Self-assembled glycopeptide nanofibers as synthetic glycoprotein mimetics to modulate lectin bioactivity

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Statement of Purpose: Carbohydrate-binding proteins, referred to as “lectins”, are extracellular signaling molecules involved in various immune processes, including inflammation, adaptive immunity, and immune privilege. Lectin bioactivity is dependent on specific non-covalent interactions with cell-surface and extracellular matrix (ECM) glycoproteins, which can enhance or inhibit signaling events that induce immune cell proliferation, differentiation, or apoptosis. Biomaterials capable of enhancing or inhibiting lectin bioactivity by mimicking natural lectin-glycoprotein interactions would likely find broad use as immunotherapeutics for various applications. However, creating biomaterials that can recapitulate lectin-glycoprotein interactions has been hindered by difficulties inherent to carbohydrate synthesis and recombinant glycoprotein expression. Here, we demonstrate that self-assembled glycopeptide nanofibers can bind to target lectins and modulate their bioactivity, analogous to natural glycoproteins.

Methods: Self-assembled glycopeptide nanofibers were formed from the beta-sheet fibrillizing peptide Q11 (Ac-QQQQFOFQQFOFQQ) and its biosynthesized analog containing an N-terminally coupled asparagine residue with an N-linked n-acetylglucosamine (GlcNAc) monosaccharide, referred to as GlcNAc-Q11. Carbohydrate content of nanofibers was varied during their assembly by mixing different ratios of both peptides, Q11 and GlcNAc-Q11, under aqueous buffered conditions. Peptide secondary structure and nanofiber morphology were assessed using circular dichroism and transmission electron microscopy, respectively. GlcNAc monosaccharides integrated into the nanofibers were enzymatically converted to the disaccharide n-acetyllactosamine (LacNAc) using the enzyme beta-1,4-galactosyltransferase (B-1,4-GalT), and the sugar donor, UDP-galactose (UDP-Gal). Enzymatic conversion was evaluated using mass spectrometry. Lectin binding to nanofibers was studied using co-precipitation assays (Co-P). Glycopeptide nanofibers with different GlcNAc or LacNAc contents were incubated with wheat germ agglutinin (WGA), a GlcNAc-binding lectin from Triticum vulgaris, for 1 h. Solutions of nanofibers bound to WGA, and that the concentration of WGA bound to nanofibers correlated with their GlcNAc content (Figure 1-a). In contrast, WGA failed to bind to LacNAc-Q11 nanofibers, (Figure 1-b), consistent with previous reports demonstrating weak or no binding between WGA and LacNAc. WGA induced apoptosis of Jurkat T cells, which was completely inhibited by nanofibers with [GlcNAc] ≥ 0.5 mM.

Conclusions: Self-assembled glycopeptide nanofibers provide a simple route to create biomaterials that can modulate the activity of immunomodulatory lectins, analogous to natural ECM glycoproteins. These glycoconjugates can provide biomaterials that present sugar ligands at precisely tunable concentrations, and which can selectively bind to target lectins according to their fine-specificity for particular glycans. The reproducible self-assembly of glycopeptides into nanofibers provides a simple route to create synthetic glycoprotein mimetics that directly address challenges associated with recombinant glycoprotein expression. Increasing the sugar content of nanofibers enhances their multivalency, and in turn affinity for target lectins, effectively recapitulating the ‘glyco-cluster effect’ provided by high carbohydrate density of natural glycoproteins. Sugar moieties are displayed along the nanofiber in such a way that they are accessible to lectin binding, as well as enzymatic treatment that can be used to precisely alter the type of sugar displayed by the nanofiber. The latter is particularly important as it will allow for a wide variety of immune-related lectin-glycoprotein interactions to be targeted by these materials. We envision that glycopeptide nanofibers that can precisely modulate natural lectin-glycoprotein interactions will provide the basis for biomaterials finding use in a broad range of immunotherapy applications.

References:

1(Zeng X. Carbohydr Res, 1998; 312.4:209-217)