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Purification, crystallization and preliminary X-ray crystallographic analysis of rice lectin from *Oryza sativa*

Lectins with sugar-binding specificity are widely distributed in higher plants and various other species. The expression of rice lectin from *Oryza sativa* is up-regulated in the growing coleoptile when anaerobic stress persists. A rice lectin of molecular weight 15.2 kDa has been crystallized using the hanging-drop vapour-diffusion method. From the diffraction of the lectin crystals at 1.93 Å resolution, the unit cell belongs to space group $P3_1$, with unit-cell parameters $a = 98.58$, $b = 98.58$, $c = 44.72$ Å. Preliminary analysis indicates that there are two lectin molecules in an asymmetric unit with a large solvent content, 70.1%.

1. Introduction

Lectins capable of binding carbohydrates are widely distributed in microorganisms, viruses, animals and higher plants (Liener *et al.*, 1986). Lectins play a role in specific recognition of and reversible binding to sugar residues and can discriminate the carbohydrates found on self glycoproteins and glycolipids from the carbohydrate patterns found on infectious non-self surfaces and perform defence behaviour (Peumans & Van Damme, 1995; Gadjeva *et al.*, 2004). Apart from their relationship to the innate immune system, lectins are used to target and deliver drugs to their site of action (Bies *et al.*, 2004). Lectins are a heterogeneous group and are classified according to their ability to recognize and to specifically bind carbohydrate ligands (Bouckaert *et al.*, 1999). In accord with their large structural differences (Barre *et al.*, 2001), plant lectins have been subdivided into seven main families: amaranthins, chitin-binding lectins comprising hevein domains, Cucurbitaceae phloem lectins, jacalin-related lectins, legume lectins, monocot mannose-binding lectins and type-2 ribosome-inactivating proteins (Peumans *et al.*, 2000). Although lectins share the common property of binding to defined sugar structures, their roles in various organisms are unlikely to be the same. Unlike animal lectins, which are known to interact with endogenous glycans and to act either within or outside the cell, most plant lectins are directed against foreign glycans and are accordingly destined to interact with another organism, either in recognition or in defence-related conditions (Van Damme *et al.*, 1998, 2004). Lectins are related to disease-resistance in plants and can prevent plants from infection by bacilli (Gaidamashvili & van Staden, 2002).

The rice (*Oryza sativa*) lectin belongs to the subgroup of mannose-binding jacalin-related lectins that can recognize and bind to sugar complexes. The protein has been characterized as a response to a salt-stress environment (Hirano *et al.*, 2000; Branco *et al.*, 2004; Zhang *et al.*, 2000; de Souza Filho *et al.*, 2003). Rice lectin is novel not only because it differs from classical plant lectins with respect to the regulation of its expression, which is induced by salt stress, desiccation and the phytohormones jasmonic acid and abscisic acid (Garcia *et al.*, 1998; de Souza Filho *et al.*, 2003), but also because it provides the first evidence of a plant lectin with an endogenous role as a cytoplasmic mannose-binding lectin (Hirano *et al.*, 2000; Zhang *et al.*, 2000), as well as being a suitable candidate to mediate essential protein-carbohydrate interactions in plants. Although the exact role of this rice lectin is not fully understood, it seems likely to play a role in the plant's response to well defined stress factors and also to infection by the pathogenic fungus *Magnaporthe grisea* (Qin *et al.*,

2003). For this reason, we have isolated the cDNA clone encoding the lectin from rice and established a successful protein-expression and single-step purification procedure to produce a large quantity of the lectin for crystallization. We found that expression of rice lectin with a molecular weight of 15.2 kDa from *O. sativa* is up-regulated in the growing coleoptile when anaerobic stress persists. To understand further the biological significance of this lectin in rice development, it is important and necessary to understand its detailed three-dimensional structure and functional relationships. Here, we report the cloning, overexpression, purification, crystallization and preliminary X-ray diffraction characterization of rice lectin.

