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Dissecting Molecular Aspects of Cell Interactions Using Glycodendrimersomes with Programmable Glycan Presentation and Engineered Human Lectins**

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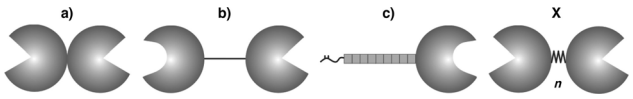
Abstract: *Glycodendrimersomes with programmable surface display of glycan, together with artificially engineered galectins, were used to understand the physiological significance of human lectins with homodimeric and tandem-repeat-type displays. The mode of topological surface presentation and the density of glycan affected vesicle aggregation mediated by multivalent carbohydrate–protein interactions. The cross-linking capacity of homodimeric lectins was enhanced by covalent connection of the two carbohydrate-binding sites. These findings highlight the value of glycodendrimersomes as versatile cell membrane mimetics, and assays provide diagnostic tools for protein functionality. This work also provides guidelines for the design of cell separators, bioactive matrices, bioeffectors, and other biomedical applications.*

Chemical synthesis has greatly advanced our understanding of the way sugar-encoded information is read and translated into cellular effects by sugar-binding proteins known as lectins.[1] Whereas our knowledge of the molecular details of glycan specificity and recognition by lectins has grown considerably, understanding their fascinating target selectivity for distinct cell surface glycoconjugates, and the structure–function relationships of lectin design, is still at an early stage. Turning to a class of adhesion/growth-regulatory tissue lectins binding to β-galactosides, that is, galectins,[2] only distinct glycoconjugates can act as functional counter receptors. In the case of the homodimeric galectin-1 (Gal-1) they are laminin in cell adhesion and CD7, the $\alpha_5\beta_1$-integrin or ganglioside GM1 in growth control of activated T cells or carcinoma/neuroblastoma cells.[3] Intriguingly, the molecular design within this class of effectors is strictly constant in vertebrate phylogenesis, with only three types of topological display of the lectin sites occurring (Scheme 1 a–c). Thus, the homodimeric (prototype) form without a covalent linkage between subunits (Scheme 1 a) must have special, so far not precisely defined physiological significance, different from a protein with a tandem-repeat-type display (Scheme 1 b). This fundamental gap in our knowledge calls for investigations in models of cell adhesion to elucidate galectin potency as cross-linkers. Clearly, this biomedically important context poses a challenge for synthetic and supramolecular chemistry.



Scheme 1. Homodimeric (a), tandem-repeat (b), and chimera-type galectin design. By turning the prototype (a) into artificial tandem-repeat-type products (X, with linker lengths of $n=2$ (GG), $n=33$ (8S), $n=42$ (4), and $n=75$ (8L)) natural and engineered proteins can functionally be compared.

To address this challenge, there is need of a programmable cell (surface) model to facilitate systematic studies. In addition to protein properties, the effect of glycan parameters such as topology of presentation and local density on lectin reactivity could then be examined. Indeed, the recently described self-assembly of dendrimersomes with predictable size from amphiphilic Janus dendrimers provides such an attractive platform.[4] Decorating their hydrophilic segment with sugars was shown to result in bioactive binding partners for lectins.[5] Thus, chemistry can now be teamed up with protein engineering. Herein we document the merits of this strategic combination for analyzing *trans*-interactions by

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answering the following three questions: Will the type of topological surface display of glycans affect lectin-mediated vesicle association? Will rational engineering of the human lectin disclose differences between the two types of bivalent design? Will alteration of surface density of cognate glycans reveal thresholds or a gradual increase?

