Structural basis of carbohydrate recognition by a Man(α1-2)Man-specific lectin from Bowringia milbraedii

Lieven Buts1*, Abel Garcia-Pino*, Lode Wyns, and Remy Loris

Laboratorium voor Ultrastructuur, Vrije Universiteit Brussel and Department of Molecular and Cellular Interactions, Vlaams Interuniversitair Instituut voor Biotechnologie, Pleinlaan 2, B-1050 Brussels, Belgium

Received on January 13, 2006; revised on March 23, 2006; accepted on March 23, 2006

The crystal structure of the seed lectin from the tropical legume Bowringia milbraedii was determined in complex with the disaccharide ligand Man(α1-2)Man. In solution, the protein exhibits a dynamic dimer–tetramer equilibrium, consistent with the concanavalin A-type tetramer observed in the crystal. Contacts between the tetramers are mediated almost exclusively through the carbohydrate ligand, resulting in a crystal lattice virtually identical to that of the concanavalin A:Man(α1-2)Man complex, even though both proteins have less than 50% sequence identity. The disaccharide binds exclusively in a “downstream” binding mode, with the non-reducing mannose occupying the monosaccharide-binding site. The reducing mannose is bound in a predominantly polar subsite involving Tyr131, Gln218, and Tyr219.

Key words: carbohydrate recognition/lectin/legume lectin/ X-ray crystallography

Introduction

Lectins are carbohydrate-binding proteins of non-immune origin that specifically recognize mono- and oligosaccharide structures. They occur in all kingdoms of life and mediate a wide variety of biological processes including microbial infections, cellular differentiation, and cancer metastasis (Taylor and Drickamer, 2003). Several families of plant lectins have been extensively studied (Peumans and Van Damme, 1995). The legume lectins in particular have become a model system for protein–carbohydrate recognition because of their wide range of specificities for monosaccharides as well as for complex carbohydrates (Sharon and Lis, 1990).

More than 100 legume lectins have been identified in species with a global geographic distribution. Crystal structures have been determined for 29 members of the family (http://www.cermav.cnrs.fr/glyco3d/index.php), most of them in complex with one or more carbohydrate ligands. From these studies, it has been established that legume lectins have a conserved monomeric scaffold but at the same time exhibit a remarkable variety of quaternary associations with important functional implications (Srinivas et al., 2001). Their carbohydrate-binding sites are formed by residues contributed by four or five loops. Three of these contain a set of very strongly conserved residues that make up the primary or monosaccharide-binding site. The fourth loop, which is highly variable in length and composition, is thought to be the prime determinant of monosaccharide specificity (Sharma and Surloria, 1997; Loris et al., 1998).

The seeds of the Nigerian legume Bowringia milbraedii, a member of the Sophoreae tribe in the legume family, contain a Man/Glc-specific lectin (Bowringia milbraedii agglutinin or BMA) that shows its highest affinity for the disaccharide Man(α1-2)Man (Animashaun and Hughes, 1989; Chawla et al., 1992). BMA is synthesized as a 29-kDa precursor that is posttranslationally cleaved into α and β chains of approximately equal size (Chawla et al., 1993). The β chain contains a single cysteine residue that participates in a disulfide bond between the two monomers. The protein has 48% sequence identity with concanavalin A (con A) from Canavalia ensiformis, which is its closest relative, and the most extensively studied legume lectin in terms of structure (Reeke et al., 1975; Hardman and Ainsworth, 1976; Naismith and Field, 1996; Moothoo et al., 1999; Sanders et al., 2001) and thermodynamics of binding (reviewed in Dam and Brewer, 2002). Here we present the crystal structure of BMA in complex with Man(α1-2)Man.

Results and discussion

Overall structure and crystal packing

The structure of the BMA:Man(α1-2)Man complex was refined against high-resolution diffraction data, resulting in a model with excellent geometry and low crystallographic R factors (Table I). The asymmetric unit contains a single BMA monomer, with clearly defined electron density for residues 1–115 and 120–236. The lack of electron density for Asn116-Ser119 is almost certainly due to a posttranslational cleavage as described by Chawla et al. (1993). Proteolytic processing occurs frequently in legume lectins (Young et al., 1995), and the pattern observed in BMA is most similar to that of Vatairea macrocarpa lectin, which may be considered an evolutionary precursor to the more extensive processing that occurs in lectins from the Phaseoleae tribe (Calvete et al., 1998). Crystallographic symmetry extends the monomer to a canonical legume lectin dimer (Figure 1a), and further to a con A-type tetramer (Figure 1b).

