Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray diffraction analysis of a galactose-specific lectin from the seeds of *Butea monosperma*

Joseph Abhilash,[a] K. Geethanandan,[a] S. R. Bharath,[b] C. Sadasivan[a,c] and M. Haridas[a,c]*

[a]Department of Biotechnology and Microbiology, Kannur University, Kannur, Kerala 670 661, India, [b]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India, and [c]Inter-University Centre for Biosciences, Kannur University, Kannur, Kerala 670 661, India

Correspondence e-mail: mharidasm@rediffmail.com

Received 25 November 2010
Accepted 22 February 2011

The galactose-specific lectin from the seeds of *Butea monosperma* has been crystallized by the hanging-drop vapour-diffusion technique. The crystals belonged to space group *P*1, with unit-cell parameters a = 78.45, b = 78.91, c = 101.85 Å, α = 74.30, β = 76.65, γ = 86.88°. X-ray diffraction data were collected to a resolution of 2.44 Å under cryoconditions (100 K) using a MAR image-plate detector system mounted on a rotating-anode X-ray generator. Molecular-replacement calculations carried out using the coordinates of several structures of legume lectins as search models indicate that the galactose-specific lectin from *B. monosperma* forms an octamer.

1. Introduction

Lectins form an important group of proteins that recognize the sugar code (Gabius, 2000). They play a central role by recognizing molecules in cell–molecule and cell–cell interactions (Sharon & Lis, 2004). Lectins are a ubiquitous and heterogeneous group of proteins and are found in organisms such as viruses, bacteria, animals and plants. They bind carbohydrates reversibly with high and wide-ranging specificity. They are noncatalytic in activity and are not produced as a response of the immune system. Lectins are important in a variety of biological processes. They mediate interactions through their carbohydrate specificities (Vijayan & Chandra, 1999; Lis & Sharon, 1998). Legume lectins may be involved in nitrogen fixation at the surface of roots, inhibition of the growth of pathological organisms and signal transduction (Goldstein & Etzler, 1983; Barondes, 1981). Plant seeds are a major source of lectins. The sequences of a large number of legume lectins are available in the sequence database and they exhibit a high degree of homology that ranges from 25–99% amongst those with similar three-dimensional structures (Manoj & Suguna, 2001). The tertiary structures of legume lectins are similar, except for some variations in the loops. Selectins, which share homology with lectin domains, are known to be involved in inflammatory processes (Kansas, 1996). This supports the use of leguminous lectins as anti-inflammatory molecules (Assreuy *et al.*, 1997; Alencar *et al.*, 1999).

2. Materials and methods

2.1. Purification and crystallization of *Butea monosperma* lectin (BML)

Dry *B. monosperma* seeds were ground in 20 m*M* PBS pH 7.4 and sonicated. The sonicated slurry was stirred overnight, centrifuged at 10 000*g* for 30 min and the supernatant was fractionated to obtain precipitate at 50–70%. The precipitate was dissolved in a minimal amount of PBS pH 7.4 and dialyzed extensively against several changes of PBS. The clear solution was subjected to chromatographic separation. The dialysed ammonium sulfate fraction (50–70%) was loaded onto a CM Sephadex C-50 column pre-equilibrated with the dialysis buffer. The resin-bound protein was eluted with a gradient buffer containing 0.1–0.5 *M* NaCl. Elution of the column took place at a flow rate of 50 ml h^{-1} and the elution profile was monitored by absorbance at 280 nm. 4 ml fractions were collected and all fractions

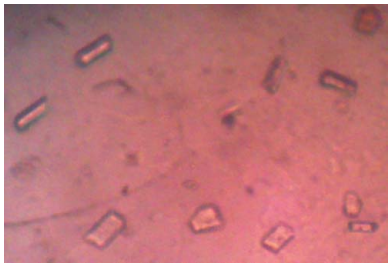

Table 1
Unit-cell parameters and X-ray data-collection statistics.

Values in parentheses are for the last resolution shell.

