Crystal Structure of Pterocarpus angolensis Lectin in Complex with Glucose, Sucrose, and Turanose*

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The crystal structure of the Man/Glc-specific seed lectin from Pterocarpus angolensis was determined in complex with methyl-α-D-glucose, sucrose, and turanose. The carbohydrate binding site contains a classic Man/Glc type specificity loop. Its metal binding loop on the other hand is of the long type, different from what is observed in other Man/Glc-specific legume lectins. Glucose binding in the primary binding site is reminiscent of the glucose complexes of concanavalin A and lentil lectin. Sucrose is found to be bound in a conformation similar as seen in the binding site of lentil lectin. A direct hydrogen bond between Ser-137(OG) to Fru(O2) in Pterocarpus angolensis lectin replaces a water-mediated interaction in the equivalent complex of lentil lectin. In the turanose complex, the binding site of the first molecule in the asymmetric unit contains the αGlc1-3βFru form of furanose while the second molecule contains the αGlc1-3βFru form in its binding site.

Lectins are carbohydrate-binding proteins other than immunoglobulins that display no enzymatic activity toward the recognized sugars. Lectins are found in all kingdoms of life ranging from micro-organisms (1–4) to plants (5–8) and animals (9–15). The biological functions associated with their carbohydrate binding activities are diverse. Many different, evolutionarily unrelated, lectin families have been identified. The legume lectin family has proven to be a very useful model system to study protein carbohydrate interactions. Their highly variable carbohydrate specificity makes them ideal to study the structural basis of carbohydrate specificity. Because they are often expressed in high yields in legume seeds, they can easily be purified in amounts suitable for experimental approaches that require large amounts of protein such as microcalorimetry and x-ray crystallography. Indeed, the crystal structures of 22 legume lectins and three other family members without lectin activity have been determined by x-ray crystallography. For most of these, complexes with one or more carbohydrates have been studied (8–9, 16–24).

The seed lectin from the tropical legume Pterocarpus angolensis (bloodwood tree) belongs to the mannose/glucose specificity group that contains several well studied members such as concanavalin A and the lectins from Lathyrus oenanthus, Lens culinaris, Pisum sativum, and Dioclea grandiflora as well as several other Canavalia and Dioclea species. Although they share a common monosaccharide specificity, all these lectins differ in the details of their specificity for oligosaccharides. In the current paper we present the crystal structures of the complexes of Pterocarpus angolensis lectin (PAL) with glucose, sucrose, and turanose, which were shown to inhibit the hemagglutination activity of this lectin.2

Crystal structures of lectin-carbohydrate complexes have contributed to our understanding of carbohydrate conformation and dynamics (25–26). Often oligosaccharide complexes of legume lectins that have been crystallized have provided the only crystal structures of the carbohydrates involved. Indeed, oligosaccharides are notoriously difficult to crystallize. Consequently only very few carbohydrates larger than a disaccharide are present in the Cambridge structural database. The current structures provide further evidence for the conformational preference of sucrose and provide the first structure of pyranose form of turanose.

MATERIALS AND METHODS

Crystallization and Data Collection—Crystallization conditions were screened using the hanging drop method with the Hampton Research Crystal Screen kit. Large single crystals of the methyl-α-D-glucopyranoside complex were obtained by equilibrating drops consisting of 5 μl of protein (10 mg/ml) and 10 mM sugar in 20 mM Tris-HCl, pH 7.45, and 5 μl of reservoir solution against 1 ml of reservoir solution (200 mM calcium acetate, 100 mM sodium cacodylate, pH 6.5, and 20% (w/v) PEG8000). Complexes of the lectin with sucrose and turanose were obtained by transferring the crystals of the methyl-α-D-glucose complex to artificial mother liquor (200 mM calcium acetate, 100 mM sodium cacodylate, pH 6.5, and 20% (w/v) PEG8000) containing increasing concentrations of the desired ligand (100 mM final concentration reached in four steps of a 5-min incubation). All x-ray data were collected at room temperature on the EMBL beamlines of the DESY synchrotron (Hamburg, Germany). Data were integrated with DENZO,

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1 The abbreviations used are: PAL, Pterocarpus angolensis Man/Glc-specific seed lectin; DESY, Deutsches Elektronen Synchrotron; ConA, concanavalin A from Canavalia ensiformis.
2 R. Loris, A. Imberty, S. Beeckmans, E. Van Driessche, J. S. Read, J. Bouckaert, H. De Greve, L. Buts, L. Wyns, unpublished results.
merged with SCALPACK (27), and converted to structure factor amplitudes using the CCP4 program TRUNCATE (28). The statistics of the data collections are given in Table I.

