X-ray Crystal Structure of a Pea Lectin-Trimannoside Complex at 2.6 Å Resolution*

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James M. Rini‡, Karl D. Hardman*‡, Howard Einspahr**, Fred L. Suddath†‡‡, and Jeremy P. Carver†

From the Departments of Molecular and Medical Genetics and Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada, the DuPont Merck Pharmaceutical Company, Wilmington, Delaware, 19880-0228, **The Upjohn Company, Kalamazoo, Michigan, 49060, and the School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia, 30322-0400

The x-ray crystal structure of pea lectin, in complex with a methyl glycoside of the N-linked-type oligosaccharide trimannosyl core, methyl 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside, has been solved by molecular replacement and refined at 2.6 Å resolution. The R factor is 0.183 for all data in the 8.0 to 2.6 Å resolution range with an average atomic temperature factor of 26.1 Å². Strong electron density for a single mannose residue is found in the monosaccharide-binding site suggesting that the trisaccharide binds primarily through one of the terminal α-linked mannose residues. The complex is stabilized by hydrogen bonds involving the protein residues Asp-81, Gly-99, Asn-125, Ala-217, and Glu-218, and the carbohydrate oxygen atoms O3, O4, O5, and O6. In addition, the carbohydrate makes van der Waals contacts with the protein, involving Phe-123 in particular. These interactions are very similar to those found in the monosaccharide complexes with concanavalin A and isolectin 1 of Lathyrus ochrus, confirming the structural relatedness of this family of proteins. Comparison of the pea lectin complex with the unliganded pea lectin and concanavalin A structures indicates differences in the conformation and water structure of the unliganded binding sites of these two proteins. Furthermore, a correlation between the position of the carbohydrate oxygen atoms in the complex and the bound water molecules in the unliganded binding sites is found. Binding of the trimannose core through a single terminal monosaccharide residue strongly argues that an additional fucose-binding site is responsible for the high affinity pea lectin-oligosaccharide interactions.

Although the biological role of plant lectins is not well understood, they have been the subject of considerable research interest. Lienert et al. (1986); Eizler, 1985; Lis and Sharon, 1991). In particular lectins of the family leguminosae have been well studied, and much is known about their carbohydrate binding specificity, primary sequence, and three-dimensional structure. Although x-ray crystallographic analysis has shown that members of this family share a highly conserved three-dimensional structure, they differ in their carbohydrate binding properties making them well suited to the study of structure-function relationships. Pea lectin, lentil lectin, favin, and LOL 1 and ConA for example, all show a monosaccharide binding specificity for mannose and glucose, while Griffonia simplicifolia lectin IV (GS4) and Erythrina corallodendron lectin (EcorL), which share a very homologous tertiary structure (Delbaere et al., 1990; Shaanan et al., 1991), bind galactose. Within the mannose/glucose-specific group, differences in the fine specificity for oligosaccharides of the N-linked type have also been found. Of particular interest is the observation that pea lectin, lentil lectin, favin, and LOL 1 require an α1,6-linked fucose moiety for high affinity binding, while ConA does not (Kornfeld et al., 1981; Debray et al., 1981; Debray and Rouge, 1984).

Structurally, the location of the monosaccharide-binding site has been known for some time based on the low resolution crystal structure complexes of ConA with methyl-α-D-mannopyranoside (Hardman and Ainsworth, 1976) and 2-deoxy-2-iodo-methyl-α-D-mannopyranoside (Becker et al., 1976). Only recently, however, have higher resolution complexes allowed for a determination of the specific lectin-carbohydrate interactions. The x-ray crystal structure of the ConA-methyl α-D-mannopyranoside complex solved at 2.9 Å resolution (Derewenda et al., 1989) showed that the carbohydrate oxygen atoms O3, O4, O5, and O6 are directly hydrogen bonded to the protein. Very similar interactions were also reported for the complexes of LOL 1 with methyl-α-D-mannopyranoside and methyl-α-D-glucopyranoside solved at 2.0- and 2.2 Å resolution, respectively (Bourne et al., 1990a). In addition, both a trisaccharide and an octasaccharide complex with LOL 1 have recently been reported (Bourne et al., 1990b, 1992). Quite remarkably, the GS4-Lewis b tetrasaccharide and EcorL-lactose complexes show that the geometry of the primary binding site is very well conserved even among the galactose binding members of the family (Delbaere et al., 1990; Shaanan et al., 1991).