No. of crystals used	1
X-ray generator	Bruker Microstar Ultra II rotating anode
Wavelength (Å)	1.5418
Detector	MAR 345
Crystal-to-detector distance (mm)	230
Rotation range per image (°)	1
Exposure time per image (s)	60
Resolution range (Å)	69.06–2.44 (2.57–2.44)
Space group	*P*1
Unit-cell parameters (Å, °)	a = 78.45, b = 78.91, c = 101.85, α = 74.30, β = 76.65, γ = 86.88
Mosaicity (°)	0.54
Total No. of measured intensities	165863 (23184)
Unique reflections	75986 (10951)
Multiplicity	2.2 (2.1)
Mean $I/\sigma(I)$	5.2 (2.3)
Completeness (%)	96.0 (94.1)
R_{merge} (%)	12.9 (34.2)
R_{meas} (%)	17.2 (43.1)
Overall *B* factor from Wilson plot (Å^2)	37.2

were checked for haemagglutination and haemagglutination inhibition (Faria *et al.*, 2004) activity. The fractions showing haemagglutination activity were pooled, dialysed extensively against PBS and subjected to further purification (Silva *et al.*, 2007).

The clear protein solution was applied onto a column of guar gum cross-linked with epichlorohydrin (Appukuttan *et al.*, 1977), equilibrated with the same buffer at a flow rate of 50 ml h^{-1}. The bound protein was then decoupled with 0.2 *M* D-galactose in PBS pH 7.4. Elution was carried out at a flow rate of 40 ml h^{-1}. Fractions containing pure protein were pooled and dialyzed extensively against PBS, pH 7.4 with several changes and were finally dialyzed against distilled water. The dialyzed protein solution was then concentrated with polyethylene glycol 20 000 and lyophilized. Purified BML was analyzed by 12.5% SDS–PAGE (Laemmli, 1970) with molecular-weight markers (Genei Bangalore, India). The purified lectin was also compared with the previously reported *Spatholobus parviflorus* seed lectin (SPL; Geethanandan, 2010). BML was analysed on a Bruker MALDI–TOF mass spectrometer. The mass spectrum was recorded on an Ultraflex TOF/TOF MALDI mass spectrometer (Bruker Daltonics) using sinapinic acid as the matrix. A 337 nm nitrogen laser was used. A total of 500 scans were averaged for the final spectrum.

The purified lectin was dialyzed against 20 m*M* phosphate buffer to remove the galactose used in the elution buffer and concentrated to 10 g l^{-1}. The hanging-drop vapour-diffusion method successfully produced diffraction-quality crystals. A precipitant consisting of 25% PEG 8000 and 5% MPD in 20 m*M* phosphate buffer pH 7.4 was used at a temperature of 293 K. A photograph of the crystals is shown in Fig. 1.

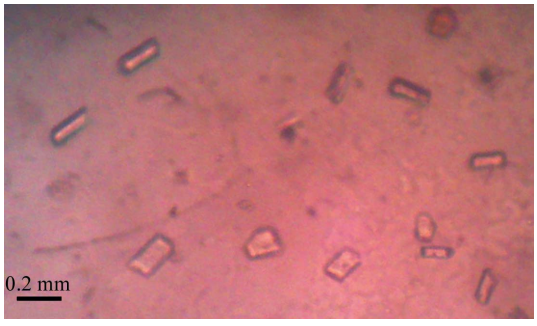

Figure 1
Crystals of *B. monosperma* lectin.

2.2. X-ray data collection and processing

Prior to data collection, individual crystals were soaked in crystallization solution containing 25%(*v*/*v*) glycerol for 1 min to achieve cryoprotection. The pre-soaked crystals were submitted to immediate flash-freezing in a cold nitrogen-gas stream. X-ray diffraction data were collected using a MAR 345 image plate. The X-ray beam (Cu $K\alpha$ radiation, λ = 1.5418 Å) from a Bruker Microstar rotating-anode X-ray generator operating at 50 kV and 100 mA was focused using an Osmic mirror system. A complete data set was collected to 2.44 Å resolution under cryoconditions (100 K). 200 frames of data were collected with 1 min exposure per frame and 1° oscillation at a crystal-to-detector distance of 230 mm. A diffraction pattern is shown in Fig. 2. The data were processed using *MOSFLM* (Leslie, 1992) and scaled using *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The crystal parameters and data-processing statistics are summarized in Table 1.