In our first series of experiments, we extended previous bioactivity testing[5b] to the tetrameric plant lectin concanavalin A (Con A), a popular workhorse when analyzing binding of α-mannosides.[1g] Janus dendrimers with different design were the building blocks for the assembly of glycodendrimersomes (Figure 1). As shown in Figure 1 (left, bottom), the presentation of noncognate lactose (Lac) led to no change of optical density, a stringent specificity control. In each test case with mannose (Man)-presenting preparations a plateau value was reached. Surface presentation of the twin-mixed headgroups turned out to be the most favorable for high-level aggregation (Figure 1). This reactivity was dependent on the lectin concentration (Figure SF1a). Thus, the way the sugar headgroup is presented modulates the extent of cross-linking vesicles by Con A. This result highlights the potential of this system and hereby encourages to proceed to a biomedically active effector, that is, assays with human hGal-1.[3] Its two contact sites for cognate sugars are at opposing sides of the ellipsoid,[3c] as shown in Scheme 1a.

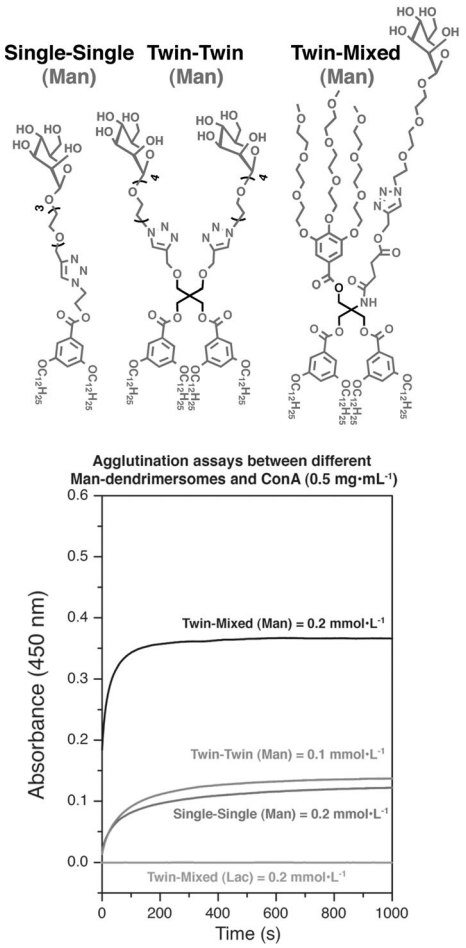

Figure 1. Agglutination with Con A (0.5 mg mL^{-1}) and Man-containing glycodendrimersomes (in mmol L^{-1}) of different topologies in HEPES buffer (10 mmol L^{-1}; with 1.0 mmol L^{-1} $MnCl_2$ and $CaCl_2$).

Using phosphate-buffered saline (PBS), first carbohydrate-independent agglutination of glycodendrimersomes was excluded (Figure 2). hGal-1 was exclusively reactive with the cognate lactose, and binding caused aggregation. Comparison of the curves of agglutination between the three different types of surface display revealed a grading (Figure 2 and SF2). The twin-mixed structure gave highest optical density (OD) values. Of note, the highest level of agglutination was subjected to a decrease over time, as also observed by cryo-TEM (Figure SF3). Because hGal-1 is known to be stable as homodimer even at μg mL^{-1} concentrations,[7] such a decrease cannot be attributed to a spontaneous dissociation into monomers. Note that this occurrence is confined to the region of highest extent of agglutination with twin-mixed type at the surface, indicating involvement of mechanical disruption. Turning hGal-1 into a covalently linked (tandem-repeat-