For five residues, the side-chain electron density was clearly in conflict with the reported amino acid sequence...
The most striking discrepancy is the conserved aspartate side chain (Asp86) in the monosaccharide-binding site, which was identified as an alanine in the published sequence. Electron-density maps for this region clearly indicate that there is an aspartate at this position, which fulfills its conserved role in binding the O3 and O4 oxygen atoms of a Man or Glc residue and fixing the orientation of the monosaccharide ring in the site (Loris et al., 1998). In two other cases (Ser125 replacing Ala and Trp207 replacing Asp), the corrections could be unambiguously identified. Residues 204 and 206, for which the electron density suggests either an aspartate or an asparagine side chain, were modeled as Asx residues.

The lattice interactions that pack the tetramers together in the crystal are all mediated by the bound ligand. As a result, the BMA:Man(α1-2)Man complex is able to adopt a crystal packing that is nearly isomorphous with that of the corresponding disaccharide complex of con A (Sander et al., 2001). This is remarkable as both proteins share only 48% sequence identity, which means that the majority of their surface residues differ.

### Quaternary structure

BMA exists in solution as an equilibrium between dimers and tetramers (Garcia-Pino et al., 2005). This equilibrium is not dependent on pH and is characterized by a dissociation constant of about 6 mM in the pH range 4–8, estimated from the observed fractions of dimer and tetramer in analytical gel-filtration profiles (Figure 2). The crystallographic 2-fold along the c axis generates a canonical legume lectin dimer, with the classical side-by-side association of the back sheets of the two monomers (Loris et al., 1998), involving the Asn2-Thr9 strand and the surface loops Ser13-Asp17 and Thr54-Arg58 (Figure 1a). It is most likely that this entity corresponds to the dimeric species observed in solution. Although the total buried surface area of the interface (910 Å² per monomer) is slightly lower than that of the corresponding con A interface (1104 Å²), the presence of a disulfide bond between the facing β strands of the monomers may be an alternative stabilizing factor. Cys5 adopts two side-chain conformations, corresponding to the reduced and oxidized states as confirmed by SDS–PAGE analysis, with and without 10 mM dithiothreitol (DTT).

### Table 1. Data collection and refinement statistics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BMA:Man(α1-2)Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>I222</td>
</tr>
<tr>
<td>Unit cell</td>
<td></td>
</tr>
<tr>
<td>a (Å)</td>
<td>66.6</td>
</tr>
<tr>
<td>b (Å)</td>
<td>86.4</td>
</tr>
<tr>
<td>c (Å)</td>
<td>91.8</td>
</tr>
<tr>
<td>Resolution range (Å)</td>
<td>15.0–1.90 (1.97–1.90)</td>
</tr>
<tr>
<td>Observed reflections</td>
<td>99,945 (7476)</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>18,493 (1699)</td>
</tr>
<tr>
<td>R&lt;sub&gt;merge&lt;/sub&gt; (%)</td>
<td>9.5 (31.0)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>87.3 (80.9)</td>
</tr>
<tr>
<td>&lt;I/σ(I)&gt;</td>
<td>13.2 (4.1)</td>
</tr>
<tr>
<td>R (%)</td>
<td>18.8 (22.7)</td>
</tr>
<tr>
<td>R&lt;sub&gt;free&lt;/sub&gt; (%)</td>
<td>23.0 (30.2)</td>
</tr>
</tbody>
</table>

Geometrical properties
- Bond length r.m.s.d. (Å): 0.006
- Bond angle r.m.s.d. (°): 1.674

Ramachandran plot
- Most favorable (%): 87.4
- Allowed (%): 12.1
- Additionally allowed (%): 0.5
- Forbidden (%): 0.0

Values between parentheses refer to the highest resolution shell.

R<sub>merge</sub> = Σ<sub>hkl</sub> |I<sub>hkl</sub> − <I>|/Σ<sub>hkl</sub> I.

---

Fig. 1. (a) Overall structure of the BMA dimer. The carbohydrate ligands and the disulfide bond connecting the monomers across the canonical interface are indicated in stick representation (green). (b) Structure of the BMA tetramer observed in the crystal context. The two canonical dimers are shown in yellow and orange.

The site (Loris et al., 1998). In two other cases (Ser125 replacing Ala and Trp207 replacing Asp), the corrections could be unambiguously identified. Residues 204 and 206, for which the electron density suggests either an aspartate or an asparagine side chain, were modeled as Asx residues.

The lattice interactions that pack the tetramers together in the crystal are all mediated by the bound ligand. As a result, the BMA:Man(α1-2)Man complex is able to adopt a crystal packing that is nearly isomorphous with that of the corresponding disaccharide complex of con A (Sander et al., 2001). This is remarkable as both proteins share only 48% sequence identity, which means that the majority of their surface residues differ.

