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Minireview

Concanavalin A Interactions with Asparagine-linked Glycopeptides. The Mechanisms of Binding of Oligomannose, Bisected Hybrid, and Complex Type Carbohydrates

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The affinity of concanavalin A (Con A) for simple saccharides has been known for over 50 years. However, the specificity of binding of Con A with cell-surface related carbohydrates has only recently been examined in detail. Brewer and coworkers [J Biol Chem (1986)261:7306-10; J Biol Chem (1987) 262:1288-93; J Biol Chem (1987) 262:1294-99] have recently studied the binding interactions of a series of oligomannose and bisected hybrid type glycopeptides and complex type glycopeptides and oligosaccharides with Con A. The relative affinities of the carbohydrates were determined using hemagglutination inhibition measurements, and their modes of binding to the lectin examined by nuclear magnetic relaxation dispersion (NMRD) spectroscopy and quantitative precipitation analyses. The equivalence zones (regions of maximum precipitation) of the precipitin curves of Con A and the carbohydrates indicate that certain oligomannose and bisected hybrid type glycopeptides are bivalent for lectin binding. From the NMRD and precipitation data, two protein binding sites on each glycopeptide have been identified and characterized. Certain bisected complex type oligosaccharides also bind and precipitate Con A, while the corresponding non-bisected analogs bind but do not precipitate the protein. The precipitation data indicate that the bisected complex type oligosaccharides are also bivalent for lectin

Abbreviations: Con A, Concanavalin A with unspecified metal ion content; CMPL, Con A with Mn^{2+} and Ca^{2+} at the S1 and S2 sites, respectively, in the locked conformation [12]; trisaccharide **1**, 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannose; α-MDM, methyl α-D-mannopyranoside; NMRD, nuclear magnetic relaxation dispersion, the magnetic field dependence of nuclear magnetic relaxation rates, in the present case, the longitudinal relaxation rate, $1/T_1$, of solvent protons.

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binding, while the nonbisected analogs are univalent. The NMRD and precipitation data are consistent with different mechanisms of binding of nonbisected and bisected complex type carbohydrates to Con A, including different conformations of the bound saccharides.

Introduction

The jack bean protein concanavalin A (Con A) is among the most widely used and studied plant lectins due to its numerous biological effects that correlate with its carbohydrate binding properties [1]. In particular, Con A has proven to be a powerful tool for studying the structure and dynamics of cell surface membranes due to its ability to bind to asparagine-linked (*N*-linked) oligosaccharides [2]. Although the specificity of Con A binding to simple mono- and oligosaccharides has been known for some time [3, 4], the binding of *N*-linked carbohydrates to Con A appears to involve additional interactions since their affinities often exceed those of monosaccharides by two orders of magnitude [5]. Thus, the mode(s) of binding of *N*-linked carbohydrates to the lectin has been an active area of investigation.

The binding specificity of Con A toward monosaccharides was shown by Makela [6] and Goldstein and coworkers [4] to be directed toward the pyranose forms of the monosaccharides glucose and mannose, which contain similar hydroxyl group configurations at the 3-, 4-, and 6-positions. The protein binds the α-anomers of these glycosides stronger than the β-anomers [3]. The binding of oligosaccharides to Con A was studied by Goldstein and coworkers who showed that there were two classes of linear oligosaccharides which differed in their affinity for the protein: the first class demonstrated no enhanced binding relative to monosaccharides; the second class showed enhanced binding. The first class included α(1-3), α(1-4), and α(1-6) oligosaccharides with a non-reducing terminal glucose or mannose residue [3]; the second class consisted primarily of α(1-2) oligomannosides [7]. α(1-2)Mannobiose and mannotriose, for example, have a 5- and 20-fold greater affinity than α-MDM, respectively [7]. Goldstein [4] also demonstrated that an internal 2-*O*-α-substituted glucose or mannose residue could bind as well as terminal non-reducing sugars. The enhanced binding of α(1-2) oligosaccharides prompted speculation that Con A possessed an extended binding site which bound more than one residue of molecules containing α(1-2)-linked glucose and mannose residues [4]. It was also suggested that the higher affinities of *N*-linked glycopeptides were due to the presence of internal 2-*O*-α-substituted mannose residues in complex type carbohydrates of the biantennary class [1, 4, 8, 9]. Binding studies of glycopeptides to Con A Sepharose affinity columns [9, 10] led to the conclusion that the minimal requirement for high affinity binding was two mannose residues in the molecules. Thus, the presence of multiple mannose residues have been implicated as important binding determinants in both oligomannose and comples type glycopeptides. However, until recently the mechanism(s) of binding of *N*-linked carbohydrates to Con A was not well understood.