Structure Determination—The structure of the complex of PAL with Me-α-D-glucose was determined by molecular replacement using the coordinates of lentil lectin (29) as starting model. Two clear solutions were found with AMORE (30) that together constructed the lectin dimer. Refinement was carried out using the maximum likelihood function target of CNS 1.0. Cross-validation, bulk solvent correction, and anisotropic B-factor scaling were used throughout. Rounding of slow cool-simulated annealing and restrained B-factor refinement using all available data were alternated with manual fitting in electron density maps using TURBO (31). At the end of the refinement simulated annealing was abandoned in favor of conventional positional refinement and water molecules were fitted into the electron density. A water molecule was added if the difference density was at least 3 σ, and at least one reasonable hydrogen bond was formed with the protein and no further unfavorable interactions were introduced. Waters were removed again if their temperature factors raised above 60 Å² or no 2Fo − Fc density remained present above 1 σ.

The structures of the PAL-sucrose and PAL-turanose complexes were solved by isomorphous substitution using the coordinates of the glucose complex as the starting model. Refinement and model building was performed as for the glucose complex. The refinement statistics for all three complexes are given in Table I.

Modeling Calculations—Energy maps of the disaccharides of interest have been calculated with the MM3 program (34). Starting conformations were taken from the crystal structure of the complex with the lectin. All hydrogen atoms have been added. Energy maps are calculated as a function of Φ and Ψ defined as: Φ = O5g–C1g–O1g–C3f and Ψ = C1g–O1g–C3f–C4f for turanose and Φ = O5g–C1g–O1g–C2f and Ψ = C1g–O1g–C2g–O5f for sucrose. The driver option of MM3 was selected, allowing for optimization of the disaccharide at each point of the grid. The dielectric constant was set to 78.0 to mimic water environment.

Calculations of the binding energy were performed on the crystal structure of the PAL dimer in two orthogonal orientations. One monomer is colored orange, the other one yellow. Manganese ions are shown as light blue spheres and calcium ions as green spheres. The bound molecules of Me-α-D-glucopyranoside are shown as CPK models.

![FIG. 1. Overall structure of the P. angolensis lectin.](https://www.jbc.org/)

**Table I**

| X-ray data collection statistics | Me-α-Glc (glucose) | Glcα1-2:Fru (sucrose) | Glcα1-3:Fru (turanose) |
|---------------------------------|--------------------|------------------------|------------------------|
| Unit cell (Å)                   | a = 56.76          | a = 56.83              | a = 56.66              |
|                                 | b = 83.55          | b = 83.55              | b = 83.23              |
|                                 | c = 122.93         | c = 123.39             | c = 123.16             |
| Beamline                        | BW7B               | BW7B                   | X31                    |
| Resolution (Å)                  | 15–20 (2.07–2.0)   | 5–21 (2.18–2.1)        | 5–22 (2.28–2.2)        |
| Nmeas                           | 173954 (13215)     | 115196 (11024)         | 107843 (9729)          |
| Nunique                         | 39215 (3190)       | 34823 (3394)           | 29133 (2875)           |
| Completeness (%)                | 97.4 (80.5)        | 99.5 (99.2)            | 95.6 (96.3)            |
| Rmerge                          | 0.090 (0.311)      | 0.084 (0.477)          | 0.149 (0.546)          |
| I/Sigf                          | 12.43 (4.65)       | 10.99 (3.87)           | 11.41 (4.05)           |
| Rf                          | 0.158 (0.222)      | 0.178 (0.240)          | 0.154 (0.238)          |
| Rfree                          | 0.210 (0.268)      | 0.217 (0.293)          | 0.238 (0.283)          |
| PDB code                        | 1N3O               | 1N3P                   | 1N3Q                   |

