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Crystallization and preliminary X-ray studies of a galactose-specific lectin from the seeds of *Spatholobus parviflorus*

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A galactose-specific seed lectin was purified from the legume *Spatholobus parviflorus* and crystallized using the hanging-drop vapour-diffusion technique. The crystals belonged to space group *P*1, with unit-cell parameters $a = 60.998$, $b = 60.792$, $c = 78.179$ Å, $\alpha = 101.32$, $\beta = 91.38$, $\gamma = 104.32^\circ$. X-ray diffraction data were collected under cryoconditions (100 K) to a resolution of 2.04 Å using a MAR image-plate detector system mounted on a rotating-anode X-ray (Cu $K\alpha$) generator. Molecular replacement using legume-lectin coordinates as a search model gave a tetrameric structure.

1. Introduction

Lectins are a heterogeneous group of proteins that are found ubiquitously in viruses, bacteria, animals and plants. They bind carbohydrates reversibly with a wide range of specificity. They are noncatalytic in activity and are important in a variety of biological processes owing to their carbohydrate specificities (Chandra *et al.*, 2006; Vijayan & Chandra, 1999; Lis & Sharon, 1998). Legume lectins may be involved in nitrogen fixation, the inhibition of pathological organisms and signal transduction (Goldstein & Etzler, 1983; Barondes, 1981). They exhibit homology ranging from 25 to 99% to lectins with similar three-dimensional structure, with some variations in the loops (Manoj & Suguna, 2001).

A galactose-specific lectin (SPL) from the seeds of *Spatholobus parviflorus*, a plant from the Fabaceae family, has been isolated by ion-exchange chromatography followed by affinity chromatography on activated guar gum. It has a molecular weight of 120 kDa and contains two different monomers of 31 and 29 kDa. The amino-terminal sequences of the two subunits are identical. They differ only in molecular weight, which may be a consequence of differences in C-terminal cleavage (Hamelryck *et al.*, 1996; Dessen *et al.*, 1995).

The crystallization and preliminary X-ray structure solution of SPL by the molecular-replacement method (Rossmann & Blow, 1962) are reported. The structure of *Dolichos biflorus* lectin (PDB code 1lu1; Hamelryck *et al.*, 1996) was used as the search model owing to its biochemical similarity.

2. Experimental procedures

2.1. Purification and crystallization

S. parviflorus seeds were extracted with 20 m*M* phosphate-buffered saline (PBS) pH 7.4 and centrifuged at 15 000*g* and the supernatant was fractionated to obtain precipitate at 50–70% ammonium sulfate. The precipitate was redissolved in buffer and dialyzed extensively against PBS. It was purified with CM Sephadex C50 and eluted with a 0.1–0.5 *M* NaCl gradient in buffer. It was further cleaned using a column containing epichlorohydrine cross-linked guar gum (Appukuttan *et al.*, 1977). The bound protein was then decoupled with 0.2 *M* D-galactose in PBS pH 7.4 and concentrated in a Centricon tube with a 10 kDa cutoff at 277 K. The results of MALDI–TOF analysis of the protein are shown in Fig. 1.

2.2. Crystallization

The purified lectin was concentrated to 8 mg ml^{-1}. Crystals of SPL were grown by the hanging-drop vapour-diffusion method from a solution consisting of 20 m*M* PBS containing 25% PEG 8000, 5% MPD and 5% 2-propanol as precipitants. The well contained 0.5 ml of this solution. 3 µl protein solution was placed on a siliconized cover slip and 3 µl well solution was added to it. The well was covered with the cover slip and sealed with silicon grease. Trials were made in the pH range 7.0–8.0 at 298 K. The best diffraction-quality crystals (Fig. 2) were obtained at pH 7.4 within two weeks.

2.3. X-ray diffraction data collection

Diffraction data were collected on a MAR 345 image plate using Cu *Kα* X-rays from a rotating-anode X-ray generator (Bruker MICROSTAR ULTRA II) operating at 50 kV and 100 mA. 2.04 Å resolution data were collected under cryoconditions using a CryoLoop (Fig. 3). The crystal-to-detector distance was 200 mm and the oscillation range was 1°. Data-intensity integration and scaling were carried out using *MOSFLM* and *SCALA*, respectively (Leslie, 1992; Winn *et al.*, 2011).

Table 1
Crystal data and data-collection statistics.

Values in parentheses are for the last resolution shell.

No. of crystals used	1
X-ray generator	Bruker MICROSTAR ULTRA II
Wavelength (Å)	1.5418
Detector	MAR 345
Crystal-to-detector distance	200
Rotation range per image (°)	1
Exposure time per image (s)	60
Resolution range (Å)	30.99–2.04 (2.14–2.04)
Temperature (K)	100
Space group	*P*1
Unit-cell parameters (Å, °)	a = 60.998, b = 60.792, c = 78.179, α = 101.32, β = 91.38, γ = 104.32
Mosaicity (°)	0.74
Total No. of measured intensities	133791 (15291)
Unique reflections	62376 (7362)
Multiplicity	2.1 (2.1)
Mean $I/\sigma(I)$	11.1 (3.1)
Matthews coefficient (Å^3 Da^{-1})	2.29
Completeness (%)	90.5 (73.2)
$R_{\rm merge}$ (%)	7.2 (21.1)
$R_{\rm meas}$ (%)	9.8 (28.7)
Solvent content (%)	46.29
No. of molecules in asymmetric unit	4