Solvent proton nuclear magnetic relaxation dispersion (NMRD) spectroscopy has proven to be an effective tool for probing the interactions of simple oligosaccharides and *N*-linked glycopeptides with Con A [5, 11]. It was shown that the binding of α-MDM to Ca^{2+}-Mn^{2+}-Con A (CMPL) resulted in a reduction in the relaxation rates across the

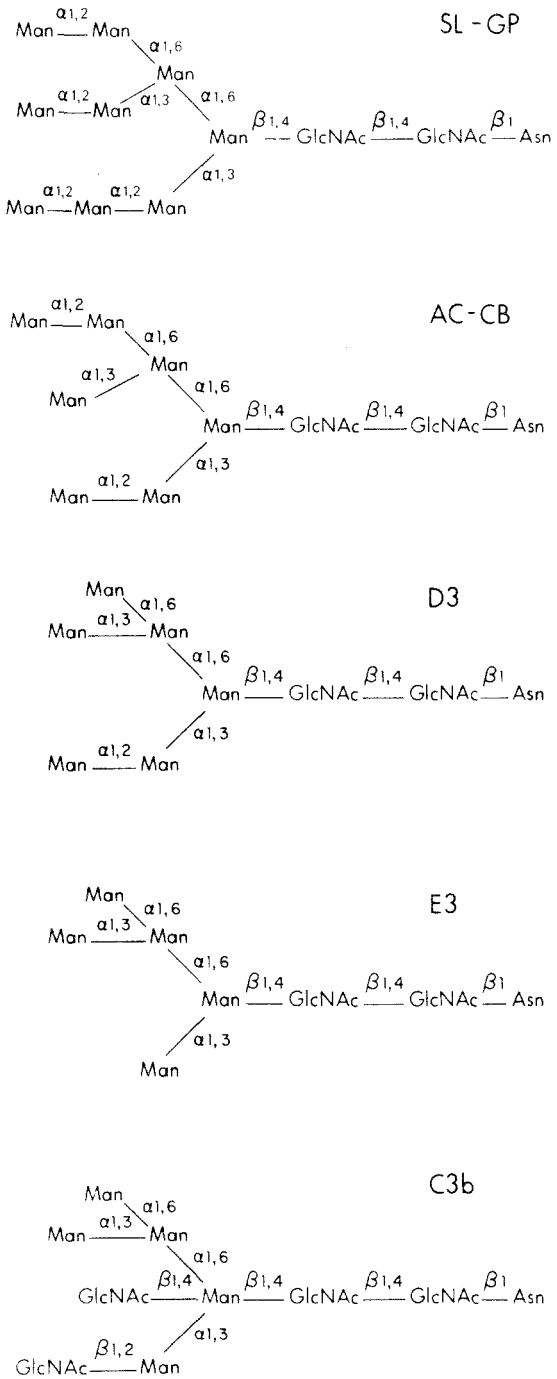

Figure 1. Structures of oligomannose and bisected hybrid type glycopeptides.

Table 1. Inhibitory potency of high mannose and bisected hybrid-type glycopeptides for Con A mediated hemagglutination of rabbit erythrocytes.

Glycopeptides or oligosaccharides[a]	Minimum concentrations required for complete inhibition of hemagglutination[b] (μM)	Relative inhibitory potency[c]
α-MDM	3100	1
Manα(1-6)Man	1600	1.9
Manα(1-3)Man	1300	2.2
1	23.8	130
SL-GP	1.0	3000
AC-CB	6.3	500
D3	44.2	70
E3	61.0	60
C3b	26.0	120

[a] See Figs. 1 and 2 for structures.
[b] The experiments were done by 2-fold serial dilution [27] with 3% rabbit RBC suspensions in 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl.
[c] All data normalized to that of α-MDM. Higher values indicate greater inhibitory potency.

NMRD profile of the lectin, and that this effect was due to a conformational change in the protein [11]. Thus, measuring the magnetic field dependence of the T_1 relaxation rates of solvent protons in the presence of the Mn^{2+} ion in Con A provides a method for monitoring the interactions of saccharides with the protein (for a review of previous NMRD studies of Con A, see [12]). Using this approach, a variety of simple mono- and oligosaccharides including the two classes of oligosaccharides observed by Goldstein and coworkers [7] were examined for their effects on the NMRD profile of the protein. The rationale was that extended site interactions might induce different conformational changes in the lectin than single site interactions, as has been observed with hen egg white lysozyme [13]. The results, however, were the same for all of the saccharides tested: saturation of the binding sites of the protein with a carbohydrate led to a uniform reduction of approximately 20% in the NMRD profile [11]. These results led to the suggestion that Con A possessed a binding site that accommodated one carbohydrate residue, and that enhanced binding of the α(1-2) oligomannosides was due to the increased probability of binding of molecules with multiple glucose or mannose residues. This conclusion was supported by stopped-flow kinetic analysis and thermodynamic measurements of the binding of fluorescent labelled and unlabelled α(1-2) oligomannosides to the protein, respectively [14, 15].

Recently the interactions of CMPL with a variety of oligomannose and bisected hybrid type glycopeptides and complex type oligosaccharides have been examined using NMRD measurements [5]. The results indicate that the modes of binding of these carbohydrates are different from simple mono- and oligosaccharides. Furthermore, quantitative precipitation studies indicate that many of these *N*-linked carbohydrates possess bivalent binding properties toward the protein [16, 17]. The results of these studies are discussed below.

