Multiple Modes of Binding Enhance the Affinity of DC-SIGN for High Mannose N-Linked Glycans Found on Viral Glycoproteins

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The dendritic cell surface receptor DC-SIGN and the closely related endothelial cell receptor DC-SIGNR specifically recognize high mannose N-linked carbohydrates on viral pathogens. Previous studies have shown that these receptors bind the outer trimannose branch Manα1–3[Manα1–6]Manα present in high mannose structures. Although the trimannoside binds to DC-SIGN or DC-SIGNR more strongly than mannose, additional affinity enhancements are observed in the presence of one or more Manα1–2Manα moieties on the nonreducing termini of oligomannose structures. The molecular basis of this enhancement has been investigated by determining crystal structures of DC-SIGN bound to a synthetic six-mannose fragment of a high mannose N-linked oligosaccharide, Manα1–2Manα1–3[Manα1–2Manα1–6]Manα1–6Man and to the disaccharide Manα1–2Manα. The structures reveal mixtures of two binding modes in each case. Each mode features typical C-type lectin binding at the principal Ca2+-binding site by one mannose residue. In addition, other sugar residues form contacts unique to each binding mode. These results suggest that the affinity enhancement displayed toward oligosaccharides decorated with the Manα1–2Manα structure is due in part to multiple binding modes at the primary Ca2+-site, which provide both additional contacts and a statistical (entropic) enhancement of binding.

The dendritic cell receptor DC-SIGN functions in the initial recognition of pathogens and also in adhesive interactions with T cells that scan the surface of dendritic cells for complementarity peptide antigen-major histocompatibility complexes (1, 2). Although the epitope for T cell interactions has not been defined, interactions with pathogens exploit the ability of DC-SIGN to recognize both branched fucosylated structures bearing terminal galactose residues and high mannose N-linked oligosaccharides (3–5). The latter specificity allows DC-SIGN to act as a receptor for several enveloped viruses that bear high mannose structures on their surface glycoproteins, most notably human immunodeficiency virus (HIV)2 (6–8). A related receptor found on endothelia in the liver, lymph nodes, and placenta, designated DC-SIGNR or L-SIGN, does not recognize fucosylated carbohydrates but shares with DC-SIGN the ability to bind tightly to high mannose N-linked carbohydrates and to serve as a viral receptor (5, 6, 9–11).

DC-SIGN and DC-SIGNR are members of the C-type lectin family of Ca2+-dependent carbohydrate-binding proteins. The two receptors have similar primary structures, each of which comprises a short N-terminal cytoplasmic tail, a transmembrane anchor, a tetramerization domain, and a C-terminal carbohydrate-recognition domain (CRD) (6). Crystal structures of the DC-SIGN and DC-SIGNR CRDs reveal the typical long form C-type lectin fold (4). There are three Ca2+ seen in these structures, of which one, designated the principal Ca2+, is common to all C-type lectins. The hallmark of sugar binding to C-type lectins is the direct coordination of the principal Ca2+ by vicinal hydroxyl groups of a pyranose ring, which also form hydrogen bonds with the amino acid side chains that serve as the other Ca2+ ligands (12). In the case of mannose-like ligands, vicinal, equatorial 3- and 4-OH groups form these coordination and hydrogen bonds. Specificity for particular oligosaccharides comes from additional contacts made to flanking regions of the C-type CRD.

Competition assays in which a test ligand is used to compete radiolabeled mannose-bovine serum albumin from immobilized CRD have been used to examine the relative affinities of mannose-containing structures for DC-SIGN and DC-SIGNR (4, 6). The trimannose core structure Manα1–3[Manα1–6]Man was found to bind 4-fold better than mannose to DC-SIGN and 2-fold better to DC-SIGNR, and the disaccharide Manα1–2Manα shows similar preferences (4). A pentasaccharide corresponding to the inner five mannoses of a high mannose structure, 

