Mannose/Glucose-Functionalized Dendrimers To Investigate the Predictable Tunability of Multivalent Interactions

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Multivalent binding between proteins and carbohydrates mediates many biological events.[1] Carbohydrate−protein interactions often occur with low monomeric binding affinities ($K_d = \sim 10^{-3}$ M);[2] a multivalent presentation of ligands is generally required to achieve physiologically relevant associations.[3] A variety of scaffolds have been used as frameworks on which to support the carbohydrate residues.[4]

Synthetic multivalent molecules are generally empirically optimized for a particular application. However, two noteworthy studies to quantify the effects one would expect from multivalent presentation have been reported. Lees and co-workers describe a binding enhancement value for divalent, pentameric, and linear polymer systems,[5] while Reinhoudt and co-workers relate the monovalent association constant to the multivalent association constant using an effective concentration value and a scaling factor.[6] Both of these methods progress the discussion of how monovalent association constants effect multivalent interactions, but both suffer from the difficulty of determining the appropriate value for the effective concentration.

Whitesides et al. suggested that polyvalent interactions should equal monovalent interactions raised to the Nth power (eq 1), where N is the number of receptor−ligand interactions. A cooperativity factor, α, is also in eq 1.[3]

$$K_N^{\text{poly}} = (K^{\text{mono}})^{\alpha N} \qquad (1)$$

The beauty of Whitesides' model as a predictive tool lies in its simplicity. If monovalent association constants can be measured, then one should be able to predict multivalent values in a straightforward fashion, regardless of the nature of the framework or the type of multivalent interaction involved. The goal of the research reported here was to determine whether monovalent differences in affinity affect multivalent association constants in predictable ways (as described by eq 1).

To investigate Whitesides' prediction regarding the relationship between monovalent and multivalent associations, we functionalized PAMAM dendrimers[7] with mannose and glucose. We evaluated the relative affinities of the dendrimers with Concanavalin A (Con A), a plant lectin that binds methyl D-mannopyrannoside 4 times better than it binds methyl D-glucopyrannoside.[2] Con A exists as a tetramer at neutral pH, with four carbohydrate binding sites located 6.5 nm apart.[8] Previously, we showed that mannose/hydroxyl-functionalized G(4)- to G(6)-PAMAM dendrimers with 50% mannose incorporation showed the highest activity in hemagglutination assays with Con A.[9] The G(4), G(5), and G(6) dendrimers are large enough to bind divalently to Con A, so these generations were chosen to study the tunability of affinity for this report. Because of the roughly spherical shape of dendrimers, the dendrimer/Con A associations that we observe are almost certainly divalent. Trivalent and tetravalent associations are not possible due

Scheme 1 [a]
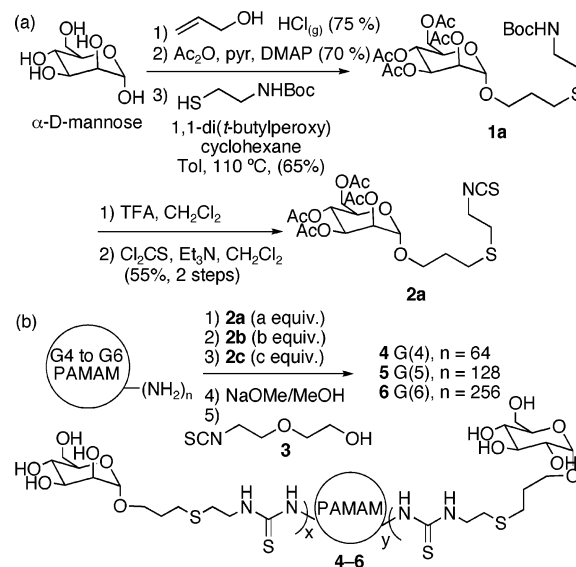
[a] (a) Synthesis of isothiocyanato carbohydrates. Mannose is shown; glucose (1b and 2b) and galactose (1c and 2c) syntheses are analogous. (b) Synthesis of mannose/glucose-functionalized dendrimers (galactose addition does not occur; see text for details). Amounts of x and y are provided in Table 1, and amounts of a, b, and c are provided in Table S1 of the Supporting Information.

to the shape and size of PAMAMs. We describe here the synthesis of mannose/glucose dendrimers and the results of hemagglutination assays with these dendrimers and Con A.

To synthesize the carbohydrate tethers, allylation of the anomeric hydroxyl of α sugar starting material,[10] peracetylation of the 2,3,4- and 6-hydroxyls[11] and thiol radical addition of Boc-protected aminoethanethiol[12] afforded intermediate 1 (Scheme 1a). Removal of the Boc group and addition of thiophosgene afforded the requisite mannose, glucose, and galactose isothiocyanates 2a−c.

Isothiocyanato carbohydrates 2a−c were sequentially added to PAMAM dendrimers. MALDI-TOF analyses were performed 24 h after each addition to determine the degree of functionalization. The acetyl protecting groups were removed under Zemplen conditions.