3. Results and discussion

S. parviflorus is a tropical plant belonging to the Fabaceae family. No reports can be found in the literature on this plant, its constituents or their bioactivity. This is the first report of any of its primary or secondary metabolites. Also, SPL is the first lectin to be purified from the genus *Spatholobus*. Purification of the lectin was effectively performed by sequential ammonium sulfate fractionation, ion-exchange chromatography (CM Sephadex C50) and affinity chromatography with activated guar gum. The galactose specificity of the lectin was exploited to decouple it from the guar gum column using D-galactose. MALDI–TOF (Fig. 1) and PAGE analyses showed that SPL is a tetramer consisting of subunits with monomeric molecular weights of 29 and 31 kDa. These results reveal that SPL is similar to the galactose-specific lectin isolated from *D. biflorus* (Hamelryck *et al.*, 1999). Diffraction-quality crystals were obtained using the hanging-drop vapour-diffusion method and they diffracted to 2.04 Å resolution. The crystals belonged to space group *P*1, with unit-cell parameters a = 60.998, b = 60.792, c = 78.179 Å, α = 101.32, β = 91.38,

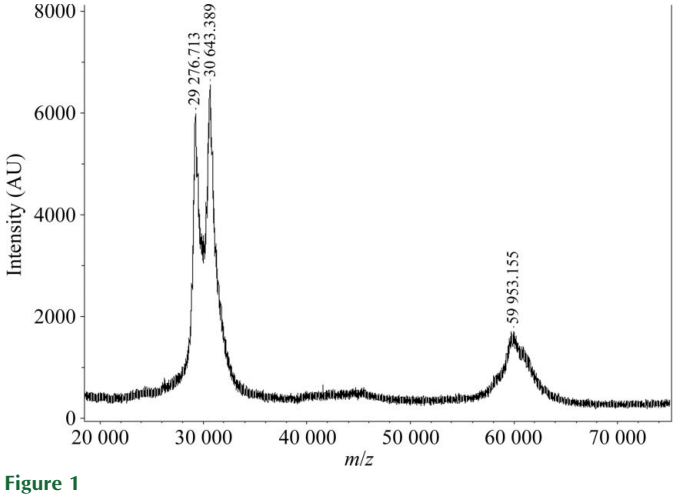

Figure 1
Determination of the molecular weight of SPL by MALDI–TOF.

Figure 2
SPL crystals.

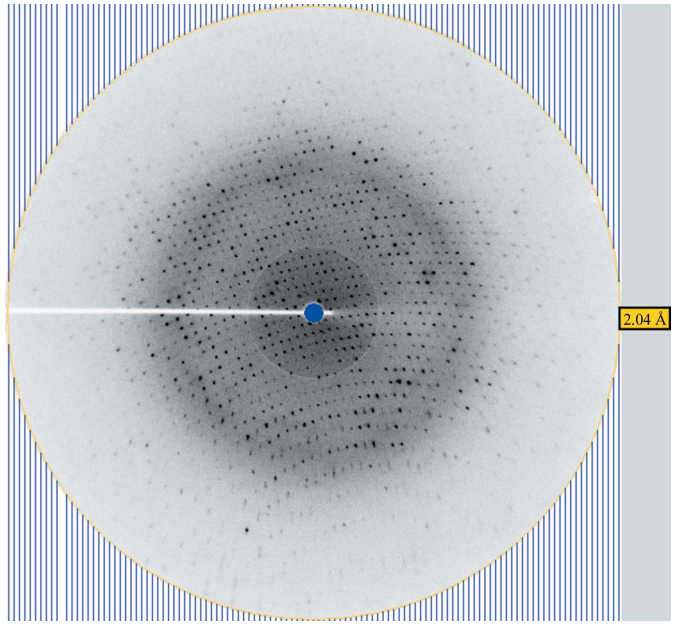

Figure 3
X-ray diffraction pattern of SPL at 2.04 Å resolution.

Table 2
Molecular-replacement data.

No.	Model used (PDB code)	θ (°)	φ (°)	χ (°)	t_x	t_y	t_z	wR_{fac}	Score
1	2sba	107.94	160.73	97.50	0.095	0.089	0.486	0.493	0.515
2	1bjq	118.24	−0.44	115.53	−0.026	−0.151	−0.469	49.6	50.9
3	1fny	1.41	20.70	149.15	0.888	0.918	0.487	0.587	0.409
4	1lu1	135.59	−132.58	173.37	0.532	0.450	0.533	0.530	0.529

$\gamma = 104.32°$. The crystal parameters and data-processing statistics are shown in Table 1. Assuming a tetramer in the crystal asymmetric unit, the calculated Matthews coefficient (Matthews, 1968) and solvent content were 2.29 Å^3 Da^{-1} and 46.29%, respectively. The structure was solved by molecular replacement using the *CCP*4 program *MOLREP* (Vagin & Teplyakov, 2010). The models used for molecular replacement were monomers, dimers and tetramers of soybean agglutinin (PDB code 2sba; Dessen *et al.*, 1995), *D. biflorus* seed lectin in complex with Forssman disaccharide (PDB code 1lu1; Hamelryck *et al.*, 1999), *Robinia pseudoacacia* lectin (PDB code 1fny; Rabijns *et al.*, 2001) and *D. biflorus* lectin in complex with adenine (PDB code 1bjq; Hamelryck *et al.*, 1999). The best results were obtained when the coordinates of 1lu1 were used, with a score of 52.9, and a w*R* factor of 53.0% (Table 2). Analysis of the structure is in progress. The final sequence has 63% homology to the starting model.

Data collection was performed at the X-ray facility for Structural Biology at the Indian Institute of Science, Bangalore, India. The infrastructure built with the support of the DBT-BIF (Government of India) at Kannur University is being used for refinement of the structure. UGC (Government of India) is acknowledged for a junior research fellowship to AJ.

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