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Crystallization and preliminary X-ray crystallographic analysis of a galactose-specific lectin from *Dolichos lablab*

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The galactose-specific lectin from the seeds of *Dolichos lablab* has been crystallized using the hanging-drop vapour-diffusion technique. The crystals belong to space group *P*1, with unit-cell parameters a = 73.99, b = 84.13, c = 93.15 Å, α = 89.92, β = 76.01, γ = 76.99°. X-ray diffraction data to a resolution of 3.0 Å have been collected under cryoconditions (100 K) using a MAR imaging-plate detector system mounted on a rotating-anode X-ray generator. Molecular-replacement calculations carried out using the available structures of legume lectins as search models revealed that the galactose-specific lectin from *D. lablab* forms a tetramer similar to soybean agglutinin; two such tetramers are present in the asymmetric unit.

1. Introduction

Lectins are a class of ubiquitous proteins that bind stereospecifically and reversibly to carbohydrates (Lis & Sharon, 1998). They are abundantly found in legumes. The legume lectins play a role in nitrogen fixation at the surface of the roots, inhibition of growth of pathological organisms and signal transduction (Barondes, 1981; Goldstein & Etzler, 1983).

A leguminous plant, *Dolichos lablab*, found in India has two distinct varieties, *D. lablab* var. *typicus* (Indian beans) and *D. lablab* var. *lignosus* (field beans). The seeds of both these plants were found to contain a glucose/mannose-specific lectin (Gowda *et al.*, 1994; Rajasekhar *et al.*, 1997). The complete primary structure of the glucose/mannose-specific lectin (DLL) from field bean seeds has been determined (Gowda *et al.*, 1994). A cDNA of a lectin FRIL (Flt3 receptor-interacting lectin) isolated from another variety of the same plant (hyacinth beans) has been shown to preserve haematopoietic progenitors in culture (Colucci *et al.*, 1999). Although some of the physicochemical and biological properties of these lectins, which have the same sugar specificity, remain the same, they differ in their sequence according to the different varieties of seeds used. The sequence of FRIL has 78% identity to DLL and 52% identity to ConA. In solution FRIL is a divalent dimer, by which it is meant that it is a dimer and binds to two carbohydrate molecules, in the presence of the trisaccharide [Man(α1–3)][Man(α1–6)][Manα(1-OMe)] (M3M6M), but in the crystal form it forms a ConA-type tetramer (Hamelryck *et al.*, 2000).

The galactose-specific lectin from the seeds of Indian beans has been isolated by affinity chromatography on Sepharose-galactose gel (Lavanya Latha *et al.*, 2006). It has a molecular weight of 120 kDa, with two subunits of 31 and 29 kDa. The lectin is a tetramer with a high content of acidic amino acids and lacks sulfur-containing amino acids. The amino-terminal sequences of both the subunits are identical, but they differ in their molecular weights, which may be a consequence of differences in C-terminal cleavage. The partial sequence of this galactose-specific lectin shows 78% similarity to that of the glucose/mannose-specific lectin.

In this paper, we report the crystallization of the galactose-specific lectin from *D. lablab*, X-ray diffraction data collection, its structure solution by the molecular-replacement method and a comparison of its quaternary structure with the known structures of legume lectins. Structure analysis of this lectin will help us to understand its functional role in the plant and its exact mechanism of action.

Table 1
Crystal data and data-collection statistics.

Values in parentheses refer to the highest resolution shell.

Wavelength (Å)	1.5418
Temperature (K)	100
Space group	*P*1
Unit-cell parameters (Å, °)	a = 73.99, b = 84.13, c = 93.15, α = 89.92, β = 76.01, γ = 76.99
No. of molecules in ASU	2
Matthews coefficient (Å^3 Da^{-1})	2.3
Solvent content (%)	45.7
Resolution (Å)	3 (3.11–3.0)
No. of observed reflections	83494 (6708)
No. of unique reflections	41323 (3450)
Completeness (%)	94.3 (78.5)
$R_{\rm merge}$ (%)	9.6 (48.3)
$\langle I/\sigma(I)\rangle$	10.2 (3.7)

2. Experimental procedures

2.1. Purification and crystallization

D. lablab seeds were purchased from Wipro Seeds, Hyderabad (Lot No. KR 306). The purification of the galactose-specific lectin was carried out as described in Lavanya Latha *et al.* (2006). Seeds of *D. lablab* were ground to a fine powder and extracted overnight in 25 m*M* Tris-buffered saline pH 7.4. The crude extract was subjected to 0–60% and 60–80% ammonium sulfate fractionation. The 60–80% ammonium sulfate fractionated pellet was applied onto an affinity chromatography column (Sepharose-DVS-galactose gel). The bound protein was eluted using 0.3 *M* galactose. This galactose-specific lectin was further purified by gel-filtration chromatography. The lectin was finally suspended in an unbuffered 0.9%(*w*/*v*) solution of NaCl and concentrated to 10 mg ml^{-1} by means of dialysis. Suitable crystallization conditions were screened using the hanging-drop method. Initial screening was performed using Hampton Research Crystal Screens I and II (Jancarik & Kim, 1991), Grid Screen PEG 6000 and Wizard screening kits. Small plate-like crystals were seen in different conditions in the pH range 6.0–8.0. The conditions under which crystals appeared (Nos. 26 and 30 of Crystal Screen 2 and A3, B3, C3, D3 and D5 of Grid Screen PEG 6000) were further optimized in an attempt to improve the quality of the crystals. Slightly larger crystals were obtained within a week when 30% PEG 6K pH 6.0 was used. The growth of the crystal was further improved by the addition of 5% MPD to the precipitant. The hanging drop consisted of 3 µl lectin solution (10 mg ml^{-1} lectin containing galactose, which was dissolved in saline and added to the protein solution such that the final concentration of galactose was 4 m*M*) and 1 µl precipitant solution. The hanging drop was equilibrated with 0.5 ml 30% PEG 6K pH 6.0 containing 5% MPD. The crystals obtained using this condition (Fig. 1) were used for data collection.

