Crystal structures of a novel anti-HIV mannose-binding lectin from Polygonatum cyrtonema Hua with unique ligand-binding property and super-structure

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ABSTRACT

Polygonatum cyrtonema lectin (PCL) is a novel anti-HIV mannose-binding lectin from Galanthus nivalis agglutinin (GNA)-related lectin family. Crystal structures of ligand-free PCL and its complexes with monomannoside and β-1-3 dimannoside have been determined. The ligand-free PCL is dimeric, with both subunits adopt the β-prism II fold. PCL subunit binds mannose using a potential bivalent mode instead of the usual trivalent mode, in which carbohydrate-binding site (CBS) I and CBS III adopt the conserved mannose-binding motif of QXDXNXVXY (X is one of any amino acid residues) as observed in other structurally characterized GNA-related lectins, while CBS II adopts a modified motif with residues Gin58 and Asp60, which are critical for mannose-binding, substituted by His58 and Asn60, respectively. As a result, CBS II is unfit for mannose-binding. In the mannoside complexes, ligand-bindings only occur at CBS I which provides the specificity for β-1-3 dimannoside. CBS II and CBS III are cooperatively occupied by a well-ordered sulfate ion, through which the individual dimers are cross-linked to form a unique super-structure of 3 helical lattice. Surveying the sequences of GNA-related lectins revealed that the modified binding motif of CBS II is widely distributed in the Liliaceae family as an intrinsic structural element. There is evidence that other GNA-related lectins will also adopt the similar super-structure as PCL. Thus PCL structure, unique in ligand-binding mode, may represent a novel type of structure of GNA-related lectins. Comparative analyses indicated that the dimer-based super-structure may play a primary role in the anti-HIV property of PCL.

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1. Introduction

Carbohydrate-binding proteins consisting of one or two-domains equivalent to the Galanthus nivalis agglutinin (GNA) form a major plant lectin family, which were originally identified from the Liliopsida (monocots) and shown an exclusive specificity towards mannose, so called “monocot mannose-binding lectins” (Van Damme et al., 1998). In the last decade, it has been found that proteins with GNA domains occur not only in monocots, but also in dicots, fishes and fungi (Peumans et al., 2002; Tsutsui et al., 2003; Machida et al., 2005; Wiens et al., 2006). Therefore it is now referred to this lectin family as “GNA-related lectins” (Van Damme et al., 2007). The majority of all characterized GNA-related lectins consists of subunits derived from primary translation products comprising a single GNA domain, and exhibits an exclusive specificity towards mannose and oligomannosides. More recently, some two-domain GNA-related lectins have been identified that are made up of subunits derived from primary translation products comprising two homologous GNA domains arranged in tandem. Unlike the single-domain GNA-related lectins, the two-domain GNA-related lectins acquire a complex specificity toward both high mannose and N-glycans (Van Damme et al., 2007). Although little is known about the physiological roles of GNA-related lectins, a number of applications based on their carbohydrate-binding specificities have been developed, such as using them as affinity supports for analysis and isolation of high mannose glycoconjugates (Shibuya et al., 1988), as potent inhibitors towards retroviruses including human immunodeficiency virus (HIV) in biomedical research (Balzarini et al., 1991), and as protecting agents against sucking insects and nematodes in plant biotechnology (Hilder...
et al., 1995). To date, a series of GNA-related lectins, such as GNA from snowdrop (Van Damme et al., 1987; Hester et al., 1995), Narcissus Pseudonarcissus Lectin (NPL) from daffodil (Van Damme et al., 1988; Sauerborn et al., 1999), Allium Sativum agglutinin (ASA) from garlic (Van Damme et al., 1992; Chandra et al., 1999), Scilla campanulata agglutinin (SCA) from bluebell (Wood et al., 1996; Wood et al., 1999) and Gastrodia antifungal protein (GAFP) from Gastrodia elata (Xu et al., 1998; Liu et al., 2005), have been biochemically and structurally characterized. All these GNA-related lectins are built from a common subunit with unique β-prism II fold, which possesses three potential carbohydrate-binding sites (CBBS). However, they adopt distinct quaternary organizations, and their biological activities are closely correlated with their respective quaternary organizations. For instance, GNA, NPL and SCA are tetrameric lectins comprising four protomers and exhibit inhibitory activities against HIV due to their ability to bind GP120, the major glycoprotein of HIV (Balzarini et al., 1991), whereas ASA, which is dimeric, does not have such property, it instead binds other glycoproteins such as alliinase and invertase with high affinity (Barre et al., 1996; Dam et al., 1998). GAFP, on the other hand, is monomeric with a strong inhibitory activity toward a wide range of phytopathogenic fungi (Xu et al., 1998). The three-dimensional structure of GAFP we reported previously provided a glimpse of a monomeric GNA-related lectin for the first time and revealed the structural mechanism governing the quaternary organizations of GNA-related lectins through “C-terminal exchange” or “C-terminal self-assembly” (Liu et al., 2005). The observations indicate that the functional ramifications of GNA-related lectins seems in correlation with certain pattern of the quaternary structure. However, some GNA-related lectins, such as ASA and PCL investigated in this study, show the similar quaternary organizations but different biological activities. It is thus interesting to have more extensive structural data so as to gain deeper insights into the possible structural mechanism by which the functional ramification of them could be achieved.

