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Three-Dimensionnal Structures of Complexes of *Lathyrus ochrus* Isolectin I With Glucose and Mannose: Fine Specificity of the Monosaccharide-Binding Site

Yves Bourne,[1] Alain Roussel,[1] Michel Frey,[1] Pierre Rougé,[2] Juan-C Fontecilla-Camps,[1] and Christian Cambillau[1]

[1]Laboratoire de Cristallographie et de Cristallisation des Macromolécules Biologiques, Faculté de Médecine Secteur-Nord, Bd Pierre Dramard, 13 326 Marseille Cedex 15, France, and [2]Laboratoire de Biologie Cellulaire, Faculté des Sciences Pharmaceutiques, Université Paul Sabatier, 35 chemin des Maraichers, 31 062 Toulouse, France

ABSTRACT **The structure of the methyl-α-D-mannopyranoside–LOL I complex has been solved by the molecular replacement method using the refined saccharide-free LOL I coordinates as starting model. The methyl-α-D-mannopyranoside–LOL I complex was refined by simulated annealing using the program X-PLOR. The final *R*-factor value is 0.182 [$F_o > 1\sigma(F_o)$]. The isostructural methyl-α-D-glucopyranoside–LOL I complex was refined by X-Ray coupled energy minimization using the methyl-α-D-mannopyranoside–LOL I structure as a starting model to an *R* factor of 0.179 (all data). In both crystal forms, each dimer binds two molecules of sugar in pockets found near the calcium ions. The two saccharide moieties, which are in the C1 chair conformation, establish the same hydrogen bond pattern with the lectin. However, the van der Waals contacts are different between the O2, C2, C6, and O6 atoms of the two molecules and the backbone atoms of residues 208–211. Mannose, due to its axial C2 conformation, encloses the backbone atoms of the protein in a clamplike way. Van der Waals energy calculations suggest that this better complementarity of the mannoside molecule with the lectin could explain its higher affinity for isolectin I.**

Key words: lectins, crystal structure, lectin specificity, mannose, glucose

INTRODUCTION

Lectins are carbohydrate-binding proteins which have been found in a wide range of species.[1] Despite the large amount of information available on lectin sequence and specificity, relatively little is known about their biological signficance in the plant kingdom. However, the abundance of plant lectins and the variety of carbohydrate specificities found among them have led to their widespread use for such purposes as the isolation and analysis of complex carbohydrates, cell separation, and studies of cell surface architecture.[2] These applications have in turn stimulated more work on the carbohydrate specificities of the lectins.

The isolectin I (LOL I) isolated from the seeds of *Lathyrus ochrus*, which is the fourth lectin containing genus of the Vicieae tribe, is a lectin specific to mannose and glucose. LOL I consists of two subunits composed of one heavy (β) and one light (α) chain. The β and α chains contain 181 and 52 amino acid residues, respectively, and the molecular weight is about 52,000 for the whole dimer. LOL I has a higher affinity for mannose or methyl-α-D-mannose than for glucose.[3] Lectins belonging to the same family, Con A,[4] pea lectin,[5] and Favin,[6] whose crystallographic structures have been determined, are also mannose/glucose specific. They reveal slight but significant differences in the dissociation constant value as compared to LOL I.[3] In the case of Favin,[6] the authors have confirmed the presence of glucose in the monosaccharide-binding site, but no details are given on the complex structure in general. More recently, the crystallization of pea lectin with a trimannoside had been reported[7] but details on the structure have not been yet communicated. Recently the X-ray structure of a methyl-α-D-mannopyranoside–Con A complex has been determined at 2.9 Å resolution.[8] We present here the X-ray structure determination of methyl-α-D-mannopyranoside–LOL I and methyl-α-D-glucopyranoside–

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Address reprint requests to Dr. Christian Cambillau, Laboratoire de Cristallographie et de Cristallisation des Macromolécules Biologiques, Faculté de Médecine Secteur-Nord, Bd. Pierre Dramard 13 326 Marseille Cedex 15, France.

Abbreviations used: Isolectin I, LOL I; concanavalin A, Con A; methyl-α-D-mannopyranoside–LOL I complex, LOLM; methyl-α-D-glucopyranoside–LOL I complex; LOLG; simulated annealing, SA; rms, root mean square.

TABLE I. X-Ray Data Collection Statistics

	LOL I—α-Met-Man	LOL I—α-Met-Glu
Number of measurements	138,159	77,034
Number of unique measurements	55,032	33,640
Percentage of total	79.5% at 1.96 Å	67.0% at 2.2 Å
R-merge (all measurements)	8.1	8.2
R-merge (after rejection)	5.6*,†	6.1‡,§

$$*R = \sum_{h}^{M}\sum_{h}^{N_h} |I_{ih} - \langle I\rangle_h| \Big/ \sum_{h}^{M}\sum_{h}^{N_h} \langle I\rangle_h$$

where N_h = number symmetry-related reflections and M = total number of equivalent hkl sets.
†After rejection for reflection with $(I_{ih} - \langle I\rangle_h) / \sigma(I_{ih}) \geq 4.6$.

$$‡\text{R-merge} = \sum^{N}\left[\sum^{n(r)}|I(i,r) - \langle I\rangle(r)|\right] \Big/ \sum^{N}[n(r)*\langle I\rangle(r)]$$

where N = number of unique reflection, $n(r)$ = number of multiple measurements for the rth reflection with the mean value $\langle I\rangle$ (r).
§Rejection ratio 0.4.

LOL I complexes at 2.0 and 2.2 Å resolution, respectively, and describe the fine specificity of the monosaccharide-binding site of this two-chain lectin.

