NANO-FLOW LC-MS COLUMNS FOR THE ANALYSIS OF OLIGOSACCHARIDES FROM GEL SEPARATED GLYCOPROTEIN CARBOHYDRATES

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ABSTRACT

Protein glycosylation is an important and interesting yet very complex field in biochemistry. The demands on LC columns (as part of a more comprehensive analysis platform) are unique in terms of selectivity because of the chemistry of the analyte as well as its complex isomeric nature.

INTRODUCTION

Glycomic and glycoproteomic research is a field of growing interest. Isolation of glycoproteins by traditional methods is time consuming, and the full characterization of oligosaccharides will require significant amounts of starting material, as well as a multidisciplinary research team. The amount of biochemical analysis possible after a traditional electrophoresis separation has been limited by the sensitivity of MS detection and efficient sample preparation techniques. Adoption of nanoflow LC-MS for oligosaccharide will make glycomic analysis applicable to gel separated proteins.

PROTEIN GLYCOSYLATION

Protein glycosylation is the most common post-translational modification in proteins. There is a variety of carbohydrate units that can be incorporated through any of its hydroxyl groups which leads to a large number of possible isomers and a very complex system to analyze.

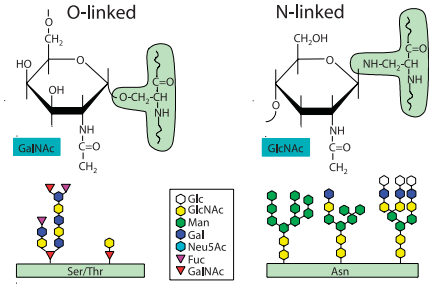


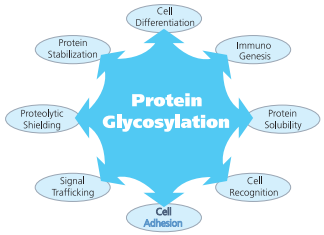
Figure 1. Possible carbohydrate structures on the protein backbone.

NUMBER OF POSSIBLE ISOMERIC PEPTIDES AND OLIGOSACCHARIDES (PYRANOSE RING ONLY)

Composition	Product	No. of isomers	
		Peptides	Oligosaccharides
X-X	dimer	1	11
X-X-X	dimer	1	176
X-Y-Z	trimer	6	1056

GLYCOSYLATION FUNCTION

Protein glycosylation is a dynamic function, which is used by the organism to regulate a number of important functions. The fact that the glycosylation can change with a disease and that unique oligo-saccharide epitopes can be found on the surface of pathogens makes these molecules particular interesting for pharmaceutical applications.



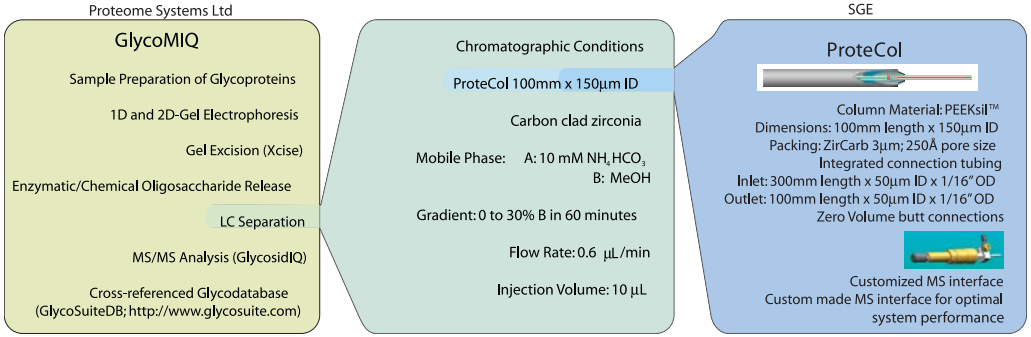
THE PROTECOL COLUMN SYSTEM

The analysis of carbohydrate structures places a special demand on the LC separation system in terms of selectivity, specificity and sensitivity. The column is packed with ZirCarb, a carbon-clad zirconia material. The graphitized carbon surface provides a unique selectivity which allows the separation of closely related isomers. High sensitivity can be achieved by keeping extra-column band broadening to a minimum. The ProteCol™ column range has integrated connection tubing to minimize void volumes. The length of the connection tubing is tailored to suit the GlycoMIQ platform to minimize the number of connections in the system. A custom built MS interface further optimizes the performance.

CONCLUSIONS

The use of ZirCarb stationary phase in a nano-LC-MS for analyzing oligosaccharides, provides both the sensitivity and isomeric resolution for glycomic and glycoproteomic applications. On-line negative ion nano LC-MS/MS also provides a mean of further characterization. With the use of intelligent software for fragmentation interpretation (GlycosidIQ), in combination with the ZirCarb ProteCol technology the automation of oligosaccharide analysis makes what is commonly perceived as too hard of an analysis more accessible to the bioanalytical chemist. The marriage of high sensitivity and automation has been one of the cornerstones in the rapid expansion of proteomic research, and the next generation of techniques is now developed to allow insight in the fundamentals of post-translational processing.

METHOD FOR THE ANALYSIS OF OLIGOSACCHARIDES



CHROMATOGRAPHIC RESULTS

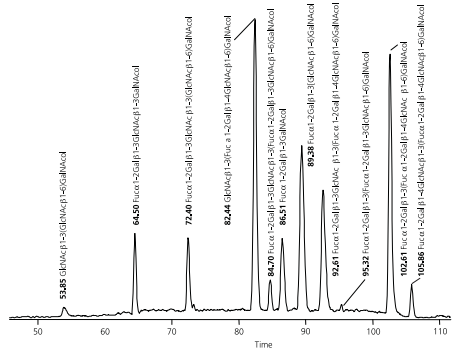


Figure 3. Separation of oligosaccharides from rat small intestine from MUC2 isolated by 1D SDS PAGE.

SENSITIVITY OF NANO-LC OF STANDARDS

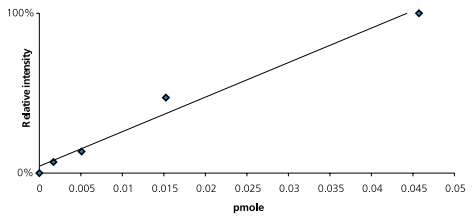


Figure 4. Sensitivity study using maltoheptaose as a standard. Concentrations of a standard were reduced to investigate the limit of detection. 2 fmole of maltoheptaose gave a signal to noise ratio of two.

MATCHING OF OLIGOSACCHARIDE STRUCTURES BY GLYCOSIDIQ

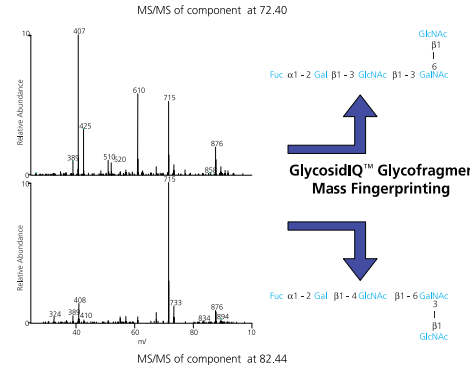


Figure 5. Example of the distinction between isomers.

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2. Wilson,N.L., Schulz,B.L., Karlsson,N.G. & Packer,N.H. Sequential Analysis of N- and O-linked Glycosylation of 2D-PAGE Separated Glycoproteins. J. Proteome Res. 1, 521-529 (2002).

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