HTP Proteome-Glycome Analysis in *Caenorhabditis elegans*

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OVERVIEW

We are uniting glycomics with the burgeoning field of proteomics, initially utilizing the nematode *Caenorhabditis elegans*. Our first step has been to characterize the N- and O-linked glycans by MALDI-TOF. Additionally, we are using a hybrid ion trap/tof instrument (MALDI-QIT-TOF) for fine structure determination. The MALDI-QIT-TOF enables MS^n for discerning structural and linkage motifs. Differential methylation/deuteration is also employed. Our next step is identification of proteins using 2D electrophoresis coupled with a robotic excision and digestion instrument that provides automated MALDI plate preparation. Proteins are identified by peptide mass fingerprinting using MALDI-TOF. Glycoproteins, identified by specific gel stains, can be released from gel spots and identified separately. This strategy can be employed using glycosylation-deficient mutant strains, highlighting affected metabolic pathways.

MATERIALS AND METHODS

PROTEIN EXTRACTION

Liquid cultures of *C. elegans* are grown to maturity then collected and washed. Worms are suspended in a lysis buffer (35 mM Tris, 8M urea, 4% CHAPS, 65mM DTT, pH 8.0) that is suitable for membrane protein extraction and placed in a specially designed pulse tube (Figure 1a). For homogenization the samples are subjected to pressure cycling treatment in a Barocycler (Boston Biomedica, Inc) using 5 cycles of 20 seconds each at 30,000 psi, room temperature. We obtained 37% higher protein yields ($t=4.71$, $p<.01$) using the Barocycler relative to sonication. Used in combination the lysis buffer and Barocycler effectively rupture the tough cuticle allowing for extraction of soluble proteins as well as membrane proteins. In 2D gels we found more high molecular weight proteins (possibly due to decreased proteolysis) as well as more high pH proteins when compared to sonication (Figure 1b). Analysis of gel spots has revealed membrane proteins including ATP synthase subunits and channel proteins.

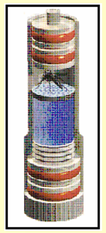

Figure 1a

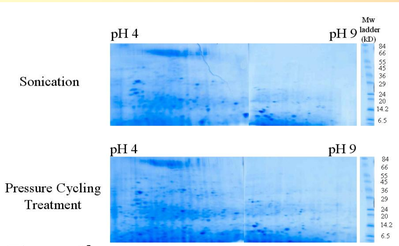

Figure 1b

PROTEOMICS

Proteins are run on 2D gels and stained with Coomassie Blue for total proteins, or Pro-Q Emerald (Molecular Probes) for glycoproteins. Proteins of interest are robotically selected, cut, and placed in a microtiter plate using the high throughput Xcise gel cutter (Proteome Systems, Figure 2). The Xcise subsequently destains, digests, and spots peptides onto a MALDI plate. The Axima-CFR MALDI-TOF (Figure 3, Shimadzu Biotech) can automatically perform peptide mass fingerprinting from up to 384 MALDI spots when interfaced with MASCOT software used to identify proteins.

GLYCOMICS

For glycome analysis glycans are released by hydrazinolysis. After permethylation or deuteration glycan profiles are examined via MALDI mass spectrometry (Axima-CFR, Shimadzu Biotech). Contrasting permethylated and deuterated spectra allows identification of endogenous methylation. MS^n further elucidates structure and linkages. For example, dissecting a single parent peak can reveal multiple fucosylation sites. The Axima-QIT is a unique hybrid instrument that combines MALDI sample introduction, a quadrupole ion trap for collision induced dissociation, and a reflectron time of flight mass analyzer (Figure 4).