MATERIALS AND METHODS

Crystallization and X-ray Data Collection

The complex of LOL I with methyl α-D-mannopyranoside

The crystallization of the LOLM complex has been described previously.[9] Crystals belong to the space group $P2_1$ with unit cell dimensions $a = 56.3$ Å, $b = 139.8$ Å, $c = 62.7$ Å, and $\beta = 91°$. There are two functional entities, a dimer, per asymmetric unit ($V_m = 2.07$ Å^3/D). To avoid crystal slippage in the glass capillary due to the weak surface tension of the MPD solution, crystals were transferred to an artificial mother liquor containing 15% PEG-6000 and 15% MPD, instead of 30% MPD, which was the original concentration in the crystallization experiment. Diffraction intensities were collected on a Xentronics/Nicolet area detector with monochromated Cu$K\alpha$/radiation from a Rigaku RU200 rotating anode X-ray generator. The data were collected by rotation around the omega axis in frames of 0.25° width, at $2\theta = 28°$, corresponding to a 2.0 Å resolution, with a crystal-to-detector distance of 160 mm. Only one crystal was used to collect the entire data set which took a total of 3 days with the generator operating at 3.2kW. The data were reduced with the XENGEN software system.[10] The statistics on the integrates intensities are indicated in Table I.

The complex of LOL I with methyl α-D-glucopyranoside

Isostructural crystals of an LOLG complex were grown under conditions similar to those of the methyl-α-D-mannopyranoside–LOL I complex.[9] Diffraction intensities were collected on film at the LURE-DCI synchrotron radiation facility in Orsay with an Arndt-Wonacott oscillation camera. The wavelength was set to approximately 1.47 Å. The crystal was maintained at 4°C during data collection. A data set extending to 2.2 Å resolution was obtained from a single crystal covering a total rotation range of 180° about the b axis, with 3° oscillations for each exposure. Films were digitalized on an Optronic P1000 densitometer at an OD range of 3.

Film integration was performed with a modified version of the program OSC.[11] Further data reduction was carried out with the PROTEIN program.[12] Statistics on integrated intensities are indicated in Table I.

Structure Determination

Methyl α-D-mannopyranoside–LOL I complex

To solve the structure we used the molecular replacement method (MERLOT[13]) using the LOL I saccharide-free atomic coordinates as the initial model. This structure, which has been solved in the laboratory,[14] belongs to a different crystal form: cell dimensions $a = 135.84$ Å, $b = 63.12$ Å, and $c = 54.54$ Å, space group $P2_12_12$. This model has been refined at 1.9 Å resolution; the final R factor is 0.185 for data with $F_o > 1\sigma(F_o)$.

A self rotation function was calculated in the 8.0 to 5.0 Å resolution range. Solutions for the two local 2-fold axes were obtained at $\psi = 76°$, $\varphi = 90°$, $\kappa = 180°$ and $\psi = 84°$, $\varphi = 120°$, $\kappa = 180°$. The first solution indicated that one of the two dimers occupied a position similar to the one in the saccharide-free crystal form. This was not surpringsly since the comparison of the saccharide-free–LOL I and saccharide–LOL I complex unit cell dimensions suggested that the packing of LOL I in the two crystal forms could be very similar.

To avoid a possible solution of the cross-rotation

function having special values for the α, β, and γ Eulerian angles, the starting dimer was rotated arbitrarly around the three Eulerian angles and placed it at the origin of a triclinic cell of dimensions $a=b=c=120$ Å, $\alpha=\beta=\gamma=90°$. Structure factors calculated from the model were used in conjunction with the observed ones in the 6.0 to 4.0 Å resolution range. Four intense peaks (5.3 standard deviations above the mean) were observed in the cross-rotation function, sampled at 5° steps. To correlate each of these peaks with the internal symmetry of each dimer, we calculated the symmetry relationships between the four solutions in polar angles and compared them to the results of the self-rotation function.

As a result, two solutions of the cross rotation function were obtained after refinement with a 1° step increments at $\alpha=109.28°$, $\beta=70.65°$, $\gamma=302.50°$ and $\alpha=69.35°$, $\beta=84.85°$, $\gamma=131.25°$, for each dimer, respectively.

The two dimers, oriented according to the results of the rotation function, were submitted to a translation function search in the 6.0 to 4.0 Å resolution range. Two high peaks (4.1 standard deviations above the mean) were found in the P(u,1/2,w) Harker section, giving the positions for x and z for the two dimers. A translation function subsequently calculated in the asymmetric unit allowed us to determine the cross-vectors between the two dimers determining their positions along the monoclinic b axis, and confirmed the previously found x and z coordinates. A three-dimensional rigid-body refinement of this solution in the 10.0 to 6.0 Å resolution range fitted precisely the two dimers in the monoclinic cell giving an R factor of 0.36. Visualization of the resulting crystal packing on a graphics display, with the TOM/FRODO[15,16] option CRYSTallize,[17] showed reasonably good contacts between symmetry-related units. It confirmed also a solution in which one of the dimers has a orientation similar to the ones in the saccharide-free orthorhombic structure.

Structure Refinement

Methyl α-D-mannopyranoside–LOL I complex

The two LOL I dimers including the metals ions and the well-defined 220 water molecules of the saccharide-free crystal form were positioned in the $P2$ unit cell with the approach indicated above. The water molecules which occupied the two monosaccharide-binding sites of the saccharide-free crystal form were removed. Solvent molecules that established bad contacts with the symmetry-related molecules were also removed. An overall temperature factor of 15 Å^2 was assigned to all nonhydrogen atoms. SA refinement of the model was carried out using the program X-PLOR.[18] Application of 150 energy minimization steps in the 8.0 to 2.2 Å resolution range resulted in a decrease in the R factor from 0.36 to 0.235. Individual temperature factors were subsequently calculated and refined at the end of the following stages.

Both electron density maps with coefficients $[2F_o-F_c]$ and difference maps calculated using phases from the protein model clearly showed the position of the four methyl-α-D-mannopyranoside molecules. Those water molecules originating from the initial model that were not found in the electron density were removed and some new solvent molecules were added. The D-mannopyranoside coordinates were obtained from the crystallographic structure of the capsular polysaccharide (entry 1CAP[19]) of the Protein Data Bank[20] and were fitted to the difference electron density using the positions of the axial and equatorial ring substituents as major criteria. The methyl group of the O1 atom was added and fitted to this electron density map on each of the monosaccharide molecules. The methyl-α-D-mannopyranoside was introduced in the X-PLOR topology and parameter files. This model was then subjected to two successive SA stages at 2.8 and 2.0 Å resolution. The resulting R factor is 0.19 with $F_o > 1\sigma(F_o)$. The electron density maps calculated with phases obtained from this model clearly showed the four methyl-α-D-mannopyranoside molecules in the C1 chair conformation and the detailed interactions of the sugars with the lectin molecules. A final energy minimization of 300 cycles optimized the stereochemistry of the previous model and converged to an R factor of 0.182 with $F_o > 1\sigma(F_o)$. The final model includes 908 residues, four methyl-α-D-mannopyranoside molecules and 300 solvent molecules. The root mean square deviation from bond distances and angles are equal to 0.0025 Å and 3.0°, respectively. Luzzati plots[21] imply that the mean positionnal coordinate error is between 0.20 and 0.25 Å.