In mechanistic terms the roles played by hydrogen bonding, van der Waals interactions, and water structure, in mediating protein-carbohydrate interactions, have been the subject of some debate (Carver, 1993; Lemieux, 1988; Quiocio, 1989). Thermodynamic analysis of a number of systems has shown, etc.

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The abbreviations used are: LOL 1, isolectin 1 of Lathyrus ochrus; ConA, concanavalin A; EcorL, Erythrina corallodendron lectin; GS4, G. simplicifolia lectin IV; triman, methyl 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside; Fuc, fucose; Man, mannose.

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However, that protein carbohydrate interactions are driven by enthalpic terms, consistent with hydrogen bond and van der Waals interactions (Carver et al., 1982; Quiocio, 1989). In addition, a loss in entropy for the system is usually observed, the origin of which has yet to be understood (Carver, 1993). Clearly, to resolve these issues a complete description of the system must be made, including an analysis of the protein conformation and water structure in the complexed and the unbound states, as well as a characterization of the carbohydrate both free in solution and in the bound form.

As part of efforts to characterize the high affinity oligosaccharide binding interactions of these lectins, we report here the x-ray crystal structure of pea lectin in complex with the α-methyl glycoside of the trimannosyl core common to all oligosaccharides of the N-linked class. The structure has allowed us to explain the carbohydrate binding specificity of pea lectin and some of the differences seen with ConA. Comparison of the complex with the unbound pea lectin and ConA structures shows differences in the conformation and water structure in the unliganded binding sites of these two proteins. Furthermore, we show a correlation between the position of hydroxyl groups of the bound saccharide and water molecules in the unliganded binding site.

**EXPERIMENTAL PROCEDURES**

**Protein Purification, Crystallization, and Data Collection—**Pea lectin was isolated from green pea seeds (Alaska variety, Smith Seeds, Toronto) by affinity chromatography on Sephadex G-100 by the method of Trowbridge (1974). The mixture of two isolectins, which differ in the length of their α-subunits by 2 amino acid residues (Rini et al., 1986a), was used in the crystallization procedure. Methyl 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside (triman) was synthesized by the method of Winnik et al. (1982) and supplied by Toronto Research Chemicals (Toronto, Ontario). Triman corresponds to the methyl glycoside of the trimannosyl core common to all N-linked type oligosaccharide structures as shown in Fig. 1. Crystals were grown from solutions containing 18 mg of pea lectin/ml, 13 mM triman, 40 mM Tris-HCl, pH 7.0, 8.6% (w/v) polyethylene glycol 4000, and 0.02% NaN3 (w/v) as described previously (Rini et al., 1986b). The crystals, which contain a dimer in the asymmetric unit grow in the orthorhombic space group, P212121, with unit cell parameters of a = 64.3 Å, b = 93.4 Å, and c = 108.5 Å. Due to difficulties in immobilizing the crystals in data collection capillaries, they were mounted in mother liquor and coated with a thin film of Formvar generated in situ as described by Raymont et al. (1977). Data collection and reduction were performed as described for the native pea lectin structure determination (Meehan et al., 1982). In brief, data were collected on a Picker diffractometer, at room temperature, using Ni-filtered Cu X-ray radiation, by the ω-step-scan procedure. Every 200 reflections, 10 standard reflections were measured and used in calculating the radiation damage correction. Integrated intensities were determined by a gaussian-fit method (Hansen et al., 1979) and corrected for Lorentz and polarization effects. Absorption corrections were applied using the method of North et al. (1988). Over 28,000 reflections to 2.5-Å resolution were collected from 14 crystals. These were reduced to 17,124 reflections with a merging R factor of 0.076. The data in the 10.0-2.6-Å resolution range is virtually complete and contains 15,931 reflections.