**RESULTS AND DISCUSSION**

Overall Structure of the P. lectin—The crystal structure of the PAL complexes with glucose, sucrose, and turanose were determined at resolutions between 2.2 and 2.0 Å. The overall structure of the PAL is shown in Fig. 1. The legume lectin fold has been described in detail many times by other authors and will not be repeated here (8). The electron density map is continuous and of high quality for residues 1–238. Tyr-239 was also fitted into the density, but the conformation of this residue remain high. The bound carbohydrates also display clear electron densities in each of the complexes (Fig. 2). Weak density is also seen for residues 240 and 241. The N terminus was clearly identified as a cyclic glutamine and contributes to dimer formation. Excess electron density extends from the side chain of Asn-118 in both molecules in the asymmetric unit of all structures, suggesting a covalent modification. The most likely modification is glycosylation, as Asn-118 lies in a glycosylation consensus sequence (NXT/S). The observed electron density is, however, not sufficiently well defined to allow fitting of a glycan.

**Fig. 1. Overall structure of the P. angolensis lectin.** Shown is a schematic representation of the PAL dimer in two orthogonal orientations. One monomer is colored orange, the other one yellow. Manganese ions are shown as light blue spheres and calcium ions as green spheres. The bound molecules of Me-α-D-glucopyranoside are shown as CPK models.
which is also the most common (8). The long version was until now observed only in two sialyllactose-specific lectins from *Maackia amurensis* (20) and in the chitobiose-specific lectin II from *Ulex europaeus* (18). In both cases, the specificity of these lectins was attributed in part to this loop. The current structure, on the other hand, shows that the backbone conformation of both loop versions is not a determinant for monosaccharide specificity. Specific side chains on this loop, on the other hand, do influence the nature of the sugar that can be accommodated in the binding site (see below).

In contrast to loops A–C, loop D does not interact directly with the structural calcium ion. It is highly variable in length, conformation, and sequence and is often referred to as the monosaccharide specificity loop (8). It is thought to be the prime determinant for monosaccharide as well as oligosaccharide specificity. In the PAL structure, the conformation adopted by this loop is identical to that found in all other known crystal structures of Man/Glc-specific lectins (19, 39, 42–44), supporting this notion.

Finally, loop E is found to interact with a bound carbohydrate in only a few cases: *M. amurensis* leukoagglutinin in complex with sialyllactose (20), *Griffonia simplicifolia* lectin IV in complex with the Leb tetrasaccharide (45) and ConA in complex with Man(α1–2)Man (46). In the current complexes of PAL, it is not involved in carbohydrate recognition in the current structures. The calcium and manganese ions are shown as large green and yellow spheres, respectively.

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**Fig. 2.** Electron densities of the sugar residues in the binding site of PAL. methyl-α-D-glucose (a), sucrose (b), Glc1–3Fru (c), and Glc1–3Fruβ (d). In each case an *F*- *F* electron density map was calculated from the refined structure with the corresponding sugar omitted. All maps are contoured at 3σ. Glc1–3Fru is taken from molecule A in the asymmetric unit of the crystal, which is involved in lattice contacts. All other densities are those corresponding to molecule B, which is not involved in crystal packing.

**Fig. 3.** Carbohydrate binding site of PAL. Shown are the five loops A–E that together form the carbohydrate binding site. Side chains that are important for sugar binding are shown in ball-and-stick and are labeled. Loop A contains the conserved cis-peptide bond preceding Asp-86; loop B contains the conserved glycine. Loop C contains a conserved asparagine and most of the residues that are important for metal binding. Loop D is the monosaccharide specificity loop. Loop E, of which Ser-45 is also shown in ball-and-stick, is not involved in carbohydrate recognition in the current structures. The calcium and manganese ions are shown as large green and yellow spheres, respectively.
Indeed the monosaccharide binding site of these lectins is very similar, and all side chain and main chain entities that interact with the glucose residue are conserved. These comprise hydrogen bonds with the carboxylate group of Asp-86, the backbone NH groups of Gly-106, Glu-221, and Gln-222 and the side chain amino group of Asn-138 as well as van der Waals interactions with Phe-132 (Table II and Fig. 4).

When comparing the two molecules present in the asymmetric unit, only minor differences are observed. These are a slightly different conformation of the side chain of Glu-221 and a small shift in the backbone conformation of residues Ser-137 to Asn-138. Molecule A in the asymmetric unit is involved in packing interactions while molecule B is not, and these small differences are entirely due to the differences in packing environment. Indeed, when one superimposes molecules B of the sucrose and turanose or sucrose complexes (see below), no relevant differences in the binding site residues are seen.