2 The abbreviations used are: HIV, human immunodeficiency virus; CRD, carbohydrate-recognition domain; Manα, Manα1–2Manα; Manα1–2Manα1–2Manα1–6Manα1–6Man; Manα1–2Manα1–3[Manα1–2Manα1–6]Manα1–6Man; Manα1–2Manα1–3[Manα1–2Manα1–6]Manα1–6Man; Manα1–2Manα1–3[Manα1–2Manα1–6]Manα1–6Man.
nose oligosaccharide but lacking all terminal α1–2-linked mannos ones binds 7- and 4-fold better than mannose to DC-SIGN and DC-SIGNR. The full N-linked high mannose oligosaccharide Man6b, however, shows much more substantial affinity enhancements (4). These data suggested that the presence of the Man6b moiety at the nonreducing termini of high mannose oligosaccharides might provide substantial affinity enhancements, perhaps by interacting with a secondary binding site for this group. The surface glycoproteins of HIV and other enveloped viruses are relatively rich in Man5, Man6 structures (13), so high affinity binding to such glycans contributes to selective interaction of DC-SIGN and DC-SIGNR with these pathogens. 

Here, the mechanism by which terminal Man1–2Man groups enhance affinity toward DC-SIGN and DC-SIGNR is investigated using synthetic fragments of the full N-linked high mannose structure in binding and structural studies. The data indicate that multiple modes of binding at the DC-SIGN carbohydrate-binding site provide a statistical enhancement of the affinity but do not account for all of the observed affinity differences. The different binding orientations feature contacts between the terminal mannose and different regions of the proteins, which likely provide the remaining component of the increased affinity for larger glycans.

**EXPERIMENTAL PROCEDURES**

**Protein Expression**—The DC-SIGN carbohydrate-recognition domain was expressed in *Escherichia coli* as described (6) and used for cocrystallization with Man1–2Man. A similar construct lacking the C-terminal 12 residue extension was used for cocrystallization with Man5. Both proteins were purified as described (6).

**Synthesis and Purification of Man5 and Man6 Oligosaccharides**—Compounds Man5 and Man6a (Fig. 1) were prepared analogously to those previously described in the literature (14, 15). O-Me protection at the reducing end was chosen to diminish the possible interference of the linker with the binding site of the protein. Compound Man6b was prepared following the same approach as Man5, using methyl 2,3,4-tri-O-benzyl-α-D-mannopyranoside (16, 17) as the core sugar unit. After removal of all protecting groups, the compounds were dialyzed 2 times each for 12 h against 2 liters of Millipore water and then lyophilized. The Man1–2Man disaccharide was purchased from Sigma.

**Binding Assays**—Solid phase competition binding assays were performed using bacterially expressed CRDs of DC-SIGN and DC-SIGNR, with 125I-Man-bovine serum albumin employed as the reporter ligand (6). The assays were performed at least twice in duplicate because only limited quantities were available. Sugar concentrations were determined using the anthrone reaction (18).

**Crystallization and Data Collection**—Crystals of both the Man6b and Man5 complexes were essentially isomorphous to the previously determined DC-SIGN CRD-Man5 complex (5), even though the latter was obtained using slightly different crystallization conditions. The asymmetric unit contains one copy of the protein-ligand complex. Rather than reindexing to allow direct rigid body refinement, the two structures were determined by molecular replacement with the DC-SIGN CRD model from the Man5 complex (Protein Data Bank ID 1SL4). The Man5 complex structure was determined with the program MOLREP (19), which gave a correlation coefficient of 70% and the *R* value of 33% for data to 3 Å. The Man6b complex was solved with the program COMO (20), which gave a correlation coefficient of 56% and the *R* value of 31% for data to 3.5 Å. Refinement and map calculations for both structures were performed with CNS (21). The maximum-likelihood amplitude target was used, with bulk solvent and anisotropic temperature factor corrections applied throughout the refinement. As refinement progressed it became clear that the ligand is bound to the site in two alternative, overlapping orientations in both structures. The two conformations were assigned occupancies of 75 and 25% based on the quality of the electron density and refined temperature factors. For each ligand orientation, Fig. 2 shows the *Fo* − *Fc* electron density calculated from coordinates omitting the indicated orientation but including the other. Water molecules were added to peaks of >3 σ in *Fo* − *Fc* maps that were within hydrogen bond distance to protein, sugar, or other water molecules. The final model of the DC-SIGN CRD-Man5 complex contains residues 253–384 of DC-SIGN, two alternative conformations of the carbohydrate ligand, 3 Ca2+, and 135 water molecules. The final model of the DC-SIGN CRD-Man6b complex contains residues 253–384 of DC-SIGN, two alternative conformations of the ligand, 3 Ca2+, and 59 water molecules. The refinement statistics are presented in Table 1.
RESULTS

