Despite the fact that complex saccharides play an importan
t role in many biological recognition processes, molecular level
descriptions of protein-carbohydrate in-
teractions are sparse. The legume lectin concanavalin A
(con A), from Canavalia ensiformis, specifically recog-
nizes the trimannoside core of many complex glycans.
We have determined the crystal structure of a con A-
trimannoside complex at 2.3-Å resolution and now de-
scribe the trimannoside interaction with con A. All three
sugar residues are in well defined difference electron
density. The 1,6-linked mannose residue is bound at the
previously reported monosaccharide binding site; the
other two sugars bind in an extended cleft formed by
residues Tyr-12, Pro-13, Asn-14, Thr-15, and Asp-16. Hy-
drogen bonds are formed between the protein and all
three sugar residues. In particular, the 1,3-linked man-
nose residue makes a strong hydrogen bond with the
main chain of the protein. In addition, a water molecule,
which is conserved in other con A structures, plays an
important role in anchoring the reducing sugar unit to
the protein. The complex is further stabilized by van der
Waals interactions. The structure provides a rationale
for the high affinity of con A for N-linked glycans.

It is well established that carbohydrates play a role in a
myriad of important biological recognition processes; infection,
the immune response, cell differentiation, and neuronal de-
velopment may all be regulated to some extent by protein-carbo-
hydrate interactions (1–4). One area of therapeutic interest in
carbohydrate recognition has relied on the their role as cell
surface receptors enabling adherence of bacteria, parasites,
and viruses in the early stages of infection (5, 6). The abnormal
structure and levels of certain tumor cell surface glycans may
also present opportunities for therapeutic intervention (7). The
notion of using oligosaccharide analogues to disrupt cell-cell
recognition is an appealing one, and is the focus of considerable
current activity in relation to the development of anti-inflam-
atory agents (8). However, the ubiquitous use of carbohy-
drates in nature potentially poses serious specificity problems.
Understanding the molecular basis of carbohydrate recognition
might provide the necessary basis on which to rationally design
biologically active saccharide analogues.

Although highly homologous, and often sharing monosacca-
ride selectivity, plant lectins exhibit exquisite oligosaccharide
specificity (9). While the function of these proteins is unknown,
their interaction with saccharides has proved a valuable source
of fundamental information. Structures of protein-saccharide
complexes have been reported for lectins from Erythina
corallodendron (EcorL) (10), Griffonia simplicifolia (GS4)
(11), Lathyrus ochrus (LOL1) (12, 42–44), pea (13), and lentil
( preliminary data only ) (14). The GS4 and EcorL lectins are
galactose-specific, while LOL1, pea, and con A are mannose-
specific. Interestingly, the overall organization of the monosac-
charide binding site is conserved among the lectins (9). In GS4
and EcorL, the galactose residue is rotated relative to mannose
in the binding site with only subtle changes in the precise side
chain organization observed. Oligosaccharide complexes of
EcorL, GS4, and LOL1 have provided structural insights into
carbohydrate recognition, and, in particular, these structures
have provided experimental evidence for the importance of
water molecules in mediating carbohydrate recognition (12).

Concanavalin A (con A)1 is the most extensively studied
member of the lectin family, and was first isolated and crystal-
lized in 1919 (15). Although the structure of the protein was
determined in the early 1970s (16), it was not until 1989 that
the 2.9-Å structure of con A-methyl α-D-mannopyranoside
complex was determined (17). This represented the first structure
of any lectin carbohydrate complex, and it explained the so-
called “Goldstein rules” for con A monosaccharide specificity
(18). The sugar was reported to be anchored to the protein by
several direct hydrogen bonds and by van der Waals interac-
tions. Subsequent extension of the resolution to 2.0 Å (19)
 permitted a more detailed description of the contacts between
the protein and the monosaccharide. However, the same depth
of understanding is not available to explain the oligosaccharide
specificity of lectins. The precise contributions of hydrogen
bonding, van der Waals interactions, and rearrangement of
bound and bulk water to the specificity of the lectin-oligosac-
charide interactions continues to be a subject of interest (20).

The oligosaccharide specificity of con A is well documented
(9). Interactions are centered on the so-called trimannoside core
(Fig. 1) found in all N-linked glycans, and it is this specific
interaction that forms the basis of con A’s use as a tool in
histochemical staining (21). Although there are several reports
of modeling studies on the con A-oligosaccharide interaction
(22, 45, 46), no crystal structure has been reported for con A
complexed to any oligosaccharide. We now report the 2.3-Å
resolution structure of con A bound to the N-linked glycan core
trimannoside Manα1–6(Manα1–3)Man.