Polygonatum cyronema lectin (PCL) is a single-domain GNA-related lectin. It was purified from the rhizomes of P. cyronema Hua, a traditional Chinese medicinal herb from Liliaceae (Bao et al., 1996). Like other single-domain GNA-related lectins, PCL is synthesized as a 160-residue polypeptide with a 28-residue N-terminal signal sequence and a 22-residue C-terminal cleavage polypeptide, which is excised in post-translation to yield the mature 110-residue polypeptide comprising a single GNA domain (An et al., 2006). In vitro, PCL exhibits a comparable inhibitory activity against HIV as that of tetrameric GNA and NPL (An et al., 2006), however, it has been characterized as a dimer in solution like ASA (Ding et al., 2008). Here we report the crystal structures of this dimeric mannoside-binding lectin with anti-HIV activities and its complexes with monomannoside and α1-3 dimannoside. The structures show an abnormal CBS which is unfit for mannoside-binding, and a unique ligand-binding mode which induces a distinct super-structure for PCL. Sequence comparisons reveal the inherent structural elements for the super-structure assembly of PCL. Based on these observations, the possible structural basis for the functional ramification of GNA-related lectins and the anti-HIV property of dimeric PCL are discussed.

2. Materials and methods

2.1. Purification and crystallization

The purification and crystallization of PCL have been described previously (Ding et al., 2008). Well diffracting crystals of both mannoside complexes were grown in a condition of 1.0 M lithium sulfate, 2% PEG8000, with the purified PCL (10 mg/ml) in 10 mM hydrochloride, 150 mM NaCl) in presence of a 100 fold molar excess of mannosides (methyl-α-D-mannoside (MeMan) for monomannoside complex, α1-3 dimannoside for dimannoside complex, both mannosides from Sigma-Aldrich). Large diamond-shaped crystals grew within four to six days.

2.2. Diffraction data collection and processing

Data collection, processing, and preliminary analysis for PCL were carried out as described previously (Ding et al., 2008), but the space group assignment was ambiguous. The data could be scaled reasonably well in a monoclinic space group, but the moderate Rmerge of 12.3% when treated as orthorhombic space group suggested possible presence of additional symmetry not accounted for by the monoclinic space group. The question about symmetry and the correct space group was resolved by calculation of the intensity distribution using the program CNS (Brunger et al., 1998), i.e., \( I(F)^2/(||F||)^2 = 1.87 \) and \( (||F||)^2/(||F||^2) = 0.84 \), implying that the true space group is monoclinic and that the data are pseudo-merohedrally twinned (Yeates, 1997).

