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THE CONFORMATION OF GLYCANS OF THE OLIGO-D-MANNOSIDIC TYPE, AND THEIR INTERACTION WITH CONCANAVALIN A: A COMPUTER-MODELLING STUDY

MARGARET BISWAS, Y CHANDRA SEKHARUDU, AND V S R. RAO*
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012 (India)
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ABSTRACT

The favored conformations of glycans of the oligo-D-mannosidic type have been determined by using empirical energy calculations. An interesting aspect is that the α-(1→3)-linked terminal D-mannose residue of the outer trimannosidic core fragment, in all the conformations which fall within 5 kcal.mol^{-1} of the global minimum, always lies close to the chitobiose core. These models are in general agreement with the available n.m.r. data. The probable modes of binding of these glycans to concanavalin A (Con A) were determined, by using a computer-modelling technique which identifies the positions for the different conformers of the carbohydrate in the binding site of Con A, based on stereochemical considerations. These studies showed that Con A can bind only to two of the three terminal D-mannose residues in these glycans, because the D-mannose residue which lies close to the chitobiose core is inaccessible for the binding of Con A. Of these two terminal D-mannose residues, the α-(1→6)-linked D-mannose may bind the more strongly. Furthermore, it is shown that the internal D-mannose residue will, at best, interact very weakly with the carbohydrate-binding site of Con A. These results rationalize well the available data on the binding affinity of these glycans to Con A. They further support the conclusion that the binding affinity of a glycan to Con A does not depend on the number in the glycan, of D-mannose residues which possess free 3-, 4-, and 6-hydroxyl groups, but, rather, on the accessibility of these residues to Con A.

INTRODUCTION

A complete understanding of the interaction between carbohydrates (glycans) and proteins (enzymes, antibodies, and lectins) is to a large extent dependent on the three-dimensional structure of both of these. Energy minimization procedures have led to conformations of various glycans[1] which are in excellent

*To whom correspondence should be addressed

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agreement with experimental data obtained from high-resolution n.m.r. data (^1H and ^{13}C) of the glycans in aqueous solution[2] and with the X-ray crystal structure[3] that is available. The glycans of the oligo-D-mannosidic type constitute a very homogeneous group which is common to numerous glycoproteins of different origins and roles. They are metabolic intermediates in the maturation of the glycans of the *N*-acetyllactosaminic type. Some observations show that oligo-D-mannosidic structures act as recognition signals in, for example, *Escherichia coli,* which binds to human epithelial cells by means of a lectin present on the pili with a specificity for oligo- or poly-D-mannosides. Also, phosphorylated glycans of the oligo-D-mannosidic type target newly synthesized, lysosomal enzymes to lysosomes by specific recognition of receptors present in Golgi and plasma membranes.

Different workers have used various n.m.r. techniques[4,5] to explore the possible spatial arrangements in glycans of the oligo-D-mannosidic type, and some progress has been made in the understanding of the three-dimensional structure of these compounds.

Experimental data are also available on the interaction between some glycans of this type with concanavalin A (Con A), a lectin, specific for α-D-mannopyranose, which is widely used and has been studied by physicochemical techniques[6]. This system is, therefore, an excellent model for a more-detailed study of glycan–protein interactions.

Con A has a high and almost equal affinity for the structures[7,8] shown in Fig. 1. The equal binding-affinity of Con A with all of these structures, which differ both in the total number of mannose residues and in the number of terminal mannose residues, is surprising in view of the earlier belief that increased numbers of α-linked mannose residues results in a greater association constant because of an increased number of possibilities of binding to Con A. This conclusion was based

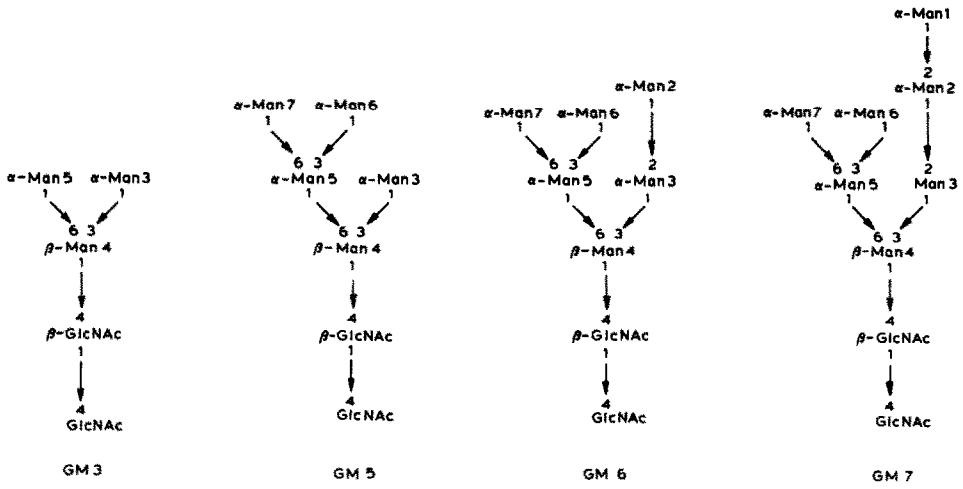

Fig. 1. Structures of the D-mannose-containing glycans studied.

on studies with linear mannobiose and mannotriose[9], and does not seem to hold good for branched structures. Attempts to interpret these binding data further have been hindered, among other things, by uncertainties as to the possible shapes of the glycans and the lack of information regarding their three-dimensional complexes with Con A.

Recently, by using a computer-modelling technique developed in our laboratory[10], we have been able to generate the probable three-dimensional structures of glycans of the *N*-acetyllactosaminic type that will complex with the crystal structure of Con A. This work was able to explain much of the experimental data, and provided valuable information about the size of the carbohydrate binding-site, and the effect of configuration, substitution, and linkage on the modes of binding of these glycans to Con A, and threw some light on the tendency of particular sugar residues in the glycan to reach the specific binding-site of Con A. We have now extended this work to complexes of Con A with glycans of the oligo-D-mannosidic type.

METHOD OF CALCULATION

(i) Energy minimization procedure

The favored conformations of a glycan containing seven D-mannose residues (GM7) (see Fig. 1) has been studied by an energy minimization procedure using empirical potential functions. The numbering of the atoms and dihedral angles

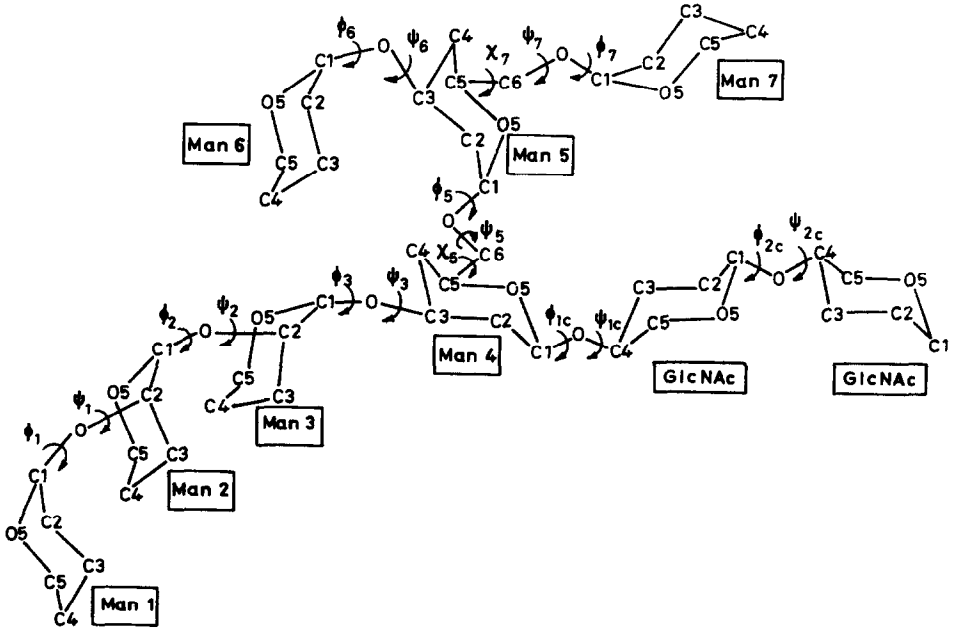

Fig. 2. Numbering of the atoms and dihedral angles used in the study The example is for GM7.