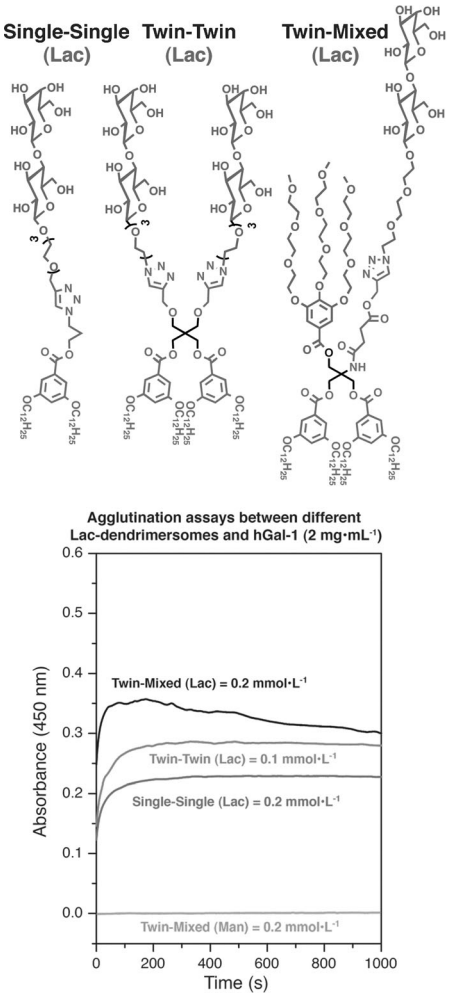

Figure 2. Agglutination with WT hGal-1 (2 mg mL^{-1}) and Man-containing glycodendrimersomes (in mmol L^{-1}) of different topologies in PBS.

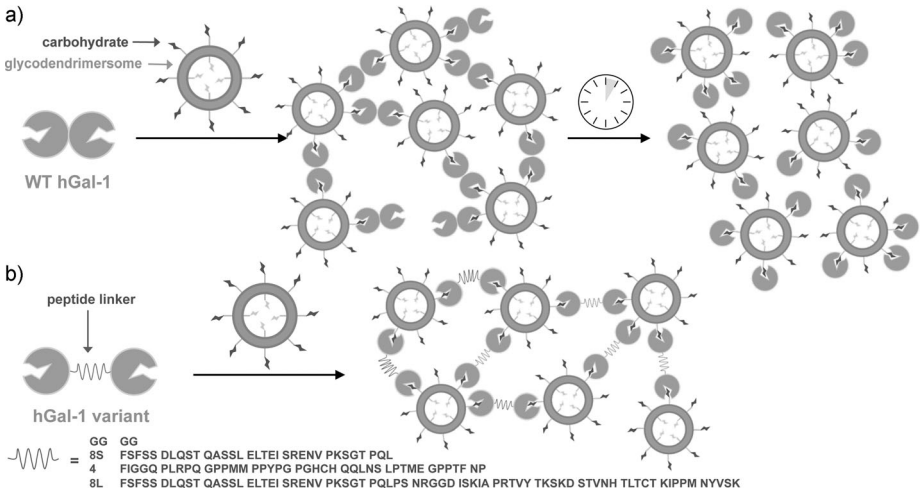

Figure 3. Scheme of (a) disreputable agglutination between noncovalently linked homodimeric WT hGal-1 and twin-mixed Lac-containing (Lac) glycodendrimersomes and (b) firm agglutination between hGal-1 variants with peptide linkers and twin-mixed Lac-containing (Lac) glycodendrimersomes. Amino acid sequences are given along with the codes of the peptide linkers (GG, 8S, 4, and 8L).

type) homodimer (Figure 3b) should create a variant forming stable aggregates. In addition, this approach will allow comparatively examination of the properties of different linkers. In fact, the physiological impact of variations in linker sequence and length, e.g., in galectins-4 and -8, are not known.[2b]

Toward this end we engineered a set of four covalently linked hGal-1 variants by using routine cDNA engineering (see the Supporting Information). This panel consists of a protein with an artificial linker of minimal length (two glycines) and three proteins with insertions of linkers between the two subunits as present in the human tandem-repeat-type galectins-4 and -8, here both forms obtained by alternative splicing (8S, 8L; see Figure 3, bottom, for sequence details). Running agglutination assays under identical conditions showed all variants to form stable aggregates, much more potent than the wild-type (WT) hGal-1 (Figure 4). There was a reproducible tendency for grading of activity depending on the nature of the linker. Obviously, the covalent tandem-repeat display turns hGal-1 into a potent cross-linker of glycodendrimersomes. A similar observation was made with agglutination of rabbit erythrocytes (data not shown), supporting the validity of the model shown in Figure 3a. In turn, this result indicates that the tandem-repeat-type organization is conducive for cell–cell/cell–matrix interactions and transport processes requiring stable associations, effectively described for galectins-4 and -8 which have two different lectin sites.[2c,3c] This cross-linking activity depended on concentration, as exemplarily shown in Figure SF4 for two variants. Testing next variations of surface density of the cognate sugar revealed a nonlinear decrease for Con A (Figure SF5). A steep decrease occurred between 12.5% and 25% Man. Such a titration course was seen for hGal-1 WT, with a step between 10% and 15% Lac and nearly no aggregation at 7.5% Lac (Figure 5). It is in accord with thresholds determined in cell adhesion assays with surface-presented glycoconjugates.[7]