Crystals of both mannoside complexes used for data collection were quickly dipped into the mother liquid supplemented with 20% PEG400 as a cryoprotectant after having been mounted in nylon cryoloops (Hampton Research) and then flash cooled in the stream of liquid nitrogen at 95 K. A 2.2 Å resolution data set for monomannoside complex was collected in-house on a Rigaku R-Axis IV++ image plate using a Rigaku MM007 rotating CuKα anode X-ray source, and a 2.0 Å resolution data set for dimannoside complex was collected at beamline 1W2B of the Beijing Synchrotron Radiation Facility, using a Mar355 detector. Both data sets were processed with the program package MOSFLM (Rossmann, 1999) and scaled with the program SCALA of the CCP4 program suite (Collaborative Computational Project, 1994). Crystals of both mannoside complexes belonged to a trigonal space group different from the monoclinic space group of PCL. Data collection statistics are summarized in Table 1.

2.3. Structure determination and refinement

In principle, structure determination by molecular replacement should not be hampered by crystal twinning (Redinbo and Yeates, 1993). Therefore, the twinned data of PCL was used to determine the structure. Structure solution of PCL was achieved using the program PHASER of the CCP4 program suite (Collaborative Computational Project, 1994), with a search model of the subunit A of GNA (PDB code 1MSA) which shares 49% sequence identity with PCL. The PCL model was then manually adjusted using the graphics package O (Jones et al., 1991), followed by structural refinement with the twinned data using the program CNS (Brunger et al., 1998). Although the electron density of the model was of high quality after CNS refinement, the R factors were unacceptable: \( R_{	ext{cst}} = 33.6\% \) and \( R_{	ext{free}} = 34.9\% \), also providing the hint that the data were twinned. Prior to refining the structure as the protocol for twinning data in the program CNS (Brunger et al., 1998), the twin fraction was estimated from a statistical comparison of twin-related reflections to be 0.399 (Yeates, 1997). Including the twinning operator \( h, -k, -l \), the estimated twinning fraction and non-crystallographic symmetry restraints in structural refinement drastically reduced the R values. The final refined structure has twinned \( R_{	ext{cst}} = 23.3\% \) and \( R_{	ext{free}} = 27.1\% \).

The structures of both mannoside complexes were subsequently determined by molecular replacement using the program Phaser of the CCP4 program suite (Collaborative Computational Project, 1994), with the refined structure of subunit A of PCL as a search model. Refinements were performed as the general protocol for structural refinement without experimental phases in the
program CNS (Brunger et al., 1998). The model of monomannoside complex was refined to 2.2 Å resolution and the model of dimannoside complex to 2.0 Å resolution. Sulfate ion and manniosides were identified at the last stage of refinement according to the electron density well accommodated to 2.0 Å resolution. The stereochemical qualities of all above models were checked using the program PROCHECK (Laskowski et al., 1993). The multisequence alignment was done by CLUSTALW (Thompson et al., 1994), and the figures were prepared using the program PYMOL (http://www.pymol.org/).

### 3. Results and discussion

#### 3.1. General fold of PCL

The three-dimensional structure of ligand-free PCL has been determined to 2.0 Å resolution. The statistics of the refinement and the final model are summarized in Table 1. Four subunits are located per asymmetric unit, forming two dimers related by a non-crystallographic 2-fold axis. The electron density well accommodates 109 amino acids for each subunit. Ala110 is missing due to the lack of electron density at the C-terminus. PCL subunit adopts the well-known β-prism II fold that is characteristic in GNA-related lectins (Hester et al., 1995). It consists of three subdomains (I, II and III), each of which is a flat four-stranded antiparallel β-sheet, arranged as the three sides of a triangular prism (Fig. 1a). All the strands are perpendicular to the quasi 3-fold axis. A number of conserved non-polar residues point their side chains to the inside of the prism, forming a hydrophobic core. Among them, the three tryptophan residues, Trp42, Trp74, and Trp103, are crucial. They are about 4 Å and 120° apart from each other, resulting in strong van der Waals interactions (Fig. 1a).