Relative Affinities of Oligomannose Structures for DC-SIGN and DC-SIGNR—To examine the contribution of the Manα1–2Man groups present on the termini of high mannose oligosaccharide to DC-SIGN and DC-SIGNR binding, three synthetic oligomannose structures corresponding to fragments of Man₉GlcNAc₂ were tested in the competition assay. Man₉ is the full 9-mannose structure that would be linked to GlcNAc-Asn in high mannose N-linked carbohydrates to DC-SIGN and DC-SIGNR binding, whereas the Man₆ compound binds roughly twice as strongly as the Man₉ glycans. The full Man₉GlcNAc₂ glycan consistently shows 2–3-fold stronger binding than Man₆ (Table 2).

Structure of Man₆b Bound to DC-SIGN—Crystallization trials of complexes between Man₆a, Man₆b, and Man₆b with the DC-SIGN CRD yielded cocrystals with the 50 mM Man₆b. The structure of this complex was determined at 2.4 Å resolution. The protein structure is identical to that previously described for complexes with Man₆aGlcNAc₂ (GlcNAcβ1–2Manα1–3[Manα1–2Manα1–3]GlcNAcβ1–2Manα1–6[Manα1–3][Manα1–3]Man) (4) and Man₆ (Manα1–3[Manα1–6]Manα1–6Man) (5). The ligand is bound in two overlapping orientations, in a mixture estimated at 75%, designated the major orientation (Fig. 3, A and B), and 25%, designated the minor orientation (Fig. 3, C and D). Of the six mannosides in the compound, only three are visible in the major orientation (Manα1–2Manα1–3Man) and two in the minor orientation (Manα1–2Man).

The penultimate α1–3-linked mannoside that forms one arm of the outer branched trimannose unit (i.e. Manα1–2Manα1–3[Manα1–2Manα1–6]Manα1–6Man) binds to the primary Ca²⁺ site and was similarly observed to bind to the Ca²⁺ in both the DC-SIGN/Man₆ (5) and DC-SIGN/Man₆aGlcNAc₂

TABLE 1

| Data collection | Man₆a | Man₆b |
|-----------------|-------|-------|
| Space group     | P4₁   | P4₁   |
| Unit cell parameters a, c (Å) | 55.64, 53.20 | 55.96, 53.26 |
| Resolution range (Å) (last shell) | 100.1–1.95 (2.06–1.95) | 100.2–2.40 (2.46–2.40) |
| R.sym (last shell) | 6.6 (20.8) | 8.5 (19.8) |
| Percent complete (last shell) | 99.9 (100) | 99.6 (99.8) |
| Average multiplicity | 4.7 | 4.6 |
| Mean I/σ (I) | 18.1 (7.2) | 15.1 (7.6) |

| Refinement | Man₆a | Man₆b |
|------------|-------|-------|
| No. reflections working set | 11379 | 6198 |
| No. reflections test set | 572 | 303 |
| No. protein atoms | 1071 | 1071 |
| No. ligand and solvent atoms | 170 | 116 |
| Rwork | 0.241 | 0.252 |
| Rfree* | 0.196 | 0.198 |
| Average B (Å²) | 25.8 | 27.3 |
| Bond length root mean square deviation (Å) | 0.005 | 0.006 |
| Angle root mean square deviation (°) | 1.22 | 1.22 |

Ramachandran plot: (% in most favored/allowed/generous/disallowed regions) 88.8/11.2/0/0 89.6/9.5/0.9/0

* Rwork = Σ||Fᵣ || – |<F(h)||)/Σ||Fᵣ ||, where |<F(h)>| = observed intensity and |<F(h)>| = mean intensity obtained from multiple measurements.

* R and Rfree = Σ||Fᵣ || – |<F(h)||)/Σ||Fᵣ ||, where |<F(h)>| = observed structure factor amplitude and |<F(h)>| = calculated structure factor amplitude for the working and test sets, respectively.