which define the conformation of the glycan are shown in Fig. 2. All of the sugar residues were assumed to be in the $^4C_1$(D) form[11] The atomic coordinates of each of the residues were based on the standard residue of Arnott and Scott[12], compiled from crystal-structure data. The 2-acetamido group was fixed by using Pauling–Corey geometry[13], so that the C-2–H-2 bond and the N–H bond are *trans*. The bond angle at the glycosidic oxygen atom was fixed at the average value of 117.5°. All possible conformations of the glycan were generated by making rotations about the inter-unit glycosidic bonds C-1–O (ϕ rotation) and O–C'X (ψ rotation) through −180° to +180° (X can be 2, 3, 4, or 6, depending on the type of linkage). The initial conformation corresponding to (ϕ,ψ) = (0°,0°) had been described earlier[14]. For the (1→6) linkage, χ represents the rotation about the C-5–C-6 bond and is zero when the C-4–C-5 bond eclipses the C-6–O bond. ψ is defined with respect to the hydrogen atom of the hydroxyl group of the hydroxymethyl group, which is *cis* to the ring-oxygen atom when χ is zero. A clockwise rotation was taken as positive.

The potential energy of the molecule was computed by using the equation, $V_{total} = V_{nb} + V_{es} + V_{tor} + V_{ano}$, where V_{nb} is the nonbonded, V_{es} is the electrostatic, and V_{tor} is the torsional contribution. V_{ano} is the contribution due to the exo-anomeric effect. To compute V_{nb}, the form of the function and the constants used were those reported by Momany *et al.*[15]. To compute V_{es}, the fractional charges on the various atoms were taken from Yathindra and Rao[16]. The contribution due to V_{ano} was calculated by using a function developed by Rao and co-workers[17,18]. The energy minimization procedure[19] was carried out by varying all the glycosidic angles simultaneously.

(ii) Computer modelling procedure

The coordinates of the Con A–methyl α-D-mannopyranoside complex at 2.4 Å resolution, deposited by Hardman and Anisworth in 1977 in the Protein Data Bank, were used in the present study. Although, recently, the native structure of Con A has been solved at high resolution[20], it was preferred for the present purpose to use the coordinates from the crystal structure of the Con A–methyl α-D-mannopyranoside complex, because this structure gives the position for the center of the pyranose ring in the binding site. Using this position as a starting point, various monosaccharides and their derivatives were fitted into the binding site[21,22] which rationalized all of the available kinetic data and spectroscopic results. Recent molecular-docking studies[23] on D-mannopyranose with Con A are also consistent with these theoretically determined positions for the monosaccharide[21].

It is gratifying to note that, for one of the possible orientations reported earlier[21] for methyl α-D-mannopyranoside in the Con A binding site (Φ = 320°, Θ = 150°, Ψ = 190°), the suggested hydrogen-bonding scheme between the glycan and the protein agrees well with that discussed by Carver and co-workers[23]. This suggests that these two orientations are very similar. Exact comparison could not be made, as Carver's group have not yet reported the coordinates of the sugar in the binding site. However, our data show that this particular orientation for the

glycan is not the best one for the high-mannose glycans which we have studied in the present work. The positions determined earlier were, therefore, used in this study for placing the sugar residue of the glycan in the binding site. The position of a sugar residue in the binding site is defined by using the Eulerian angles and translational parameters. The initial orientation defined by the Eulerian angles $\Phi = 0°$, $\Theta = 0°$, and $\Psi = 0°$ was described earlier[24]. Amino acid residues that fall within a sphere of 25-Å radius from this reference point (the center of the pyranose ring in the binding site of Con A) were used. The bivalent-metal ions (Ca^{2+} and Mn^{2+}) were also included in the molecular-fitting studies. The atoms in the amino acid side-chains beyond C^{β} were treated as flexible, with the exception of the side chains which form the coordination bonds with metal ions[20,25].

As a way of simulating conformational changes in the glycan, all of the probable conformations within 5 kcal.mol^{-1} of the minimum energy conformer were made to interact with Con A. Since 3-*O*- and 4-*O*-substituted sugar residues do not bind[26] to Con A (and, as we had shown earlier, cannot be accommodated in the carbohydrate-binding site[21,24] of Con A) only those residues which possess free 3-, 4-, and 6-hydroxyl groups were placed in the binding site during the modelling procedure. While studying the interactions of glycan with Con A, either the terminal or the internal mannose residues were placed in the binding site. When fitting the terminal mannose residues, possible binding orientations were restricted to the allowed orientations for methyl α-D-mannopyranoside[21], and the binding orientations for the internal mannose residues were restricted to the allowed orientations of methyl 2-*O*-methyl-α-D-mannopyranoside[21] in the carbohydrate-binding site of Con A. The allowed orientations for the glycans of the oligomannosidic type in the binding site were determined by using stereochemical contact criteria[27]. Possible noncovalent interactions between the glycan and Con A were also determined, as previously described[10,22].

RESULTS AND DISCUSSION

(i) Conformation of the glycan GM7, containing seven D-*mannose residues*

The conformations that fall within 5 kcal.mol^{-1} of the minimum are shown in Table I. The chitobiose core generally favors one conformation, with (ϕ_{1C},ψ_{1C}) and (ϕ_{2C},ψ_{2C}) around (60°,20°) and (70°, −5°) respectively. Another set of conformational angles (−3°, −36°) is also possible, but is more than 3 kcal.mol^{-1} higher in energy compared to the minimum-energy conformation. This indicates that the chitobiose core is fairly rigid, in agreement with conclusions drawn earlier[1].

The α-Man-(1→2)-α-Man-(1→2)-Man trisaccharide fragment strongly favors a conformation with both (ϕ_1,ψ_1) and (ϕ_2,ψ_2) assuming values close to (−40°,−20°). These dihedral angles can also take values close to (20°,30°), but this increases the energy of the molecule by 2 kcal.mol^{-1} or more (not shown in Table I) and has little effect on the overall shape of the molecule. The α-(1→3) linkage which joins this trisaccharide to the core mannose residue (Man4) is also fairly rigid, and