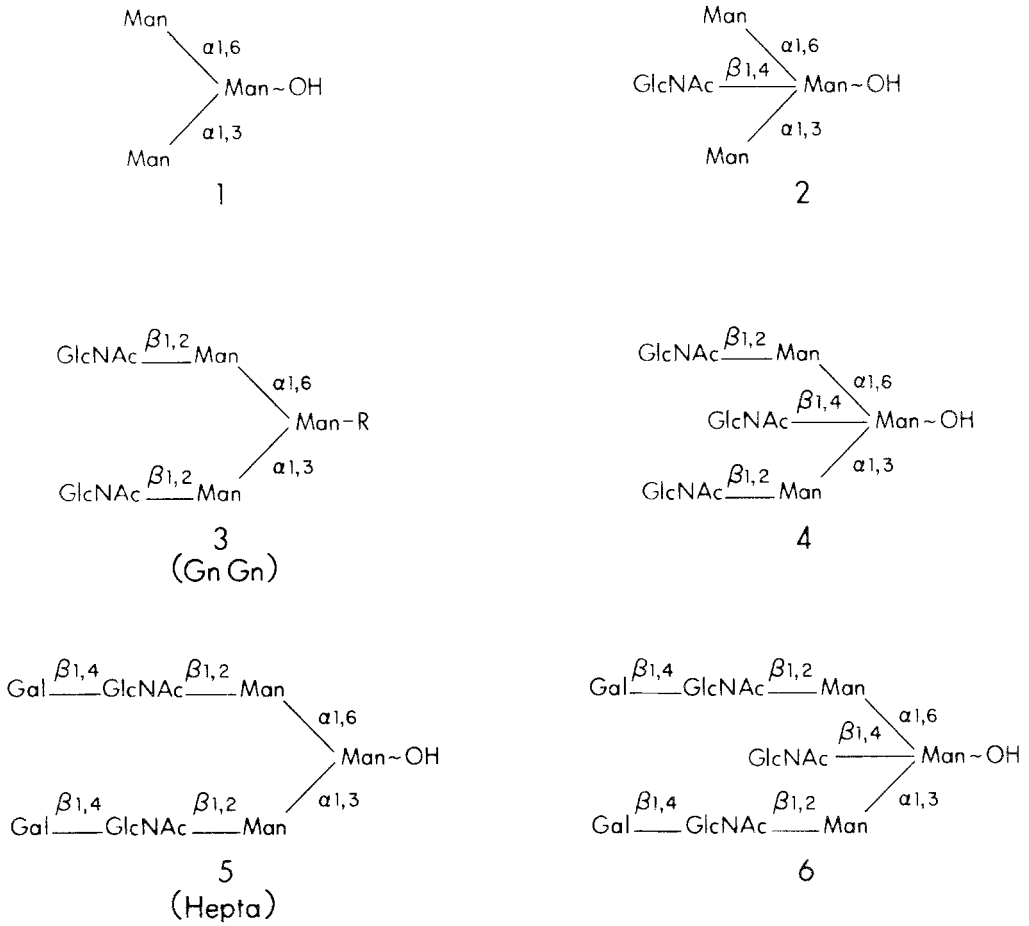

Figure 2. Structures of non-bisected (**1**, **3**, **5**,) and bisected (**2**, **4**, **6**) complex type glycopeptide, oligosaccharides, and related analogs.

Interactions of Oligomannose and Bisected Hybrid Type Glycopeptides with Con A

Relative Affinities

Fig. 1 shows the structures of the oligomannose glycopeptides, SL-GP, AC-CB, D3, and E3, and the bisected hybrid type glycopeptide, C3b. The relative affinities of the glycopeptides with respect to α-MDM, as determined by hemagglutination inhibition measurements, are shown in Table 1. Where comparable, the affinities of the glycopeptides agree with the values obtained by frontal affinity chromatography [18].