Two neighboring subunits assemble into a dimer by exchanging their C-terminal β-strands. This mode is often referred to as “C-terminal exchange” in GNA-related lectins (Liu et al., 2005). A considerable surface area is buried in the dimeric interface, conferring stability on the dimer (Fig. 1b). To achieve the strand exchange, the two-residue loop connecting the last two strands of the subunit adopts an extended conformation in which the peptide bond between Gly99 and Pro100 adopts an unusual cis-conformation.

The β-prism II fold adopted by PCL subunit is quite different from the β-prism I fold adopted by jacalin-like lectins, such as banana lectin from Musa paradisiacal (Singh et al., 2005), where all the strands of β-prism I fold are arranged parallel to the quasi 3-fold axis. Although the jacalin-like lectins often assemble into various oligomers similar to GNA-related lectins, the modes of their quaternary organizations are not highly conserved and quite different from that of the GNA-related lectins (Singh et al., 2005).

#### 3.2. PCL subunit possesses an abnormal CBS

The subunits of many GNA-related lectins possess three potential CBSs, located in subdomains I, II and III, respectively, and each contains a consensus signature sequence QXDXNXVXY (X is one of any amino acid residues). The conserved polar residues Gln, Asp, Asn, and Tyr bind O2, O3 and O4 of mannose through a network of four hydrogen bonds. Another conserved hydrophobic residue Val interacts with C3 and C4 of mannose through hydrophobic interactions (Fig. 1c) (Hester et al., 1995; Sauerborn et al., 1999; Chandra et al., 1999; Liu et al., 2005). Therefore, the subunits of these lectins bind mannose using a trivalent mode. However, several GNA-related lectins that do not possess three active mannose-binding sites have also been reported (Barre et al., 1996), including the two-domain GNA-related lectins such as Arum maculatum agglutinin (AMA) from wild arum (Van Damme et al., 1995) and Tulipa hybrid lectin I from tulip which has broad carbohydrate specificities (TxLc-I) Van Damme et al., 1996a. In these lectins, mutations at the CBSs abolish their affinities toward mannose, but the structural basis of the loss of affinities has not been characterized.

Here we report the structural properties of such a modified CBS as observed in the structure of PCL. In the structure of PCL subunit, CBS I and CBS III are strictly conserved, whereas the potential CBS II has some distinct features. The unambiguous density map provides the direct evidence that the critical residues Gln58 and Asp60 for mannose-binding are substituted by His58 and Asn60, respectively, which coincides well with the deduced amino acid sequence from cDNA sequence analysis (An et al., 2006). Structural superposition shows that despite the high similarity of the main chain geometry of CBS II between PCL subunit and ASA subunit, the side chains of His58 and Asn60 in PCL do not interact with O2 and O3 of mannose because they are rather far from each other (Fig. 1c). As a result, the modified CBS II of PCL becomes unfit for mannose-binding. Therefore, PCL subunit possesses an abnormal CBS and uses a potential bivalent mode to bind mannose.

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**Table 1**

Data collection and structural refinement statistics:

<table>
<thead>
<tr>
<th></th>
<th>PCL</th>
<th>PCL-monomannoside complex</th>
<th>PCL-dimannoside complex</th>
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<tbody>
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<td><strong>Data collection</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Space group</td>
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<td>P3₂2₁</td>
<td>P3₂2₁</td>
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<tr>
<td>α, β, γ (°)</td>
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<td>76.46, 76.46, 61.75</td>
<td>76.66, 76.66, 61.40</td>
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<tr>
<td>Resolution (Å)</td>
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<td>50–2.20 (2.32–2.11–2.00)</td>
<td>2.20</td>
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<tr>
<td>No. of unique reflections</td>
<td>99,043</td>
<td>67,425</td>
<td>200,279</td>
</tr>
<tr>
<td>No. of reflections</td>
<td>28,647</td>
<td>10,885</td>
<td>14,437</td>
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<td>Completeness (%)</td>
<td>99.7 (99.7)</td>
<td>99.8 (100)</td>
<td>100 (100)</td>
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<tr>
<td>Redundancy</td>
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<td>6.2 (6.1)</td>
<td>13.9 (14.0)</td>
</tr>
<tr>
<td>R/σ(I)</td>
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<td>20.1 (4.5)</td>
<td>38.2 (8.0)</td>
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<tr>
<td>Rsym (%)</td>
<td>8.4 (25.8)</td>
<td>8.0 (34.9)</td>
<td>7.6 (26.5)</td>
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</table>