TABLE I

MINIMIZED CONFORMATIONS[a] OF GM7

Conformer number	*Dihedral angles in degrees*																		*Relative energie in kcal mol*$^{-1}$
	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_{1C}	ψ_{1C}	ϕ_{2C}	ψ_{2C}	χ_5	ψ_5	ϕ_5	ψ_6	ϕ_6	χ_7	ψ_7	ϕ_7	
1	−44	−17	−46	−25	−48	−21	59	20	67	−4	−179	61	−57	−25	−49	−62	74	−49	0.0
2	−44	−17	−47	−25	−48	−21	70	17	71	−9	−176	64	−49	−24	−47	57	61	−53	1.2
3	−44	−17	−47	−25	−48	−21	56	17	65	−5	180	62	−57	35	22	−63	77	−50	1.3
4	−44	−17	−47	−25	−48	−21	70	18	72	−9	−174	67	−50	−24	−47	−174	70	−49	1 4
5	−43	−13	−40	−9	−38	−17	67	−7	58	−1	69	59	−39	−23	−50	−62	67	−50	2.6
6	−44	−17	−47	−25	24	29	51	16	61	−3	180	65	−56	−14	−43	−62	77	−50	2.7
7	−44	−17	−47	−25	−48	−21	−3	−36	58	−1	175	82	−56	−26	−53	−68	73	−54	3 2
8	−44	−17	−47	−25	−47	−21	57	16	66	−5	176	63	−58	39	9	−175	78	−49	3 6
9	−44	−17	−48	−25	−48	−21	56	20	64	−5	180	67	−57	35	22	56	66	−53	3 6
10	−43	−17	−45	−23	−44	−19	69	−7	58	−2	70	59	−42	−23	−51	−173	78	−47	3 8
11	−44	−17	−47	−25	24	29	70	17	71	−9	−176	64	−49	−24	−47	57	61	−53	3 8
12	−44	−17	−47	−25	24	29	56	17	65	−5	180	63	−56	35	22	−62	77	−50	3 9
13	−44	−17	−47	−25	24	29	70	18	72	−9	−174	67	−50	−24	−47	−174	69	−49	4.0
14	−44	−17	−48	−25	−47	−23	71	−8	58	−1	67	64	−43	−23	−51	58	64	−52	4.5
15	−44	−17	−47	−25	−48	−21	−3	−35	57	−1	171	82	−57	−23	−51	−172	77	−49	4 7

[a] (ϕ_1,ψ_1) and (ϕ_2,ψ_2) can also take values close to (20°,30°) This increases the energy of any particular conformation by about 2 kcal mol^{-1}, and hence are not separately given in the Table The possible conformations for GM6 and GM5 are similar to those given here and are, therefore, not shown separately

(ϕ_3,ψ_3) takes values close to (−50°,−20°), although values around (20°,30°) are also possible. Such a highly restricted rotation about the bonds involved in this linkage has been previously discussed for some of the *N*-acetyllactosaminic type of glycans[1]. Thus, the rigidity in the α-Man-(1→2)-α-Man-(1→2)-Man trisaccharide, and the rigidity of the α-(1→3) linkage, are extended into the rigid chitobiose core, making this complete fragment of the glycan fairly rigid.

The other α-(1→3) linkage is also extremely rigid, since, for those conformations which fall within 5 kcal.mol^{-1} of the minimum, (ϕ_3,ψ_3) is always close to (−50°,−20°).

Flexibility of GM7, therefore, seems to reside entirely in the two α-(1→6) linkages and, in particular, depends on the χ angles (χ_5 and χ_7). χ_5 strongly favors values of ~180°, although values of ~+60° are also possible. The outermost α-(1→6) linkage is more flexible, with χ_7 taking all three staggered orientations corresponding to values of −60°, +60°, and 180°. The values of other dihedral angles in the α-(1→6) linkage are also less variable. Values observed for ϕ_5 and ϕ_7 fall between −40° and −60°, and, for ψ_5 and ψ_7, fall in the range 60° to 80°.

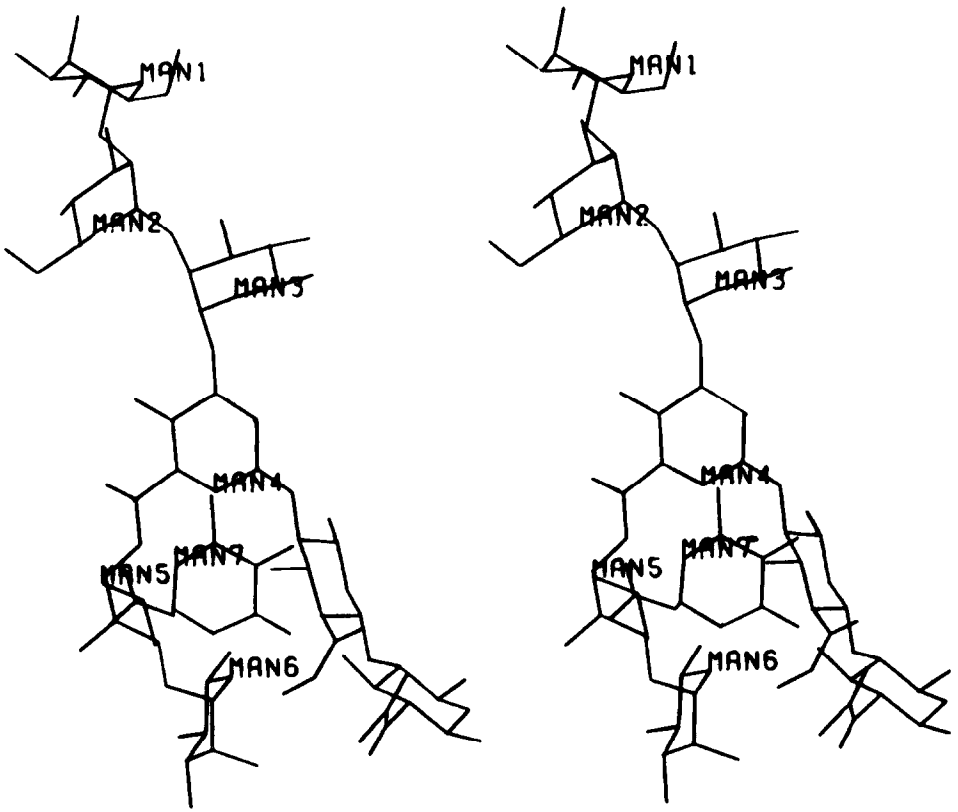

Fig 3 Stereoscopic projection of conformer 1 of GM7. This is the minimum-energy conformation for which X_5 is 180° and X_7 is −60°. In this conformation, the primary binding residue, Man7, cannot be placed in the sugar binding site of Con A.

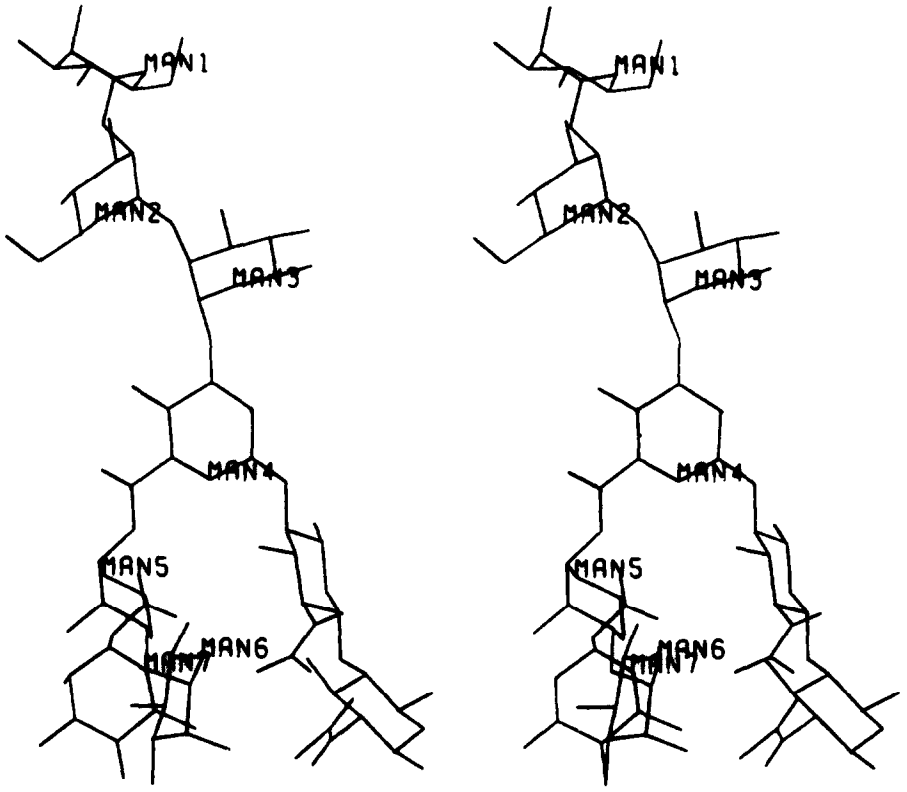

Fig. 4 Stereoscopic projection of conformer 2 of GM7 This conformer is similar to that of conformer 1, except that, in this case, X_7 is +60° The change in the orientation of Man7 can be clearly seen (*cf*, Fig 1) Such a change allows Man7 to reach the sugar binding site of Con A (see Fig 7)

