Utilization of an Isotopically Labelled Glycoprotein Internal Standard to Enable Comparison of Glycan Quantitation

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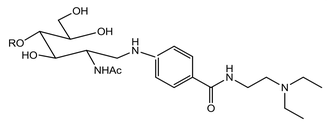
Abstract

The ability to quantitatively determine the glycan composition of a sample is an essential element of comparative glycomics. Identifying changes in glycan expression has the potential for multiple practical applications, such as an investigative tool for medical and scientific professionals exploring various disease states. For the potential biomedical applications of glycan analysis to reach fruition, analytical strategies need to be developed that provide consistent interlaboratory results. This study investigates the ability of various approaches, including label-free and the use of an isotopically labeled internal standard, to provide consistent glycan quantitation across multiple methodologies, as a step towards interlaboratory consistency.

Materials and Methods

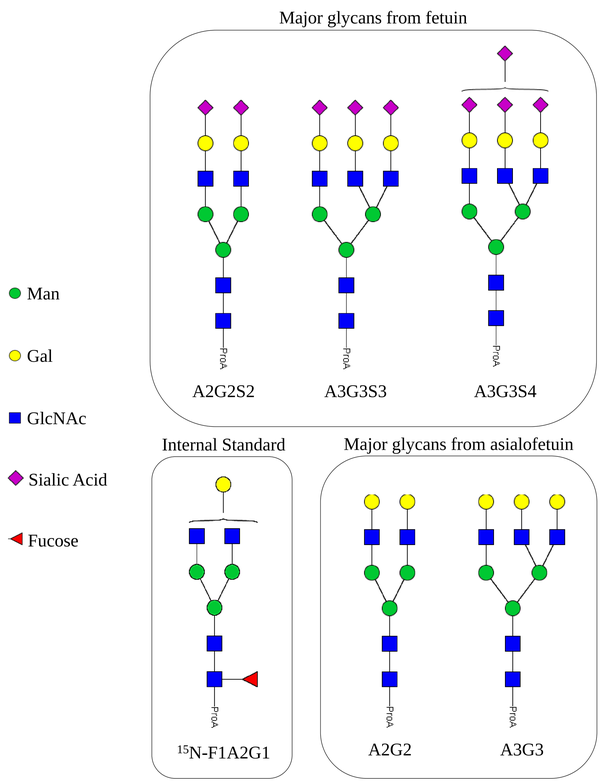
Samples of varying percent concentrations of bovine fetuin (15.84%, 48.48%, and 82.47%) in asialofetuin were prepared and then spiked with an internal standard. The internal standard used was a mouse IgG labeled with ^{15}N and was obtained from Glycoscientific (Athens, GA). The reference material contains labeled glycans that are attached to the protein backbone, therefore this standard can be mixed with the sample prior to release of the glycans. Each sample underwent enzymatic digestion with trypsin and deglycosylation with PNGase F, then the released N-linked glycans were tagged with procainamide and analyzed via LC-MS.

Oligosaccharides with Procainamide (ProA) labeling:



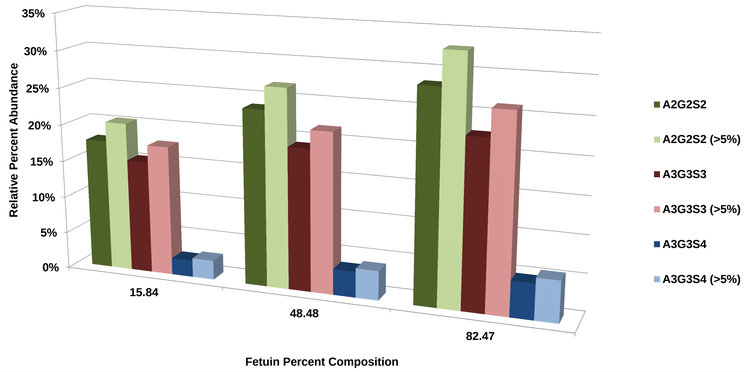
Experimental conditions:

- LC: 1100 series (*Agilent*)
- MS: Finnegan LTQ (*Thermo Scientific*)
- Column: Halo Penta-HILIC column 0.2 x 150 mm (*Advance Materials Technology*)
- Mobile phase A: 95% H_2O/ACN with 50 mM Ammonium Formate and 1% Formic Acid
- Mobile phase B: Acetonitrile
- Column temperature: 25ºC
- Flow rate: 2 µL/min



Results

Effect of low abundance exclusion on relative percent abundance



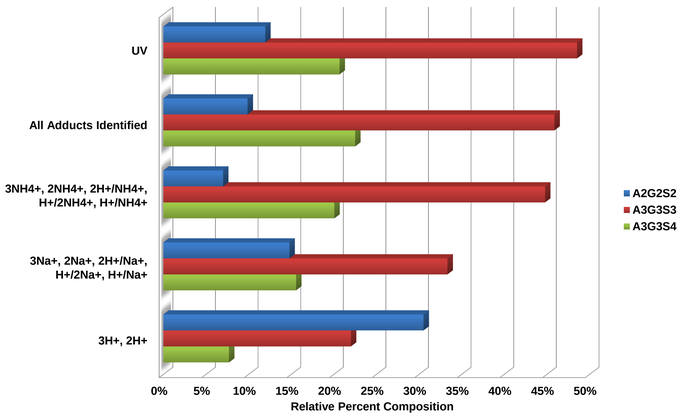
All ions identified in the solutions containing 15.84%, 48.48%, and 82.47% fetuin were summed for each sample, and the relative percent abundance for the three major glycans for fetuin (A2G2S2, A3G3S3, and A3G3S4) was calculated. When the signals for glycans identified to have a prevalence less than 5% of each sample were removed from the calculation, the relative abundance calculations of the three major glycans resulted in higher percentages. The more prevalent the glycan, the greater the discrepancy.

Effect of adduct inclusion on relative percent abundance

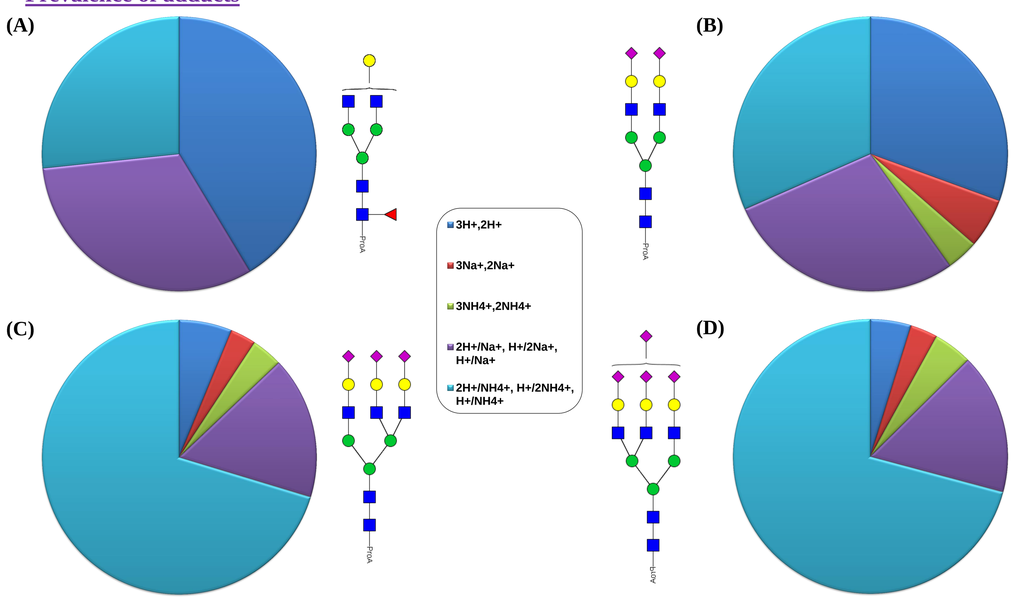
All molecular ions identified by LTQ for the 48.48% fetuin concentration sample were summed, and the relative percent abundance for the three major glycans for fetuin (A2G2S2, A3G3S3, and A3G3S4) was calculated. The relative percent abundance was also calculated via UV for comparative purposes.