**Structure Determination—**The structure was determined by molecular replacement using the unliganded pea lectin structure as search model. A comparison of pea lectin and ConA as molecular replacement models for this structure has also been made and is reported elsewhere (Rini et al., 1988). A single monomer of the pea lectin dimer (Einspahr et al., 1986) was used in both the rotation and translation searches. The model contained 1769 atoms made up of residues 1-179 (β-chain) and 180-234 (α-chain) (numbering based on the pea lectin cDNA clone, Higgin et al., 1983) as well as the manganese and calcium ions. The monomer was oriented in a rectangular cell with dimensions a = 65.0 Å, b = 90.0 Å, and c = 110.0 Å so that the 2-fold rotation axis, relating the two monomers of the dimer, was aligned along the y axis of this cell. Structure factors were then calculated in space group P21 using the program written by Argawal (1978), which utilizes the Winograd discrete Fourier transform procedure, with an overall temperature factor of 13.0 Å². The Crowther (1972) fast rotation function, as implemented in the program package MERLOT (Fitzgerald, 1989), was then evaluated in the 6.0-5.0-Å resolution range with a Patterson cutoff radius of 17.0 Å. The first (100%, 4.7e) and second (85%) highest peaks in the map were related by an approximate 2-fold relationship and were located on the same β-section of the map consistent with the fact that the 2-fold rotation axis of the dimer was oriented along the y axis of the P1 cell (Dereewoda et al., 1981). Using data in the 6.0-5.0-Å resolution range, these positions were refined using the rigid body rotation search (1.0° grid in the final cycle) implemented in MERLOT.

The transforms required for the translation searches were calculated in a cell with dimensions 185 × 185 × 185 Å³, to 5.6-Å resolution, with an overall temperature factor of 13.0 Å². The T; translation function of Crowther and Clinton (1987) was then evaluated in the 6.0-5.0-Å resolution range. Translation parameters for both monomers were solved independently. To define a common origin, one set of Patterson vectors was calculated between the non-crystallographically related monomers. For both monomers the correct peak was the highest peak in the map on two of the Hex axes and contained a structure which gave an R factor of 0.46 for all data in the 10.0-2.5-Å resolution range. Subsequent refinement of the rotation and translation parameters of each monomer using an R factor based grid search (MERLOT) followed by rigid body refinement with CORELS (Sussman, 1985) lowered the R factor to 0.26 for these data.

**Structure Refinement—**Initial structure refinement was performed using the fast Fourier transform based version (PROMPT) of the Hendrickson and Konnert restrained refinement program (Finzel et al., 1985). Using the target restraints described by Hendrickson (1985) and a structure factor weighting term of approximately 0.5 (|F̃|/|F̄|), the R factor was reduced to 0.28 for all data in the 10.0-2.6-Å resolution range with restrained atomic temperature factors. A difference electron density map calculated at this stage revealed clear density for a single monosaccharide residue in each binding site of the dimer. A single mannose residue, taken from one of the terminal regions of the NMR-derived mannan structure (Borriss et al., 1983), was then positioned in the electron density at each site, optimizing in both cases the O6 hydroxyl rotamer. The structure was then refined using the high temperature simulated annealing protocol in X-PLOR (Brünger, 1990). The X-PLOR parameter files were modified to incorporate mannosyl and a nonproline ε-amino residue and to incorporate the changes in energy parameters suggested by Weis et al. (1989). The entire structure was then checked using 10% omit maps and rebuilt where necessary using the interactive graphics program PRODOR (Jones, 1985). In calculating these maps, bias was minimized by refining the phasing model for 40 cycles of positional refinement in X-PLOR, with a region of interest had been deleted from the model. Restrained individual atomic temperature factors were refined and 79 well ordered water molecules were incorporated into the model. As described by Kundrot and Richards (1987), water molecules were given an occupancy of 0.6. At all stages of refinement both monomers in the asymmetric unit were treated independently. Model alignments were performed by a least-squares procedure using the program SUPER (written by J. Rini).