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FIGURE 2. Electron density maps for bound ligands. The indicated bound ligand orientation is shown superimposed on the Fₐ – Fᵣ electron density map (green, 2 σ contour) calculated from a model from which the indicated orientation was omitted but which included the alternative orientation. A, Man₆a major orientation. B, Man₆a minor orientation. C, Manα₂ major orientation. D, Manα₂ minor orientation.
TABLE 2
Competition data for ligand binding to DC-SIGN and DC-SIGNR CRDs

| Ligand     | DC-SIGN |                  | DC-SIGNR |                  |
|------------|---------|------------------|----------|------------------|
|            | $K_i$   | fold increase    | $K_i$    | fold increase    |
|            | $\mu M$ |                  | $\mu M$  |                  |
| Man        | 2300 ± 100 | 1.0 ± 0.0 | 2500 ± 200 | 1.0 ± 0.0 |
| $\text{Man}_{1–2}$Man$^*$ | 183 ± 18 | 12 ± 3 | 277 ± 22 | 10 ± 2 |
| $\text{Man}_{1}$ | 157 ± 17 | 14 ± 1 | 251 ± 28 | 11 ± 2 |
| $\text{Man}_{6}$ | 73 ± 6 | 32 ± 4 | 128 ± 17 | 20 ± 5 |
| $\text{Man}_{9}$GlcNAC$^a$ | 26 | 88 | 54 | 43 |

$^*$ Relative values of $K_i$ vs. mannose for Man$\text{1–2}$Man taken from Ref. 4. Note that the absolute values of $K_i$ in those experiments cannot be compared with the values shown in the rest of the table because the solid phase competition assays were done with a different batch of iodinated Man-bovine serum albumin.

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The major orientation corresponds to the arrangement seen in these earlier crystal structures (Fig. 3E). The $\alpha1–6$ branch of the oligosaccharide is not visible, however, which is surprising given the shape complementarity and specific hydrogen bonds between the $\alpha1–6$-linked mannose and Phe$^{133}$, Ser$^{360}$, and other residues in DC-SIGN that were observed in the earlier structures (4, 5). The reason for this difference is obscure, especially considering the fact that the crystal is essentially isomorphous to the Man$_6$ complex (5). In the Man$_4$ and Man$_3$GlcNAC$_2$ structures, the $\alpha1–6$-linked mannose has higher temperature factors than the $\alpha1–3$-linked mannose, suggesting that it may be more weakly bound. The $\alpha1–2$-linked mannose at the nonreducing terminus directly contacts Val$^{351}$.

In the second, less populated orientation, the same mannose residue is bound to the Ca$^{2+}$, but its orientation is reversed by a 180° rotation about a line bisecting the pyranose ring through the C-3–C-4 bond. This rotation exchanges the positions of the 3- and 4-OH groups so that they still form the Ca$^{2+}$ coordination and hydrogen bonds characteristic of C-type lectin-mannose interactions (Fig. 3, B and D). A similar situation was observed in complexes of mannose-binding proteins with various ligands (22). In this orientation, only two sugars are visible: the mannose at the Ca$^{2+}$ site and the nonreducing terminal $\alpha1–2$-linked mannose, which forms hydrogen bonds with Glu$^{358}$ and Ser$^{360}$ and which also interacts with the face of the Phe$^{133}$ ring (Fig. 3C). Thus, it appears that the Phe$^{133}$ side chain has important roles in the recognition of ligand in either orientation. Because only Man$_6$ is visible, it is not possible to distinguish whether these residues correspond to the $\alpha1–3$ or $\alpha1–6$ arms of Man$_9$ or whether they represent a mixture of both (Fig. 1). An overlay of the major and minor orientations is shown in Fig. 3F.