Sucrose Binding—Sucrose is bound to PAL with its glucose moiety in the primary binding site. The fructose residue makes only few interactions with the protein (Fig. 5 and Table II). A strong hydrogen bond is made between O3-f and Ser-137(OG) in addition to a weaker one between O1-g (the oxygen of the glycosidic linkage) and the same side chain. The binding mode of the sucrose molecule is very similar to that observed in the complex with lentil lectin (48), except that in the present structure there is a direct interaction between the fructose residue and the protein. Lentil lectin has a shorter metal binding loop, and hence there is no structural equivalent for Ser-137 of PAL (Fig. 5b). Interestingly, OG of Ser-137 is mimicked by a water molecule in lentil lectin (Wat1 in Fig. 5b). Similar to Ser-137(OG), this water molecule not only hydrogen bonds to fructose but also to the side chain NH2 group of Asn-138 (Asn-138 in PAL).

Sucrose in the crystalline state adopts a folded conformation with two intramolecular hydrogen bonds (49–50). This conformation is known to deviate significantly from the experimen-

### Table II

Hydrogen bonds between sugar and protein in the different complexes

A dash indicates that no equivalent hydrogen bond exists.

| Atoms involved in hydrogen bond | Glucose Me-Glc | Sucrose Glc(α1→2)Fruf | Turanose Glc(α1→3)Frup | Turanose Glc(α1→3)Prupβ |
|--------------------------------|---------------|-----------------------|------------------------|------------------------|
| Glc O3 Gly-106 N               | 2.71/2.85     | 3.12/2.78             | 2.80                   | 2.96                   |
| Glc O4 Asp-86 OD1              | 2.56/2.64     | 3.12/2.70             | 2.52                   | 2.63                   |
| Glc O4 Asn-138 ND2             | 3.01/2.92     | 2.44/2.93             | 3.15                   | 3.06                   |
| Glc O5 Glu-221 N               | 2.90/3.09     | 3.38/3.15             | 3.20                   | 3.23                   |
| Glc O6 Asp-86 OD2              | 2.69/2.75     | 2.90/2.76             | 2.52                   | 2.72                   |
| Glc O6 Glu-221 N               | 3.05/3.20     | 2.78/3.09             | 3.06                   | 3.22                   |
| Fru O1 Glu-222 NE2             | ...           | ...                   | ...                    | ...                    |
| Fru O1 Wat (→ Glu-221 OE2)     | ...           | 3.01/...              | ...                    | ...                    |
| Fru O2 Ser-137 OG              | ...           | 3.25/3.37             | 3.14                   | 3.17                   |
| Fru O2 Asp-136 OD2             | ...           | ...                   | 2.62                   | ...                    |
| Fru O2 Wat (→ Asp-136 OD1/OD2) | ...           | ...                   | 2.67/2.94              | 2.85                   |
| Fru O3 Ser-137 OG              | ...           | ...                   | ...                    | ...                    |
| Fru O5 Wat (→ Glu-221 OE2)     | ...           | 2.92/...              | ...                    | ...                    |
| Fru O4 Glu-221 OE2             | ...           | ...                   | ...                    | ...                    |
tally determined one in solution (51–52) as well as from theoretical prediction from molecular modeling (53). Also, a series of sucrose-containing oligosaccharides have been crystallized, and rather different conformations of the \( \alpha \)H9251 Glc1–2\( \beta \)Fru\( f \) linkage have been observed (54). These experimental data have been reported on the energy map of the sucrose molecule together with the lowest energy conformation (Fig. 6a). The lentil lectin-sucrose complex was the first protein-sucrose complex to be determined by x-ray crystallography. The conformation observed in the lentil lectin complex (\( \Phi = 107^\circ \), \( \Psi = -58^\circ \)) is rather similar to the one in the sucrose crystal structure (\( \Phi = 108^\circ \), \( \Psi = -45^\circ \)), albeit with no intramolecular hydrogen bond. In this complex, the sucrose molecule is directly involved in crystal packing interactions. The conformation observed in the sucrose-PAL complex (\( \Phi = 118^\circ \), \( \Psi = -47^\circ \)) belongs to the same conformational family as the one observed in the lentil lectin complex.

