

IDAWG: A Novel Quantitative Method for Glycomics

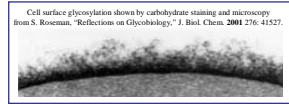
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Overview

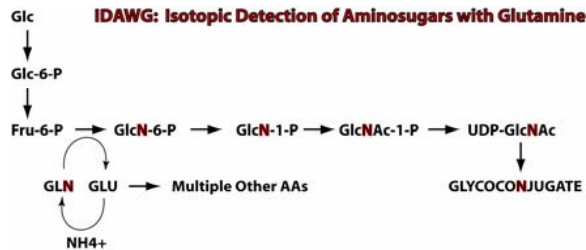
One of the major challenges in the -omics field is the development of technologies that allow for comparative, relative-quantitative analysis between samples. In proteomics, stable isotope approaches, such as SILAC and ICAT, have been developed to address this need. Here we report a methodology that takes advantage of stable isotope labeling of glycans in cell culture for performing quantitative glycomics. This methodology termed IDAWG, isotopic detection of aminosugars with glutamine, relies on the hexosamine biosynthetic pathway that uses the side-chain of glutamine as its sole donor source of nitrogen for aminosugars in the production of sugar nucleotides.

Introduction

- Glycosylation is one of the most common post-translational modifications encountered in eukaryotic systems -- estimated that 60-90% of mammalian proteins are glycosylated
- Glycans play critical roles such as:
 - mediating biological recognition events
 - cell-cell recognition
 - inflammatory reactions
 - tumorigenesis
 - cell development and differentiation
- The repertoire of glycans change based on the condition of the cell, in particular between normal and transformed (malignant) cells and thus may be good markers of tumorigenesis.
- Identifying glycan structures and how these structures change is the focus of an emerging field called glycomics.



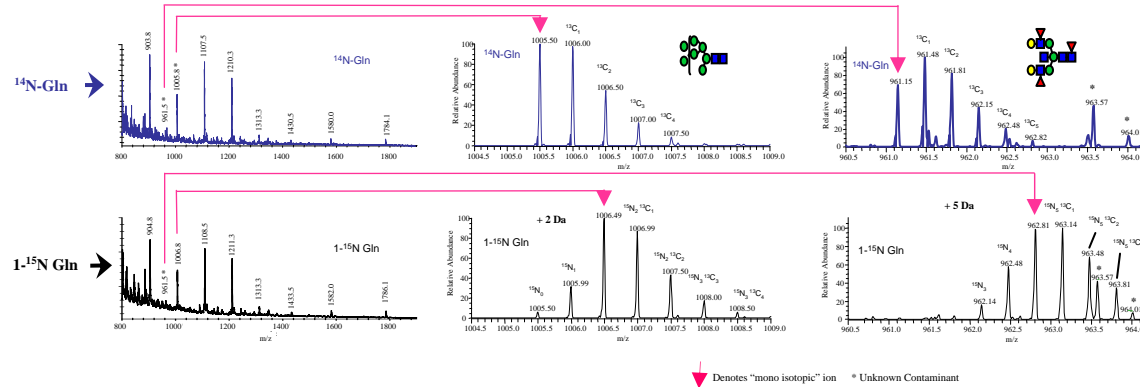
The IDAWG Concept



IDAWG relies on the hexosamine biosynthetic pathway that uses the side-chain of glutamine as its sole donor source of nitrogen for aminosugars in the production of sugar nucleotides. Thus, introduction of glutamine with an ¹⁵N labeled side-chain (1-¹⁵N-Gln) into Gln-free media allows for all aminosugars, such as GlcNAc, GalNAc, and sialic acids to become labeled with ¹⁵N. These isotopically labeled sugars become incorporated into various glycoconjugate, such as N- and O-linked glycans, glycolipids, and extra cellular matrix polysaccharides, and thus the mass of these glycans are increased by Dalton per aminosugar

Results: ¹⁵N Incorporation into Glycoprotein Glycans

Initial experiments were performed on mouse embryonic stem cells to demonstrate that ¹⁵N from the side chain of glutamine was rapidly and completely incorporated into GlcNAc, GalNAc, and sialic acids. This incorporation is shown by comparing the FT-MS spectra (below) of two of N-linked glycans released from mES cells grown with ¹⁴N-Gln (top row) and 98% isotopic labeled 1-¹⁵N-Gln for 48 hours (bottom row).



Extent of ¹⁵N Incorporation into Glycoprotein Glycans

These FTMS experiments revealed 99% incorporation of ¹⁵N into N-linked glycans under these conditions, as shown by the table below. Similar results were obtained for O-linked glycans. Although we did not perform FTMS analysis on other types of glycans, we predict that these glycans will also be similarly labeled with this procedure.

Labeling Efficiencies for a Variety of N-linked Glycans

# of Nitrogens	Composition	% ¹⁵ N Incorporation
2	(Hex) ₄ - (Man) ₃ (GlcNAc) ₂	98.1
	(Hex) ₂ - (Man) ₂ (GlcNAc) ₂	98.9
	(Hex) ₃ - (Man) ₂ (GlcNAc) ₂	98.7
4	(Hex) ₂ (HexNAc) ₂ (Deoxyhexose) ₁ - (Man) ₃ (GlcNAc) ₂	98.5
	(Hex) ₂ (HexNAc) ₂ (Deoxyhexose) ₂ - (Man) ₃ (GlcNAc) ₂	98.0
	(Hex) ₃ (HexNAc) ₂ (Deoxyhexose) ₁ - (Man) ₃ (GlcNAc) ₂	98.8
5	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₁ - (Man) ₃ (GlcNAc) ₂	100.3
	(Hex) ₂ (HexNAc) ₂ (Deoxyhexose) ₂ - (Man) ₃ (GlcNAc) ₂	100.6
	(Hex) ₁ (HexNAc) ₂ (Deoxyhexose) ₂ - (Man) ₃ (GlcNAc) ₂	100.3
Average		99.1

Conclusions:

IDAWG is introduced as an *in vivo* isotopic labeling strategy for quantitative - comparative glycomics/glycoproteomics.

IDAWG is expected to be applicable to a variety of glycans

Currently, the use of IDAWG in other cell lines, including those that are post mitotic, is being explored to optimize labeling times.

Half-life studies are being performed on glycan structures by switching a cell population from heavy to light labeling conditions and harvesting and analyzing the glycans by LC-MS² approaches at multiple time points afterwards.

In conclusion, the IDAWG approach is an easily applied and powerful new tool in the glycomics toolbox.

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