Structure of Man$_1$–2Man Bound to DC-SIGN—To assess whether DC-SIGN might have additional subsites for the Man$_1$–2Man residues found at the nonreducing termini of high mannose oligosaccharides, the CRD was co-crystallized with 25 mM Man$_1$–2Man. The structure of the disaccharide complex reveals binding only in the principal Ca$^{2+}$ site; no
other carbohydrate molecules were observed even at low electron density map contour levels. Man$_{1-2}$Man binds at the principal Ca$^{2+}$ site in two orientations, again related by a 180° rotation about the C-3—C-4 bond bisector. The major orientation is virtually identical to that of the Man$_{1-2}$Man moiety in the minor Man$_{ab}$ ligand orientation and forms the same contacts with DC-SIGN, including the contact with Phe$_{313}$ (Fig. 4, A and B). In the minor orientation, only a single sugar is visible and forms the typical Ca$^{2+}$ coordination and hydrogen bonds (Fig. 4, C and D). This mannose is oriented identically to the Ca$^{2+}$-bound mannose in the major Man$_{ab}$ orientation. The electron density maps, however, do not make clear whether the sugar bound at the Ca$^{2+}$ site is the reducing or nonreducing end of the disaccharide; it is possible that there is a mixture of the two. In particular, unlike the Man$_{ab}$ structure, the nonreducing α1-2-linked mannose is not visible. The electron density for this sugar is not especially well defined in the Man$_{ab}$ complex, so the lack of density for this residue in the Man$_{ab}$ complex could be due to its low occupancy. It is also possible that the favorable interaction with Val$_{351}$ seen in the Man$_{ab}$ structure does not compensate for the loss of entropy required to form this contact in the disaccharide.

**Models of Man$_{a}$ Binding**—To assess whether the two modes of binding observed in the Man$_{ab}$ and Man$_{a}$ structures are relevant to a full, 9-mannose oligosaccharide, coordinates for Man$_{a}$GlcNAc$_2$ obtained by NMR analysis of the free glycan (23) were superimposed on the two orientations of Man$_{ab}$. As noted previously (4), superposition of the outer branched trimannose moiety reveals no significant steric clashes between the rest of the oligosaccharide and the protein (Fig. 5, A and B), with only the side chain rotamers of Leu$_{171}$ needing adjustment to avoid clashes. Superposition of the second orientation, which places the terminal α1-2-linked mannose of the outer α1-3 arm near Phe$_{313}$, also reveals no clashes with the protein, with the possible exception of interference between Arg$_{245}$ and the innermost GlcNAc residue (Fig. 5C). Further modeling, in which the terminal Man$_{1-2}$Man groups of the other arms were superimposed, shows no steric clashes (Fig. 5, D and E). Similar results were obtained with crystallographic coordinates of Man$_{a}$GlcNAc$_2$ derived from the complex with a neutralizing anti-HIV antibody (24), although in this case some minor adjustments to the carbohydrate were required when the outer branched mannose units were superimposed (data not shown).

**DISCUSSION**

The structure of the Man$_{ab}$-DC-SIGN complex reveals two significantly populated binding modes for the ligand. The major binding mode corresponds to that observed in previous crystal structures, featuring a specific site for the outer branched trimannose unit of high mannose N-linked carbohydrates. In this orientation, additional contacts are formed between a nonreducing α1-2-linked terminal mannose and Val$_{351}$. This region of DC-SIGN is also important in binding to fucosylated sugars and a terminal GlcNAc in the GlcNAc$_2$Man$_3$ complex (4). The latter compound binds 17-fold more strongly to DC-SIGN relative to mannose and 4-fold more than the trimannose core, suggesting that the additional interactions contribute significantly to overall specificity. Surprisingly, a second binding orientation was observed in which the mannose at the principal Ca$^{2+}$ site is reversed, thereby generating new interactions between the nonreducing terminal mannose and the region around Phe$_{313}$. Modeling indicates that this orientation would be able to bind to the protein as part of a full 9-mannose structure (Fig. 5, C–E).

The relevance of the dual binding modes of the Man$_{ab}$ compound was confirmed by the structure of the Man$_{1-2}$Man disaccharide, which shows the same interactions. In this case, the preferred binding mode leaves the nonreducing end near the Phe$_{313}$ site. This probably reflects a different energetic balance of the Man$_{a}$ and Man$_{ab}$ compounds, but in any case it is clear that this binding mode can be significantly populated.
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fact that no other binding sites for this ligand were observed suggests that there are no other secondary subsites that interact with terminal Manα1–2Man disaccharides in larger N-linked high mannose oligosaccharides that might account for enhanced binding to such glycans.