MATERIALS AND METHODS

Con A and Manα1–6(Manα1–3)Man were purchased from Sigma
(Poole, United Kingdom) and Dextra Laboratories (Reading, United
Kingdom), respectively.

Crystals (dimensions: 0.3 mm × 0.4 mm × 1.2 mm) of the protein-
carbohydrate complex were obtained, after 2 weeks, from a hanging
drop of 8 mg/ml protein and 7 mM trimannoside equilibrated against
20% polyethylene glycol (M, 6000), pH 9.0. All diffraction data were

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† The abbreviation used is: con A, concanavalin A.

‡ Recipient of Equipment Grant 043586/Z/95/Z/MP/RF/PK from the
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collected at room temperature on a crystal mounted in a glass capillary using the Enraf-Nonius/MacScience DIP2000 dual image plate. X-rays were generated using an Enraf-Nonius FR591 rotating anode generator and focused using the MacScience mirror system. The fresh crystal diffracted to 2.1 Å; however, crystal decay limited the effective resolution to 2.3 Å. Data were recorded as 252 non-overlapping 12.5-min 0.5° oscillations and processed using DENZO and SCALEPACK (23). The crystal was a primitive unit cell of dimensions a = 81.65 Å, b = 66.68, c = 108.32, α = γ = 90.0°, β = 97.79°. The asymmetric unit contains a tetramer; Matthew’s number 2.9 Da Å⁻³, approximately 52% solvent. A k + 2n systematic absence was visible, and the space group was assigned as P2₁. A summary of the data is given in Table I. The structure was determined using the molecular replacement procedure AMORE (24) as implemented in the CCP4 package (25). The 2.4 Å structure of the methyl α-D-mannopyranoside-con A complex (Protein Data Bank code 1CVN) was used as the search model (with metal ions, sugars, and waters removed). A random subset of data (10%) was omitted from all refinement calculations in order to provide an unbiased assessment of the refinement (26). The raw molecular replacement solution in P2₁ was rigid body refined to 2.7 Å and gave a free R-factor of 34.0% and an R-factor of 34.6%. An Fₚ - Fc electron density map was generated with phases calculated from the rigid refined model, strong density (>5σ) was observed for all 8 metal ions and for 8 of the 12 possible sugar units, with weak but convincing density for the remaining 4. This was taken as confirmation of the correctness of the space group assignment and the molecular replacement solution. Refinement proceeded with X-PLOR (27) (restrained positional and thermal factor) alternating with manual intervention using O (28). Non-crystallographic positional and thermal factor restraints were maintained throughout the refinement. The metal ions were included in the refinement with zero electrostatic charge, and the trimannoside molecules were included when a difference Fₚ - Fc map showed 3σ density for all 12 mannose residues (Fig. 2). Water molecules were added to the model, if (a) they corresponded to peaks with magnitudes greater than 3.5σ in the Fₚ - Fc map, (b) they made physically reasonable hydrogen bonds with the protein (or other ordered water molecules), (c) they subsequently reappeared in peaks of 1.0σ in the FC - Fc map, and (d) a drop in the free R-factor was observed. The final model comprises 948 residues and has an R-factor of 20.5% and a free R-factor of 25.5% for data between 8 and 2.3 Å with F > 2.5σ for all data in the range from 8 to 2.3 Å, the R-factor is 21.7%. Table II gives a summary of refinement results. A Ramachandran plot (not shown) revealed 1 residue in a generously allowed region and no residues in disallowed regions, 86.5% of the residues were in the most favored regions and the remaining 13.5% in additionally allowed regions. All stereochemical parameters measured by PROCHECK version 3.3 (29) were better than average for a structure at 2.3 Å (data not shown). Co-ordinates (code 1CVN) and structure factors (code R1CVNSF) have been deposited with Protein Data Bank (30). Coordinates will be available 6 months after the date of this publication, and structure factors after 1 year.

