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Crystallization and preliminary X-ray diffraction analysis of the seed lectin from *Parkia platycephala*

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The crystallization and preliminary X-ray diffraction analysis of the seed lectin of *Parkia platycephala*, a *Mimosoideae*, regarded as the most primitive group of the *Leguminosae* plants, are reported. Its amino-acid sequence consists of three tandemly arranged jacalin-related β-prism domains, which is a novel fold for a leguminous lectin. Furthermore, no other lectin structure with this arrangement of domains has been described. $P2_12_12_1$ crystals (unit-cell parameters a = 63.6, b = 68.5, c = 208.5 Å), which diffract to a maximum resolution of 2.2 Å, were obtained in hanging drops at pH 8 and 293 K by the vapor-diffusion method using 10% 2-propanol and 20% polyethylene glycol 4000 as precipitants. The asymmetric unit contains two lectin molecules and has a solvent content of 46%. Only a single β-prism domain could be located by molecular replacement using the structure of the *Helianthus tuberosus* lectin (PDB code 1c3k) as the search model. Isomorphous heavy-atom derivatives are currently being produced to solve the complete structure of the *P. platycephala* seed lectin.

1. Introduction

Lectins are a structurally heterogeneous group of carbohydrate-binding proteins of non-immune origin comprising distinct families of evolutionarily related proteins (Van Damme *et al.*, 1998). Sugar-recognition mechanisms have evolved independently in diverse protein frameworks and lectins are ubiquitous in animals, plants and microorganisms. Lectins play biological roles in many cellular processes, such as fertilization, cell communication, differentiation and development, host defence, parasitic infection, tumour metastasis *etc.* by deciphering the glycocodes encoded in the structure of glycans attached to soluble and integral cell-membrane glycoconjugates (Gabius & Gabius, 1997).

Although the past few years have witnessed the elucidation of a variety of novel lectin structures from monocotyledonous (mannose-specific bulb lectins from *Amaryllidaceae*, *Liliaceae*, *Alliaceae* and *Orchidaceae*) and dicotyledonous plants (GalNAc-specific lectins from *Moraceae* and *Amaranthus* species and mannose/maltose-specific lectins from Convolvulaceae) (Wright, 1997; Lee *et al.*, 1998; Chandra *et al.*, 1999; Sauerborn *et al.*, 1999; Wood *et al.*, 1999; Bourne *et al.*, 1999), the largest and best characterized lectin family is still that from seeds of leguminous plants (consult the 3D Lectin Database at http://www.cermav.cnrs.fr/databank/lectine). Most studies on Leguminoseae lectins involve members of the Papilionoideae subfamily, while investigations on lectins of the other two subfamilies, *Caesalpinioideae* and *Mimosoideae*, are scarce. The *Mimosoideae* subfamily of leguminous plants comprises six tribes (*Adenanthereae*, *Mimoseae*, *Mimozygantheae*, *Parkiae*, *Acacieae* and *Ingeae*) which include 56 genera. At present, the tribe *Parkiae* is the only taxon from which lectins have been biochemically characterized. These include the seed lectins from *P. speciosa* (Suvachittanont & Peutpaiboon, 1992), *P. javanica* (Utarabhand & Akkayanont, 1995), *P. platycephala* (Cavada *et al.*, 1997; Ramos *et al.*, 1999; Mann *et al.*, 2001) and *P. discolor* (Cavada *et al.*, 2000).