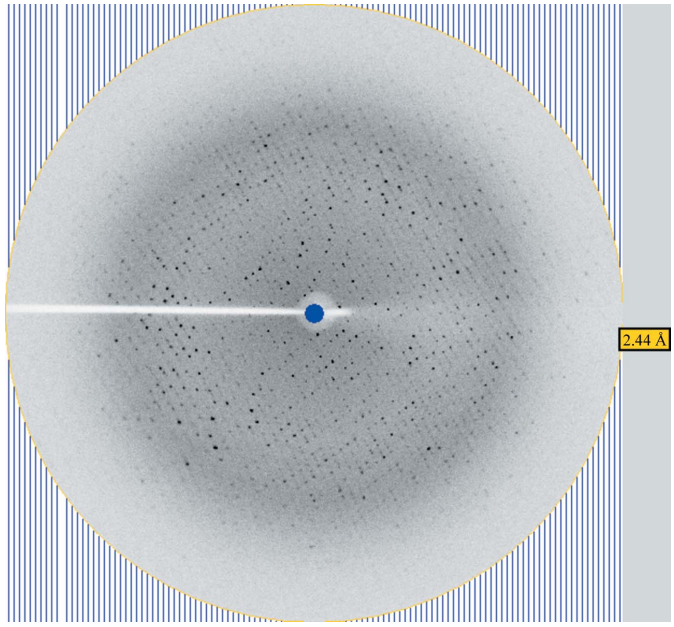

Figure 2
Diffraction pattern of *B. monosperma* lectin.

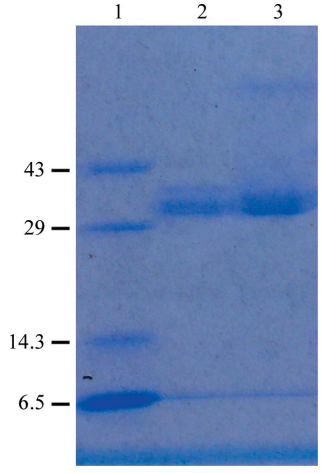

Figure 3
SDS–PAGE. Lane 1, molecular-weight markers (labelled in kDa): ovalbumin (~43 kDa), carbonic anhydrase (~29 kDa), lysozyme (~14.3 kDa) and aprotinin (~6.5 kDa). Lane 2, BML; Lane 3, SPL.

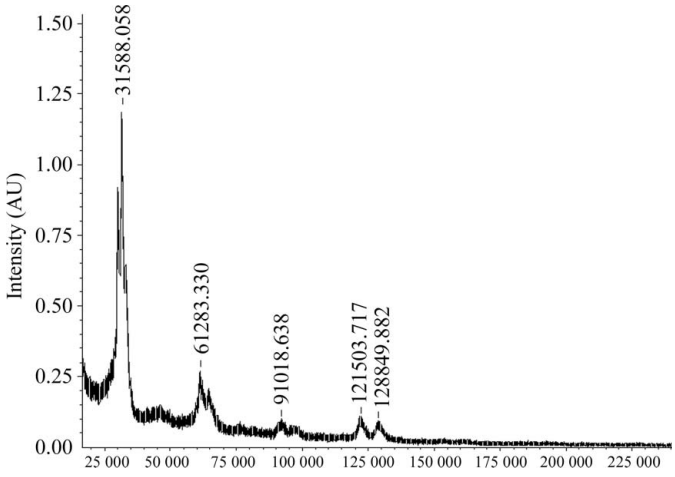

Figure 4
MALDI–TOF spectrum of BML.

3. Results and discussion

In the presence of 10 m*M* β-mercaptoethanol, two closely spaced bands of 30 and 31 kDa were observed on 12% SDS–PAGE (Fig. 3). SPL was used for comparison and gave bands with molecular masses of 29 and 31 kDa. The MALDI–TOF mass spectrum of the protein showed prominent peaks at 31, 61, 91, 121 and 128 kDa (Fig. 4). However, the spectrum cannot be explained with confidence. All of the recorded MALDI–TOF peaks, apart from the peak at 128 kDa, seem to be multiples of the first peak at 31 kDa. The prominent peak at 31 kDa shows a strongly split character, presumably owing to the presence of a lower molecular-weight associate. The haemagglutination test confirmed that the protein has affinity towards erythrocytes.