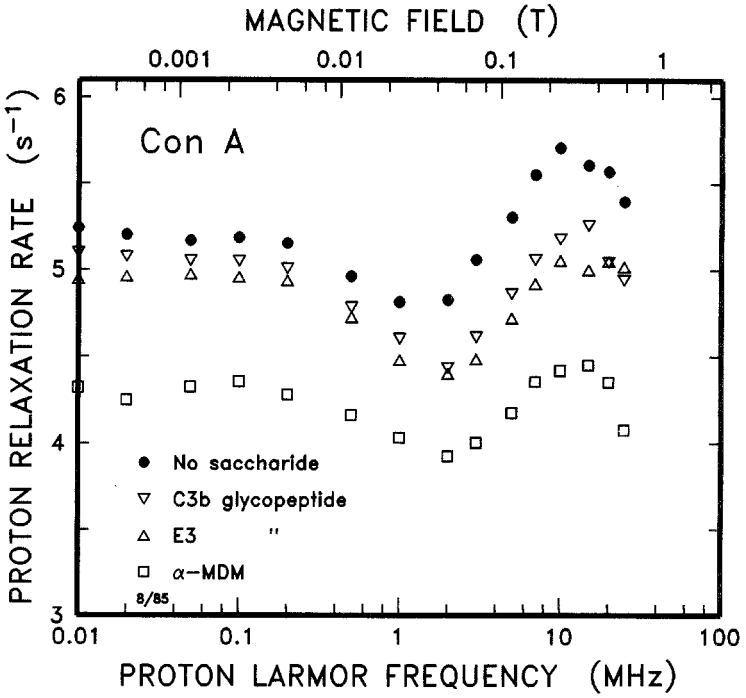

Figure 3. NMRD profiles of CMPL (●), CMPL with 1 mM E3 (△), CMPL with 1 mM C3b (▽), and CMPL with 10 mM α-MDM (□) at 25°C in pH 5.6 buffer. The concentrations of CMPL was 0.40 mM. The concentration of carbohydrates used were sufficient to saturate the binding sites of the protein. A pH 5.6 buffer of 0.1 M potassium acetate and 0.9 M KCl was used. The volume of each sample was 0.1 ml, and the temperature was 25°C.

NMRD Studies

Recent studies using NMRD have provided evidence that the oligomannose and bisected hybrid type glycopeptides in Fig. 1 bind to Con A primarily by extended site interactions of the outer trimannosyl moiety on the α(1-6) arms of the glycopeptides [5]. These conclusion are based mainly on the observation that D3, E3, and C3b all produce changes in the NMRD profile of CMPL (cf. Fig. 3) similar to that induced by the binding of the synthetic trisaccharide, 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannose (**1**) (Fig. 4). Trisaccharide **1** produces a smaller change in the NMRD profile of CMPL, compared to that induced by α-MDM binding (Fig. 4) [11]. Furthermore, **1** has 130-fold greater affinity than α-MDM [5], which is comparable to the affinities observed for D3, E3, and C3b, within the limits of experimental error (Table 1) [5]. These results indicate that trimannoside **1** and the glycopeptides induce a similar conformational change in the protein which differs from that induced by the binding of α-MDM (as well as simple mono- and oligosaccharides). The two disaccharides, 3-*O*-(α-D-mannopyranosyl)-D-mannose and 6-*O*-(α-D-mannopyranosyl)-D-mannose, which are part of the structure of trisaccharide

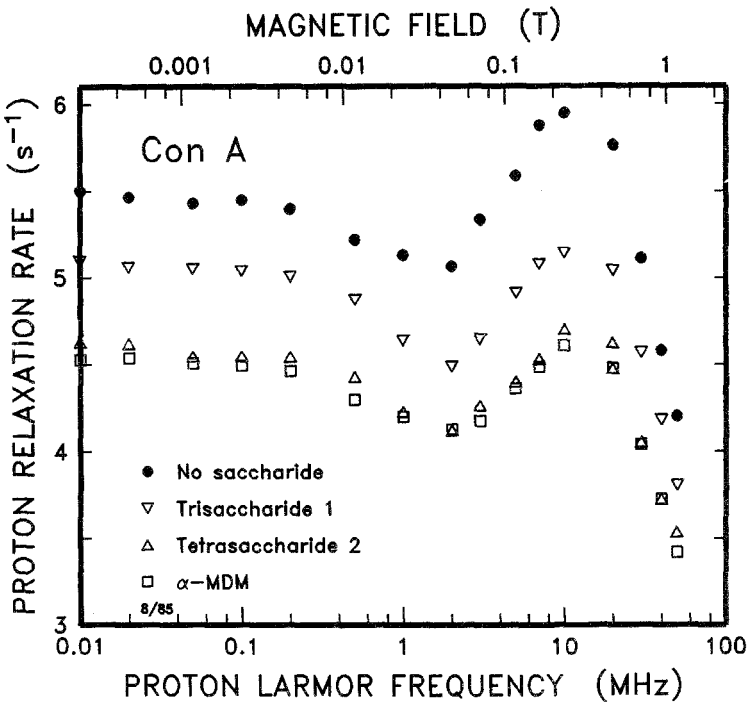

Figure 4. NMRD profile of CMPL (●), CMPL with 10 mM α-MDM (□), CMPL with 14 mM trisaccharide **1** (▽), and CMPL with 10 mM tetrasaccharide **2** (△). The conditions are the same as in Fig. 3.

1, do not possess high affinity binding (Table 1), and induce a change in the NMRD profile of CMPL similar to α-MDM [5]. These results therefore suggest that the two nonreducing mannose residues of trisaccharide **1** undergo extended site interactions with the protein. The fact that tetrasaccharide **2** (Fig. 2), which is a bisected analog of **1**, produces a change in the NMRD profile of CMPL similar to α-MDM and not **1** (Fig. 4), provides direct evidence that the protein binds to the outer trimannosyl moiety of C3b and not to the inner trimannosyl moiety which contains a bisecting *N*-acetylglucosamine, since the NMRD profile of C3b resembles **1** and not **2** [5]. These conclusions are supported by the glycopeptide binding studies of Ohyama *et al.* [18] and Carver *et al.* [19].