**Refinement statistics**

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<th>Ligand</th>
<th>Ion</th>
<th>Water</th>
<th>Average B-factors (Å²)</th>
<th>Protein</th>
<th>Ligand</th>
<th>Ion</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of atoms</td>
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<td>843</td>
<td>13</td>
<td>101</td>
<td>32.1</td>
<td>21.2</td>
<td>18.3</td>
<td>50.1</td>
<td>32.1</td>
</tr>
<tr>
<td>R cryst [%]/R free [%]</td>
<td>23.3/27.1</td>
<td>19.8/21.7</td>
<td>9.0/21.2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>R.m.s deviations</td>
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<td>0.005</td>
<td>0.05</td>
<td>0.005</td>
<td>1.44</td>
<td>1.43</td>
<td>1.47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are for highest-resolution shell.
3.3. PCL–mannoside complexes show the unique carbohydrate-binding property

The structures of PCL complexes with monomannoside and α1-3 dimannoside have been determined to 2.2 Å resolution and 2.0 Å resolution, respectively (Table 1). Because of the different packing mode, both mannoside complexes contain only one subunit per asymmetric unit. All the 110 amino acids of PCL subunit were built into the high-quality electron density map. Least-square superpositions of 109 Cα atoms of the subunits of PCL–mannoside complexes with those of ligand-free PCL yield a root-mean-square deviation of about 0.6 Å, indicating the protein structure remains essentially unchanged in different mannose-binding states. The corresponding dimer is assembled by two crystallographic 2-fold symmetry related subunits using the same assembly mode of “C-terminal exchange”. Thus, based on the structures of ligand-free PCL and its complexes with mannoside ligands reported here, it could be concluded that a stable homo-dimer is the basic structural unit of PCL. This is consistent with the result from size-exclusion chromatography analysis which PCL is a homo-dimer in solution (Ding et al., 2008).

In both mannoside complexes, only CBS I of PCL is occupied by the ligand. The binding of monomannoside involves an extended network of hydrogen bonds (Fig. 2a), in which two hydrogen bonds, formed by O2 of mannose with the side chains of Asp92 and Asn94, constrain this hydroxyl group to the axial epimer and make this site exclusively specific for mannose. Val96 further immobilizes the monomannoside through hydrophobic interactions with C4 of mannose. In addition, the C-terminal polypeptide of the dimer-related subunit further stabilizes the monomannoside by a number of auxiliary interactions. For example, the side chains of His84 and Pro109 interacts with mannose through extensive stacking interactions, and the side chains of Val101 and Ala104 interact with C6 and C1 of mannose through non-polar van de Waals interactions. When α1-3 dimannoside is utilized as the ligand, the non-reducing mannose moiety binds to this site and the reducing mannose moiety extends to the solvent region. As expected, the binding manner of the non-reducing mannose moiety is identical to that of the monomannoside (Fig. 2a). Furthermore, two pieces of evidence suggest that CBS I of PCL has a specific affinity for α1-3 dimannoside. First, the reducing mannose moiety of the dimannoside forms the stacking interactions with the side chains of His84 and Pro109. This observation is also supported by the well defined electron-density of the reducing mannose moiety (Fig. 2b). Second, the molecular surface at CBS I shows that the binding pocket agrees well with α1-3 dimannoside but cannot accommodate other dimannosides such as α1-2 and α1-6 dimannoside in their low energy conformations without steric clash (Fig. 2b).