### Quaternary structure

BMA exists in solution as an equilibrium between dimers and tetramers (Garcia-Pino et al., 2005). This equilibrium is not dependent on pH and is characterized by a dissociation constant of about 6 mM in the pH range 4–8, estimated from the observed fractions of dimer and tetramer in analytical gel-filtration profiles (Figure 2). The crystallographic 2-fold along the c axis generates a canonical legume lectin dimer, with the classical side-by-side association of the back sheets of the two monomers (Loris et al., 1998), involving the Asn2-Thr9 strand and the surface loops Ser13-Asp17 and Thr54-Arg58 (Figure 1a). It is most likely that this entity corresponds to the dimeric species observed in solution. Although the total buried surface area of the interface (910 Å² per monomer) is slightly lower than that of the corresponding con A interface (1104 Å²), the presence of a disulfide bond between the facing β strands of the monomers may be an alternative stabilizing factor. Cys5 adopts two side-chain conformations, corresponding to the reduced and oxidized states as confirmed by SDS–PAGE analysis, with and without 10 mM dithiothreitol (DTT).
Through the crystallographic 2-fold along the b axis, two canonical dimers associate into a con A-like tetramer. This type of tetrameric arrangement, although the first one to be discovered (Reece et al., 1975; Hardman and Ainsworth, 1976), is relatively uncommon within the large family of quaternary arrangements that have been described for legume lectins (Srinivas et al., 2001). Except for con A and its close relatives from different Canavalia and Dioclea species (Sanz-Aparicio et al., 1997; Rozwarski et al., 1998; Wah et al., 2001; Gadelha et al., 2005), it has until now only been observed in the crystal structure of the Flt3 receptor-interacting lectin (FRIL) from Dolichos lablab (Hamelryck et al., 2000). The tetramer-generating interface of BMA buries only 732 Å² per monomer, compared with 1051 Å² in con A and 843 Å² in FRIL, which is dimeric in solution but has the potential to form highly organized con A-type tetramers in specific conditions. The formation of the con A-type tetramer in the BMA crystals is reminiscent of what is observed for the vegetative legume lectin DB58 from Dolichos biflorus. Although DB58 occurs as a dimer in solution, the crystal structure reveals a PHA-L-type tetramer that is very similar to the tetramer formed by the seed lectin DBL from the same plant (Buts et al., 2001).

Several lectins from the Diocleinae subtribe (including con A) that associate into a tetramer similar to BMA have been found to exhibit pH-dependent oligomerization (Calvete et al., 1999). At pH values below 5.5, con A is almost exclusively dimeric, whereas above pH 7.0 it is almost exclusively tetrameric (Senear and Teller, 1981; Wah et al., 2001). For other lectins from the same group, including the Dioclea grandiflora seed lectin, the equilibrium is strongly shifted toward the tetrameric form over the corresponding pH range. For BMA, no pH dependence was observed, which is in agreement with the absence of ionisable groups in close proximity in the interface.

Carbohydrate binding

The electron density for the carbohydrate ligand is very clear (Figure 3a). The non-reducing Man residue of the Man(α1-2) Man disaccharide binds in the primary- (or monosaccharide-) binding site (Figure 3b), which is formed by a number of residues from four loop regions A–D (Sharma and Surolia, 1997). The lengths and conformations of these loops in BMA are most similar to those in the binding site of con A. The conserved triad consisting of the side-chain carboxylate group of Asp86 (in loop A), the side-chain amide group of Asn133 (loop C), and the backbone amino group of Arg106 (loop B) corresponds exactly to Asp208, Asn14, and Arg228 in the binding site of con A. Tyr131 in the metal-binding loop is the commonly observed aromatic side chain which provides a hydrophobic contact with the B face of the sugar ring in the primary-binding site. Loop D, often referred to as the specificity loop, has an identical conformation and very similar sequence in con A and BMA, with Leu99 in con A corresponding to Gln216 in BMA.

The reducing mannose is bound in a subsite, previously labeled the downstream subsite “+1” (Figure 3b; Loris et al., 2004). This subsite in BMA is predominantly of a polar nature, involving hydrogen bonds with Tyr131 and Gln218.

Additional van der Waals interactions occur with Tyr219, but the major role of this residue seems to be the correct positioning of Gln218 via a parallel π-stacking interaction. Figure 3c compares the conformations of the Man(α1-2)Man glycosidic bond in the BMA complex with those observed in other legume lectin complexes. All conformations fall in the single low-energy region for the Man(α1-2)Man disaccharide, which is fairly restrictive for the Phi angle, but rather more tolerant of variations in the Psi angle (70–180°).