**RESULTS AND DISCUSSION**

Overall Structure of the Protein in Con A-Trimannoside Complex—In the crystal of the con A- Trimannoside complex, con A is a tetramer with each monomer consisting of a sandwich of two β sheets (Fig. 3). The overall fold of the protein is identical to the native structure (16). Residues 118–123 are almost completely disordered in this structure; the loops at Ser-161 and Ser-204 are also in poor density. The remainder of the structure is well ordered, and apart from the loops mentioned above, the 2Fₚ - Fc electron density is unbroken for the backbone at a contour level of 1.0σ. Comparing this structure to the 1.75-Å native structure (31) shows an average root mean square deviation for all CA atoms of 0.47 Å; excluding flexible and disordered loops reduces this value by more than half. Comparing the trimannoside complex reported here to the 2.0-Å methyl

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**TABLE I**

| Crystallographic data collection statistics | | |
| --- | --- | --- |
| Quantity | Resolution range | |
| | B-2.3 Å | 2.4-2.3 Å | |
| Number of reflections | 48,025 | 5025 | |
| Average redundancy | 2.3 | 1.5 | |
| R-merge⁵ | 8.5% | 22.2% | |
| % complete⁶ | 95% | 80% | |
| % complete (F > 2.5σF)⁷ | 88% | 67% | |

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**TABLE II**

| Final model of the trimannoside-con A complex | | |
| --- | --- | --- |
| Number of protein atoms | 7236 | |
| Number of sugar atoms | 136 | |
| Number of water molecules | 273 | |
| Number of metal ions | 8 | |
| Root mean square deviations from | | |
| Ideal bond lengths⁸ | 0.009 Å | |
| Ideal bond angles⁹ | 1.78° | |
| Perfect non-crystallographic symmetry⁸ | 0.11 Å | |
| Mean temperature factor | | |
| Main chain | 16.9 Å² | |
| Side chain | 18.4 Å² | |
| Sugar atoms | 20.31 Å² | |
| Water molecules | 26.82 Å² | |
| Crystallographic R-factor (F > 2.5σF) | 20.5% | |
| Free crystallographic R-factor (F > 2.5σF) | 25.5% | |

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⁵ R-merge = Σ||Ii - I(hkl)|| / ΣI(hkl), (hkl).
⁶ Number of reflections/total number of possible reflections.
⁷ Number of reflections (F > 2.5σF)/total number of possible reflections.
complex (13) only one mannose residue was visible and the remaining two sugars units disordered (in effect directly analogous to structures of the con A and LOL1 monosaccharide complexes). The direct recognition of all three sugar units of trimannoside by con A is in marked contrast to the extensive complexes). The direct recognition of all three sugar units of trimannoside by con A is in marked contrast to the extensive water network surrounding the sugar was not observed.

Conformation of Bound Trimannoside—NMR studies on N-linked glycans indicate two principle conformations for the α-1,6-anomers of the trimannoside component, with the α-1,3-antenna being conformationally invariant (except where the β-Man unit is substituted) (32, 33). In solution, the α-1,6-antenna exists predominantly in an extended "forward" form. This arrangement is also evident from the crystal structure of con A-bound trimannoside, and is one of the conformations predicted by Imberty and Perez (46) from molecular modeling studies on the binding of GlcNAcβ1–2Manα1–6Man to con A. In effect, con A binds the trimannoside in its solution conformation without substantial conformational perturbation of the saccharide.

Since O4 of the reducing sugar unit is in contact with Tyr-12, substitution at this position is expected to abolish ligand binding in the mode observed for the unsubstituted trimannoside. Such a steric clash accounts for the reduced binding (34) of con A by N-linked glycans possessing a bisecting GlcNAc residue at O-4 of the trimannoside unit.

Specific Protein-Carbohydrate Contacts—All three sugar residues make contacts with the protein by way of hydrogen bonds and/or van der Waals interactions (Tables III and IV, Fig. 4).

1,6-Linked Mannose—The 1,6-linked mannose residue sits in the monosaccharide binding site of con A, and interactions between the protein and this sugar unit are essentially those observed in the con A-methyl α-mannoside complex (17).

Reducing Mannose—The binding site for the reducing man-

nose residue is bounded by Tyr-12, which forms a hydrogen bond to mannose O-4. In addition, O-2 is hydrogen-bonded to a structurally conserved bridging water molecule, which is also present in both native con A and the methyl α-mannoside-con A complex. While the trimannoside used in this study contains a free reducing terminus, which can equilibrate between α- and β-anomers, well defined density is observed for the α-configured hemiacetal. The anomeric hydroxyl group makes no contact with protein. A β-configuration, as present in the N-linked oligosaccharide ligands for con A (Fig. 1), would also make any contact with protein.

1,3-Linked Mannose—The 2- and 6-hydroxyl groups of the 1,3-linked mannose residue are not in contact with either protein or bridging water. OH-3 forms a strong hydrogen bond to the to the protein backbone (Thr-15) and is also hydrogen-bonded to the side chain of Thr-15; O-4 is also hydrogen-bonded to the side chain of the same residue. Work reported by Mandal and co-workers (35), which employed a systematically modified series of monodeoxytrimannosides, demonstrated that the 2,4- and 6-hydroxyl groups of the 1,3-linked mannose unit of trimannoside are not essential for inhibition of con A-mediated hemagglutination of rabbit erythrocytes. In contrast the corresponding 3-deoxy sugar was 10-fold less effective. The apparent recognition recorded here of OH-4 of the 1,3-linked mannose unit of trimannoside by Thr-15 was therefore somewhat unexpected. It should be noted, however, that the observed Thr-15OG1-O-4 heteroatom distance of 3.1 Å is at the longer end of what one might consider a strong hydrogen bond.