Projections of some of the possible conformations are shown in Figs. 3, 4, and 5. In conformers 1 and 2 (Figs. 3 and 4), the branched α-(1→6) arm and the chitobiose core come close together. In conformer 1 ($\chi_5 = 80°$, $\chi_7 = -60°$) both Man6 and Man7 are near the core, whereas in conformer 2 ($\chi_5 = 180°$, $\chi_7 = +60°$) Man7 swings out a little away from the core. When $\chi_5 = +60°$ and $\chi_7 = -60°$, as in conformer 5 (Fig. 5), the branched α-(1→6) arm swings out, so that Man7 now lies close to the α-(1→2)-linked trisaccharide. However, even in this conformation, Man6 is close to the chitobiose core.

The possible conformations for the other two glycans (GM6 and GM5) are not reported separately, as they are in general very similar to those described for GM7, without (ϕ_1,ψ_1) in the case of GM6, and without (ϕ_1,ψ_1) and (ϕ_2,ψ_2) in the case of GM5 (see Table I).

The n.m.r. data provided by various workers[4,5] has invariably indicated that the residues in the branched α-(1→6) arm will be in close proximity to the chitobiose core, and the α-(1→2)-linked trisaccharide will be in an extended con-

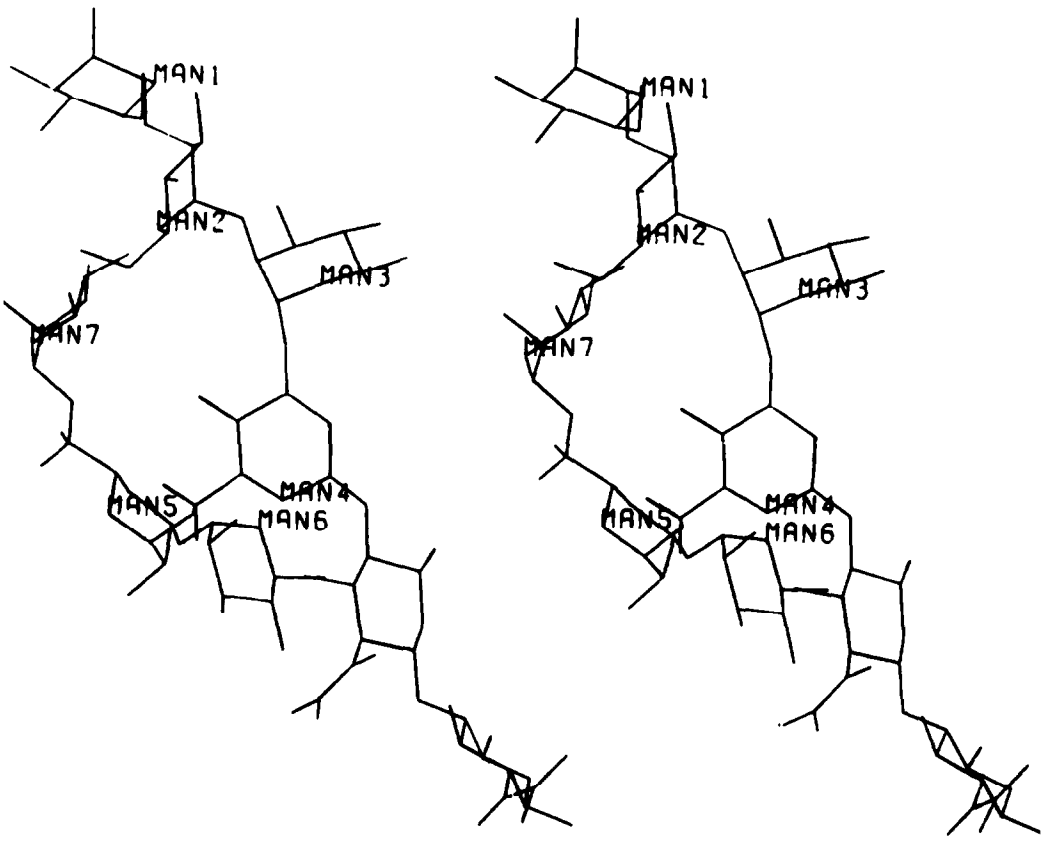

Fig. 5. Stereoscopic projection of conformer 5 of GM7. In this case, X_5 is +60° and X_7 is −60° The change in the dihedral angle, X_5, dramatically alters the orientation of the outer trimannosidic fragment

formation, in good agreement with the present data. The most likely values, shown in Table I, for the conformational angles agree well with the values given by n.m.r. spectroscopy coupled with h.s.e.a. calculations[5]. The value (30°, −44°) reported by Carver and Brisson[5] for ϕ_{2C},ψ_{2C} differs significantly from the one reported here. The value given by them[5] has never been observed for the crystalline state, and falls in the disallowed region of the steric maps. Furthermore, for χ_7, they[5] assigned the single value of 60° (ω^* = 180°), whereas the present study indicates that the terminal α-(1→6) linkage is flexible, and χ_7 can take all three of the staggered positions. χ_7 = 60° is, however, favored for the glycan to interact with Con A (as discussed later). Thus, from n.m.r.-spectral studies, the possibility of other likely conformations which differ in the values of one or two dihedral angles have not been explored. Such conformations may be important when considering the

*Our values for χ correspond to Carver's ω His values can be obtained by adding 120° to our χ values

TABLE II

INTERACTIONS OF GM7 WITH Con A WHEN Man1 IS PLACED IN THE BINDING SITE

Orientation of Man1 in the binding site (in degrees)			*Conformers of GM7 which can enter the binding site*	*Possible hydrogen bonds between GM7 and Con A*	
				Man1	*α-(1→2) arm*
Φ	*Θ*	*Ψ*			
270	160	120	1 to 4 and 6 to 15	O2···ND2(14) O3···COO(208) O4···N(99),COO(208) O5· NH1,NH2(228) O6···NH1,NH2(228)	Man2(O6)···OH(100)
280	160	120	1 to 4 and 6 to 15	O2 ND2(14) O3 COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6· NH1,NH2(228)	Man2(O6)· ·OH(100)
280	150	130	1 to 4 and 6 to 15	O2·· ND2(14) O3· COO(208) O4·· N(99),COO(208) O5·· NH1,NH2(228) O6·· NE(228),O(226)	—
290	140	130	1 to 4 and 6 to 15	O2 ND2(14) O3 · COO(208) O4· ·N(99),COO(208) O5 ·NH1,NH2(228) O6 ·NH1,NH2(228), O(226)	—
320	150	190	1 to 4 and 6 to 15	O2···ND2(14), NE, NH2(228) O3···COO(208) O4 · N(99),N(100), OD1(208) O6 O(226)	Man2(O3)·· NH2(228) Man2(O2)···OH(100)
330	150	200	1 to 4 and 6 to 15	O2· ·NE,NH2(228) O3···COO(208) O4 ·N(99), N(100), OD1(208) O6 ·O(226)	Man2(O6)···NH2(228)
310	80	290	1 to 15	O2 ··COO(208) O3···OD1,ND2(14), NE(228) O4· ·ND2(14) O5 N(99)	Man2(O6) ··NH2(228)
320	100	290	1 to 15	O2· OD1(208), N(228) O3···OD1,ND2(14), OD2(208) O4· ND2(14) O5·· N(99) O6·· N(100)	Man2(O6)· ·NH2(228)
340	100	290	1 to 4, 6 to 9, 11 to 15	O2· ·N(228) O3 OD1,ND2(14), OD2(208) O4·· ND2(14) O6·· N(99),N(100)	Man2(O6) ··NH1(228) Man2(O5)· NH2(228)