The observation of dual binding modes, each resulting in unique contacts with DC-SIGN, permits a semi-quantitative explanation of the affinity enhancements observed when high mannose structures are decorated with α1–2-linked mannose residues at the nonreducing termini. Using \([P]\) and \([L]\) to denote the concentrations of free protein and ligand, respectively, and \([PL_n]\) for the concentration of the \(n^{th}\) distinct protein-ligand complex with an association constant \(K_{a(n)}\), the measured affinity constant is related to the affinity constants of the individual binding modes by the equation \(K_{a(meas)} = ([PL1] + [PL2] + \ldots)/[P][L] = K_{a1} + K_{a2} + \ldots\). Using the definition \(x_n = K_{a(n)}/K_{a1} = [PLn]/[PL1]\), this relationship can be restated as \(K_{a(meas)} = K_{a1}(1 + x_2 + \ldots)\). Because \(\Delta G_{(meas)} = -RT\ln K_{a(meas)} = -RT\ln K_{a1} - RT\ln(1 + x_2 + \ldots) = \Delta G_1 - RT\ln(1 + x_2 + \ldots)\), \(\Delta G_1 = -RT\ln K_{a1}\) and \(\Delta G_n = -RT\ln K_{a(n)}\), it follows that \(x_n = \exp(-\Delta G_n - \Delta G_1)/RT\).

Thus, for two binding modes of equal energy, \(\Delta G_2 - \Delta G_1 = 0, x_2 = 1\), and \(\Delta G_{(meas)} = \Delta G_1 - RT\ln 2\), so the ability to bind in two equally energetic modes provides an additional RT\ln 2 of free energy, corresponding to 0.41 kcal mol\(^{-1}\) at 25°C. For \(n\) isoenergetic binding modes the observed association constant will be \(n\) times the intrinsic association constant, whereas additional weaker binding modes will increase the association by less than a factor of \(n\). The effect of this statistical factor can be illustrated by comparing the binding of Manα1–2Man with the binding of mannose.

With free 3- and 4-OH groups, mannose could bind in either of two orientations related by a 180° rotation that interchanges the 3- and 4-OH groups, as described above. In the disaccharide, each residue can in principle bind in either orientation, giving a total of four binding modes. Thus, if all of these modes were strictly equivalent, the relative \(K_i\) for the disaccharide would be twice that of mannose. This argument ignores the possible contribution of an alternative binding arrangement involving the 1- and 2-OH groups, which has only been observed in the case of galactose binding to mannose-binding C-type lectins (25). The predicted ratio is seen for DC-SIGNR, but the ratio is about 4 for DC-SIGN, which probably indicates that there are additional, favorable interactions with DC-SIGN.
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made by the second sugar of the disaccharide. It is also possible that free mannose can bind in only one of two modes, as seen in crystal structures of mannose-binding proteins bound to monosaccharides, which generally show a single orientation rather than a mixture in the binding site (22, 25).

For more complex ligands, we can consider the trisaccharide binding mode observed in Man$_6$GlcNAc$_2$ and Man$_9$ as a relatively high affinity mode. The 5-mannose core of the full Man$_9$ structure, which lacks all Man$_1$–2Man groups, binds to DC-SIGN 7-fold better than mannose and 4-fold better in the case of DC-SIGNR. Man$_5$ also possesses the inner branched trimannose unit in the core that in the absence of the β-linked GlcNAc (4) might also bind. The ability to bind to either the inner or outer branched trimannose units likely explains the enhancement of Man$_5$ over Man$_3$ (4). Man$_{6b}$ binds 14-fold better than mannose to DC-SIGN and 12-fold better than mannose to DC-SIGNR (Table 2). Man$_9$ lacks the inner trimannose, but the outer branched trimannose binding mode and the “reversed” mode in which the nonreducing terminal Man is bound near Phe$^{113}$ are present. The 2- and 4-fold enhancement of Man$_{6b}$ over Man$_5$ binding to DC-SIGN and DC-SIGNR, can be accounted for in part by the second binding mode. In the major orientation, the α1–2-linked terminal mannose on Man$_{6b}$ forms additional interactions relative to Man$_5$, which might make this orientation inherently stronger. In principle the Man$_1$–2Man group present on the termini of both the α1–3 and α1–6 branches of Man$_{6b}$ can bind in this second orientation, but they cannot be distinguished in the structure (see “Results”), potentially providing three distinct modes at the principal Ca$^{2+}$ site (when the outer branched trimannose moiety plus the two termini of the two branches are considered).