Methyl α-D-glucopyranoside–LOL I complex

Structure factors calculated from the refined LOLM complex and scaled against the observed data of the isostructural LOLG complex gave an R factor of 0.24 (all data) in the 8.0 to 2.2 Å resolution range. After 200 cycles of energy minimization, the R factor value was reduced to 0.18. The resulting electron density map clearly showed the position of the four glucose molecules and the equatorial position of the O2 atoms. At this stage, the four methyl-α-D-mannopyranoside molecules were substitued by four methyl-α-D-glucopyranoside molecules from entry 1CAP[19] of the Protein Data Bank[20] and were fitted interactively to the electron density map.

A final energy minimization procedure of 300 cycles gave an R factor of 0.179 (all data) including refinement of individual temperature factors. This final model includes 908 residues, 4 methyl-α-D-glucopyranoside molecules, and 300 water molecules. The root mean square deviation from bond distances and angles are of 0.0025 Å and 3.0°, respectively.

Luzzati plots[21] imply that the mean positional coordinate error is between 0.25 and 0.30 Å.

RESULTS

Crystal Packing Comparison to the Saccharide-Free LOL I Crystal Form

As mentioned above, the close similarities of saccharide-free–LOL I and saccharide–LOL I unit cell dimensions suggested that LOL I might have the same location in both unit cells. In fact, only one of the two dimers present in the saccharide–LOL I asymmetric unit is positioned in a way similar to that of the saccharide-free crystal form. The analysis of the residues involved in the protein–protein interactions shows that quite a few of them are different. Among these contacts, there is the first intermolecular stacking that has been detected in LOL I crystals [Arg-38 (β) and Tyr-25 (β)]. Thirty-nine percent of the residues which participate to the saccharide-free–LOL I crystal packing are also involved in the protein–protein interactions in the saccharide–LOL I complexes. As opposed to the case of the methyl-α-D-mannopyranoside–Con A complex, where sugar–water contacts are essential for the crystal packing, the stability of the crystals of both LOLM and LOLG complexes does not seem to depend directly on the presence of mannoside or glucoside. Only the C7 and O1 atoms of one saccharide molecule (see S1 in Table III) participates in van der Waals contacts with an aspartic acid and a proline residues of a symmetry-related lectin.

Overall Structure Description

Comparison of the backbone atoms of the two saccharides–LOL I complexes shows that they are quite similar. The rms deviations between them is 0.23 Å.

When the two complex structures are compared to that of the saccharide-free-LOL I, the rms deviation between backbone atoms is 0.37 and 0.32 Å for the two dimers, respectively. These values are smaller (0.28 Å) when the four monomers are considered separately. Therefore, the differences are probably due to a flexible dimer–dimer interaction. Figure 1a–d shows the mean deviation for main-chain atoms between the saccharide-free–LOL I model and the LOLM model, on one hand, and the LOLG model on the other. The most substantial positional differences are localized in the segments corresponding to residues 208–211 in the four monomers and residues 27–30 in the C monomer. The latter corresponds to a disordered region, which has been extensively rebuilt in the refined saccharide-free–LOL I structure[14] and in pea lectin.[5] As in the case of the saccharide-free–LOL I structure, no clear electron density appears beyond Gly-49 for the four C-terminal residues of the four α-chains.

The refined average temperature factors for main-chain and side-chain atoms of the LOLM complex are 18.3 and 22.2 Å^2, respectively. In the case of the LOLG complex these values are 26.4 and 29.0 Å^2, respectively. Figure 2a–d displays the refined temperature factor (B) values of the LOLM and LOLG complexes for main-chain atoms. It is apparent from these plots that the B factors are comparable for the four monomers with different values in a few equivalent regions, which is due to the specific environments in the crystal lattice. These areas include residues 27–30, 55, 77, and residues belonging to the C-terminal of the β-chain, which are also disordered in the saccharide-free–LOL I crystal form. The averaged B factor for the four mannoside molecules is 21.6 Å^2, which is of the same order of magnitude as the values observed for the side-chain atoms of the lectin. On the other hand, the four glucoside molecules have an average B value of 34.0 Å^2, slightly higher than the side-chain value of LOLG. This may indicate a higher mobility of glucose moiety compared to mannose, and may be correlated to its lower affinity for LOL I. Table II summarizes the refined B factor values for each saccharide molecule individually (S1 to S4) and the averaged value for the four saccharide molecules present in the asymmetric unit of the two complexes. In both cases, the S1 sugar moiety has the lowest B factor. This is not surprising since it is the only one to establish van der Waals contacts with a symmetry-related lectin molecule.

The Ca^{2+} and Mn^{2+} sites are also highly conserved in Con A, pea lectin, and Favin. The difference in the metal ion positions between these lectins and LOL I are small and comparable to the positional errors of our complexes. The *cis*-peptide bond between residue 80 and 81, also present in pea lectin, Favin (residues 81 and 82), and Con A (residues 207–208), was clearly visible during the refinement, and has been introduces as so in the X-PLOR dictionary. One of the two water ligands of Ca^{2+} is involved in hydrogen bonding to the O and OD2 atoms of Asp-81, a residue involved in the binding of monosaccharides, and may stabilize the *cis*-peptide conformation between the the residues 80 and 81 (Fig. 3). Its presence seems to be essential for the correct geometry of the Ca^{2+} site and for sugar binding, as described by Reeke et al.[22,23]