interactions of these glycans with Con A, as the glycan in solution may assume different conformers to different extents.

(ii) Computer modelling of Con A–GM7 complex

In GM7, there are three terminal mannose residues (Man1, Man6, and Man7) and two internal mannose residues (Man2 and Man3) which are likely candidates to reach the carbohydrate-binding site of Con A. Hence, each of these residues was placed, in turn, in the binding site in all of the conformations of GM7 which fall within 5 kcal.mol^{-1} of the minimum.

Binding of terminal mannose residues. — (a) Man1. Man1 of GM7 in almost all of the favored conformations can be placed in the carbohydrate-binding site of Con A (see Table II). The major exception is conformer 5 (see Table I), in which χ_5 and χ_7 are $+60°$ and $-60°$, respectively. In this particular conformation, the two α-(1→6) linkages bring Man7 close to the α-(1→2) arm (see Fig. 5) in such a way that Man7 gives rise to a number of severe steric contacts with Con A.

Possible orientations that the Man1 residue can assume in the binding site (see Table II) are typical of those previously reported for the nonreducing mannose residue in α-(1→2)-linked mannobiose[18]. The 2-, 3-, 4-, and 6-hydroxyl groups in Man1 form, in the binding site, hydrogen bonds with Con A. These interactions with the protein are very similar to those previously reported[21] for methyl α-D-mannopyranoside. An additional hydrogen bond is also possible with any one of the hydroxyl groups, OH-2, OH-3, or OH-6, of Man2, depending on the orientations of Man1 in the binding site (see Table II). Hydrogen bonds can also be formed with the 3- and 4-hydroxyl groups of Man3 in those conformations for which (ϕ_1,ψ_1)

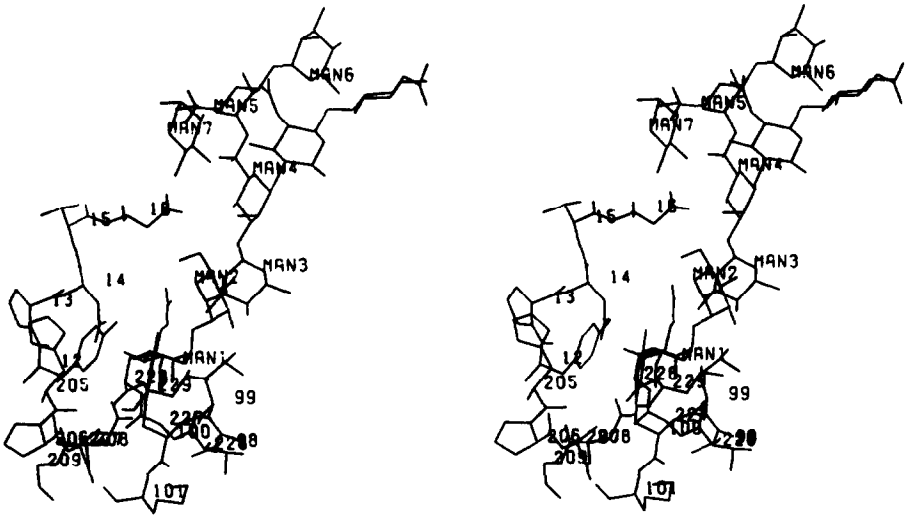

Fig 6 Stereoscopic projection of GM7 in its minimum-energy conformation, with Man1 placed in the binding site of Con A in one of the allowed orientations (Φ = 340, Θ = 100, and Ψ = 290°) Both the α-(1→6) arm and the chitobiose core are placed outwards from the binding site and away from the protein surface.

is close to (20°,30°). However such a conformation is ~2 kcal.mol^{-1} above the global minimum. A stereoscopic projection (see Fig. 6) shows GM7 in its minimum-energy conformation with Man1 placed, in one of the allowed orientations, in the Con A binding site. Both the α-(1→6) arm and the chitobiose core are placed outwards from the binding site, and away from the protein surface. The trimannosidic core (Man5, Man6, and Man7) in the minimum-energy conformation of GM7 is generally placed farther away from the protein surface than the chitobiose core. As the chitobiose core extends away from the protein, it appears that extension of this core through an *N*-glycosylic linkage may not affect the accessibility of Con A to Man1, as the peptide fragment would be farthest from the surface of the lectin. The possible interactions between Con A and GM7, through Man1, did not seem to be altered drastically when different conformers of GM7 were allowed to interact with Con A, as the overall shape of the glycan depends on χ_5, which is rather restricted. However, owing to some rotational freedom for Man1 in the binding site, some changes in the placements of the core and the trimannosidic core with respect to the protein are seen. It is interesting that, although there are in the glycan many sugar residues which could form hydrogen bonds with

TABLE III

INTERACTIONS OF GM7 WITH Con A WHEN Man7 IS PLACED IN THE BINDING SITE

Orientation of Man7 in the binding site (in degrees)			*Conformers of GM7 which can enter the binding site*	*Possible hydrogen bonds between GM7 and Con A*	
				Man7	*Trimannosidic core*
Φ	Θ	Ψ			
290	140	130	2, 9, 11, and 14	O2· ND2(14) O3···COO(208) O4···N(99),COO(208) O5· NH1(228) O6·· NH2(228),OH(226)	—
310	80	290	2, 11, and 14	O2 ·COO(208) O3 OD1,ND2(14),NE(228) O4· ND2(14) O5 N(99)	Man5(O2)·· NH1(228) Man5(O2)···NH2(228) Man6(O3)· ·OD1(16) Man6(O4)· OD2(16)
340	100	290	2, 11, and 14	O2 N(99) O3···N(288) O4· OD1,OD2(14),COO(208) O6 · COO(208)	Man5(O2) ·NH1(228) Man5(O5)···NH2(228) Man5(O6) ··NH2(228)
360	120	310	14	O3 ·N,NE(228) O4 ·OD1,ND2(14),COO(208) O5· ·N(99) O6· N(99),N(100)	Man5(O2) NH2(228) Man5(O3) OH(12) Man5(O5)···NH2(228) Man6(O2)· OH(12) Man6(O3)···O(13)

Con A, in most of the cases, only the immediate neighbor forms an additional hydrogen-bond with the protein, just as in the case[18] of the disaccharide α-Man-(1→2)-Man.