The *P. platycephala* seed lectin is a non-glycosylated single-chain polypeptide with a molecular mass of 47.9 kDa. Its primary structure is made up of three homologous repeats (Mann *et al.*, 2001), each of which exhibits sequence similarity with jacalin-related lectin monomers from *Asteraceae*, *Convolvulaceae*, *Moraceae*, *Musaceae*, *Gramineae* and *Fagaceae* plant families. It is noteworthy that all the 22 legume lectin crystal structures reported to date (http://www.cermav.cnrs.fr/databank/lectine) have the highly conserved jelly-roll topology first observed in concanavalin A (Srinivas *et al.*, 2001; Wah *et al.*, 2001; Rabijns *et al.*, 2001). Thus, the structure of the *P. platycephala* lectin represents a novel fold for a leguminous lectin. Moreover, the *P. platycephala* lectin shows also

sequence similarity with stress- and pathogen-upregulated defence genes of a number of different plants, suggesting a common ancestry for jacalin-related lectins and inducible defence proteins. Here, we report the crystallization and preliminary X-ray diffraction analysis of the seed lectin from *P. platycephala*.

2. Materials and methods

2.1. Isolation of *P. platycephala* lectin

Mature seeds from *P. platycephala* collected in the state of Ceará (Northeast Brazil) were ground in a coffee mill and the flour was defatted with *n*-hexane and air-dried at room temperature. Soluble proteins were extracted overnight at room temperature by continuous stirring with 1:50(*w*/*v*) 50 m*M* sodium acetate buffer pH 4.0 containing 150 m*M* NaCl. Insoluble material was pelleted by centrifugation at 10 000*g* at 278 K for 20 min. The supernatant was fractionated by ammonium sulfate precipitation (40% saturation). The resulting precipitate, recovered by centrifugation at 10 000*g* at 277 K for 20 min, was resuspended in and dialyzed exhaustively against 50 m*M* Tris–HCl pH 8.0 and applied to a Sephadex G100 column (2.5 × 30 cm) equilibrated and developed with the same buffer. Unbound material was eluted by washing with equilibrium buffer and the bound lectin was desorbed from the column with equilibrium buffer containing 0.1 *M* D-glucose. The lectin was freed from hapten by dialysis against 1 *M* acetic acid and then dialyzed against 100 m*M* Tris–HCl pH 7.6 containing 150 m*M* NaCl. Insoluble material was removed by centrifugation (as above) and the lectin in the clear supernatant was purified by affinity chromatography on a mannose–agarose column equilibrated with 100 m*M* Tris–HCl pH 7.6 containing 150 m*M* NaCl. Mannose-bound lectin, recovered with running buffer containing 0.1 *M* D-mannose, was dialyzed against MilliQ water and lyophilized. The apparent molecular mass and homogeneity of the purified *P. platycephala* seed lectin were estimated by SDS–PAGE and MALDI-TOF mass spectrometry (Mann *et al.*, 2001).

2.2. Crystallization, data collection and processing

The sparse-matrix method (Jancarik & Kim, 1991) using the Crystal Screen I and II formulations supplied by Hampton Research (California, USA) was utilized to perform initial screening of the crystallization conditions. Crystals were grown at 295 K by the vapour-diffusion method using hanging drops composed of equal volumes of protein solution (~2–4 mg ml^{-1} in 50 m*M* Na HEPES pH 8) and reservoir buffer (100 m*M* Na HEPES pH 7.5, 10% 2-propanol and 20% polyethylene glycol 4000). Crystals suitable for diffraction experiments (maximal dimensions of 0.6 × 0.3 × 0.1 mm) grew within four weeks. For the X-ray diffraction studies, crystals were flash-frozen at 100 K in a nitrogen-gas stream. Reservoir buffer containing 20% glycerol proved to be a suitable cryoprotectant. The crystals were transferred directly from the drop to the cryoprotectant solution and were allowed to equilibrate for approximately 1 min. Thereafter, single crystals were mounted in nylon loops and rapidly transferred to the cryostream. The cryoprotected crystals were analysed on an in-house SMART CCD detector mounted on a Bruker AXS X-ray generator operated at 50 kV and 90 mA using cross-coupled Göbel mirror-monochromated Cu $K\alpha$ radiation from a rotating anode. Diffraction data were collected as a series of discrete frames, each comprising 0.3° oscillation and an exposure time of 80 s, using a crystal-to-detector distance of 70 mm. Another data set was collected at the EMBL Outstation (Grenoble) on beamline ID14. The data were indexed, integrated and scaled using *SMART*, *SAINT* and *PROSCALE* (Bruker AXS), *MOSFLM* (Leslie, 1997) and *SCALA* (Collaborative Computational Project, Number 4, 1994). The self-rotation functions (Rossmann & Blow, 1962) were calculated using the program *POLARRFN* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

