Exploring Glycan Binding Interactions on Murine Myoblasts and Myotubes via Fluorescence Microscopy and an Enzyme Linked Lectin Assay (ELLA)

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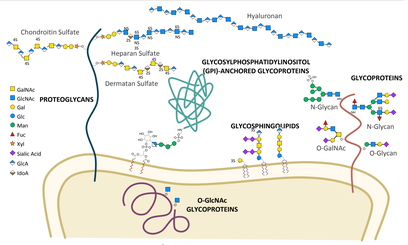
Abstract

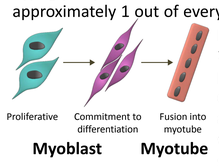
Glycans are an essential building block in all organisms. They participate in crucial cellular processes, especially those on the cell surface.[1] Therefore, it is important to interrogate their binding in a biological setting so that they may be accessed as potential treatment targets. Looking specifically at the human body, one area in which glycans have been implicated are muscular diseases including muscular dystrophies.[2,3] We looked at the presence of a subset of glycans on a mouse muscle cell line using glycan binding proteins, otherwise known as lectins. We then optimized the lectin binding concentration for muscle precursor cells (myoblasts) and compared their binding affinities to differentiated myotubes. We also visualized the binding via fluorescence microscopy.

Background

Carbohydrates, also known as glycans, are among the four major classes of biomolecules found in living cells. Although they are commonly recognized for their role in energy production, energy storage, the building of macromolecules, sparing protein, and assisting in lipid metabolism, they are also involved in several cell surface interactions.[1] They cover these cell surfaces and participate in cellular communication, serve as individual and cooperative binding partners, and are implicated in countless other cellular processes.[4] Glycans are structurally diverse due to their complex chemical structure and variety of possible linkages to one another, complicating scientists' ability to investigate them. As glycan binding proteins, lectins have proven to be a very useful tool for glycan detection and interrogation.[5] In this project we asses the ability of four lectins to bind the surface of mouse muscle cells *in vitro*.

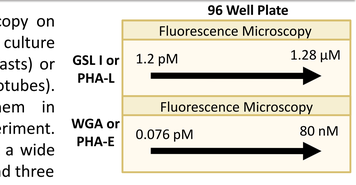


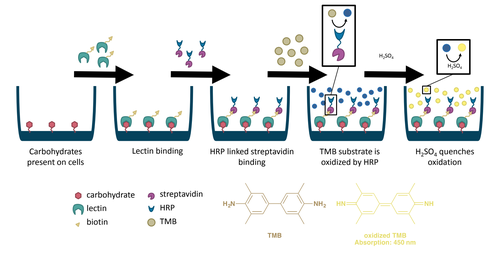
A growing body of work implicating glycans in disease has illuminated the capability to harness their potential as treatment targets. Muscular dystrophies (dystroglycanopathies) are characterized by hypoglycosylation of the a-dystroglycan protein.[3] Duchenne and Becker muscular dystrophy affects approximately 1 out of every 7250 males between the ages of 5 to 24 years old.[2] Cell surface glycans play a key role in the pathologies of these diseases and are potential targets for treatment. For this project we specifically looked at glycans present on the surface of C2C12 mouse muscle cells. Under the right conditions, this muscle progenitor (myoblast) cell line can fuse together and differentiate into myotubes, *in vivo* this is referred to as myogenesis with final maturation into myofibers. The glycan profile on the cell surface varies during these different stages of cell development.[4] While previous work in the lab has revealed that several lectins bind the C2C12 cell surface, we focused on a select few and found the optimal concentration range to reach binding saturation. To do so, we used an enzyme linked lectin assay (ELLA) which provides a quantitative absorbance read out proportional to lectin binding on the surface. In addition, we used fluorescence microscopy to produce a visual representation of the lectin binding under the same experimental conditions.

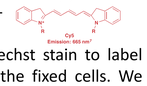


Methods

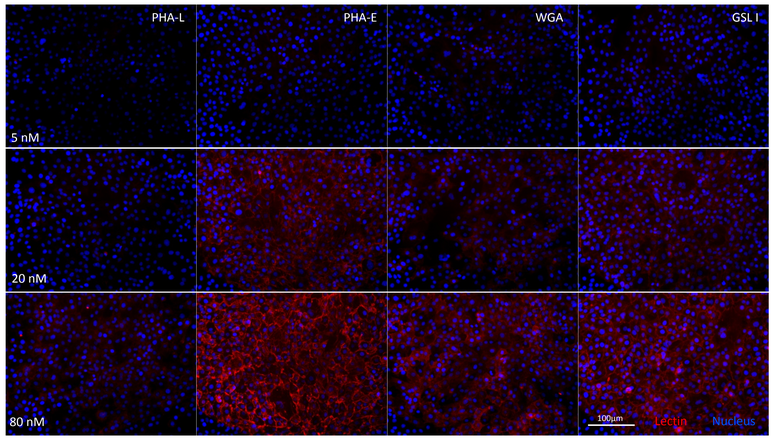
We performed the ELLA and fluorescence microscopy on fixed C2C12 cells. We plated live cells in a 96 well culture plate. They grew for 2 days and were fixed (myoblasts) or were switched to differentiation conditions (myotubes). After 6 days of differentiation we fixed them in paraformaldehyde and performed our binding experiment. First we added four different biotinylated lectins at a wide concentration range. After one hour of incubation and three washes we added streptavidin linked horseradish peroxidase (HRP). After another hour long incubation and three washes, we introduced tetramethylbenzidine (TMB) which, when oxidized by HRP, turns from colourless to blue. We added sulfuric acid to denature the peroxidase and cease oxidation. The absorbance of the resulting yellow solutions was measured and directly correlated to the amount of bound lectin on the cell surface. We reserved one well of each experimental condition for microscopy to which we added Cy5-streptavidin, as well as hoechst stain to label the nuclei of the fixed cells. We examined the plates under a fluorescent microscope to obtain the fluorescence images.