(b) Man6. In GM7, in all the conformations which fall within 5 kcal.mol^{-1} of the minimum (see Table I), the terminal mannosyl group (Man6) cannot enter the binding site of Con A in any of the orientations which are possible for methyl α-D-mannopyranoside[21]. Mainly, unfavorable steric contacts between the core residues and the protein hinder the binding of GM7 through this residue. This indicates that GM7 cannot bind to Con A through the terminal Man6 group.

(c) Man7. Man7 can enter the binding site in only some of the favored conformations of GM7. Conformers 2, 11, and 14 are the main ones which allow Man7 to enter the binding site (see Table III). A common feature of these three conformers is that χ_7 is +60°. χ_5 can be either 180° (conformers 2 and 11) or +60° (conformer 14). In the binding site, Man7 is more restricted than Man1 (see Tables II and III). Man7 forms hydrogen bonds with Con A which, in a way similar to that described for the binding of Man1, involve the 2-, 3-, 4-, and 6-hydroxyl groups. Hydrogen bonds with hydroxyl groups, one each from Man5 and Man6, are also possible with Con A (see Table III). In addition to the possibility of these hydrogen bonds, the two hydrogen atoms attached to the C-6 atom involved in the α-(1→6) linkage are placed close to the side chain of leucine 99, leading to possible hydrophobic interactions. Such hydrophobic interactions would further stabilize the binding of GM7 through Man7. The possibility of this kind of hydrophobic interaction has been invoked to account for the higher binding activity of methyl pyranosides to Con A when compared to the free sugars[21]. In fact, it has also been observed[28] that α-(1→6)-linked mannobiose is twice as active as α-(1→3)-mannobiose in binding to Con A. This may be due to the possibility of additional hydrophobic interactions in the α-(1→6)-mannobiose.

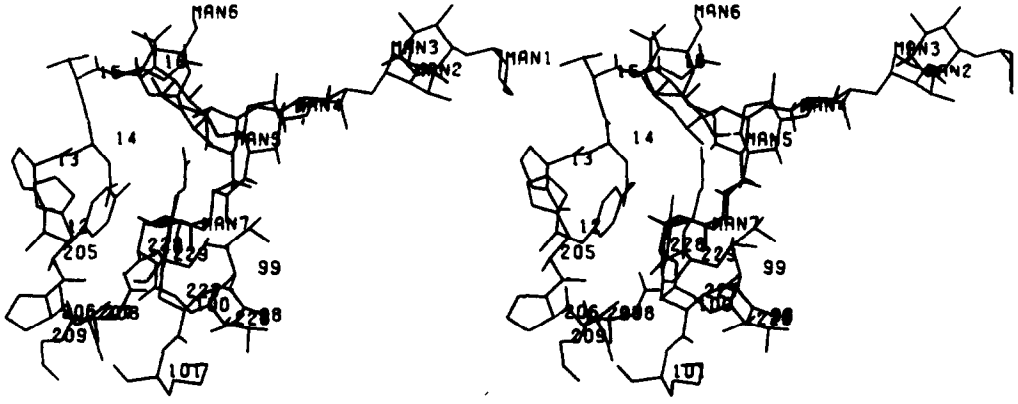

Fig 7 Stereoscopic projection of conformer 2 of GM7, with Man7 placed in the binding site of Con A in one of the allowed orientations (given in legend to Fig 6) Man6 is level with His (205), and the chitobiose core comes close to Tyr (100)

A stereoscopic projection (Fig. 7) shows GM7 in one of its minimum-energy conformers (conformer 2), with Man7 placed in the Con A binding-site in one of the allowed orientations. It may be seen that, in the trimannosidic core (Man5, Man6, and Man7), Man5 and Man6 are placed close to the surface of the protein. The chitobiose core is also placed near the surface of the protein. However, the α-(1→2) trisaccharide fragment is away from the protein surface. Interestingly, Man6 shows a great propensity to point towards the protein, and is situated at the same level as histidine 205. The chitobiose core is placed in the vicinity of tyrosine 100. Thus, a large part of the glycan is favorably placed close to the protein surface, and it appears that the glycan GM7 has greater complementarity to the sugar-binding site of Con A when Man7 is placed in the binding site.

Thus, of the three terminal D-mannosyl groups in GM7, computer-modelling experiments revealed that, constrained by the stereochemistry, only two of them (Man1 and Man7) can enter the Con A binding-site. However, it may be concluded that the glycan may interact the more favorably with Con A through Man7, in view of the possibility of additional hydrophobic interactions and the greater complementarity of the glycan to the protein. In such a case, the terminal α-(1→6) arm may form a highly favored binding-site on the glycan for Con A interaction.

Binding of the internal mannosyl residues. — Man2 and Man3. GM7, in only a few of the possible conformers, can place the internal mannosyl residue Man2 in the binding site in only a few orientations. However, this residue cannot be placed exactly as are the terminal mannosyl groups, and has to be moved outwards from the binding site by ~1 Å, which results in the loss of a possible hydrogen-bond between the O-2 atom of the mannosyl residue and the protein. When Man2 is placed in the binding site, the relative placement of the glycan with respect to the protein is very similar to the case wherein Man2 of GM6 is placed in the binding site (see the following section). Man3 in GM7, on the other hand, cannot reach the binding site in any of the conformers which fall within 5 kcal.mol^{-1} of the global minimum, suggesting that this residue may not be a site of interaction for Con A.

(iii) Computer modelling of complexes of Con A with the glycans GM6 and GM5

In both GM6 and GM5, there are three terminal mannosyl groups, and in GM6, there is also one internal mannosyl residue; these are possible candidates to interact with the carbohydrate-binding site of Con A.

Man6, in both GM6 and GM5, also cannot reach the binding site, as has already been discussed in the case of GM7. On the other hand, the terminal mannosyl group Man7 can reach the binding site in conformers 2, 11, and 14 (see Table I), as in GM7. The freedom of rotation for Man7 of GM6, and the nature of hydrogen bonds with the protein that are possible are also similar to the case of Man7 of GM7 This means that the hydrogen atoms attached to the C-6 atom in the α-(1→6) linkage are involved in the hydrophobic interactions with leucine 99, and the hydroxyl groups OH-2, OH-3, and OH-4 of Man7 may also be involved in the

TABLE IV

INTERACTIONS OF GM6 WITH Con A WHEN Man2 IS PLACED IN THE BINDING SITE

Orientation of Man2 in the binding site (in degrees)			*Conformers of GM6 which can enter the binding site*	*Possible hydrogen bonds between GM6 and Con A*	
				Man2	*α-(1→2) arm*
Φ	*Θ*	*Ψ*			
270	160	120	1 to 15	O2···ND2(14) O3···COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NH1,NH2(228)	Man3(O6)···OH(100)
280	160	120	1 to 4 and 6 to 15	O2···ND2(14) O3· ·COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NH1,NH2(228)	Man3(O6)···OH(100)
280	150	130	1 to 4 and 6 to 15	O2···ND2(14) O3 ··COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NE(228),O(226)	—
290	140	130	1 to 4 and 6 to 15	O2···ND2(14) O3···COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NH1,NH2(228),O(226)	—
320	150	190	1 to 15	O2···ND2(14),NE,NH2(228) O3···COO(208) O4·· N(99),N(100),OD1(208) O6···O(226)	Man3(O3)···NH2(228)
330	150	200	1 to 15	O2···NE,NH2(228) O3···COO(208) O4···N(99),N(100),OD1(208) O6· O(226)	Man3(O6)···NH2(228)
310	80	290	1 to 4, 6 to 9 and 11 to 15	O2···COO(208) O3···OD1,ND2(14),NE(228) O4···ND2(14) O5·· N(99)	Man3(O6)···NH2(228)
320	100	290	1 to 4, 6 to 9, 11 to 13 and 15	O2·· OD1(208),N(228) O3···OD1,ND2(14),OD2(208) O4 ··ND2(14) O5···N(99) O6 ··N(100)	Man3(O6)···NH2(228)
340	100	290	1 to 4, 6 to 9, and 11 to 15	O2···N(228) O3· OD1,ND2(14),OD2(208) O4 ··ND2(14) O6···N(99),N(100)	Man3(O6)···NH1(228) Man3(O5)·· NH2(228)