**RESULTS AND DISCUSSION**

**Structure Description—**Comparison of the pea lectin complex with that of the unliganded structure shows that they are very similar. To maximize alignment of the structures, however, a 2.2° rotation of one of the monomers must be made. This relatively small shift in domain disposition can presumably be explained by differences in the crystallization
The geometry of the calcium-
and manganese ion-binding sites, including the metal-coordinating water molecules, is also very similar to that seen in the unliganded lectin (Fig. 2). Furthermore, the cis peptide linkage between residues Ala-80 and Asp-81 is conserved. In both monomers no electron density is seen for the COOH terminus of the β-chain (residues 182-187), presumably as a result of the COOH-terminal processing described by Rini et al. (1986a). The α-chains show no density beyond residues 234 and 236 for monomers 1 and 2, respectively. The current model has an R factor of 0.183 for all data in the 8.0-2.6-Å resolution range with root-mean-square deviations on bond lengths and angles of 0.013 Å and 2.9°, respectively. The final overall temperature factor is 26.1 Å² with average backbone and side chain temperature factors of 24.8 and 28.0 Å², respectively. The average water temperature factor is 16.5 Å² with an occupancy of 0.6.

Carbohydrate-binding Site—Fig. 3 shows the omit electron density in the monosaccharide-binding site of monomer 1, contoured at 3σ. The electron density for monomer 2 is almost identical. Although some additional density is seen extending out into solution, at lower contour values, this density is uninterpretable even after symmetry averaging over both monomers. A single mannose residue has been modeled and refined in each site. Furthermore, using the NMR-derived trisman structure (Brisson and Carver, 1983), it was shown that either terminal mannose residue could be accommodated in the binding site. Conceivably, both binding orientations occur further contributing to the poor quality of the density beyond the monosaccharide-binding site. The binding site of each monomer is exposed to solvent, and there are no crystal contacts in the immediate vicinity of either. Based on inhibition of agglutination assays (Stubbs et al., 1986), trisman has been found to bind to pea lectin with an affinity approximately three times greater than that seen for d-mannose ($K_d = 0.7 \times 10^{-3}$ M; Trowbridge 1974).

The average temperature factors for the mannose residues are 25.9 and 27.6 Å² for monomers 1 and 2, respectively, close to the average value for the protein. The mannose residue in the pea lectin monosaccharide-binding site makes six hydrogen bonds with the protein as shown in Fig. 4 for monomer 1. The geometry of the site is virtually identical in monomer 2 and a list of the hydrogen bond interactions for both monomers is shown in Table I. Asp-81 presumably plays a central role in binding the mannose residue as its carboxylate oxygen atoms simultaneously accept hydrogen bonds from both the O4 and O6 hydroxyl groups of the monosaccharide. Based on very high resolution protein-carbohydrate complexes, Vyas (1991) has noted that the strongest hydrogen bond interactions are those in which the carbohydrate hydroxyl groups serve as hydrogen bond donors. Glu-218, Ala-217, and Gly-99, which bind to the O6, O5, and O3 oxygen atoms, respectively, all interact by donating hydrogen bonds through their backbone NH groups. Asn-125 also serves as a hydrogen bond donor, in this case through its side chain ND2 atom. The C6 hydroxyl is found in the gg conformation (Cummying and Carver, 1987) with ω equal to -47° and -49°, respectively, in monomers 1 and 2. In this conformation the O6 hydroxyl group, like that of O4, can simultaneously serve as a hydrogen bond donor and acceptor. This geometry has been exploited by the high affinity group-I carbohydrate-binding proteins, typified by the periplasmic carbohydrate receptors, which essentially bury their carbohydrate ligands (Vyas et al., 1991; Quiocho, 1986). In the pea lectin-trisman complex, only the O4 and O6 hydroxyl groups, which are most deeply buried in the carbohydrate-binding site, show this hydrogen bonding pattern. Neither the O1 oxygen atom nor the O2 hydroxyl group is involved in a hydrogen bond. In addition, extensive van der Waals interactions between the protein and carbohydrate are made. On average each mannose residue makes 59 contacts involving 9 different protein residues (Table II). Phe-123 is particularly noteworthy, making a stacking interaction with the mannose carbon atoms C5 and C6 not unlike that described in other protein-carbohydrate complexes (Vyas, 1991; Quiocho, 1986). Of the hydroxyl bonds made with the carbohydrate, only those involving Asp-81 and Asn-125 are to side chain atoms. In both cases these side chains are stabilized either directly or through water by interactions with the calcium ion (see Fig. 2). In addition the carboxyl oxygen atom of Phe-123 makes a direct interaction with the calcium ion confirming the importance of the metal-binding site in maintaining the carbohydrate binding properties of these lectins.