The crystals belonged to space group *P*1, with unit-cell parameters $a = 78.45$, $b = 78.91$, $c = 101.85$ Å, $\alpha = 74.30$, $\beta = 76.65$, $\gamma = 86.88^\circ$. The presence of an octamer in the asymmetric unit leads to a Matthews coefficient of 2.17 Å^3 Da^{-1} and a solvent content of 43.38%, which is consistent with Matthews V_M statistics (Matthews, 1968). The structure was solved by the molecular-replacement method using the *CCP*4 program *MOLREP* (Vagin & Teplyakov, 2010). Monomers, dimers and tetramers of SPL (PDB entry 3ipv; K. Geethanandan, S. R. Bharath, J. Abhilash, C. Sadasivan & M. Haridas, unpublished work), soybean agglutinin (PDB entry 2sba; Dessen *et al.*, 1995), *Dolichos biflorus* seed lectin in complex with Forssman disaccharide (PDB entry 1lu1; Hamelryck *et al.*, 1999), *Robinia pseudoacacia* lectin (PDB entry 1fny; Rabijns *et al.*, 2001) and *D. biflorus* lectin in complex with adenine (PDB code 1bjq; Hamelryck *et al.*, 1999) were used as search models. The best results were obtained when the coordinates of SPL were used. After molecular replacement, the correlation coefficient and *R* factor were 56.0 and 41.8%, respectively, for two tetramers in the asymmetric unit. Some of the subunits are related by a noncrystallographic twofold rotation of 180 $\pm$ 1°: *a*–*b*, *a*–*d*, *a*–*f*, *a*–*g*, *b*–*d*, *b*–*g*, *c*–*e*, *c*–*f*, *c*–*h*, *d*–*e*, *d*–*g*, *e*–*f*, *e*–*h* and *f*–*h*. It would be interesting to observe the structural features that direct the protein to crystallize in space group *P*1 only. Refinement of the structure is in progress.

The data used in the present work were collected at the X-ray facility for Structural Biology at the Indian Institute of Science, supported by the Department of Science and Technology (DST) and the Department of Biotechnology (DBT), India. Professor M. R. N. Murthy, MBU, IISc Bangalore and his colleagues are acknowledged for the X-ray diffraction data collection.

References

Alencar, N. M., Teixeira, E. H., Assreuy, A. M., Cavada, B. S., Flores, C. A. & Ribeiro, R. A. (1999). *Mediators Inflamm.* **8**, 107–113.
Appukuttan, P. S., Surolia, A. & Bachawat, B. K. (1977). *Indian J. Biochem. Biophys.* **14**, 382–384.
Assreuy, A. M., Shibuya, M. D., Martins, G. J., De Souza, M. L., Cavada, B. S., Moreira, R. A., Oliveira, J. T., Ribeiro, R. A. & Flores, C. A. (1997). *Mediators Inflamm.* **6**, 201–210.
Barondes, S. H. (1981). *Annu. Rev. Biochem.* **50**, 207–231.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Dessen, A., Gupta, D., Sabesan, S., Brewer, C. F. & Sacchettini, J. C. (1995). *Biochemistry*, **34**, 4933–4942.
Faria, R. A., Andrade-Neto, M., Pinto, L. S., Castellón, R. R., Calvete, J. J. & Cavada, B. S. (2004). *Arch. Latinoam. Nutr.* **54**, 349–353.
Gabius, H.-J. (2000). *Naturwissenschaften*, **87**, 108–121.
Goldstein, I. J. & Etzler, M. E. (1983). *Chemical Taxonomy, Molecular Biology and Function of Plant Lectins.* New York: Alan Liss.
Geethanandan, K. (2010). PhD dissertation. Kannur University, Kerala, India.
Hamelryck, T. W., Loris, R., Bouckaert, J., Dao-Thi, M.-H., Strecker, G., Imberty, A., Fernandez, E., Wyns, L. & Etzler, M. E. (1999). *J. Mol. Biol.* **286**, 1161–1177.
Kansas, G. S. (1996). *Blood*, **88**, 3259–3287.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **26**.
Lis, H. & Sharon, N. (1998). *Chem. Rev.* **98**, 637–674.
Manoj, N. & Suguna, K. (2001). *Protein Eng.* **14**, 735–745.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Rabijns, A., Verboven, C., Rougé, P., Barre, A., Van Damme, E. J., Peumans, W. J. & De Ranter, C. J. (2001). *Proteins*, **44**, 470–478.
Sharon, N. & Lis, H. (2004). *Glycobiology*, **14**, 53R–62R.
Silva, J. A., Damico, D. C., Baldasso, P. A., Mattioli, M. A., Winck, F. V., Fraceto, L. F., Novello, J. C. & Marangoni, S. (2007). *Protein J.* **26**, 193–201.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.
Vijayan, M. & Chandra, N. (1999). *Curr. Opin. Struct. Biol.* **9**, 707–714.