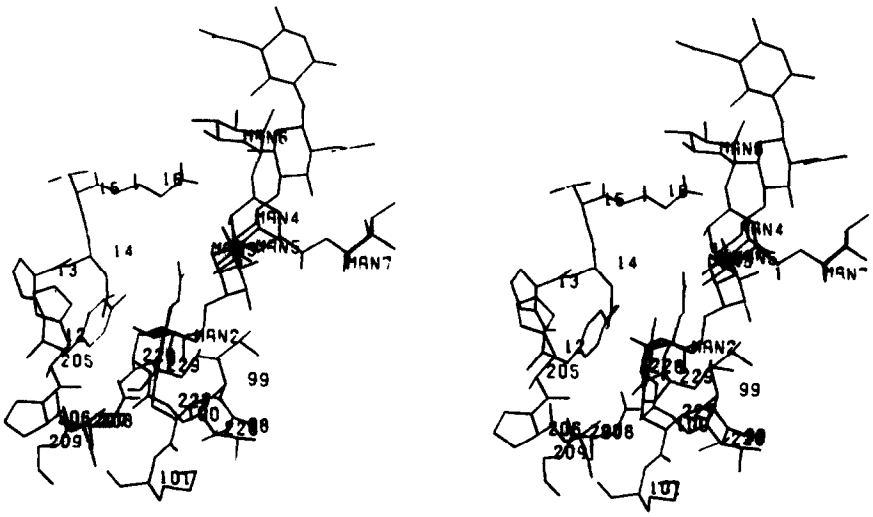

Fig 8 Stereoscopic projection of GM6 in its minimum-energy conformation, with Man2 placed in the binding site of Con A in one of the allowed orientations (given in legend to Fig 6) Both the α-(1→6) arm and the chitobiose core are placed outwards from the binding site, away from the protein

formation of hydrogen bonds with Con A (see Fig. 7). This indicates that the length of the α-(1→2) arm has very little effect on the accessibility of Con A to Man6 and Man7 of these glycans.

In GM6, Man2 can enter the binding site in almost all of the favored conformations. The possible hydrogen-bonding scheme is similar to that of methyl α-D-mannopyranosides[21]. A stereoscopic projection (see Fig. 8) shows GM6 in its minimum-energy conformation (similar to conformer 1 of GM7) with Man2 placed in the sugar-binding site of Con A in one of the allowed orientations. It may be seen that both the α-(1→6) arm and the core are placed outwards from the binding site and away from the protein surface. When Man2 of GM6 is placed in the binding site, the fragment α-Man2-(1→2)-Man3 is placed similarly to α-Man1-(1→2)-Man2 of GM7. However, the trimannosidic core (Man5, Man6, and Man7) and the chitobiose fragment are placed differently in each (see Figs. 6 and 8). In GM5, Man3 can enter the binding site of Con A in several of the allowed conformations. Again, the binding orientations and the hydrogen-bonding scheme are similar to those described for methyl α-D-mannopyranoside[21] Additional hydrogen-bonds are possible, mainly with Man4, the residue to which Man3 (placed in the binding site) is attached (see Table V). A stereoscopic projection of GM5 with Man3 in the binding site is shown (see Fig. 9) in one of the possible orientations. When Man3 is placed in the binding site, the glycan is situated differently with respect to the protein than in the previous cases. Man6 and Man7 are downward in the projection, and Man7 is placed close to aspartic acid 16.

Thus, the removal of mannose residues in the α-(1→2)-linked trisaccharide fragment (going from GM7 to GM5 *via* GM6) does not alter the accessibility of the

TABLE V

INTERACTIONS OF GM5 WITH Con A WHEN Man3 IS PLACED IN THE BINDING SITE

Orientation of Man3 in the binding site (in degrees)			*Conformers of GM5 which can enter the binding site*	*Possible hydrogen bonds between GM5 and Con A*	
				Man3	*Man4 and trimannosidic core*
Φ	Θ	Ψ			
270	160	120	1 to 4 and 6 to 15	O2···ND2(14) O3···COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NH1,NH2(228)	—
280	160	120	1 to 4, 7 to 10 and 13 and 14 6, 11 and 12	O2···ND2(14) O3···COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NH1,NH2(228)	— Man4(O4)···OH(100) Man4(O6)···OH(100)
280	150	130	1 to 3, 7, 9, 10, 14 and 15 4 and 8 6, 11, 12 and 13	O2···ND2(14) O3···COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NE(228),O(226)	— Man7(O3)···OH(100) Man4(O4)···OH(100)
290	140	130	1 to 4, 6, 7 and 9 to 14 8	O2···ND2(14) O3···COO(208) O4···N(99),COO(208) O5···NH1,NH2(228) O6···NH1,NH2(228),O(226)	— Man7(O3)···OH(100)
320	150	190	1 to 9, and 11 to 15	O2···ND2(14),NE,NH2(228) O3···COO(208) O4···N(99),N(100),OD1(208) O6···O(226)	Man4(O4)···NH2(228)
330	150	200	1 to 4, 6 to 9 and 11 to 15	O2···NE,NH2(228) O3···COO(208) O4···N(99),N(100),OD1(208) O6 ··O(226)	— Man4(O4)···NH2(228)
310	80	290	1 to 4, 7 to 10 and 15 6 and 11 to 13	O2· ·COO(208) O3···OD1,ND2(14),NE(228) O4 ··ND2(14) O5···N(99)	Man4(O4)···NH2(228)
320	100	290	1 to 4, 7 to 9 and 14 and 15 6 and 11 to 13 10	O2···OD1(208),N(228) O3· ·OD1,ND2(14),OD2(208) O4···ND2(14) O5···N(99) O6···N(100)	— Man4(O4)···NH2(228) Man6(O2)···OD2(16)

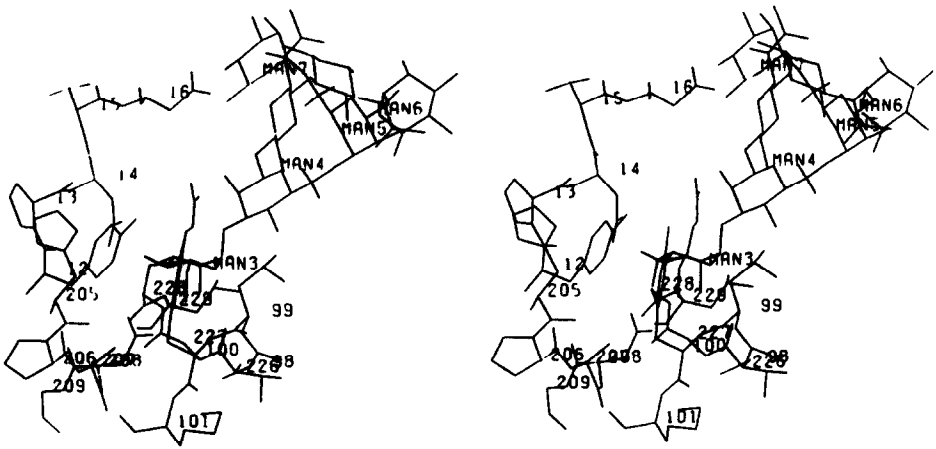

Fig 9 Stereoscopic projection of GM5 in its minimum-energy conformation, with Man3 placed in the binding site of Con A in one of the allowed orientations (given in legend to Fig 6) Man7 lies close to Asp (16), while the chitobiose core and Man6 are away from the protein surface

terminal residue on this fragment, although the placement of the rest of the glycan with respect to the protein varies considerably. Of the two possible binding-sites on these glycans, the terminal α-(1→6)-linked mannose (Man7) forms the primary binding-site for Con A, due to the additional possibility of hydrophobic interactions (as already discussed for GM7). The other mannose residue, Man2 in GM6 and Man3 in GM5, will be a secondary binding-site for Con A. This is consistent with the recent report of Brewer and Bhattacharyya[8], who arrived at the same conclusions from quantitative, precipitation analysis.