The trisaccharide studied by Bourne et al. (1990b) differs from trisman in that it lacks Man4' and includes GlcNAc2 (see Fig. 1). Based on the results presented here and the fact

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**Fig. 2. Calcium- and manganese ion-binding site.** The calcium and manganese coordinating ligands are connected by dashed lines. Small open circles correspond to water molecules. For clarity only a selected subset of the possible hydrogen bonds are shown. The hydrogen bond between Asp-81 and one of the calcium coordinating water molecules is also shown.
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FIG. 3. Stereo view of the carbohydrate electron density for monomer 1. A single mannose residue is shown in the omit electron density from a $F_o - F_i$ map contoured at 3σ.

FIG. 4. Stereo view of the hydrogen bond interactions between the carbohydrate and pea lectin. Protein residues (closed bonds) in the carbohydrate-binding site are shown making hydrogen bonds (dashed lines) with the bound mannose residue (open bonds). Single digit numerals label the mannose oxygen atoms. Residues Gly-99, Asn-125, Ala-217, and Glu-218 donate hydrogen bonds to the carbohydrate while Asp-81 serves as a hydrogen bond acceptor.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Lectin-carbohydrate hydrogen bond interactions</th>
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<tr>
<td>Donor atom</td>
<td>Acceptor atom</td>
</tr>
<tr>
<td>Man O6</td>
<td>Asp-81 OD1</td>
</tr>
<tr>
<td>Man O4</td>
<td>Asp-81 OD2</td>
</tr>
<tr>
<td>Gly-99 N</td>
<td>Man O3</td>
</tr>
<tr>
<td>Glu-218 N</td>
<td>Man O6</td>
</tr>
<tr>
<td>Ala-217 N</td>
<td>Man O5</td>
</tr>
<tr>
<td>Asn-125 ND2</td>
<td>Man O4</td>
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<table>
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<th>Table II</th>
<th>Lectin-carbohydrate contacts (&lt;4.0 Å)</th>
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<td>C1</td>
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</tr>
<tr>
<td>O1</td>
<td>0</td>
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<tr>
<td>C2</td>
<td>0</td>
</tr>
<tr>
<td>O2</td>
<td>2</td>
</tr>
<tr>
<td>O3</td>
<td>2</td>
</tr>
<tr>
<td>O4</td>
<td>5</td>
</tr>
<tr>
<td>C4</td>
<td>10</td>
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<tr>
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<td>C6</td>
<td>12</td>
</tr>
<tr>
<td>O6</td>
<td>15</td>
</tr>
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that the amino acid residues in the monosaccharide-binding sites are conserved between pea lectin and LOL 1, it is somewhat surprising that electron density is observed for all 3 carbohydrate residues in the LOL 1 complex. However, it should be noted that in the LOL 1 complex both Man3 and GlcNAc2 only interact with the protein through bound water molecules. Furthermore, since the trisaccharides in both monomers are also involved in crystal contacts it is likely that these water-mediated interactions do not represent important binding modes in solution. This suggestion is consistent with the results of the LOL 1-octasaccharide complex (Bourne et al., 1992) which shows that Man3 and GlcNAc2 now interact with the protein in an entirely different manner.

Unliganded Pea Lectin and ConA—Comparison of the pea lectin complex with both crystallographically independent monomers of the native structure refined at 1.7-Å resolution shows that on ligand binding the 216-218 loop shifts toward the carbohydrate resulting in displacements of up to 1.1 Å in backbone atom positions (Fig. 5). The accommodation in loop position is almost certainly the result of hydrogen bond formation between the backbone NH groups of Ala-217 and Glu-218 with the carbohydrate oxygens O5 and O6, respectively. A similar displacement was observed in the LOL 1-carbohydrate complexes (Bourne et al., 1990b). In addition, alignment

*The pea lectin coordinates used are those refined at 1.7-Å resolution by F. L. Suddath, S. R. Phillips, and H. Einspahr which appear in the Brookhaven Protein Data Bank (Bernstein et al., 1977) as entry 2LTN.
of the structures shows that four water molecules, common to both monomers, would have to be displaced on carbohydrate binding. Two of the water molecules are very close to the positions of the O3 and O4 oxygen atoms of the mannose residue in the complex and in fact make the same hydrogen bond interactions with the protein (see Fig. 5). Although the third and fourth water molecules are hydrogen bonded to the carbohydrate binding groups Asp-81 OD1 and Ala-217 N, due to the 216-218 loop displacement, they do not occupy positions analogous to the corresponding hydroxyl groups of the bound carbohydrate. Although the thermodynamic significance of displacing these water molecules is unclear, the conservation in geometry with respect to the O3 and O4 groups certainly reflects the preformed character of the site in this region.