Monosaccharide-Binding Site

The two monosaccharide-binding sites are located at the ends of the longest axis of the molecule (Fig. 4a). The potential sites for binding a biantennary glycan of the *N*-acetyllactosaminic type are also clearly visible in Figure 4b: two clefts are present above and below the monosaccharide-binding site. The two saccharide molecules bind to the protein in the same way, adopting the C1 chair conformation. Only two side chains of LOL I, which also bind the Ca^{2+} metal ion Asp-81 and Asn-125, are involved in the hydrogen-bonding scheme between the sugar and the protein. The other hydrogen bonds involve

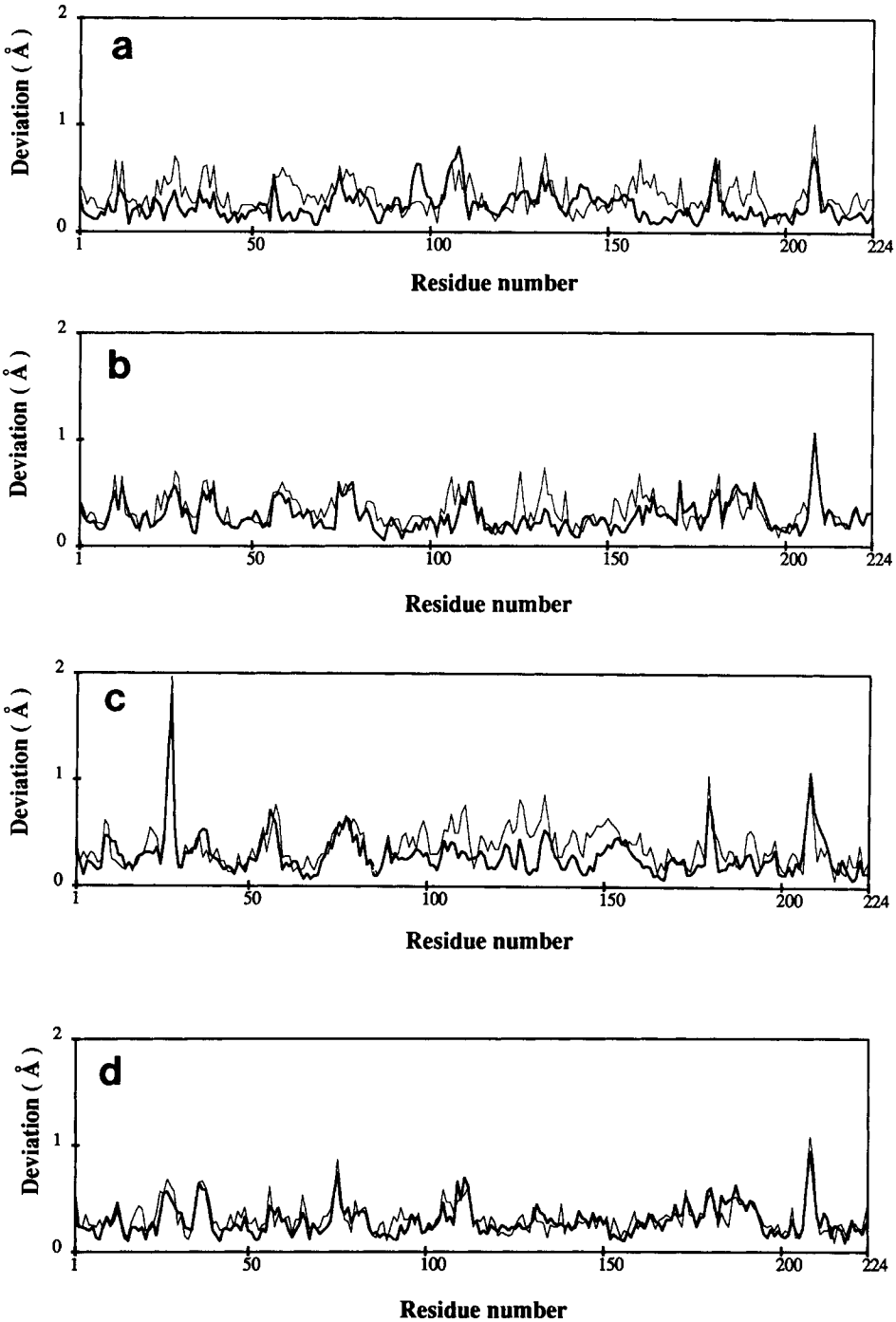

Fig. 1. Average deviation (Å) between backbone atoms of saccharide-free–LOL I model and (**a**) LOLM model (thick line) and LOLG model (thin line) (monomer A); (**b**) LOLM model (thick line) and LOLG model (thin line) (monomer B); (**c**) LOLM model (thick line) and LOLG model (thin line) (monomer C); (**d**) LOLM model (thick line) and LOLG (thin line) (monomer D). This and all other molecular illustrations in this paper were prepared with a POSTCRIPT® output file from the molecular modeling program TURBO-FRODO written by A. Roussel.[26]

backbone nitrogen atoms belonging to the loops 97–100 and 208–211 of LOL I. Loop 97–100 contains three consecutive glycine and loop 208–211 contains one. Glycine-rich loops have also been implicated in the binding of carbohydrate to pig pancreas α-amylase.[24] These glycine-containing loops are probably very flexible and could be involved in the binding of various molecules.