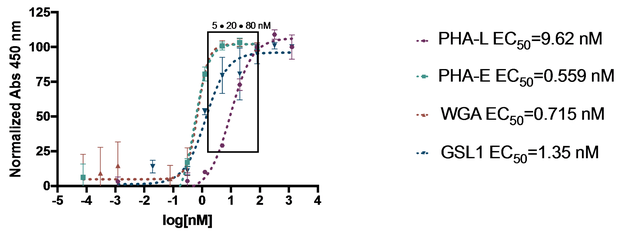




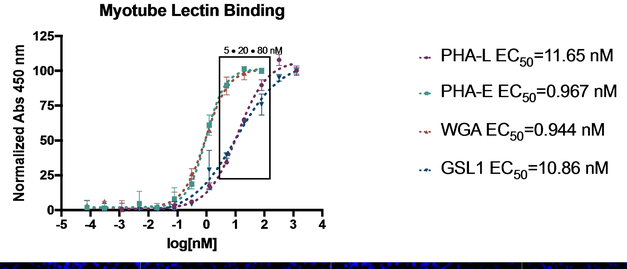


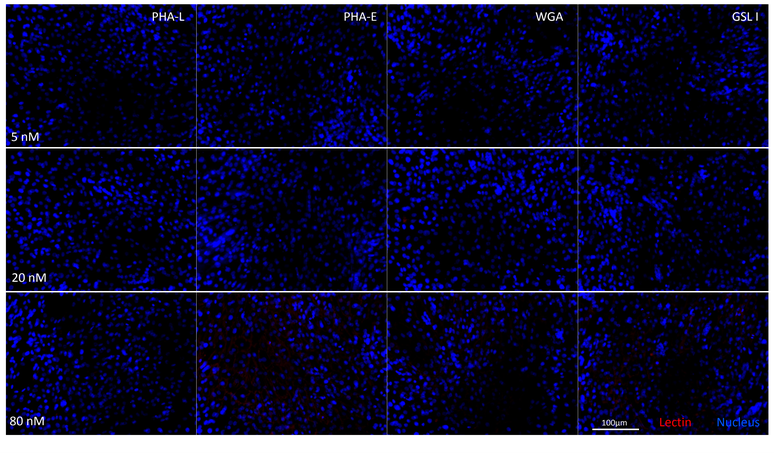
Lectin Binding – C2C12 Myoblasts





Lectin Binding – C2C12 Myotubes

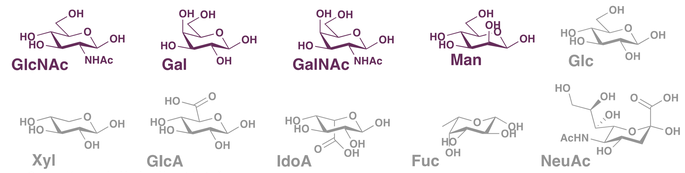




Lectins & Sugars

Lectin	Common Abbreviation	Source	Mol. Wt. (kDa)	Number of Subunits	Glycoprotein	Preferred Sugar Specificity
Phaseolus vulgaris Erythroagglutinin	PHA-E	Phaseolus vulgaris (Red Kidney Bean) seeds	126	4	yes	Galβ4GlcNAcβ2Manα6 (GlcNAcβ4) (GlcNAcβ4Manα3) Manβ4
Phaseolus vulgaris Leucoagglutinin	PHA-L	Phaseolus vulgaris (Red Kidney Bean) seeds	126	4	yes	Galβ4GlcNAcβ6(GlcNAc β2Manα3)Manα3
Wheat Germ Agglutinin	WGA	Triticum vulgaris	36	2	no	GlcNAc
Griffonia simplicifolia I	GSL I	Griffonia simplicifolia seeds	114	4	yes	αGal, αGalNAc

Four lectins used for this study[8]



Common Monosaccharides found in vertebrates[9]

Discussion and Conclusions

After conducting several ELLAs, we found the optimal binding range for these lectins on myoblasts. We discovered that this range is not much different than that for myotubes. According to the microscopy images as well as the EC_{50} values determined for each binding process, it appears that across the board, the lectins bound more to the myoblasts. From the data gathered we were able to determine some of the sugars present on the C2C12 cell surface due to the lectins' preferred sugar specificity: N-Acetyl-D-glucosamine (GlcNAc), D-Galactose (Gal), N-Acetyl-D-galactosamine (GalNAc), and complex N(nitrgoen)-linked glycans containing GlcNac and Gal, as well as D-Mannose (Man). GSL I in particular saw a 10 fold increase in EC_{50} on myotubes compared to myoblasts. This indicates that there may be a decrease or masking of terminal galactose and/or N-acetylgalactosamine upon differentiation. We only performed one repeat of each experiment and more repetitions would be needed to confirm the aforementioned data before moving onto the next steps. Since sialic acid is often added to the end of glycan chains,[10] the logical next step would be to use SNA, a sialic acid binding lectin,[8] to interrogate a change in terminal sialic acid during both cellular stages. Furthermore, a wider panel of lectins should be screened to investigate the presence of other common terminal monosaccharides.

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