The Mechanism(s) of High Affinity Binding of SL-GP and AC-CB

The oligomannose type glycopeptides AC-CB and SL-GP (Fig. 1) with 500- and 3000-fold higher affinities than α-MDM, respectively. Both glycopeptides produce a drop in the NMRD profile of CMPL similar to those produced by D3, E3, C3b, and trisaccharide **1** [Bhattacharyya L, Brewer CF, unpublished results]. The NMRD data suggest that the trimannosyl moiety on the α(1-6) arm of both AC-CB and SL-GP is the primary site of

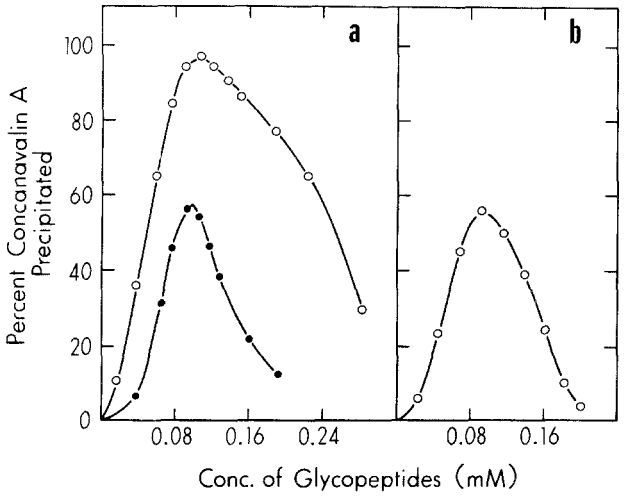

Figure 5. Precipitin curves for precipitation of Con A by high mannose type glycopeptides D3(a) and E3(b) in pH 7.2 (○) and 5.6 (●) buffers at 21°C. See Table 3 for conditions of the experiments.

binding, as observed for the other oligomannose type glycopeptides. The only structural differences at the primary sites of AC-CB and SL-GP compared to D3, E3, and C3b is the addition of extra α(1-2) mannose residues on the α(1-6) arms of the former two glycopeptides.

The enhanced affinities of AC-CB and SL-GP of approximately 5- and 30-fold, respectively, relative to D3, E3, C3b are similar to the 5- and 20-fold increase in affinities observed for α(1-2) mannobiose and mannotriose for Con A, respectively, relative to α-MDM [7]. The increase in affinities of the latter oligosaccharides has been attributed to a statistical increase in the probability of binding due to the presence of multiple mannose residues with free 3-, 4-, and 6-hydroxyl groups in the oligosaccharides, that are individually capable of binding to the protein [11]. These findings suggest that a similar mechanism may occur in AC-CB and SL-GP. The enhanced affinities of the glycopeptides appear to be due to the higher probability of binding at the primary sites because of the presence of the outer α(1-2) mannose residue(s). This may be envisioned, in part, as a decrease in the dissociation rate of the complex due to "diffusional jumps" between the trimannosyl moiety on the α(1-6) arm and the α(1-2) mannose residue(s) linked to the trimannosyl group, without complete dissociation of the complex. The increased affinity of SL-GP relative to AC-CB is due to the additoinal α(1-2) mannose on the α(1-3) arm of the outer trimannosyl moiety (Fig. 1). Thus, each α(1-2) mannose linked to the outer trimannosyl moiety produces a 5- to 6-fold increase in affinity by a "statistical" mechanism similar to that observed for the binding of α(1-2) oligomannosides.

Table 2. Inhibitory potency of complex-type oligosaccharides and glycopeptides for Con A mediated hemagglutination of rabbit erythrocytes.

Glycopeptides or oligosaccharides	Minimum concentrations required for complete inhibition of hemagglutination[a] (μM)	Relative inhibitory potency[b]
1	23.8	130
2	450	7
3	26.0	120
4	310	10
5	150	20
6	10 000	—[c]

[a] The experiments were done by 2-fold serial dilution [27] with 3% rabbit RBC suspensions in 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl.
[b] All data normalized to that of α-MDM. Higher values indicate greater inhibitory potency.
[c] No inhibition.

Precipitation Studies

Fig. 5 shows that oligomannose type glycopeptides AC-CB, D3 and E3 precipitate the lectin under appropriate conditions [16]. Similar data are obtained for SL-GP and C3b. The data are similar to antigen-antibody and lectin-polysaccharide precipitin profiles [20, 21], and suggest multivalent interactions between the glycopeptides and Con A. The data for D3 in Fig. 5b show that the Con A tetramer which exists at pH 7.2 results in stronger precipitation than the Con A dimer which exists at pH 5.6 [22]. These results are consistent with the greater valency of the tetramer [20]. The ratio of the concentrations of glycopeptides to Con A monomer at the equivalence point (maximum precipitation) of the respective precipitation profile indicates approximately 1:2 stoichiometries (Table 3). Since each monomer of Con A possesses one sugar binding site (see [1]), the results indicate that SL-GP, AC-CB, D3, E3, and C3b are bivalent in binding the lectin.

The precipitation and NMRD data lead to the conclusion that the α(1-6) arms of the glycopeptides constitute a single high affinity binding site for Con A [5, 16]. Since trisaccharide **1** binds 130 times greater than α-MDM, and α-MDM has a dissociation constant of 150 μM at 25°C, the dissociation constant of the trimannosyl binding moiety in D3, C3b, and E3 (the primary site) can be assigned a value of approximately 1.2 μM. In SL-GP and AC-CB, the dissociation constants of the primary site are therefore approximately 50 nM and 0.3 μM, respectively.