Since the monosaccharide binding interactions with ConA (Derefendida et al., 1989) are similar to that reported here for pea lectin a similar analysis of the ConA-binding site from the 1.75-Å resolution structure (Hardman et al., 1982) was made. Due to small differences in the relative disposition between the β-sheets in ConA and pea lectin, the binding sites were aligned using only backbone atoms in the vicinity of the carbohydrate-binding residues. Using this subset of alignment atoms ConA and both crystallographically independent monomers of the native pea lectin structure were superimposed on monomer 1 of the pea lectin complex (Fig. 6). Although the alignment of the native pea lectin-binding sites differ slightly from that obtained with the β-sheet alignment residues, the 216-218 loop perturbation is not affected. Remarkably, however, the corresponding loop in the unliganded ConA structure is seen to be quite well aligned with that of the pea lectin complex. Furthermore, the bound water molecules in the ConA site reflect this similarity, with water molecules now found at positions corresponding to those of the mannose O5 and O6 oxygen atoms in the pea lectin complex (Fig. 7). Since the 96-100 loop (216-218 in pea lectin) is involved in a lattice contact in the ConA structure, it is not possible to conclude that this conformation necessarily represents the unbound ConA structure in solution. However, if it does, the ConA-binding site is significantly more preformed and may in part be responsible for the increase in affinity shown by ConA over pea lectin for monosaccharides such as α-methyl-D-mannopyranoside (Kaifu et al., 1975). Interestingly, the importance of this loop in determining binding specificity is suggested by the diffraction analysis of the GS4-Lewis b tetrasaccharide (Delbaere et al., 1990) and EcorL-lactose (Shaanan et al., 1991) complexes. The galactose moiety, which occupies the monosaccharide-binding site in these lectins, is rotated so that the O3 and O4 hydroxyl groups now interact with the equivalent of Asp-81. As pointed out by Shaanan et al. (1991) the O6 hydroxyl is accommodated in this orientation by residues around Ala-218 and Gln-219 (EcorL numbering) which differ significantly in conformation from that found in the mannose/glucose-binding lectins.

Carbohydrate Binding Specificity—The hydrogen bonding scheme described for the pea lectin complex is in complete agreement with binding studies utilizing selectively fluorinated glucose analogs (Van Wauwe et al., 1975). The observation that 4-deoxy-4-fluoro and 6-deoxy-6-fluoro glucose are not bound by pea lectin confirms the importance of the hydrogen bonds involving these hydroxyl groups and supports the suggestion that they serve as hydrogen bond donors. Similarly, the involvement of the O3 hydroxyl oxygen atom as a hydrogen bond acceptor is consistent with the observation that 3-deoxy-3-fluoro glucose binds to pea lectin, albeit with one-third of the affinity obtained for glucose. Furthermore, the relative insensitivity of pea lectin to the O2 hydroxyl orientation, is consistent with the lack of any obvious interaction involving the O2 position.

Differences in binding specificity among pea lectin, lentil lectin, fain, and ConA for 3-O-methyl and 3-O-benzyl derivatives of glucose (Van Wauwe et al., 1975; Allen et al., 1976; Goldstein and Hayes, 1978) are also explained by the model. Although these substituents enhance monosaccharide binding to pea lectin, lentil lectin, and fain severalfold over that of unsubstituted glucose, ConA cannot bind the 3-O-methyl derivative and binding is reduced with 3-O-benzyl substitution. As shown in the complex, the O3 hydroxyl group of the bound carbohydrate accepts a hydrogen bond from the backbone NH group of Gly-99 which in ConA corresponds to Arg-228 (see Fig. 6). In all likelihood the O3 substituents make unfavorable contacts with the bulky Arg-228 side chain in ConA. In the other lectins the conserved glycine residue at position 99 would not present such a constraint. In fact, in these cases the substituents would be directed toward Tyr-100 and Trp-128, residues conserved among those lectins which show enhanced binding, and it has been suggested that a favorable interaction with these residues is responsible for the enhanced binding affinities shown by these lectins (Rini, 1987; Bourne et al., 1990a).