Binding of the internal mannose residue. — Man3. GM6 can place the internal mannose residue (Man3) in the binding site in only a few orientations for which the position for the center of the sugar residue is moved outwards from the binding site by just less than 1 Å from the position which is possible for the terminal mannosyl group. These orientations are the same as those described for the internal mannose (Man2) in GM7, which enters the binding site and, in this case also, results in the loss of the hydrogen bond between the 2-hydroxyl group of Man3 and the protein. The minimum-energy conformations of GM6 do not allow binding in this mode, but values for (ϕ_3,ψ_3) of around $(-40°,-60°)$, which increase the energy of the glycan by 3 kcal.mol^{-1} or more, allow Man3 to reach the binding site

(iv) Complexes of Con A with GM3

In GM3, there are only two terminal mannosyl groups, Man3 and Man5 (see Fig. 1), which may possibly interact with Con A. The possible modes of binding of this glycan to Con A have been described[10]. Man3 of GM3 can enter the binding site of Con A in many of the allowed conformations of GM3. The hydrogen-bond scheme and possible binding orientations are similar to those of Man1 of GM7, Man2 of GM6, and Man3 of GM5. Man5 of GM3 can enter the binding site in

orientations similar to those of Man7 of GM7, GM6, and GM5. The hydrogen-bonding scheme when this residue is placed in the binding site has been described[10], and it is similar to Man7 of the other glycans. In this case also, the hydrogen atoms attached to the C-6 atom involved in the linkage may be engaged in hydrophobic interactions with the side chain of leucine 99. Thus, the two terminal groups, Man5 and Man3, of GM3 are the primary and secondary binding sites for Con A in a way similar to the two terminal groups of the other glycans that interact with Con A, namely, in GM7, Man7 and Man1, in GM6, Man7 and Man2, and, in GM5, Man7 and Man3.

CONCLUSIONS

Our modelling data clearly show that it is not the number of mannosyl residues in the glycan that determines its binding properties but the accessibility of the mannosyl residues to the binding site of Con A. This is dependent on the conformation of the glycan, and is beautifully illustrated here, where, although GM7, GM6, and GM5 have three terminal mannosyl groups, only two of these can be placed in the binding site of Con A. Of these two, the terminal α-(1→6)-Man (Man7) may bind more strongly to Con A because, in addition to the hydrogen bonds, there is a possibility of hydrophobic interactions in this mode of binding. This group may, therefore, be the primary binding-site on the glycan.

The other terminal mannosyl group that interacts with Con A will be the less favored, or weak, site of attachment for Con A. These results are in general agreement with those of Brewer and Bhattacharyya[8], but differ from those of Carver *et al.*[5,23], who considered the terminal α-(1→6)-linked mannose as the only possible binding site for Con A.

Previous studies indicated that the carbohydrate-binding site of Con A is small, accommodating for the most part a single sugar residue. However, specificity of Con A for a particular glycan structure is dependent on the accessibility of the mannosyl residues, in the glycan, that have free hydroxyl groups at C-3, C-4, and C-6 of the glycan, but is independent of the total number of mannosyl residues. It also depends on the noncovalent interactions possible between the glycan and Con A. Generally, the amino acids 12, 14, 16, 99, 100, 208, 226, and 228 interact with the mannosyl residues in the binding site.

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REFERENCES

1 M Biswas, Y. C Sekharudu, and V S R Rao, *Int. J. Biol Macromol*, 8 (1986) 2–8
2 J R. Brisson and J. P Carver, *Can J Biochem. Cell Biol*, 61 (1983) 1067–1078

3 J DEISENHOFER, *Biochemistry*, 20 (1981) 2361–2369.
4 J MONTREUIL, *Pure Appl Chem.*, 56 (1984) 859–877
5 J. P. CARVER AND J R. BRISSON, in V GINSBERG AND P. ROBBINS (Eds), *Biology of Carbohydrates*, Wiley, New York, 1984, Ch 5
6 H. BITTIGER AND H. P SCHENBLI, *Concanavalin as a Tool*, Wiley, New York, 1976
7 J. U. BAENZIGER AND D. FIETE, *J Biol Chem.*, 250 (1979) 2400–2407.
8 C F. BREWER AND L BHATTACHARYYA, *J. Biol Chem* , 261 (1986) 7306–7310
9 A. V LANDSCHOOT, F. G LOONTIENS, AND C K. DE BRUYNE, *Eur J Biochem* , 83 (1978) 277–285
10 Y C. SEKHARUDU, M BISWAS, AND V S R RAO, *Int J Biol Macromol.*, 8 (1986) 9–19
11 J. F. STODDART, *Stereochemistry of Carbohydrates*, Wiley, New York, 1971, p 50
12 S. ARNOTT AND W. E SCOTT, *J Chem Soc , Perkin Trans 2*, (1972) 324–335
13 R B COREY AND L PAULING, *Proc R Soc London, Ser. B*, 141 (1953) 10–20
14 N YATHINDRA AND V S R RAO, *Biopolymers*, 9 (1970) 783–790
15 F A. MOMANY, R F MCGURIE, A W BURGESS, AND H A SCHERAGA, *J. Phys Chem.*, 79 (1975) 2361–2381
16 N YATHINDRA AND V S R RAO, *Carbohydr Res* , 25 (1972) 256–260.
17 S PRAKASH, Ph D Thesis, Indian Institute of Science, Bangalore, India, 1980
18 Y C SEKHARUDU AND V S R. RAO, *J Biosci* , 8 (1985) 389–401
19 R FLETCHER AND M J D POWELL, *Comput. J.*, 6 (1963) 163–168.
20 K. D HARDMAN, R C. AGRAWAL, AND M. J FREISER, *J Mol. Biol.*, 157 (1982) 69–86
21 Y C SEKHARUDU AND V S R RAO, *Int J Biol. Macromol* , 6 (1984) 337–347
22 Y C SEKHARUDU AND V S R RAO, *Curr Sci* , 53 (1984) 403–406.
23 J P CARVER, A. E MACKENZIE, AND K. D HARDMAN, *Biopolymers*, 24 (1985) 49–63.
24 Y C SEKHARUDU AND V. S. R RAO, *J Biomol. Struct Dyn* , 2 (1984) 41–54
25 G N. REEKE, JR , J W BECKER, AND G. M EDELMAN, *J Biol Chem* , 250 (1975) 1525–1547
26 R. D. PORETZ AND I J GOLDSTEIN, *Biochemistry*, 9 (1970) 2890–2896
27 G N RAMACHANDRAN AND V SASISEKHARAN, *Adv Protein Chem.*, 23 (1968) 283–437
28 I J GOLDSTEIN AND C. E HAYES, *Adv Carbohydr Chem. Biochem* , 35 (1978) 127–340.