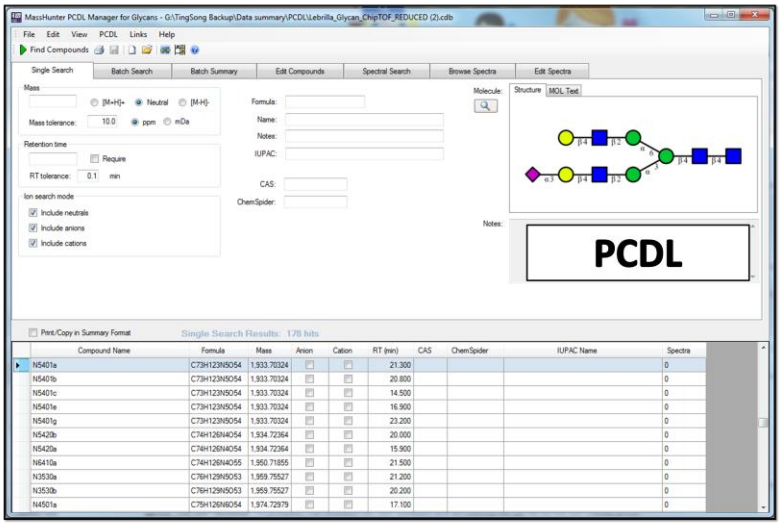

Figure 1: Accurate mass and retention time database displayed in PCDL Manager software. Users can search, browse, add and delete compounds and compound information (e.g. RT) with the PCDL Manager user interface.

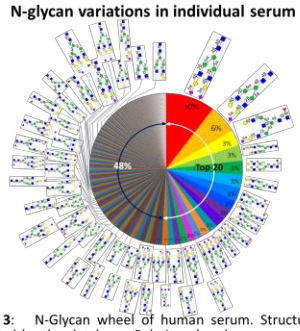

Figure 3: N-Glycan wheel of human serum. Structures are identified by the database. Relative abundances are averaged from nine individuals.

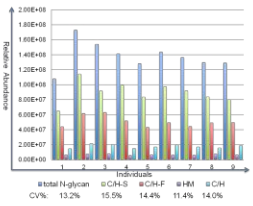

Figure 4: Variations of subclasses of N-glycans in nine individual sera.

Acknowledgments

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