Given the unequal occupancies of the two orientations observed in the Man$_{6b}$ complex, it is likely that an inherently stronger interaction of the major, trimannose binding mode and the statistical effect of the second mode both contribute to the observed affinity enhancements. If the major orientation is of higher affinity than the nonreducing Man$_1$–2Man binding mode, the affinity enhancements provided by the latter will be less than a factor of $n$ modes. If we assume that the observed occupancies reflect the relative affinities of the two binding modes, with the estimated 3:1 ratio of occupancy in both the Man$_5$ and Man$_9$ structures, $x = \frac{3}{7}$, so $\Delta G = 0.65 \text{ kcal mol}^{-1}$ would be the energy difference between these modes. If we further assume that the observed minor mode has an equal mixture of the two different α1–2 termini, then the ratios are 3:0.5:0.5, and the energy difference is 1.06 kcal mol$^{-1}$. This result illustrates that small differences in energy caused by differences in contacts combined with entropy losses caused by conformational immobilization can give rise to preferred binding orientations and likely explains why all possible modes are not allowed even though the binding site requires only vicinal, equatorial OH groups for Ca$^{2+}$ ligation.

The affinities of Man$_{6a}$ for DC-SIGN and DC-SIGNR are enhanced to a similar extent as for Man$_{6b}$. In this case, the outer branched trimannose unit is present, but no Man$_1$–2Man moieties are appended to these branches. However, two Man$_1$–2Man groups present on the α1–3 arm of the inner branched trimannose would provide two more binding modes. It is also possible that the inner branched trimannose unit could bind. Thus, this compound would appear to have a similar number and kind of binding modes as Man$_{6b}$, despite their different covalent structures. In the full Man$_9$ structure, the inner and outer branched trimannose units are present, as well as the Man$_1$–2Man groups attached to the branches. If we assume that the Man$_1$–2Man group on the α1–3 branch of the inner trimannose structure can provide two more modes, we have a total of six modes, which would explain its further enhancement relative to Man$_5$.

Although this analysis makes several assumptions about which modes of binding might or might not occur, it is clear that the ability of high mannose oligosaccharides to interact with DC-SIGN and DC-SIGNR in multiple orientations can give rise to statistical affinity enhancements that are consistent with the measured values. Energetic differences among the different binding modes also play an important role in determining the affinity of each compound. Nonetheless, the 2–3-fold increase in affinity displayed by the full Man$_9$GlcNAc$_2$ structure versus Man$_5$ is difficult to understand. Perhaps the inner GlcNAc residues restrict the conformation of nearby sugar groups such that there is a smaller entropy penalty for binding, or alternatively, novel contacts are formed between these residues and the surface of the protein.

DC-SIGN and DC-SIGNR serve as receptors for HIV and several other enveloped viruses by binding to the high mannose oligosaccharides present on viral surface glycoproteins. The CRD of DC-SIGN specifically recognizes an internal portion of the carbohydrate, namely the outer branched trimannose unit unique to these carbohydrates. The presence of Man$_1$–2Man enhances the affinity of oligomannose toward these receptors, even though by itself this disaccharide binds only slightly more strongly than mannose. The CRD has an intrinsically high affinity for oligomannose structures, and tetramerization likely provides further avidity enhancements for arrays of such structures (6, 26). The ability of DC-SIGN and DC-SIGNR to bind high mannose glycans in multiple orientations may facilitate this multivalent binding of clusters of CRDs to glycans displayed in various arrangements on the surface of the virus, as proposed previously for cell surface recognition by mannose-binding proteins (22). There are some parallels with the mechanism by which a neutralizing antibody to HIV, 2G12, binds specifically to the terminal Man$_1$–2Man groups present on high mannose carbohydrates (13, 27). The binding site of 2G12 appears to recognize specifically a single orientation of Man$_1$–2Man present on the nonreducing termini of Man$_5$, but at least two of the three branch termini bind to this antibody, which would contribute some statistical enhancement of affinity. High avidity is provided in this case by the unusual domain-swapped dimeric antibody structure, which is proposed to display appropriately spaced binding sites that match the spacing of these structures on the viral surface (24, 28).

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