A key feature of the lentil lectin-sucrose complex was a water bridge between Glc(O2) and Fru(O3) (Wat2 in Fig. 5b) This bridging water was also observed in a molecular dynamics simulation of sucrose in explicit water (55–56). The PAL-sucrose complex does not show this water, despite that it would sterically be possible to fit one. The reason for this can be 2-fold. The resolution of the PAL-sucrose complex (2.1 Å) is not as good as the resolution of the lentil lectin-sucrose complex (1.9 Å). More important is probably the crystal environment. In the lentil lectin-sucrose structure, the sugar is tightly packed in crystal lattice interactions including a well defined water network. Lattice interactions in monomer A of the PAL-sucrose complex are less abundant, and they are completely absent for the binding site of monomer B. It is thus possible that the bridging water is indeed present a fraction of the time (as was suggested by the molecular dynamics study (55–56)), but to be observed in the crystal it needs to be stabilized by additional interactions.

FIG. 5. Binding of sucrose to PAL and lentil lectin. a, complex of sucrose with PAL. b, equivalent complex with lentil lectin shown in the same orientation. Both complexes are very similar, but the OH group of Ser-137 of PAL is replaced by Wat1 in the lentil lectin structure. Wat2 corresponds to a water molecule that is also present in simulations of the structure of sucrose in explicit water.

FIG. 6. Energy maps of sucrose (\( \alpha \)Glc1–2\( \beta \)Fru\( f \)) (a), turanose (\( \alpha \)Glc1–3\( \beta \)Fru\( p \)) (b), and turanose (\( \alpha \)Glc1–3\( \beta \)Fru\( f \)) (c). Iso-energy contours have been drawn by interpolation of 1 kcal/mol above the global minimum of each map. The lowest energy conformations is indicated by a star. Black circle, conformation observed in complex with PAL; open circle, conformation observed in complex with lens lectin; black square, conformation in the crystal structure of the disaccharide; cross, conformations in crystal structure of sugar derivatives or oligosaccharides containing this linkage.
Turanose—Turanose, or Glc(\(1\)–3)Fru, occurs in solution in three major isomeric forms that are in equilibrium via the open chain form of the fructose residue: Glc1–3Fru (20%), Glc1–3Frup (41%), and Glc1–3Frup (39%) (57–58). The crystal structure of the pyranose form of turanose is known (59). As for the furanose form, it has not been crystallized as a disaccharide but only as a fragment of the melezitose trisaccharide (60–61).

Unexpectedly, turanose is bound as Glc1–3Fruf in the binding site of molecule A and as Glc1–3Frup in the binding site of molecule B. In both cases, the fructose interacts with the protein via hydrogen bonds (Table II and Fig. 7). In its pyranose form, the fructose ring adopts a \(\alpha\)C\(_5\) conformation, whereas in its furanose form, the ring shape is an \(\alpha\)E envelope that corresponds to the lowest energy region as defined by molecular mechanics study (62).

The conformations observed at the glycosidic linkage are plotted on the corresponding energy maps (Fig. 6, b and c). The conformational analysis of turanose in its pyranose form has been performed earlier (63), and the potential energy surface was very similar to the one reported here. As for the turanose in its furanose form, the energy map was not calculated earlier and it does not present major differences from the pyranose form. In both forms, the conformation observed in the crystal binding site belongs to the same conformational family as the calculated global minimum although with some differences in the value of the \(\Psi\) torsion angle.

The electron densities in both sites are very clear (Fig. 2, c and d) and suggest that at least 80% of the sugar is in the Glc1–3Fruf and Glc1–3Frup isomer, respectively. In absence of other factors, the reason for this phenomenon can only be the difference in crystal environment. In monomer B the binding site is not involved in lattice contacts. It may thus be assumed that the lectin prefers the pyranose form over the furanose one in solution.

To better understand the selection for the furanose and pyranose isomers, modeling calculations were performed with Glc1–3Fruf and Glc1–3Frup in the binding sites of molecules A and B, with and without taking into account crystal lattice contacts. The results of these calculations are shown in Table III. There are no meaningful differences between the calculated interaction energies \(\Delta H_{\text{inter}}\) for the furanose and pyranose form in site B. However, in site A, the additional symmetry-related contacts indeed seem to stabilize the furanose form.
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