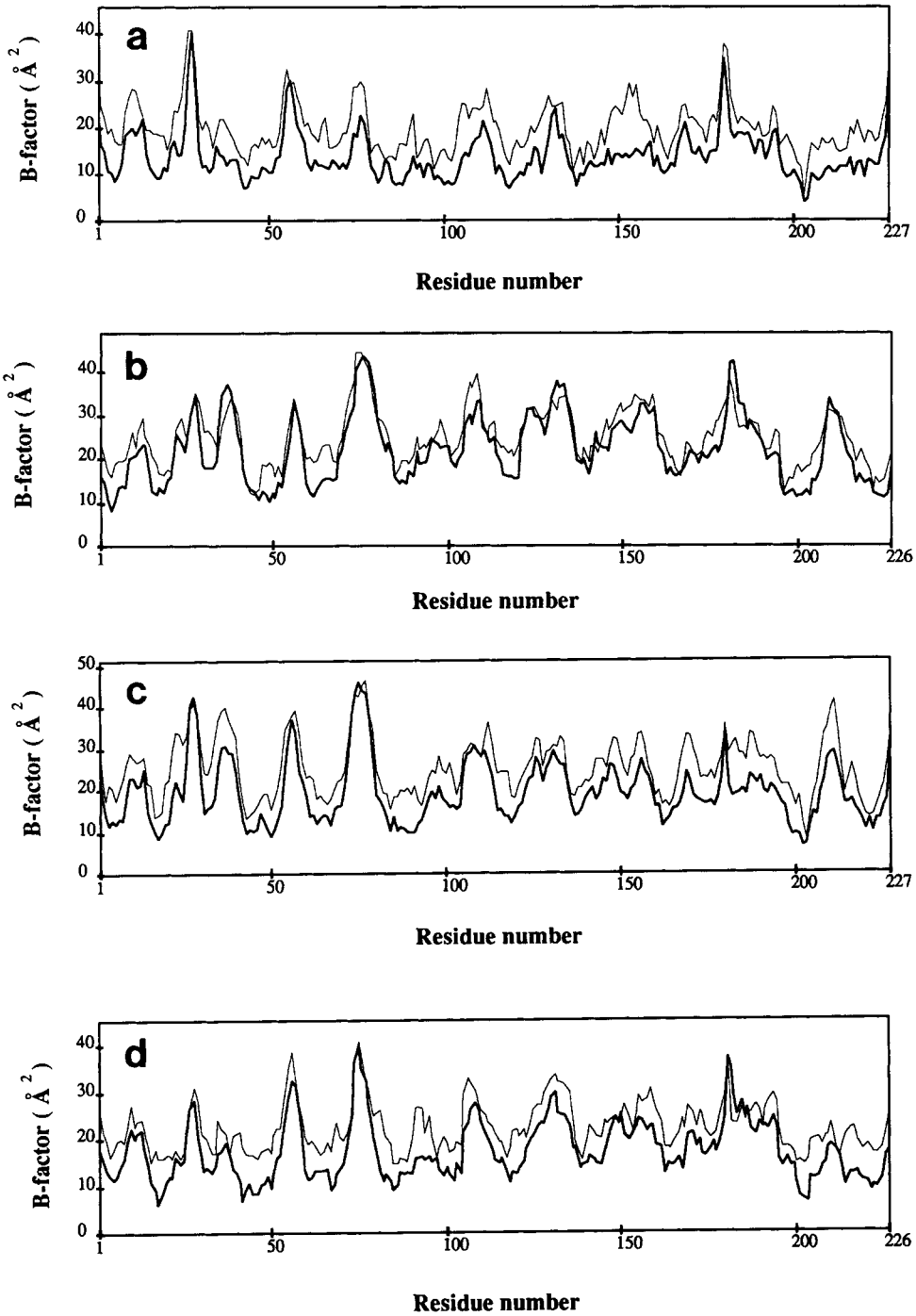

Fig. 2. Variation of the mean temperature factor value for main-chain atoms of the four LOLM monomers (**a** to **d**; thick line) and the four LOLG monomers (**a** to **d**; thin line).

The *B* factor values of the residues involved in the binding of the two monosaccharides are weak, indicating that these residues are stabilized by the presence of the sugars (Fig 2a–c). The hydrogen-bonding schemes for mannose and glucose are summarized in Table III. Figure 5 presents a schematic diagram of the interaction between the monosaccharide and the lectin. The hydrogen bond geometry is satisfied for the O4 and O6 atoms of the sugar moieties, as illustrated in Figure 6.

TABLE II. Comparison of the Temperature Factor Values of the Glucose and the Mannose Molecules*

	S1	S2	S3	S4	All
Mannose	13.6 (0.6)	30.0 (1.0)	25.3 (0.8)	17.6 (0.9)	21.6
Glucose	23.1 (1.0)	44.4 (1.0)	39.8 (1.0)	30.8 (1.1)	34.0

*The values of B factors (Å^2) are summarized for each of the four monosaccharides (S2, S2, S3, S4) present in the asymmetric unit of the monoclinic crystal form, and averaged (all) in the two structures. The movement of the loop 208–211 between the saccharide-free–LOL I model and the two saccharide–LOL I models is also given in parentheses for each sugar molecule (value in Å).

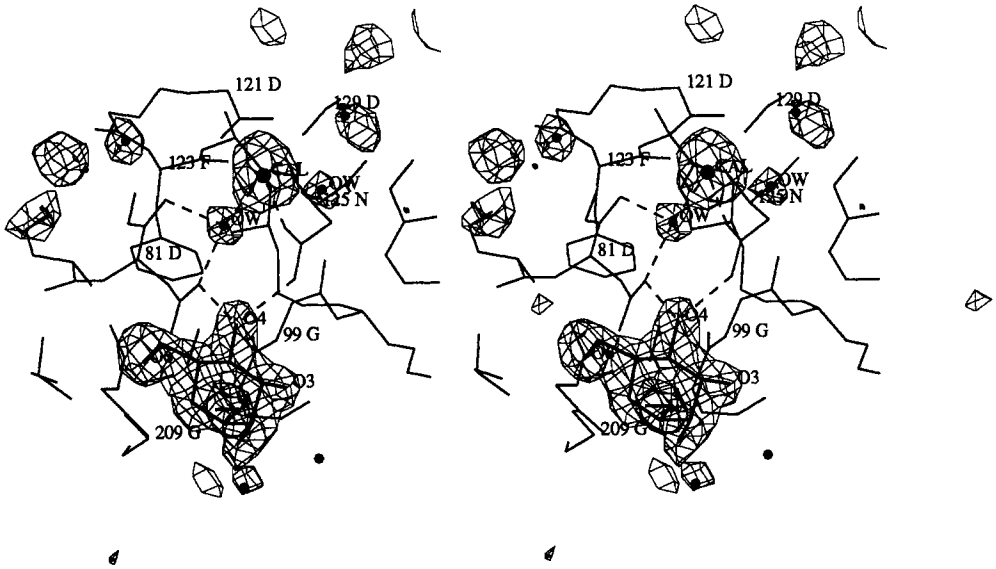

Fig. 3. Stereoscopic view of the monosaccharide-binding site showing the network of hydrogen bonds which stablize the *cis*-peptide conformation between the residue 80 and 81. The electron density from an $[F_o-F_c]$ map is displayed, in which atomic parameters for this sugar residue, the calcium ion, and the water molecules were excluded from phase calculation.