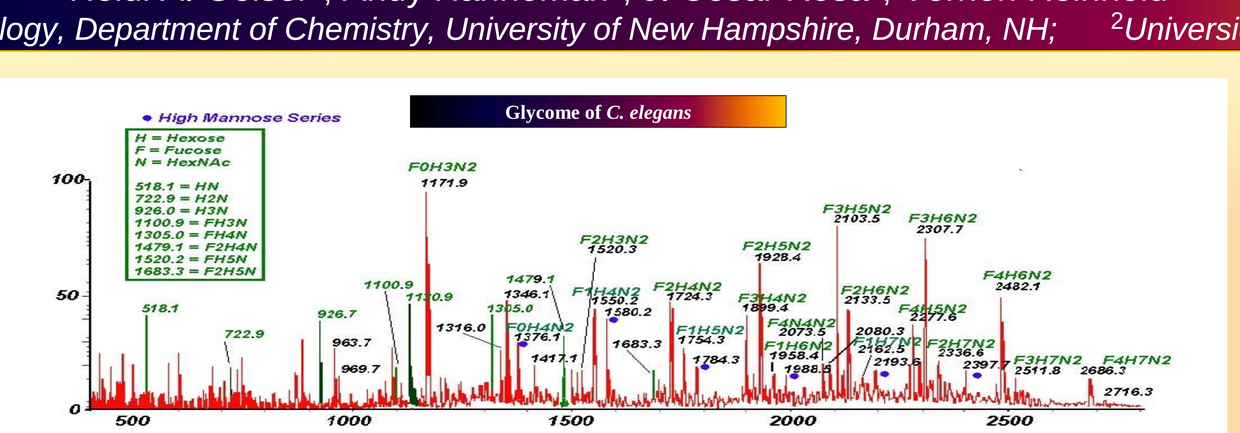

Figure 5

Figure 2

Figure 3

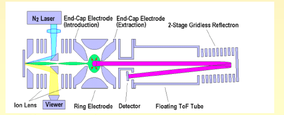

Figure 4

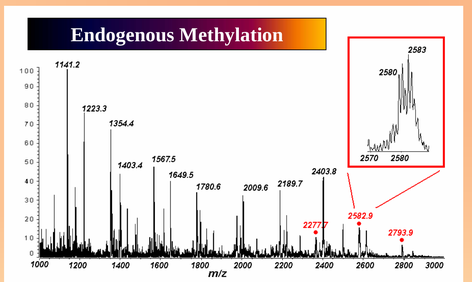

Figure 6

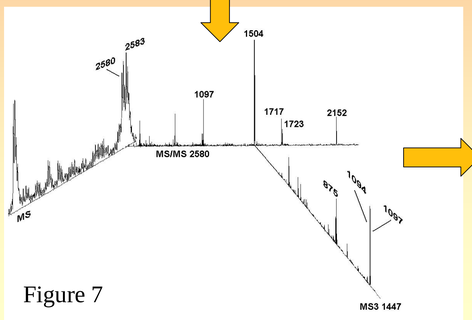

Figure 7

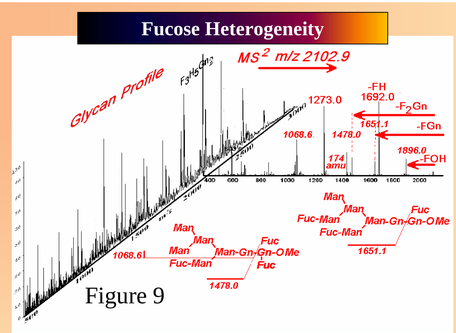

Figure 9

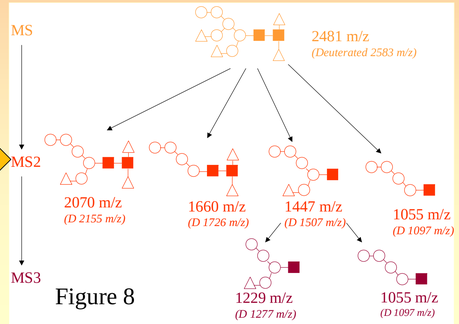

Figure 8

RESULTS

Using MALDI-TOF mass spectrometry we have obtained a complete profile of N-and O-linked glycans from *C. elegans* (Figure 5. The O-linked glycan peaks are highlighted in green and listed on the inset table). This profile demonstrates an abundance of highly fucosylated glycans, with fucose appearing on antenna hexose residues as well as on the core. *C. elegans* glycans contain up to four fucose residues and up to 8 hexoses. Using an MS^n approach on a MALDI-QIT-TOF instrument we have obtained evidence for endogenous methylation on hexose and possibly on fucose residues (Figure 6, 7, 8). Isomeric relationships of fucose residues are common, necessitating MS^n dissection for positive structural determination (Figure 9). This approach has revealed the presence of multiple isobaric high-fucose and endogenously methylated glycans. We have not seen evidence of phosphoryl choline linkages.

CONCLUSIONS

The glycome of *C. elegans* provides a basis for working in the area of glycoproteomics. This data set becomes relevant to *C. elegans* physiology when coupled with high throughput proteomics. Identification of individual glycoproteins and an associated backdrop of non-glycosylated proteins ultimately allows for structure-function relationships to be drawn. By analysis of glycosylation-deficient mutants we aim to elucidate roles for specific glycoproteins and proteins involved in glycosylation.

Future work on *C. elegans* glycans will focus on distinguishing isobaric glycan structures including composition, linkage and O-methylation sites. Concurrent efforts are targeted at identification and analysis of glycoproteins from 2D gels for high throughput glycoproteomics capability.

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ACKNOWLEDGEMENTS

We thank John Collins for providing the knowledge and equipment for growing *C. elegans*, and Joyce Reinhold and Dominique Kief for help with growing and collecting the worms.

This work was supported by an NIH-BRIN grant.