Analyses of the precipitation data of a variety of oligomannose and bisected hybrid type glycopeptides [16] indicate that the second binding site(s) on SL-GP, AC-CB, D3, E3, and C3b exist on the α(1-3) arm of the core β-mannose residue (secondary site). For D3 and AC-CB the secondary site is the α(1-2)mannobiosyl moiety on the α(1-3) arm, for E3 and C3b it is the mannosyl and GlcNAcβ1-2Man moieties, respectively, and for SL-GP it is the α(1-2)mannotriosyl moiety. An estimate of the K_d values of these secondary sites has

Table 3. Stoichiometry of precipitin reaction between Con A and glycopeptides and oligosaccharides.[a]

Glycopeptide or oligosaccharide	Concentration of glycopeptide at equivalence point (μM)		Protein concentration (μM)		Ratio of conc. of glycopeptide to protein monomer	
	pH 7.2	pH 5.6	pH 7.2	pH 5.6	pH 7.2	pH 5.6
SL-GP	19		38		1:2.0	
AC-CB	110		210		1:1.9	
D3	110	100	200	170	1:1.8	1:1.7
E3	90		180		1:2.0	
C3b	100		190		1:1.9	
2	140		200		1:1.4	
4	120	130	220	240	1:2.0	1:1.8

[a] The experiments were done in 0.1 M Tris-HCl, 0.9 M KCl, pH 7.2, or 0.1 M potassium acetate, 0.9 M KCl, pH 5.6, buffers at 21°C. Both buffers contain 1 mM Mn^{2+} and 1 mM Ca^{2+}.

been made according to the values of the corresponding mono- and oligosaccharides [7]. Thus, at 25°C the K_d value of the α(1-2)mannotriosyl group of SL-GP is approximately 8 μM, the α(1-2)mannobiosyl group of AC-CB and D3 is about 30 μM, and the mannosyl residue in E3 is 150 μM. The K_d of the GlcNAcβ1-2Man moiety of C3b is estimated to be between the latter two values. Differences in the affinities of the residues at the secondary sites have been accounted for by a statistical mechanism of enhanced binding [11], as discussed above. The higher affinity of the primary sites compared to the secondary sites of the glycopeptides explains why the NMRD studies, done in the presence of excess glycopeptides, reveal only binding of the primary sites.

Interactions of Nonbisected and Bisected Complex Type Glycopeptides and Oligosaccharide with Con A

Relative Affinities

Fig. 2 shows a series of non-bisected and bisected complex type oligosaccharides and a glycopeptide. Trimannosyl oligosaccharide **1** is the common structural element present in all complex type glycopeptides and is responsible for their high affinity binding to Con A [5, 17]. The relative binding affinities of **1**, **3**, and **5** for Con A, as measured by hemagglutination inhibition, are 130, 120, and 20, respectively, with respect to α-MDM (Table 2). The bisected complex type carbohydrates, **2** and **4**, have 7- and 10-fold greater relative affinities for the lectin, respectively, than that of α-MDM (Table 2). Oligosaccharide **6** binds too weakly to detect. Thus, the affinities of the non-bisected complex type oligosaccharides for Con A are generally greater, often by an order of magnitude, than those of the corresponding bisected analogs.

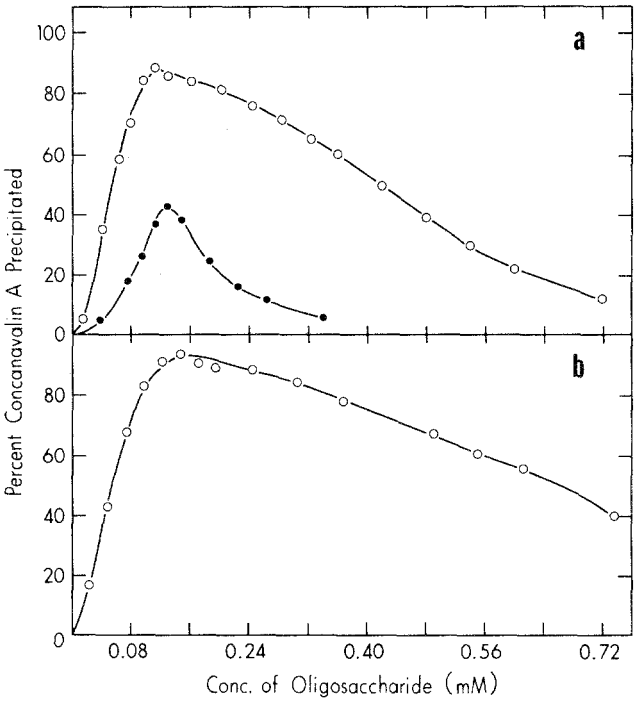

Figure 6. Precipitin curves for precipitation of Con A by bisected complex type oligosaccharides **4** (a) and **2** (b) in pH 7.2 (○) and 5.6 (●) buffers at 21°C. See Table 2 for conditions of the experiments.