The presence of a Fuc1 in the N-linked type oligosaccharides has been shown to be a necessary requirement for high affinity binding to glycoproteins, glycopeptides, and oligosaccharides by pea lectin, lentil lectin, fain, and LOL 1 (Korn-
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FIG. 6. Stereo view of the unliganded ConA and pea lectin-binding sites superimposed on the pea lectin complex. The pea lectin complex with bound monosaccharide is shown in heavy lines. ConA is shown with open bonds and both monomers of the unliganded pea lectin structure are shown in thin lines. The molecules were aligned using the 28 backbone atoms from residues 80–82, 99, and 121–123 for pea lectin and 207–209, 228, and 10–12 for ConA. The root-mean-square deviation on test atoms was 0.22 Å for ConA, and 0.17 and 0.20 Å for monomers 1 and 2 of the unliganded pea lectin structure. For clarity only the pea lectin residues have been labeled. The pea lectin/ConA sequence correspondence is as follows: Asp-81/Asp-208, Gly-99/Arg-228, Phe-123/Tyr-12, Asn-125/Asn-14, and Glu-218/Tyr-100.

FIG. 7. Stereo view of the unliganded ConA structure and its binding site waters superimposed on the pea lectin complex. The pea lectin complex with bound monosaccharide is shown in heavy lines and the ConA structure (Hardman et al., 1982) is shown in open bonds. Superimpositions were performed as described in Fig. 6. Water molecules a–d correspond to the four water molecules in the ConA-binding site which would be displaced on carbohydrate binding. Water molecules a, b, and d are close to the analogous positions occupied by the mannose oxygen atoms O4, O6, and O5 in the pea lectin complex.

This observation suggests that in addition to the monosaccharide-binding site there is an additional site of interaction for the fucose moiety. Consistent with this suggestion is the fact that the tightest pea lectin binding biantennary glycopeptides terminate in the trimannose core (Yamamoto et al., 1982) studied in this complex, and the results presented here which show that only a single residue of the core interacts with pea lectin. ConA differs from the above-mentioned lectins in that it binds the trimannose core with an affinity similar to that of biantennary glycopeptides. In fact, spectroscopic evidence implicates both arms of the trisaccharide in this interaction (Carver et al., 1985).

Bourne et al. (1992) have proposed a model describing the interaction of LOL 1 with a glycoprotein containing a fucosylated biantennary N-linked glycosaccharide. In their model the trimannose core and GlcNAc5′ play the primary role in binding the lectin, and it is suggested that Fuc1′, which does not interact with the lectin in this model, serves to modulate the conformation of GlcNAc1 and GlcNAc2. However, the model was based on their complex with an unfucosylated oligosaccharide which may not be representative of the high affinity interaction. Certainly the results reported here and the observation that the tightest binding pea lectin glycopeptides terminate in the trimannose core would suggest that, for pea lectin at least, the fucose makes a direct binding interaction with the lectin.

CONCLUSIONS

As the first step in characterizing the high affinity pea lectin-oligosaccharide interaction, we have determined the structure of pea lectin with the trimannosyl core of the N-linked type structure. The trisaccharide binds to the lectin through a single terminal mannose residue strongly suggesting a direct role for fucose binding in the high affinity oligosaccharide interactions. Comparison with the unbound structure shows that on ligand binding the 216–218 loop undergoes a shift in position to maximize hydrogen bond interactions with the carbohydrate. A similar analysis with ConA shows that this loop may be in the carbohydrate binding conformation even in the absence of bound carbohydrate. Differences in the conformation of the unliganded binding sites of these two proteins are also reflected in the water structure within the binding site. Furthermore, a correspondence between the positions of these water molecules and the oxygen atoms in the bound carbohydrate has been illustrated. Clearly, the challenge that remains is to determine in quantitative terms, the role played by each of these components in defining the
affinity and specificity of these interactions.

Acknowledgment—We thank Dr. Ian Wilson for the use of computer and graphics facilities during part of the refinement of this structure.

REFERENCES