Excluding one or more groups of the adducts from this calculation resulted in an altered percent abundance result for that glycan, as well as a dramatic shift in overall composition results.

Most notably, when only the fully protonated species was included, A2G2S2 was calculated to be the most abundant glycan, while it was the least abundant when all adducts were considered. This suggests that the more sialylated the glycan, the more apt it is to form adducts.



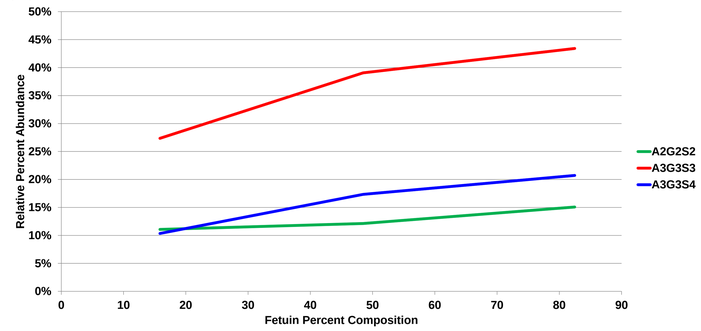
Prevalence of adducts



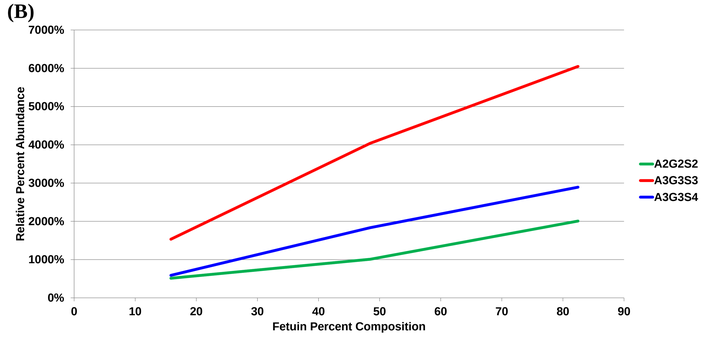
The intensities of the molecular ion species for each glycan were grouped into subsets, the intensities of all ions in each subset were summed, and divided by the total ion intensities for all molecular ion species to yield the relative percent abundance. These values are shown for (**A**) ^{15}N-F1A2G1 internal standard (**B**) A2G2S2 (**C**) A3G3S3 and (**D**) A3G3S4. As the degree of sialyation increased, the propensity to form ammoniated and natriated adducts also increased.

Relative quantitation utilizing an internal standard

(A)



(B)



The percent abundance of A2G2S2, A3G3S3, and A3G3S4 from each of the solutions containing 15.84%, 48.48%, and 82.47% fetuin were calculated **(A)** relative to the summed response for all ions identified via LTQ and (**B**) relative to the response of the ^{15}N-F1A2G1 internal standard ions. Linearity in response to increasing fetuin concentrations and agreement between the experimental and theoretical level of increase are improved with the use of an internal standard.

Conclusions and Discussions

➢ Utilization of an internal standard compensates for random and systematic errors, allowing for more consistent analytical results.

➢ The presence of an internal standard permits relative quantification of selected targets of interest in complex samples without the need for complete sample analysis.

➢ Percent abundance relative to an internal standard exhibits a greater linearity across analyte concentration shifts, and results provide a closer correlation to expected trends.

➢ The more highly sialylated a glycan, the greater the affinity for ammonium and natrium ions, therefore complete analysis of the target analyte is required.

Forward Thinking

➢ Due to differences in ionization efficiency and adduct affinity between various N-glycan species, the employment of an isotopically labeled version of the analyte of interest would be ideal.

Acknowledgements

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