Hydrogen HO6 is bonded to OD1 atom of Asp-81. Oxygen O6 is bonded to the peptide NH atoms of Gly-209, Ala-210, and Glu-211. In a similar way, O4 is bonded to the peptide NH atoms of Gly-99 and amide NH atom of Asn-125. Hydrogen HO4 is bonded to OD2 atom of Asp-81. Moreover, both O5 and O3 atoms are bonded to the peptide NH atoms of Gly-99 and Ala-210, respectively. The other oxygen atoms of the two sugar molecules are not involved in hydrogen bonds to the lectin; they are bonded to solvent molecules, like the O1 atom in mannose and the O2 atom in glucose. The other sugar–protein contacts involve close hydrophobic interactions between the ring atoms of residue Phe-123 and the C5 and C6 atoms of the two sugar moieties.

Comparison of the Monosaccharide-Binding Site Between the Saccharide-Free–LOL I and the Saccharide–LOL I Structures

The structures of the saccharide-free and saccharide–LOL I are extremely similar with the exceptions of the loop 208–211 (Fig. 1a–d), which is slightly displaced toward the cavity where it establishes three hydrogen bonds with the saccharide molecules. The movement of this loop is variable in the four monomers, and is correlated with the B factor value of each sugar molecule (Table II). Both parameters, which are the sugar B factor value and the loop movement, seem to depend highly on the packing environment in the case of the mannose moiety.

Comparison of the Monosaccharide-Binding Site Between Concanavalin A and LOL I

The Con A monomer consists of a single polypeptide chain, while the LOL I monomer consists of β- and α-chains and the amino acid sequence of Con A is circularly permuted with respect to LOL I. The homology with Con A is obtained by aligning, with few deletions, the amino ends of LOL I with residue 123 of Con A, proceeding to the COOH-terminal of the latter lectin, and continuing along their NH_2-terminal region. Contacts between the O2 atom of the sugar moiety and a symmetry-related water molecule are very important in the crystal stability of the mannoside–ConA complex structure.[7]

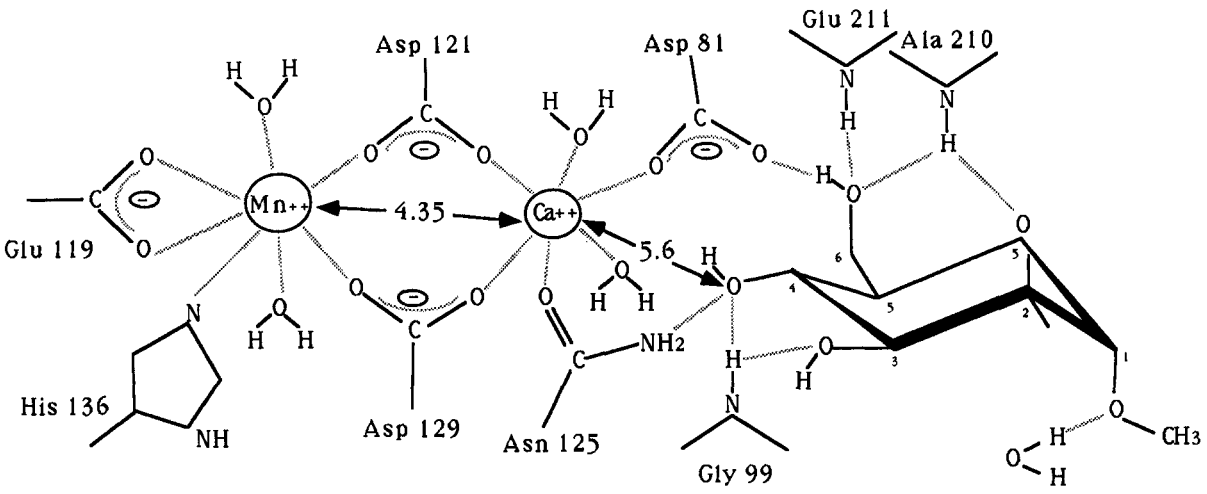

Fig. 4. Schematic diagram of the interaction between the monosaccharides and the lectin. The sugar is represented with both the axial and the equatorial C2 atom conformation.

TABLE III. The Hydrogen-Bonding Scheme of the Two Monosaccharides*

Donor	Acceptor	Distance (Å)
OH2 symm	O1	2.81
Gly-99 N	O3	2.85
Gly-99 N	O4	3.23
Asp-125 ND2	O4	2.80
O4	Asp-81 OD2	2.78
Ala-210 N	O5	2.93
O6	Asp-81 OD1	2.91
Ala-210 N	O6	3.01
Glu-211 N	O6	3.01
Gly-209 N	O6	3.28
OH2 symm	O2	2.80
Gly-99 N	O3	2.82
Gly-99 N	O4	2.10
Asn-125 ND2	O4	2.96
O4	Asp-81 OD2	2.90
Ala-210 N	O5	3.12
O6	Asp-81 OD1	2.50
Ala-210 N	O6	3.01
Glu-211 N	O6	3.17
Gly-209 N	O6	3.27

*The interactions are summarized in the case of mannose (top) and glucose (bottom).

For this reason the glucoside, which differs only in the C2 atom conformation, cannot stabilize these crystals. In our case, only one sugar moiety is involved in crystal packing interactions and involves the anomeric methyl group, which is constant in mannose and glucose. Despite these differences, the hydrogen-bonding schemes in the case of the saccharide–ConA and saccharide–LOL I complexes are similar. Nevertheless, some of the residues involved in sugar–lectin contacts differ from those of Con A: Tyr-12, Arg-228, Leu-99, and Tyr-100 of Con A, which in LOL I are equivalent to Phe-123, Gly-99, Ala-210, and Glu-211. These differences are probably responsible for the different affinity for mannose and glucose displayed by these two lectins. The residues involved in the binding of the monosaccharide in the case of LOL I and Con A are listed in Table IV. The putative residues in the case of pea lectin and Favin are also represented. The methyl substituent of O1 atom, which was not apparent in the mannoside–Con A structure, is clearly visible in the LOL I complexes; it is directed toward the solvent, making no contacts with the lectin.