Intriguingly, comparison between the WT and variant proteins disclosed a marked difference at low surface density of cognate sugar (Figure 5, Figure SF6 for GG, and Figure SF7 for 8S). Presence of the linker enabled the lectin to maintain activity at Lac surface loading below 7.5%. To explore whether this difference is confined to our supramolecular model, we tested a series of cell lines by cytofluorometry with fluorescent galectin. As shown in Figure SF8, binding was blocked by presence of the hapten, and osmolarity increases by noncognate sugar (Man) did not impair binding. In all cell systems, surface reactivity for the WT protein was markedly lower than for the variant

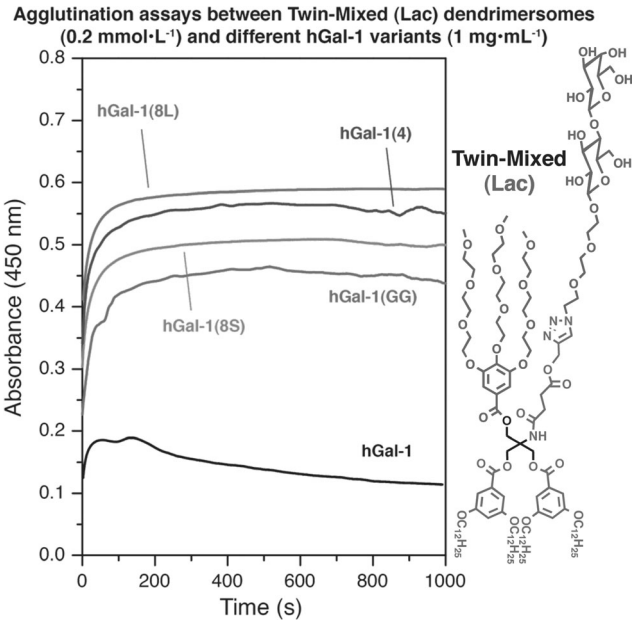

Figure 4. Agglutination with twin-mixed Lac-presenting (Lac) glycodendrimersomes (0.2 mmol L^{-1}) and different hGal-1 variants (1.0 mg mL^{-1}) in PBS.

(Figures 6 and SF9), in full agreement to glycodendrimersome-based assays. Thus, the insertion of a linker into hGal-1 WT increases its cross-linking capacity at low ligand density.

In summary, this study demonstrates the principal value of glycodendrimersomes as tools to study *trans*-interactions of plant and human lectins. The mode of glycan presentation and the surface density influenced carbohydrate-dependent aggregation, underscoring the assumed model character. Regarding the protein, the cross-linking capacity was enhanced and aggregates stabilized by introducing a covalent

