Glycosylated polypeptide nanofibers as polyvalent lectin inhibitors with enzymatically-tunable binding specificity

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Statement of Purpose: The role of lectin-glycan interactions in pathological immune responses (e.g. chronic inflammation and tumor immune privilege), and viral infection, has led to a growing interest in molecules that inhibit these interactions. However, creating effective lectin-glycan inhibitors is challenged by the relatively low affinity of monovalent glycans for target lectins, difficulties associated with polyvalent glycan synthesis and purification, and the need to precisely modify glycan chemistry to vary lectin-binding specificity. Peptides that self-assemble into supramolecular structures, such as β-sheet nanofibers, provide biomaterials with a high density of pendant ligands,[1] suggesting their potential for creating polyvalent glycans as lectin inhibitors. Here, we proposed that a variant of the β-sheet fibrillizing peptide, Q11 (QQKFQFQFEQQ) terminated with a monosaccharide, n-acetylglucosamine (GlcNAc) will assembly into nanofibers that bind to target lectins and inhibit their activity. In addition, we hypothesized that lectin-binding specificity can be tuned via enzymatic conversion of nanofiber-bound GlcNac to a different glycan, such as the disaccharide n-acetyllactosamine (LacNAc). This general approach to prepare polyvalent glycans with easily modifiable composition is likely to be broadly applicable for creating therapeutic inhibitors of specific lectin-glycan interactions that underlie a wide variety of diseases.

Methods: Q11 and Asn(GlcNAc)-Ser-Gly-Ser-Gly-Q11 (GlcNAc-Q11) were synthesized using a standard solid-phase peptide synthesis protocol for Fmoc-protected amino acids, by conjugating Fmoc-Asn(Ac_3AcNH-β-GlcNAc)-OH to resin-bound NH_2-SGSG-Q11. GlcNAc-Q11 was cleaved from resin using a standard trifluoroacetic acid-based protocol, and GlcNAc was deprotected via methanolysis. To form nanofibers, dry lyophilized peptide was dissolved in DI H2O, and then diluted 10-fold in 1x phosphate-buffered saline (pH 7.4) or 20 mM HEPES + 10 mM $MnCl_2$ (pH 7.4). Nanofibers were visualized with transmission electron microscopy (TEM) by adsorption onto lacey carbon grids and uranyl acetate negative staining. Nanofiber secondary structure was analyzed via circular dichroism (CD). Lectin binding to nanofibers was assessed via tryptophan fluorescence quenching (ex 280 nm/em 325 nm) using a plate reader. Jurkat T cell apoptosis was assayed by adding 10 µg/mL wheat germ agglutinin to 20000 cells in RPMI 1640 + 10% FBS with or without GlcNAc nanofibers, culturing cells for 4 h, and then assaying for cell metabolic activity with the CellTiter assay, according to manufacturer's instructions. GlcNAc was converted to LacNAc by incubation for 18 h at 37°C in the presence of β-1,4-galactosyltransferase and uridine diphosphate galactose (UDP-Gal). GlcNAc to LacNAc conversion was analyzed with MALDI-TOF mass spectrometry.

Results: GlcNAc-Q11 assembled into nanofibers rich in β-sheets, as determined by TEM and CD (data not shown). GlcNAc nanofibers bound the lectin, wheat germ agglutinin (WGA), in a GlcNAc dose-dependent manner (Fig 1A). These nanofibers also inhibited WGA-mediated apoptosis of Jurkat T cells (Fig. 1B), an in vitro model for lectin-mediated T cell apoptosis during pathological immune responses.

Nanofiber-bound GlcNAc was efficiently converted to LacNAc by β-1,4-galactosyltransferase and UDP-Gal (Fig. 1C), and this reaction did not significantly alter nanofiber morphology as determined by TEM and CD (data not shown). LacNAc nanofibers bound the lectin, galectin-1, with higher affinity than GlcNAc nanofibers (data not shown) or soluble β-lactose (Fig. 1D), a known galectin-1 ligand. Together, these results demonstrated the feasibility of using a chemoenzymatic approach to alter lectin-binding specificity of glycosylated nanofibers.

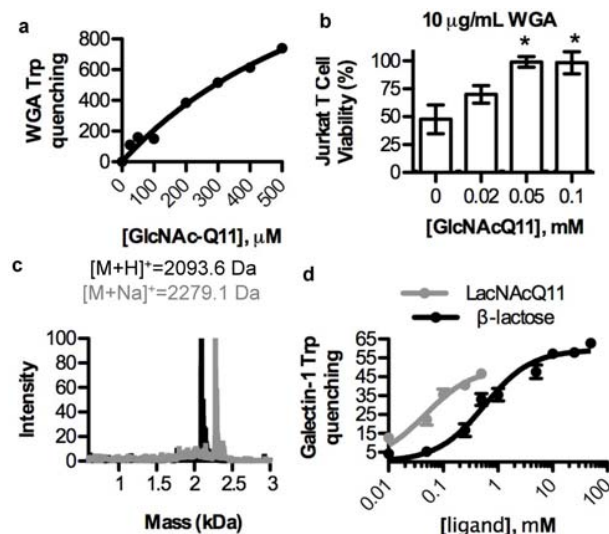

Figure 1: GlcNAc-Q11 nanofibers (a) bound WGA in a GlcNAc dose-dependent manner, and (b) inhibited Jurkat T cell apoptosis by WGA. Nanofiber-bound GlcNAc was (c) enzymatically converted to LacNAc, and (d) LacNAc nanofibers bound galectin-1 with higher affinity than soluble β-lactose. * $p<0.05$, ANOVA, Tukey's post-hoc.

Conclusions: Glycosylated peptides that self-assemble into a supramolecular structure provide polyvalent glycans. These polyvalent glycans bind to target lectins and inhibit their biological activity. Lectin-binding specificity of glycosylated nanofibers is easily varied via a generalizable chemoenzymatic synthesis approach. Together, these data suggest the therapeutic potential of glycosylated nanofibers as polyvalent lectin inhibitors with tunable binding specificity.

References: 1) Collier JH, Rudra JS, Gasiorowski JZ, Jung JP. Chem Soc Rev. 2010 39(9):3413-24.

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