As described by Reeke and Becker,[22] Con A differs from pea lectin, lentil, and Favin in being less inhibited by saccharides with small substituents (e.g., methyl) on oxygen 3 than by the unsubstituted sugars. These experiments have not been made in the case of LOL I. Nevertheless, from our models, it is possible to explain this sugar specificity difference between Con A and the other three lectins. The two aromatic residues (Tyr-100 and Trp-128) are located near the monosaccharide-binding site at approximately 5.5 Å from the oxygen 3 of the sugar moiety. Hydrophobic interactions between the ring atoms of these residues and the CH_3 of the O3 atom are likely to be established. These residues are highly conserved in the three lectins, but are subsituted by glycine in the Con A amino acid sequence, which prevents the above mentioned hydrophobic interaction to be present.

Fine Sugar Specificity

The hydrogen-bonding schemes for the mannose and the glucose in the binding site of LOL I are nearly identical. The O2 atom, which is the only atom to have a conformational difference between the mannose the the glucose moieties, is not involved in a hydrogen bond with the lectin. Therefore, it is not likely that electrostatic forces are responsible for the fine specificity of LOL I toward

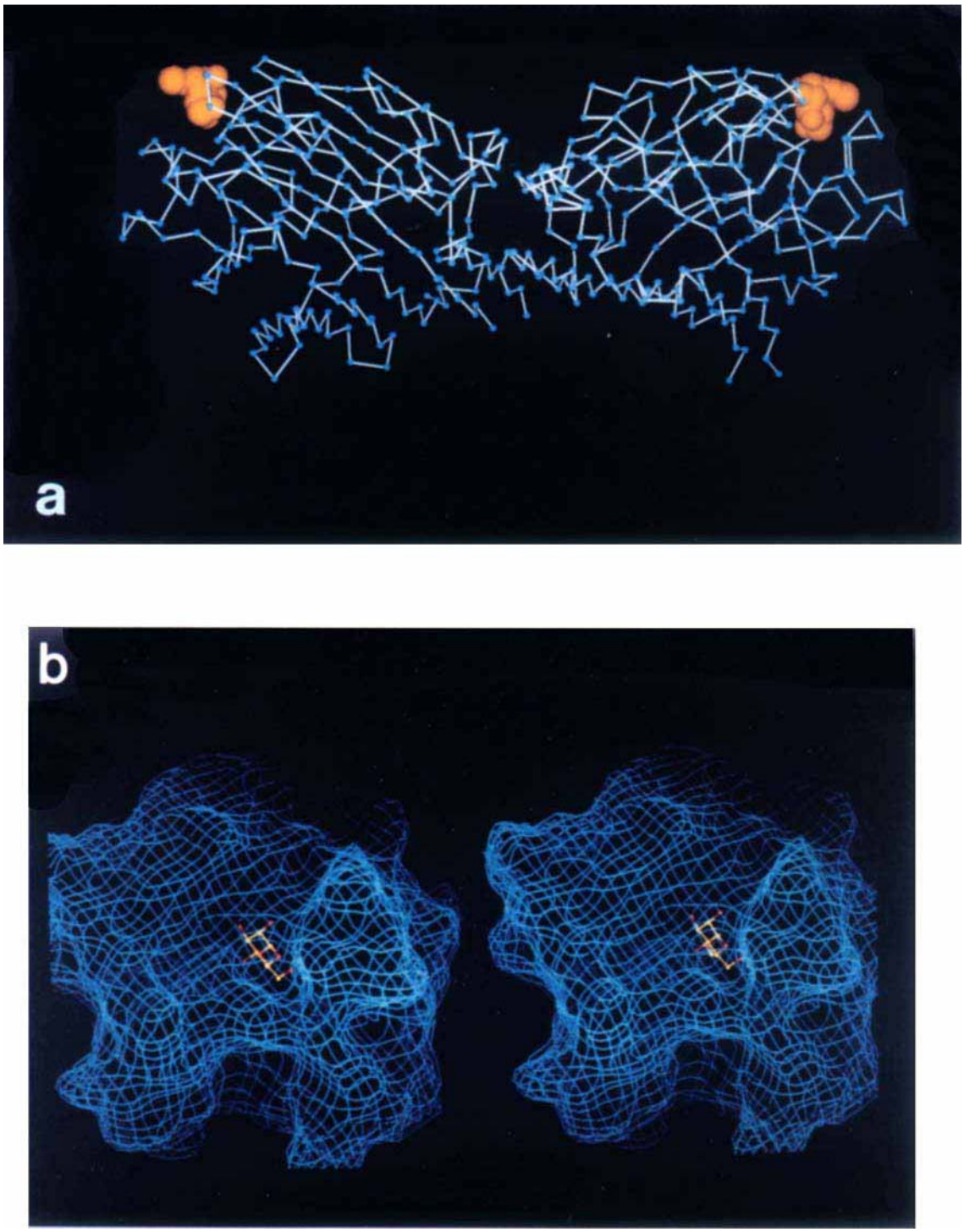

Fig. 5. **(a)** View of the LOL I Cα-carbon tracing of LOL I (ball and stick representation) with the two mannoside moieties (space-filling model). **(b)** Stereoscopic view of the water-accessible Connolly surface[27,28] of LOL I showing the mannoside moiety (ball and stick representation) and the probable extension for the binding of a biantennary glycan of the *N*-acetyllactosaminic type.

mannose and glucose.[3] Nevertheless, due to its axial C2 conformation, the mannose molecule establishes in a clamplike way extensive hydrophobic interactions with the lectin molecule through the O6, C6, C2, and O2 atoms and residues 208–211 of LOL I. An estimate of the free-energy difference between