NMRD Studies

As discussed above, the NMRD data of oligosaccharide **1**, which can be considered as the simplest analog of non-bisected complex type carbohydrates, in the presence of CMPL indicates that the two non-reducing terminal mannose residues of the trimannoside bind to an extended binding site in Con A [5]. Essentially the same NMRD profiles are observed for glycopeptide **3** and oligosaccharide **5** [5], suggesting similar mechanisms of binding.

On the other hand, oligosaccharide **2**, which is the bisected analog of **1**, shows a change in the NMRD profile of CMPL similar to that induced by α-MDM (Fig. 4) [5]. These results suggest that, unlike **1**, **2** does not bind by extended site interactions, but rather by a single mannose residue. The reduced affinity of **2**, relative to **1**, is consistent with this conclusion (Table 2).

The NMRD profile of CMPL in the presence of **4** does not resemble those for **1**, **3** or **5**, but rather shows an even larger drop in the profile compared to that for α-MDM (not shown). It is clear that **4** does not bind by the same mechanism as the non-bisected complex type carbohydrate analog **3**. Rather, its affinity is nearly the same as **2**, and, like **2**, it results in a larger drop in NMRD profile than that induced by **1**, **3**, or **5**.

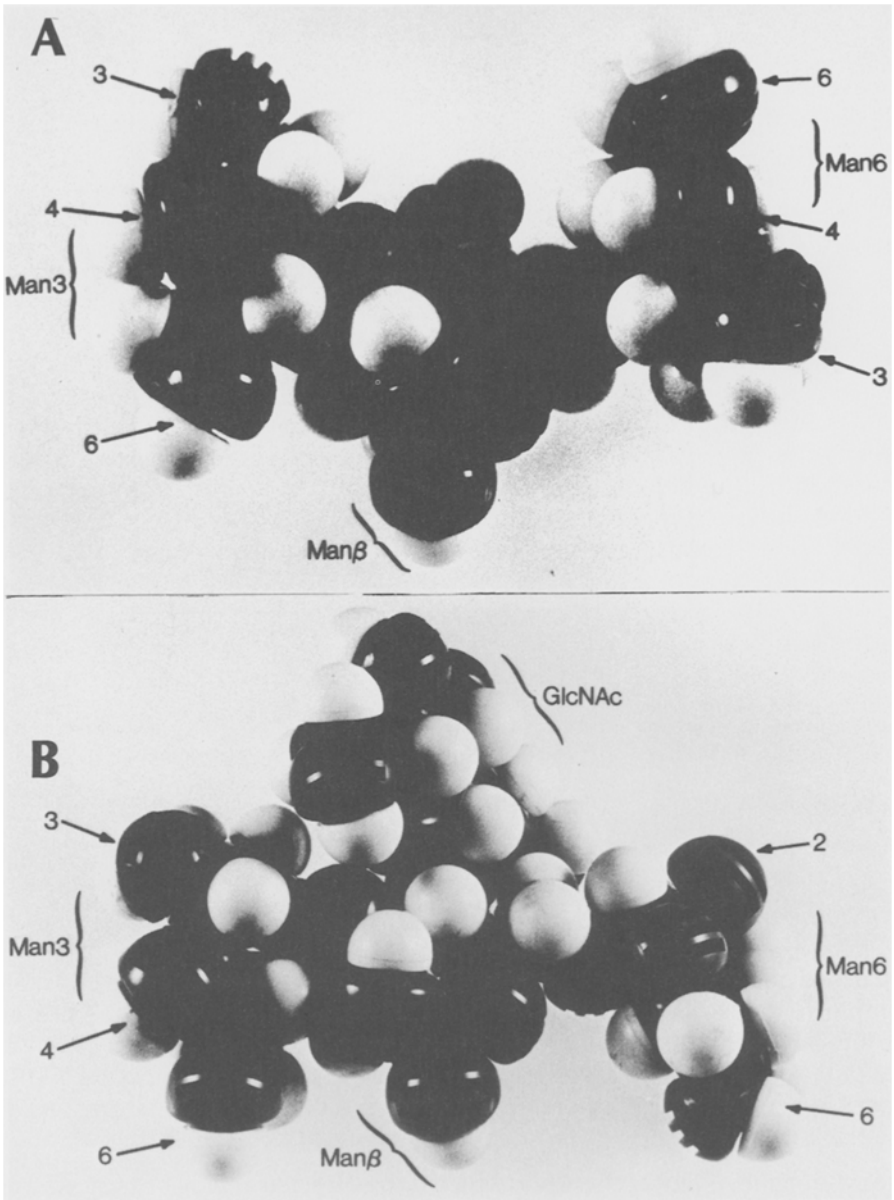

Figure 7. Corey-Pauling-Koltun space-filling models of **1** and **2** (Fig. 1). (a) **1** at the rotation angle of α(1-6) set to $\omega = 180°$, and (b) **2** at $\omega = -60°$ [23, 24]. The angle, ω, is the dihedral angle formed by the H-5, C-5, C-6, and O-6 atoms of the core β-mannose residue. The numbers 2, 3, 4 and 6 indicate 2-, 3-, 4- and 6-hydroxyl groups of mannose residues. Man3 and Man6 stand for mannose residues on α(1-3) and α(1-6) arms of core β-mannose (Manβ). GlcNAc represents the bisecting *N*-acetyl-D-glucosamine residue linked β(1-4) to core β-mannose.