2. Materials and methods

2.1. Protein cloning, expression and purification

Rice seeds were washed and soaked in sodium hypochlorite (1%, 1 h) to sterilize their surface. After being washed with distilled water, the seeds were soaked in distilled water for germination. The germinated seeds were selected and transferred into another tank and then submerged in distilled water (depth 2 cm) for another 8 d. The coleoptiles were harvested for tRNA isolation. The total mRNA of the rice coleoptile was extracted (RNeasy Plant Mini Kit, Qiagen) and the cDNA library was synthesized (SuperScript III, Invitrogen). The DNA segment coding for residues 1–145 of the rice lectin protein was amplified by the polymerase chain reaction (PCR) from the cDNA library with the forward primer 5'-ATGACGCTGGTGAA-GATTGGTCC-3' and the reverse primer 5'-TCAAGGTGGAC-GTAGATGCCAATTGC-3'. The PCR products were cloned into the yT&A (Yeastern Biotech Co.) cloning vector and verified by nucleotide sequencing. After confirming the gene sequence, we subcloned the gene into the *Bam*HI and *Kpn*I sites of a pQE30 expression vector that contains an N-terminal 6×His-tag fusion protein. The recombinant plasmid was transformed into *Escherichia coli* strain JM109.

For expression of rice lectin, we inoculated one colony of the bacteria into Luria–Bertani (LB) broth (3 ml) containing ampicillin ($50\text{ }\mu\text{g ml}^{-1}$) for overnight incubation at 310 K. The culture was then transferred into fresh LB medium (500 ml, with $100\text{ }\mu\text{g ml}^{-1}$ ampicillin). The culture was grown at 310 K with moderate shaking (140 rev min^{-1}) and incubation was continued for another 12–16 h. The cells were then harvested by centrifugation ($6800g$) at 277 K for 30 min. The medium was discarded and the cell pellet was resuspended in a binding buffer (10 ml) containing 20 mM Tris–HCl, 0.5 M NaCl and 5 mM imidazole pH 7.9. The resuspended cells were then sonicated four times (Dr Hielcher UP 200S, 80 s each, cycle 0.3,

amplitude 40) on ice and the supernatant was collected by centrifugation ($18\text{ }000g$) at 277 K for 30 min. The soluble protein extract was then passed through an Econo-Column (Bio-Rad, length 10 cm) with 10 ml His-Bind Resin (Novagen) pre-equilibrated with 50 ml Tris buffer. The column was washed with ten volumes of binding buffer containing 5 mM imidazole and then washed with six volumes of washing buffer containing 60 mM imidazole. The purified protein was finally eluted with three volumes of elution buffer containing 250 mM imidazole. The protein sample was concentrated using an Amicon concentrator (Millipore) and the buffer was concurrently changed to 20 mM Tris buffer pH 7.0. The yield of protein was approximately 10 mg; the purity was greater than 95% according to analysis with SDS–PAGE (15%) and UV–Vis spectra.

2.2. Crystallization

Prior to crystallization trials, the protein sample was ultracentrifuged to a concentration of 5 mg ml^{-1} in 20 mM Tris buffer pH 7.0. Crystallization was performed by the hanging-drop vapour-diffusion method at 291 K. Small crystals were observed from a condition using PEG 400 and calcium chloride within 1 d of initial screening using the Crystal Screen kits (Hampton Research Co.). This condition was further refined to produce larger lectin crystals using 2 μl hanging drops containing equal volumes of protein solution and a reservoir solution containing 30% (v/v) PEG 400, 20 mM CaCl_2 and 0.1 M sodium acetate buffer pH 4.6. Crystals of diffraction quality were used for the collection of X-ray data (Fig. 1).

2.3. X-ray data collection and processing

The protein crystals were initially screened and characterized using synchrotron radiation at the protein crystallographic beamline BL17B2 equipped with a Q210 CCD (ADSC) detector at the National Synchrotron Radiation Research Center (NSRRC) in Taiwan. Data collection was completed at BL12B2 equipped with a Quantum 4R CCD (ADSC) detector at SPring-8 in Japan. The crystal was transferred into a cryoprotectant solution containing glycerol (20%), mounted in a glass loop (0.4–0.5 mm, Hampton Research) and flash-cooled in liquid nitrogen at 100 K. For complete collection of high-resolution data, two 360° rotations with 0.5° oscillation were measured using X-rays of wavelength $1.00\text{ }\text{\AA}$ at 110 K in a nitrogen stream using a cryosystem (X-Stream, Rigaku/MSC Inc.). The first rotation was to measure the high-resolution diffraction to $1.93\text{ }\text{\AA}$ with an exposure time 40 s and a crystal-to-detector distance of 150 mm. Some low-resolution ($>5\text{ }\text{\AA}$) reflections saturated the scanner electronics during recording at $1.93\text{ }\text{\AA}$ resolution and were not measured. The second rotation was to measure diffraction at low to medium resolution ($25\text{--}2.37\text{ }\text{\AA}$) with a crystal-to-detector distance of 240 mm and a shorter exposure time of 10 s, which allowed measurement of the few reflections that remained saturated at low resolution in the first data set. The overlapping reflections between the first and second data set were used for subsequent data merging. All data were indexed, integrated, scaled and merged using the HKL2000 program suite (Otwinowski & Minor, 1997).