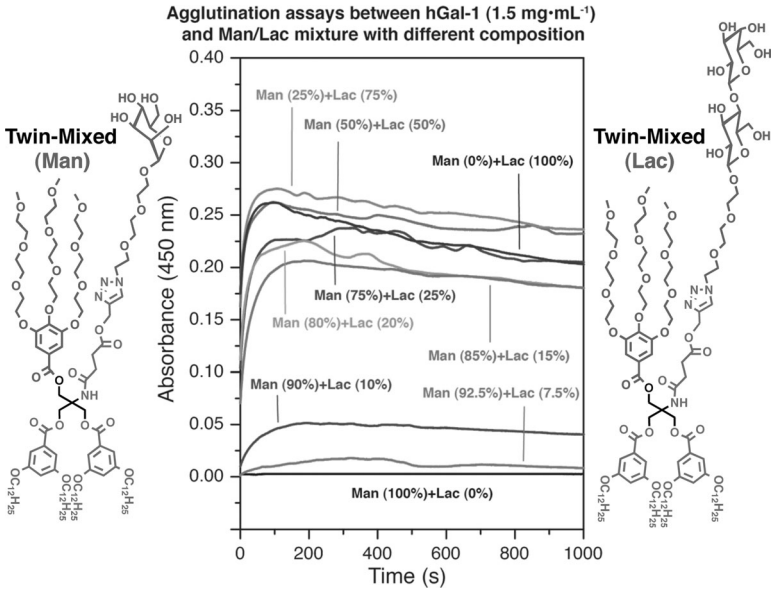

Figure 5. Agglutination with WT (1.5 mg mL^{-1}) and mixtures of twin-mixed Man-containing (Man) and Lac-containing (Lac) glycodendrimersomes of different surface composition (Man + Lac = 0.2 mmol L^{-1}) in PBS.

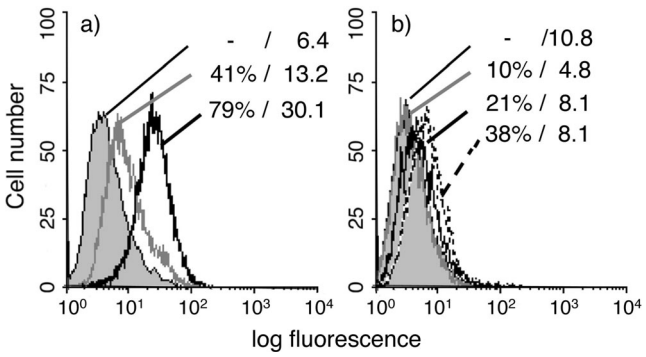

Figure 6. Fluorescent staining of pancreatic (a) and colon (b) adenocarcinoma cells by WT and variant (8S) Gal-1 proteins. Gray area documents background, percentage of positive cells/mean fluorescence intensity are listed. a) Staining by WT (gray) and variant (black) proteins used at 2 μg mL^{-1}; b) staining by WT protein at 1 μg mL^{-1} (top)/10 μg mL^{-1} (middle) and by variant protein at 1 μg mL^{-1} (dashed, bottom). Note that even a 10-fold higher concentration of WT protein resulted in lower percentage of positive cells than obtained with variant.

connection between the two subunits of hGal-1. On the contrary, this alteration might impair capacity for transient contacts in cell migration, a typical function of hGal-1. The type of linker (4, 8S, 8L) had a minor influence. Equally important, WT hGal-1 requires a surface density of ligand presentation above a threshold, in contrast to the linker-containing variants. Remarkably, corresponding differences were seen with tumor cells. Clustered ligand presentation will thus establish sites for high-affinity binding of the WT protein. Fittingly, an approximately 10-fold decrease in affinity was observed by harming microdomain integrity in neuroblastoma cells.[8]

These present insights highlight the value of this model as a diagnostic tool for unraveling protein functionality and of the glycodendrimersomes as versatile programmable models. Systematically applying bioinspired modifications of glycans and other rationally designed variants is thus a promising approach to extend our understanding of structure–activity correlations in *trans*-interactions.[9] As a consequence, these results will provide guidelines for optimizing design of new lectin variants for specific practical aims, for example, to separate cells, to establish a bioactive matrix for tissue engineering or to serve as highly potent and selective bioeffector, here building on known activity of Gal-1 as inducer of anoikis/apoptosis in autoimmune disorders or tumors.[2c,3c]

Keywords: adhesion · agglutination · dendrimers · lectin · vesicles

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