None of the CBS II sites in PCL–mannoside complexes has been observed to bind a mannoside ligand. Instead, a sulfate ion and several ordered water molecules have been found and hydrogen bond interactions are formed between them (Fig. 2c). This observation confirms that this site has lost its specificity for mannoside ligand. However, it may accommodate some other ligand that could fit the modified stereochemistry of this site, like sulfate ion. Sequence alignments show that CBS II of PCL adopts a modified sequence H58XN60XN62XV64XY66 (sequence number according to PCL) which is different from the consensus sequence motif Q58XD60XN62XV64XY66 (Fig. 3) at the first two positions. The observation indicates that the lack of mannose-binding ability of CBS II is an inherent property of PCL. Surveying the sequences of GNA-re-
lated lectin family, it is found that the sequence deviations of CBS II are widely observed in the Liliaceae family, especially in the Polygonatum genus (Van Damme et al., 1996b; Sun et al., 2008) (Fig. 3). All three GNA-related lectins from Polygonatum genus with known sequences have a common modified sequence motif of H58XN60XN62XV64XY66 that is unfit for mannoside-binding (Fig. 3). In addition, the crystal structure of a lectin from Scilla campanulata (SCA) with mutations of critical residues (N62V and Y66I) has been determined (Wood et al., 1999) and it also shows a modified CBS II unfit for mannose-binding.

Although CBS III of PCL is strictly conserved in both sequence and structure as a usual binding site, mannoside ligand has not been observed in either mannose complex. Instead, this site is occupied by a well-ordered sulfate ion, interacting with the conserved residues through a network of several hydrogen bonds similar to that of monomannoside binding (Fig. 2d). The observation is quite different from those of other GNA-related lectins structurally characterized so far, in which monomannoside could bind to CBS III, despite the monomannoside affinity of this site is severely reduced compared with that of CBS I due to the absence of the auxiliary interactions from the dimer-related subunit (Hester et al., 1995; Chandra et al., 1999). Modeling a monomannoside at CBS III instead of the sulfate ion in both mannose complexes was unsuccessful because an integral monomannoside cannot be accommodated without severe steric clashes with the residues of the crystallographic 3-fold-related symmetric subunit.

Thus, based on the unique carbohydrate-binding property we observed in the structures of PCL–mannoside complexes, it is reasonable to propose that the potential bivalent mannose-binding mode of PCL may be adopted by some other GNA-related lectins, and the modified CBS II may be intended for some specific, yet still unknown, ligands other than sulfate, giving rise to biological implications. It has been shown previously that GNA-related lectins may interact with high mannose or complex N-glycans other than mannose (Van Damme et al., 2007). The observations in the structures of PCL–mannoside complexes further reveal the possible structural basis for such ligand-binding property.

3.4. Dimer-based super-structure mediated by unique ligand-binding mode

Interestingly, the well-ordered sulfate ion at CBS II also interacts with CBS III of crystallographic 3-fold-related symmetric molecule (Fig. 4a). In this way, the sulfate ions simultaneously bind to CBS II and CBS III located at dimer–dimer interface and form a dimer-based super-structure for PCL (Fig. 4b). Here, PCL dimers are arranged along a 32 screw axis, resulting in a helical cross-linkage (Fig. 4b). As a result of such helical cross-linkage, both mannose complex crystals have high solvent content of 72% and a channel of 33 Å in diameter. In addition, the lattice contacts consist primarily of the sulfate ion-mediated dimer–dimer interactions.
ASA is a GNA-related lectin with trivalent mannose-binding property and similar dimeric organization as PCL. However, sequence and structure comparisons between PCL and ASA show that dimeric ASA is unable to form the similar super-structure observed in PCL. First, CBS II of ASA has a conserved sequence and stereochemistry for mannose-binding, consequently it cannot accommodate ligand other than mannose, such as sulfate ion, to induce the dimer-dimer interactions in a way as observed in PCL. Second, superposition of two ASA subunits upon two cross-linked PCL subunits in the super-structure shows that a loop (abbreviated as IL), which comprises residues between Gly46 and Gly52 and is located at the cross-linked interface, has a significant difference between PCL and ASA (Fig. 4c). IL of ASA adopts an extended conformation, resulting in severe steric clash between the neighboring cross-linked subunits (Fig. 4d). The observation indicates that the dimer-based superstructure of PCL should not be a mere consequence of crystal packing, but instead resulted from its inherent structural elements, especially its unique ligand-binding mode.