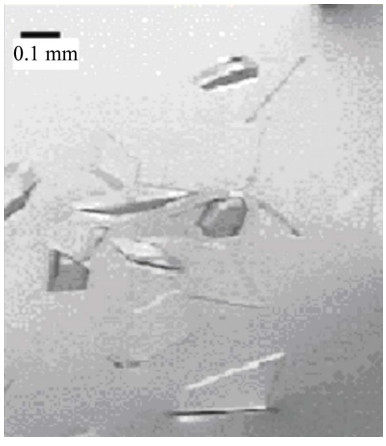

Figure 1
Crystals of the galactose-specific lectin from *D. lablab* obtained from 30% PEG 6K pH 6.0 containing 5% MPD.

2.2. Data collection

X-ray diffraction data were collected using a MAR 345 imaging plate. The X-ray beam (Cu $K\alpha$ radiation, λ = 1.5418 Å) from a Rigaku Ultrax-18 rotating-anode X-ray generator operating at 50 kV and 100 mA was focused with an Osmic mirror system. When we attempted to collect data at room temperature, it was observed that the crystals were radiation-sensitive. Hence, a complete data set was collected under cryoconditions (100 K) to 3 Å resolution. Crystal freezing included soaking the crystal in a cryoprotectant solution consisting of the original crystallization solution. The pre-soaked crystal was then submitted to immediate flash-freezing in a cold nitrogen-gas stream. Only 327 of the intended 360 frames could be collected owing to an interruption in data collection, with 15 min exposure per frame and 1° oscillation at a crystal-to-detector distance of 180 mm. The data were processed with *DENZO* and *SCALEPACK* from the *HKL*2000 package (Otwinowski & Minor, 1997).

3. Results and discussion

Crystals of the galactose-specific lectin were grown by the hanging-drop method and diffraction-quality crystals were obtained from a crystallization condition with pH 6.0 buffer. The crystals belonged to space group *P*1, with unit-cell parameters a = 73.99, b = 84.13, c = 93.15 Å, α = 89.92, β = 76.01, γ = 76.99°. The data could only be processed in space group *P*1. Although the program *DENZO* suggested a fewer higher space groups, we were unsuccessful in processing the data in these space groups as the unit-cell angles deviated significantly from 90°. Statistics of data collection and processing to 3 Å resolution are summarized in Table 1. Assuming the presence of two tetramers in the asymmetric unit leads to a Matthews coefficient of 2.3 Å^3 Da^{-1} and a solvent content of 45.7%, which is consistent with Matthews $V_{\rm M}$ statistics (Matthews, 1968).

The structure was solved by the molecular-replacement method using the *CCP*4 program *AMoRe* (Navaza, 1994). Dimers and tetramers of FRIL (PDB code 1qmo), soybean agglutinin (SBA; PDB code 2sba), phytohaemagglutinin (PHA-L, PDB code 1fat), *Ulex europaeus* lectin-II (UEA-II; PDB code 1qoo) and *Maackia amurensis* leukoagglutinin (MAL; PDB code 1dbn) were used as search models. The best results were obtained when the coordinates of PHA-L were used. The correlation factor and the *R* factor were 56.0 and 41.8%, respectively, for data between 15 and 3.8 Å resolution for two tetramers in the asymmetric unit. When the two tetramers were superposed using the program *ALIGN* (Cohen, 1997), they superpose with an r.m.s.d. of 0.8 Å for the main-chain atoms and are related to each other by a rotation of 152°, thus ruling out the possibility of a higher space group.

Each tetramer has 222 symmetry and is formed by two canonical dimers containing a 12-pleated β-sheet with six strands from each subunit. The tetrameric association was found to be similar to the Gal/GalNAc-binding tetrameric legume lectins SBA, PHA-L, UEA-II, MAL, the *D. biflorus* lectins DBL (PDB code 1lu1) and DB58 (PDB code 1lul), *Robinia pseudoacacia* lectin (PDB code 1fny) and *Vicia villosa* lectin (PDB code 1n47) and is distinctly different from the structures of either the Glc/Man-binding lectins FRIL and ConA or the Gal-specific peanut agglutinin (PDB code 2pel). Model building and refinement of the structure are in progress.

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