His-205 and Trimannoside Recognition—Carver and co-workers (22) have suggested that His-205 is located near to the 1,3-arm of the bound trimannoside, and may contribute to its recognition. This model has been further commented on by Chervenak and Toone (36). Data reported here do not support a role for His-205 in trimannoside recognition, since it is located at least 6.5 Å from the bound ligand.

Overall Picture—Unlike most other mannose-specific lectins, con A specifically recognizes all three sugar units of the N-linked glycan trimannoside core. Data reported here confirm a single high affinity site on con A for the 1,6-linked mannose unit of the trimannoside, together with an extended binding

![Image](https://example.com/image.png)
site that forms specific direct and indirect (via structural water) contacts with both the reducing and 1,3-linked mannose units.

Structural Basis of the High Affinity for Trimannoside by Con A and Dioclea grandiflora Lectin, but Not Pea Lectin or LOL1—The extended con A binding site for trimannoside results in specific interactions with all three sugar units. As noted above, the 1,6-linked sugar is recognized by the single high affinity monosaccharide binding site of con A (17). Similar monosaccharide recognition sites are found on both pea lectin and LOL1 (42, 13). However, neither of the latter two lectins bind trimannoside with high affinity, indicating that the specificity of con A for trimannoside resides in the extended binding site, which recognizes both the reducing unit and 1,3-linked mannose residue.

The reducing mannose residue makes good hydrogen bonds to both Tyr-12 and a bridging water molecule, which is in turn ligated by Asn-14, Asp-16, and Arg-228. In both pea lectin and LOL1 (42) the con A Tyr-12 is replaced by Phe, which is incapable of making the hydrogen bond to the reducing mannose O-4 observed in the con A-trimannoside complex. In addition, two of the three residues that act as ligands to the structural water are replaced by non-polar residues: Asp-16 by Ala (37) and Arg-228 by Gly (42). Although a water is found in the same position in LOL1 (but not in pea lectin), it is only ligated by one Asn residue. The two key recognition elements for the reducing unit of trimannoside are therefore substantially different in pea lectin and LOL1. The 1,3-linked mannose residue forms a strong hydrogen bond to the main chain of the protein, with additional hydrogen bonds to O-3 and O-4 from Thr-15.

**Table IV**

| Sugar Residue                   | Residue                      |
|---------------------------------|------------------------------|
| 1,6-Terminal mannose            | Tyr-12, Asn-14, Gly-98, Leu-99, Tyr-100, Ala-207, Asp-208, Gly-227, Arg-228 |
| Reducing sugar                  | Tyr-12, Asp-16, Leu-99, Arg-228 |
| 1,3-Terminal mannose            | Tyr-12, Pro-12, Asn-14, Thr-15, Asp-16 |

**Figure 4.** The extended trimannoside binding site of con A. Panel A, a close-up view of the saccharide in the binding site. The amino acids are shown as space-filling spheres with side chains colored by residue type; the trisaccharide is shown in stick format. (Figure was generated by RASTER3D (41, 47)). Panel B, a schematic representation of the hydrogen bonds between the sugar and protein. The distances for these hydrogen bonds are given in Table III.
residue is conserved in LOL1, but is replaced by Ala in pea lectin (37). We have already mentioned that the contribution of Thr-15 to trimannoside recognition warrants further investigation.

We note that the amino acids we have identified as being important for recognition of trimannoside by con A (Tables III and IV, Fig. 4B) are conserved in the Diocleagrandiflora lectin (38), which also has a high affinity for trimannoside (36).

Summary—The con A-trimannoside structure reveals a highly specific interaction based on hydrogen bonds to the main chain and/or side chain of the protein from all three sugar residues. A structurally conserved water molecule appears to play a crucial role in recognition.

A comparison between the native and mannoside complexes of con A at 2.0-Å resolution shows that trimannoside binds to con A at a relatively rigid and preformed extended binding site from which water molecules are displaced. Specific direct and indirect interactions with amino acids, which are not conserved in LOL1 and pea lectin, account for the high affinity and specificity of con A for the trimannosyl core of N-linked glycans.

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