A series of cross-linked complexes between different lectins and oligosaccharides have been structurally characterized at atomic resolutions (Weis et al., 1992; Wright, 1992; Rini, 1995), and these structures demonstrate that the structural property of a particular multivalent oligosaccharide determine the symmetry of the cross-linked complex. Thus, the unique super-structure formed by a particular lectin depends on the composition of the bound oligosaccharides. The PCL structures described here show a unique ligand-binding mode, by which the bivalent binding of PCL dimers with sulfate ions mediates a helical cross-linked super-structure.

3.5. Possible structural basis of anti-HIV property

It has been widely accepted that lectins usually form different oligomers bearing multivalent CBSs. Furthermore they form
ordered arrays of complexes when they bind multivalent glycoconjugates, similar to the lattices formed by antibodies and multivalent antigens. Lectin-induced cellular bioactivities are believed to involve the cross-linkage of lectin-receptor complexes on the cell surface. The detailed structural analyses of GNA complex provides a glimpse of a possible molecular assembly, which may play a role when GNA binds glycans on cell membranes and, as a result of the binding, block the viral fusion events (Wright and Hester, 1996).

High resolution crystal structures of lectin–oligosaccharide complexes have shown the super-structures unique to each class of lectin complex. The super-structure of PCL–mannoside complex presents a special type of lattice among the GNA-related lectins in that dimeric PCL utilizes the modified CBS II and normal CBS III to bind a special ligand and assemble into a $3_2$ helical lattice (Fig. 4b). It is plausible to propose that the extended binding region in CBS I confers PCL the affinity for $\alpha1$-$3$ dimannoside, and as a consequence of this affinity, the ability to recognize the high-mannose glycans of the HIV surface envelop glycoprotein GP120. Furthermore, the unique super-structure of PCL–mannoside complex may disturb or destabilize the binding of GP120 to specific receptors on cell membranes and block the subsequent viral fusion. Therefore, the unique super-structure of PCL–mannoside complex would be a useful tool for the development of anti-HIV drugs.

Fig. 4. Dimer-based super-structure mediated by unique ligand-binding mode. (a) The well-ordered sulfate ion simultaneously binds to CBS II and CBS III located at the symmetric related dimer–dimer interface. (b) Sulfate ions induce the crystallographic symmetric PCL subunits assembling into a $3_2$ helical cross-linkaged super-structure. Sulfate ions are shown as sphere models. (c) Superpositions of two ASA subunits to the cross-linked PCL subunits mediated by the sulfate ion. The red arrowhead points out a significant deviation of loop IL at the cross-linked interface from ASA to PCL. (d) The detailed loop IL of ASA. It has a unique insertion of Lys51 in sequence, in turn, to take an extended conformation, resulting in severe steric clash with the cross-linked subunit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
presented in this paper is probably one of the possible structural bases for the anti-HIV property of PCL. Of course, for direct identification, more experimental observations are needed. Interestingly, ASA possesses the same subunit structure and the similar dimeric organization as PCL, but it is unable to assemble into the PCL-like super-structure due to the intrinsic structural element as mentioned in previous paragraphs (Figs. 3 and 4d). Correspondingly, there is no report to show ASA has any anti-HIV activity so far. It supports the point of view proposed above.

3.6. Protein data bank accession codes

The atomic coordinates have been deposited in the RCSB Protein Data Bank, with the accession code 3AOC for PCL, 3AOD for PCL complex with monomannoside and 3AOE for PCL complex with \( \alpha (1-3) \) dimannoside.

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