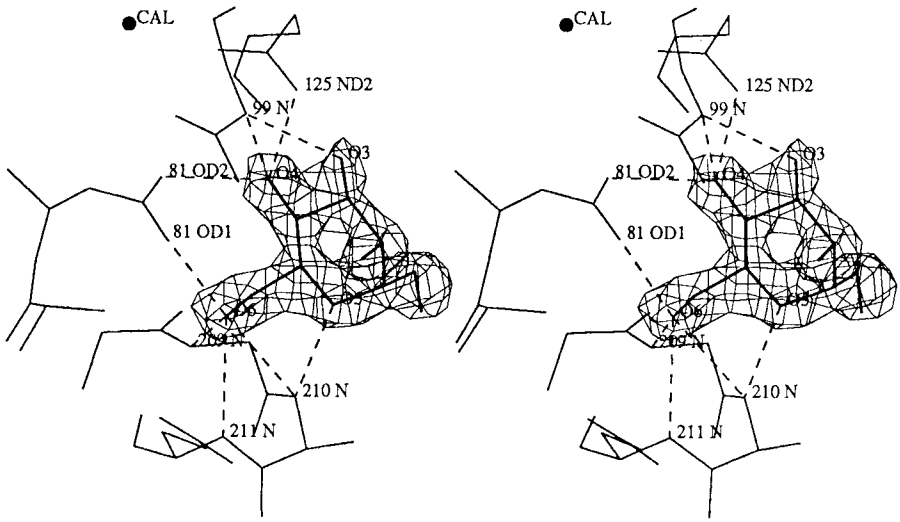

Fig. 6. Stereoscopic view of the monosaccharide-binding site showing the network of hydrogen bonds the mannose moiety and LOL I. The electron density from an $[F_o-F_c]$ map is displayed, in which atomic parameters for this sugar residue were excluded from phase calculation.

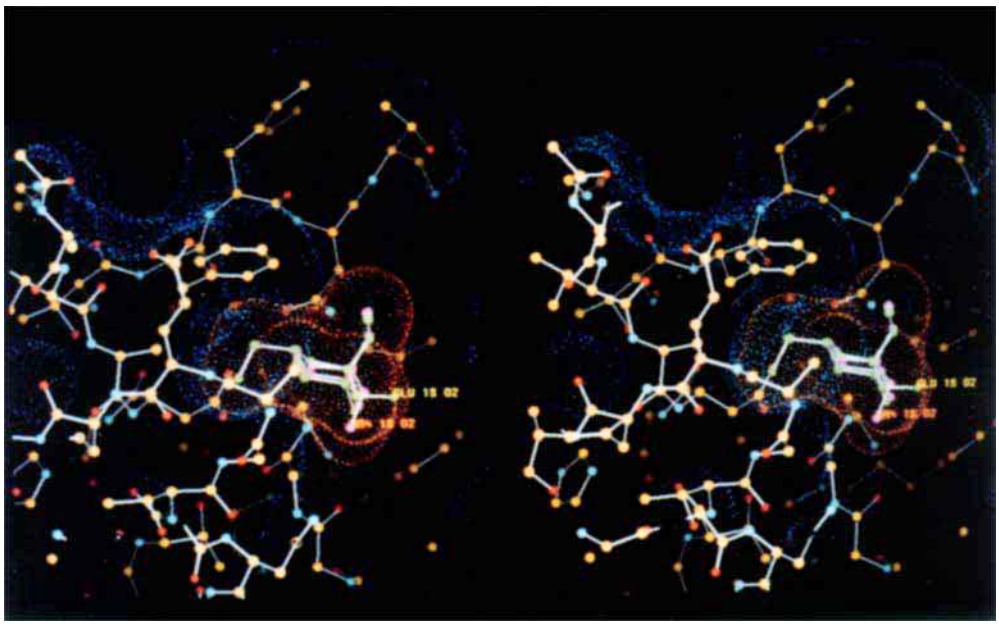

Fig. 7. Stereoscopic view of the monosaccharide-binding site showing the Connolly surfaces[28] complementarity between the mannose moeity (van der Waals surface, gold dots) and the lectin molecule (water-accessible surface, blue dots). The mannose (magenta) and the glucose (green) moieties are superimposed in the monosaccharide-binding site of LOL I. The O2 atom is labeled in the axial and equatorial conformation corresponding to the mannose and the glucose, respectively.

the binding of mannoside and glucoside molecules can be obtained from their minimal concentrations giving a complete inhibition of hemagglutination.[3] The free-energy of binding of the mannoside is approximately 0.8 kcal/mol better than that of the glucoside. This value is consistent with the geometry of

TABLE IV. Corresponding Residues Involved in the Binding of the Monosaccharides of LOL I and Con A*

LOL I	Con A	pea lectin	Favin
Asp-81	Asp-208	Asp-81	Asp-82
Gly-99	**Arg-228**	Gly-99	Gly-100
Asn-125	Asn-14	Asn-125	Asn-126
Ala-210	Leu-99	Ala-210	Ala-211
Glu-211	Tyr-100	Glu-211	Glu-212
Phe-123	Tyr-12	Phe-123	Phe-124

*The mode of superposition of Con A with the three other lectins is explained in the text. The amino acid sequence of Favin contains one more amino acid at the NH_2-terminal end of the β-chain. Arg-228 (in bold type) does not exist in the amino acid sequences of LOL I, pea lectin, and Favin.

binding of the two saccharide molecules. As shown in Figure 7, a more extensive interaction of the mannoside moiety with the lectin molecule is evident. After superimposing the equivalent carbon atoms of the glucose with the mannose in the structure of the LOLM complex the same order of magnitude of van der Waals energy between the monosaccharides and the lectin was calculated with the program X-PLOR.[18]

CONCLUSION

Derewenda et al.[8] have described the monosaccharide-binding site of the single chain concanavalin A lectin. This site is now structurally known for the two chain-lectin LOL I. In this case, the higher affinity of LOL I toward the mannoside moiety can be rationalized. Differences found in the sequence alignment of the residues involved in the binding of the monosaccharides between Con A and LOL I are probably responsible for the different affinity for mannose and glucose molecules displayed by these two lectins.

Further investigations have been made with LOL I in order to find others monosaccharide-binding sites. Recently, we have cocrystallized LOL I with a trisaccharide and also with a biantennary octosaccharide.[25] The refinement of the trisaccharide–LOL I complex is underway, as well as the structure determination of the octosaccharide–LOL I complex by the molecular replacement method.

The list of atomic coordinates for the LOLM and the LOLG complexes will be deposited with the Protein Data Bank.[20]

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