For con A, three distinct binding modes have been observed; binding modes 1 and 3 have the same internal glycosidic bond angles but opposite orientations for the disaccharide in the binding site.
The binding affinity of this disaccharide (Brewer and Brown, 1979) and the Me complex have been considered to contribute to the high binding modes observed in the con A:Man(1-2)Man complex of BMA and con A. Each panel is a stereo superposition of the binding sites of BMA and con A. The four hydrogen bonds which stabilize this conformation in con A (shown in black) cannot be formed in BMA.

Fig. 4. Comparison of the binding modes of Man(1-2)Man in the binding sites of BMA and con A. Each panel is a stereo superposition of the BMA-binding site (colored according to atom type) and one of the binding modes observed in con A (grey and purple), with the disaccharide ligand and side chains in stick representation. BMA side chains are labeled in a regular font, whereas those of con A are in italic. (a) Comparison with binding modes 1 (grey) and 2 (purple). Observed hydrogen bonds between the disaccharide and the BMA-binding site are shown (black). (b) Comparison with binding mode 3 observed in the D-site of P212121. The four hydrogen bonds which stabilize this conformation in con A (shown in black) could fulfill a similar function.

Binding mode 3 is observed in the D chain of the P212121. Here, the reducing mannose is found in the upstream “–1” subsite, which involves mainly residues from loop B (Figure 4b). This situation is similar to the Man(1-2)Man complex of the Pterocarpus angolensis seed lectin (PAL; Loris et al., 2004). Although the crystal packing of BMA does not allow for this binding mode, it would become sterically permissible in the absence of symmetry-related molecules. Nonetheless, Thr226 and Ser168, which form a total of four hydrogen bonds with the non-reducing mannose in con A, are replaced by Gly104 and Gly46 in BMA. This limits the additional interaction of the non-reducing residue in this binding mode to three van der Waals contacts, severely reducing the stabilization of this binding mode. The alternative binding modes observed in the con A:Man(1-2)Man(1-0) Me complex have been considered to contribute to the high binding affinity of this disaccharide (Brewer and Brown, 1979; Moothoo et al., 1999). This effect seems unlikely to be important in the case of BMA.

Materials and methods

Purification, crystallization, and structure solution

The purification of BMA by affinity chromatography and the subsequent crystallization of the BMA:Man(1-2)Man complex have been described (Garcia-Pino et al., 2005). Crystals were flash-frozen using a 100 mM MES (pH 6.5), 32.5% (w/v) PEG20000 solution for cryoprotection. Diffraction data were collected at EMBL beamline X11 at the DESY synchrotron (λ = 0.8123 Å) and processed using the HKL package (Otwinowski and Minor, 1997). The structure was solved by molecular replacement using Phaser (Storoni et al., 2004; McCoy et al., 2005) with a PAL monomer (chain A from PDB entry 1Q8S; Loris et al., 2004) as the search model. A single clear solution was found, which was refined using the MLF target of CNS 1.1 (Brunger et al., 1998), alternated with manual editing steps using Turbo-Frodo (Roussel and Cambillau, 1989) and Coot (Emsley and Cowtan, 2004). Cross-validation, bulk solvent correction, and overall anisotropic temperature factor scaling were used throughout. Final coordinates and structure factors were deposited in the Protein Data Bank with accession codes 2FMD and r2FMDsf. Figures were made with PyMOL (DeLano, 2002), MolScript (Kraulis, 1991), and Raster3D (Merritt and Murphy, 1994).

Structural analysis

Hydrogen bonds were determined using HBPLUS (McDonald and Thornton, 1994). Van der Waals contacts were analyzed using the CCP4 program CONTACT (Collaborative Computational Project, Number 4, 1994) with a cut-off distance of 4Å. Contact surfaces were calculated using NACCESS (Hubbard and Thornton, 1993). All energy calculations were performed using the Tripos force-field (Clark et al., 1989) together with the PIM (Pérez-Imbert-Mazeau) parameterization (Imbert et al., 1999) developed for carbohydrate–protein interaction. Energy maps were calculated as a function of the Φ and Ψ dihedral angles defined as Φ = O5-C1-O1-C2′ and Ψ = C1-O1-C2′-C3′ for the α(1-2) linkage, according to the crystallographic “+1” convention (Imbert and Perez, 1994).

Supplementary material

Supplementary data are available at Glycobiology online (http://glycob.oxfordjournals.org/).

Acknowledgments

We thank Theresa Animashaun for kindly providing seeds of Bowringia milbraedii. The authors acknowledge the use of synchrotron beamtime at the EMBL beamlines at the DORIS storage ring (Hamburg, Germany). This work was funded by the Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), the Onderzoeksfonds of the Vrije Universiteit Brussel (OZR), and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO).

Conflict of interest statement

None declared.
References


Hubbard, S.J. and Thornton, J.M. (1993) NACCESS [computer program]. Department of Biochemistry and Molecular Biology, University College London.