3. Results and discussion

SDS–PAGE showed a single band at about 15 kDa under SDS denaturing and reducing conditions. Protein crystals of hexagonal shape appeared in several hours and continued to grow to a final size $0.1 \times 0.1 \times 0.5\text{ mm}$ within 1 d in an incubator at 291 K. The protein crystals are sensitive to the change in precipitant concentration while being transferred to the cryoprotectant solution containing glycerol

Figure 1
A single crystal of rice lectin grown by the hanging-drop method.

Table 1

Diffraction statistics of rice lectin.

Values in parentheses are for the highest resolution shell (2.00–1.93 Å).

Wavelength (Å)	1.00 (SPring-8, BL12B2)
Temperature (K)	100
Resolution range (Å)	25.0–1.93
Space group	$P3_1$
Unique reflections	35801
Completeness (%)	98.6 (96.8)
$I/\sigma(I)$	19.4 (6.0)
Average redundancy	8.57
$R_{\text{sym}}^{\dagger}$ (%)	6.5 (23.2)
Mosaicity	0.761
Unit-cell parameters (Å)	$a = 98.58, b = 98.58, c = 44.72$
No. of molecules per ASU	2
Matthews coefficient (Å ³ Da ⁻¹)	4.19
Solvent content (%)	70.1

 $\dagger R_{\text{sym}} = \sum_h \sum_i [|I_i(h) - \langle I(h) \rangle| / \sum_i I_i(h)]$, where I_i is the i th measurement and $\langle I(h) \rangle$ is the weighted mean of all measurements of $I(h)$.

Figure 2

Molecular-packing diagram for the lectin crystals viewed down the 3_1 screw axis. The lectin dimers related by a pseudo-twofold form large solvent channels running along the c axis.

(20%). Several crystals were tested prior to data collection owing to a relatively high ($>1^\circ$) mosaicity that caused overlap of diffraction spots in the high-resolution regions. Radiation damage was observed after protracted exposure during data collection, which caused a decrease in $I/\sigma(I)$ and an increase in R_{sym} . Thus, although data for a total of 360° rotation were collected, only a 310° range was selected for high-resolution data processing after an inspection of data statistics with regard to crystal decay. Analysis of the diffraction pattern indicates that the crystals exhibit trigonal symmetry and systematic absences indicated that the space group is $P3_1$ or $P3_2$. Assuming the presence of one or two molecules per asymmetric unit, we estimated the Matthews coefficient to be 4.19 or $2.10 \text{ Å}^3 \text{ Da}^{-1}$, corresponding to a solvent content of 70.1 or 40.3% (Matthews, 1968), which are located at the two limits of the general range for protein crystals. Details of the data statistics are given in Table 1.

Initial attempts to solve the crystal structure of rice lectin were performed by molecular replacement using the structure of the lectin from *Helianthus tuberosus* (PDB code 1c3k; Bourne *et al.*, 1999; only 35% sequence identity) with the nonconserved residues changed to alanines as a search model. A molecular-replacement solution was found using the *CNS* v1.1 program (Brünger *et al.*, 1998) and confirmed the space group to be $P3_1$ and that there are two molecules in an asymmetric unit. The crystal packing (Fig. 2) with a large solvent content provides an explanation for the anisotropic diffraction of the fairly large crystals. After refinement as a rigid body in the resolution range 25–3.0 Å, the R factor was 42.4%. Model building and refinement of the lectin structure to a resolution of 1.93 Å is in progress and structural details will be described in a separate paper.

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