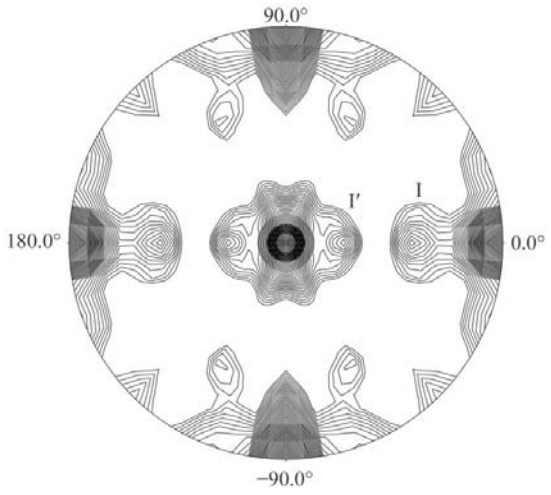

Figure 1
Representation of the $\kappa = 180°$ section of the self-rotation function from the *P. platycephala* seed lectin crystals. The largest peaks correspond to the twofold crystallographic axes along the three directions. A non-crystallographic dyad axis (indicated as I, I′ in the figure) is also clearly defined.

3. Results and discussion

Crystals of the *P. platycephala* seed lectin were grown at pH 8 in hanging drops by the vapour-diffusion method, using a mixture of 10% 2-propanol and 20% polyethylene glycol 4000 as precipitant. Autoindexing and consideration of systematically absent reflections revealed that the crystals belong to the orthorhombic space group $P2_12_12_1$ (unit-cell parameters a = 63.6, b = 68.5, c = 208.5 Å). The final 2.2 Å resolution data set is 91% complete and has an overall $R_{\rm sym}$ of 6.3%. Patterson maps computed in several resolution shells did not yield any significant peak. However, the self-rotation function (Rossmann & Blow, 1962) calculated using the program *POLARRFN* (Collaborative Computational Project, Number 4, 1994) revealed a prominent peak with a height 31.6% of that obtained for the crystallographic 2_1 axis (Fig. 1). Considerations of the packing density and the results from the self-rotation function support the presence of two subunits in the asymmetric unit, which would correspond to a Matthews coefficient ($V_{\rm M}$) of 2.30 Å^3 Da^{-1} and a solvent content of 46% (Matthews, 1968). This reflects the situation in solution, where the *P. platycephala* lectin exists as a non-pH-dependent dimer as judged by equilibrium sedimentation analytical ultracentrifugation (Mann *et al.*, 2001).

The primary structure of the *P. platycephala* lectin consists of three tandemly arranged homologous domains, each of which shares the signature of the β-prism fold (Mann *et al.*, 2001) similar to that observed in the crystal structure of the lectin from *H. tuberosus* (Bourne *et al.*, 1999). Using the structure of the *H. tuberosus* lectin (PDB code 1c3k) as the search model in *AMoRe* (Navaza, 1994), possible molecular-replacement solutions were found, but only for a single β-prism domain. The best solution has a final correlation coefficient of 0.34 after rigid-body refinement. This low coefficient reflects the fact that the search model represents only one third of a complete *P. platycephala* lectin molecule. Although the molecular-replacement solution leads to acceptable crystal packing, it leaves large

voids that may be filled by the other two domains of each lectin molecule in the asymmetric unit. We are currently searching for suitable isomorphous heavy-atom derivatives to solve the complete crystal structure of the three tandemly arranged β-prism domains of the *P. platycephala* lectin.

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