We functionalized 50% of the dendrimers' amino endgroups with mannose and glucose since our previous work indicated that 50% mannose functionalization caused the highest activity in the hemagglutination assay.[9] After mixtures of mannose and glucose were added to the dendrimers in varying ratios to total 50% (Table 1), galactose (which does not bind to Con A) was added. Although galactose additions of about 50% were intended, galactose addition was negligible. Warming the galactose additions to 40 °C with both

Table 1. Hemagglutination Assay Results

	No. mannose residues[a]	No. glucose residues[a]	relative activity per active sugar[b]
4a	30	0	3820 ± 1650
4b	24	7	2660 ± 0
4c	18	13	2260 ± 780
4d	10	26	1090 ± 380
4e	0	29	260 ± 110
5a	44	0	4830 ± 2090
5b	38	13	3040 ± 0
5c	16	40	1270 ± 440
5d	0	45	310 ± 130
6a	53	0	5350 ± 0
6b	34	35	3510 ± 1220
6c	16	50	2150 ± 0
6d	0	77	470 ± 0

[a] No. of sugar residues was determined using MALDI-TOF MS data after deacetylation (M_W = 168 g/mol for 4 Ac) and after addition of tethered sugar (M_W = 507 g/mol per tethered sugar). See the Supporting Information for details. [b] Active sugar = man + glc. Standard deviation values are large because of serial 2-fold dilutions. For standard deviation = 0, all inhibitory concentrations were equal. All values represent at least three trials. Relative activity of methyl mannose = 1.
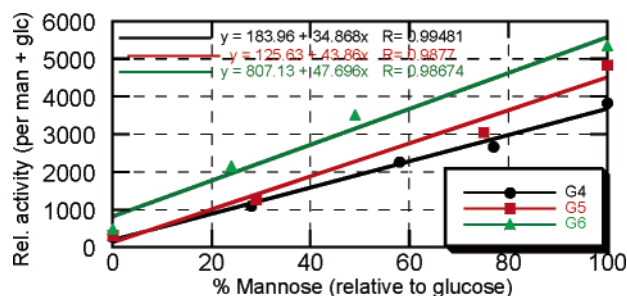

Figure 1. Percent mannose of the glucose/mannose mixture versus relative activity (per glucose + mannose).

acetylated and deacetylated mannose/glucose dendrimers and adding isothiocyanatoethoxyethanol **3**[9] failed to cause significant galactose loadings.

Hemagglutination assays were performed to evaluate the relative activities of **4**–**6** with Con A.[12] Control assays with PAMAM and galactose-functionalized PAMAMs showed no nonspecific dendrimer–lectin association. The relative activity numbers in Table 1 are on a per carbohydrate (glc + man) basis and are relative to methyl mannose. The relative amounts of glucose and mannose induce a linear change in the relative activity for all three generations (Figure 1).

The difference in relative activity between glucose-functionalized and mannose-functionalized dendrimers in the G(4) series is 14.7, and the difference for G(5) dendrimers is 15.6 and the difference for G(6) dendrimers is 11.4. Using eq 1 and assuming a cooperativity constant α of 1, one would predict that exchanging mannose for glucose would cause a 4^2- or 16-fold reduction in binding to Con A since the dendrimer–Con A association is a divalent interaction. (Rationales for $\alpha = 1$ and $N = 2$ are provided in the Supporting Information.) The G(4) and G(5) differences (14.7 and 15.6) are very near 16, while the G(6) value (11.4) is slightly lower. Perhaps the larger size of G(6) allows for a compensatory effect due to increased sugar clustering around the binding sites.[13] Alternatively, the curvature of the G(6) dendrimers may be different enough from G(4) and G(5) to change the shape complementarity

between Con A and the dendrimer, which can significantly change the association motif.[14] The linearity of the fit shown in Figure 1 suggests that man/glc-functionalized dendrimers bearing varying ratios of mannose to glucose also exhibit the affinity changes predicted by eq 1.

The results reported here with two ligands (mannose and glucose) that vary by a factor of 4 in the strength of their monovalent associations to Con A but vary by almost a factor of 16 in their divalent dendrimer/Con A associations indicate that multivalency can be influenced in predictable, and therefore, tunable ways. Monovalent differences are amplified by multivalent associations, and mixtures of low and high affinity ligands can be used to attenuate multivalent affinities.

In summary, hemagglutination assays with Con A and mannose/glucose-functionalized dendrimers **4**–**6** indicate that multivalent affinities can be predicted based on monovalent association constants. The glucose and mannose monomers differ in binding strength only by a factor of 4; multivalent association amplifies this difference. Transposition of the observed relationship between monovalent and multivalent association constants into more complex systems (for example, polyvalent rather than divalent complexes and nondendritic frameworks) should reasonably follow and is currently being explored. Further evaluation of mannose/glucose dendrimer–Con A complexes using the hemagglutination and precipitation assays is also underway. That multivalent affinity can be attenuated by mixing ligands of varying binding strengths provides a new element of control and predictability to the design of synthetic multivalent molecules for biological applications.

Supporting Information Available: Experimental procedures and characterization data for **1**–**6**, hemagglutination assay procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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