Precipitation Studies

Fig. 6 shows that bisected complex type oligosaccharides, **2** and **4**, can precipitate Con A, as observed with certain oligomannose and bisected hybrid type glycopeptides. The data in Table 3 indicate that **4** is bivalent for Con A. Similar data for **2** show a stoichiometry of 1:1.4 of oligosaccharide to Con A monomer (Table 2), which is lower than that observed for **4**. This appears to be due to the weaker binding of **2** [20]. Nevertheless, the results are similar to **4**, indicating that **2** is also bivalent for Con A binding. In contrast, **1, 3**, and **5** do not precipitate the lectin, which indicates that they are univalent ligands.

Mechanisms of Binding

The present results indicate that the non-bisected and bisected complex type carbohydrates in Fig. 2 bind to Con A *via* different mechanisms. Insight into these differences comes from comparing the affinity, NMRD, and precipitation data for **1** and **2**. Oligosaccharide **1** undergoes extended site interaction with the protein which accounts for its high affinity and NMRD profile [5]. These results are consistent with its univalence, since both of its non-reducing mannose residues are simultaneously bound to the same Con A monomer. Bisected analog **2**, on the other hand, provides an NMRD profile of the protein which is indistinguishable from that for α-MDM (Fig. 4), suggesting that **2** binds to a monomer of Con A by only *one* of its non-reducing mannose residues. This leaves the other non-reducing mannose residue of **2** free to bind to a second Con A molecule, which, under the appropriate conditions, results in precipitation of the protein (Fig. 6b).

Analysis of Corey-Pauling-Koltun space-filling models of **1** and **2** (Fig. 7) indicates that the orientations of their α(1-6) arms when bound to the protein must be different in order to be consistent with the NMRD and precipitation data. Evidence from proton NMR studies and minimum energy calculations suggests that the α(1-6) arm of the methyl α-glycoside of **1** exists predominantly in two rotamer conformations with values of ω = -60° and 180° [23, 24]. Since the data for **1** are consistent with both non-reducing terminal mannose residues of the oligosaccharide binding to an extended binding site of Con A, this requires that the α(1-6) arm possess a value of ω = 180° for binding, as shown in Fig. 7a. In this conformation, both the α(1-3) and α(1-6) mannose residues of **1** have their 3-, 4-, and 6-hydroxyl groups facing the same direction, which is required for binding to a common protein surface. This would therefore appear to be the conformation of **1** bound to the protein. (The high energy barriers to rotation about the α(1-3) arm preclude alterations in the conformation of this portion of the molecule [23, 24]).

On the other hand, data for **2** are consistent with only one of its mannose residues binding to one Con A molecule. Rotation of the α(1-6) arm of **2** to a value of ω = -60° results in the two non-reducing terminal mannose residues facing away from each other, as shown in Fig. 7b. This allows either the α(1-3) or α(1-6) mannose residue to bind to one protein molecule, while the other non-reducing mannose residue of **2** can bind to a second Con A molecule from the opposite side. It appears, then, that the preferred binding conformation of the α(1-6) arm of **2** is with ω = -60° (Fig. 7b). Thus, the binding conformations of **1** and **2** appear to be different. These conclusions also apply to the other non-bisected and bisected complex type oligosaccharides in Fig. 3, in which their binding determinants are the same as those of **1** and **2**, respectively.

It is important to note that the reason for the different binding mechanisms of **1** and **2** is not known. It is possible that the rotamer population of the α(1-6) arm of **2** is affected by the presence of the bisecting *N*-acetylglucosamine, or that unfavorable steric interactions occur between the *N*-acetylglucosamine residue of **2** and the protein binding site when the rotamer angle about the α(1-6) arm is $\omega = 180°$.

Biological Implications of the Bivalency of Oligomannose, Bisected Hybrid, and Bisected Complex Type Glycopeptides

The present findings indicate that certain oligomannose, bisected hybrid, and bisected complex type glycopeptides are bivalent for Con A. These observations may relate to the ability of so-called "Con A receptors" on the surface of cells to undergo "patching and capping" in the presence of the lectin [25]. Evidence also suggests that microaggregation of cell surface glycoconjugates appears to be a key step in many of the biological responses of cells to Con A binding [25, 26]. In this regard, Bhattacharyya *et al.* [Bhattacharyya L, Kahn MI, Brewer CF; unpublished results] have recently shown that bivalent *N*-linked glycopeptides selectively form homogeneous cross-links with Con A in the precipitation reactions. These findings suggest that the degree of binding specificity between multivalent lectins such as Con A and multivalent carbohydrates is much higher in the lattices that are formed in the cross-linking reactions than in the corresponding non-aggregated or solution complexes. The intrinsic ability of asparagine-linked glycopeptides to function as bivalent cross-linking molecules for binding proteins such as Con A may relate to the role of these carbohydrates as receptors on the surface of cells.

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