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Crystallization and preliminary structural studies of champedak galactose-binding lectin

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Galactose-binding lectin from champedak (*Artocarpus integer*) consists of two chains: α and β (133 and 21 amino acids, respectively). It has been shown to recognize and bind to carbohydrates involved in IgA and C1 inhibitor molecules. The protein was purified and crystallized at 293 K. Crystals were observed in two space groups, $P2_1$ and $P2_12_12$, and diffracted to 1.65 and 2.6 Å, respectively.

1. Introduction

Champedak (*Artocarpus integer*) is a fruit that is found throughout Malaysia. The seed of this fruit has been reported to contain a galactose-binding lectin (CGB; champedak galactose-binding lectin) that is similar to the lectin jacalin found in jackfruit (Hashim *et al.*, 1991). CGB recognizes and binds the T-cell antigenic determinant Galβ1-3GalNaC in IgA and C1 inhibitor molecules (Hashim *et al.*, 1991, 1993). This binding has been utilized as a tool to study the carbohydrate structures of IgA1 from patients with IgA nephropathy (Shuib *et al.*, 1998; Hashim *et al.*, 2001). CGB binding specificity has also been utilized in two-dimensional gel-based proteomic analyses to study the differential expression of several serum glycoproteins in selective cohorts of patients with cancers (Abdul-Rahman *et al.*, 2007; Mohamed *et al.*, 2008).

Like jacalin, CGB consists of a propeptide, cleavage of which gives the α and β chains. Although the sequence of CGB is not fully known, recent work has revealed a high degree of similarity between this lectin and jacalin (10% difference between the first 47 residues of the α chain and the β chain of CGB and jacalin; Abdul Rahman *et al.*, 2002). The structure of jacalin has been solved in complex with a number of carbohydrates and is described as a tetramer (Arockia Jeyaprakash *et al.*, 2005; Jeyaprakash *et al.*, 2003). It is likely that the structure of CGB will be similar. Here, we describe the crystallization of CGB in two different space groups using very similar conditions.

2. Material and methods

2.1. Protein preparation and crystallization

CGB lectin was isolated from 40 g of champedak seeds. The powdered seeds were suspended in 400 ml phosphate-buffered saline (PBS) pH 7.4 and stirred for 24 h at 277 K. The homogenate was centrifuged at 8000*g* for 15 min at 277 K and the resulting supernatant was collected, subjected to 60% ammonium sulfate precipitation and left to stir for 2–3 h at 277 K. The solution was then centrifuged at 8000*g* for 15 min at 277 K. The resulting pellet containing the crude extract was collected, dissolved in cold PBS and dialysed against several changes of PBS for 48 h. CGB lectin was further purified using a galactose-affinity chromatography column and eluted in 0.8 *M* galactose in PBS pH 7.4. The bound fractions with high absorbance (A_{280} between 0.4 and 1.3) were pooled and dialysed against water. The dialysed bound fraction was freeze-dried to concentrate the CGB lectin prior to storage at 253 K; it was resolubilized in 20 m*M* Tris pH 7.5 prior to use.

The galactose column was prepared using the method of Hermanson *et al.* (1992). In brief, Sepharose 4B was activated using divinyl sulfone (DVS) to introduce reactive vinyl groups into the gel. The DVS-activated gel was suspended in an equal volume of 20%(*w*/*v*) galactose in 0.5 *M* sodium carbonate. The mixture was stirred at room temperature (RT) for 24 h before the gel was washed successively with 2 l water and 0.5 *M* sodium bicarbonate. The gel was subsequently resuspended in 0.5 *M* sodium bicarbonate containing 2 ml β-mercaptoethanol and stirred for 2 h in a fume cupboard to block excess reactive vinyl groups. The gel was then washed with 2 l water followed with the same volume of PBS. The resulting galactose-coupled Sepharose 4B gel was packed into polypropylene columns of 2.8 cm in diameter with a packed gel volume of 16 cm in height.

The estimated purity of the purified CGB lectin was 95% (from SDS–PAGE; Hashim *et al.*, 1991, 1993). Initial crystallization screens were set up using sitting-drop vapour diffusion, with drops consisting of 500 nl protein and 500 nl reservoir. The trays were incubated at 293 K. Crystals were observed in Cryo I screen (Emerald BioSystems) condition No. 39 (40% PEG 600, 100 m*M* phosphate/citrate buffer pH 4.2). Crystals grew within two weeks to dimensions of approximately 0.15 × 0.15 × 0.1 mm (Fig. 1*a*).

A second crystal form consisting of small rectangular rods (Fig. 1*b*) was observed in PEG/Ion Screen 2 (Hampton Research) condition No. 28, but these were not followed up as they diffracted to no further than 10 Å resolution.

2.2. Data collection and processing

The crystals were plunged directly into a stream of cooled nitrogen gas (100 K; Oxford Cryosystems) without any further cryoprotection. Data were collected in-house on a MAR 345 detector using a Cu $K\alpha$ source on a Rigaku MicroMax-007 X-ray generator. Data were collected in 1° oscillations over a total rotation of 163° and crystal diffraction was observed to beyond 2.6 Å resolution. A second data set was collected at Diamond Light Source station I02 (Fig. 2). A total of 702° of data, split into two passes, one to collect high-resolution data and the second to collect complete low-resolution data (detector distances of 257.4 and 427.485 mm, respectively), were collected in 0.5° oscillations for 5 and 1 s, respectively. Bragg diffraction was observed to beyond 1.3 Å resolution (at the detector corners) and the data were cut off at 1.65 Å based on the statistics following scaling, in particular the poor completeness and merging statistics for higher resolutions (for details, see Table 1). The data sets were processed and scaled using *MOSFLM* (Leslie, 1992) and *SCALA* (Evans, 1993) and the space groups were confirmed by *POINTLESS* (Evans, 2006), all of which are from the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

Table 1
Data-collection statistics for the two crystals.

Values in parentheses are for the highest resolution shell.

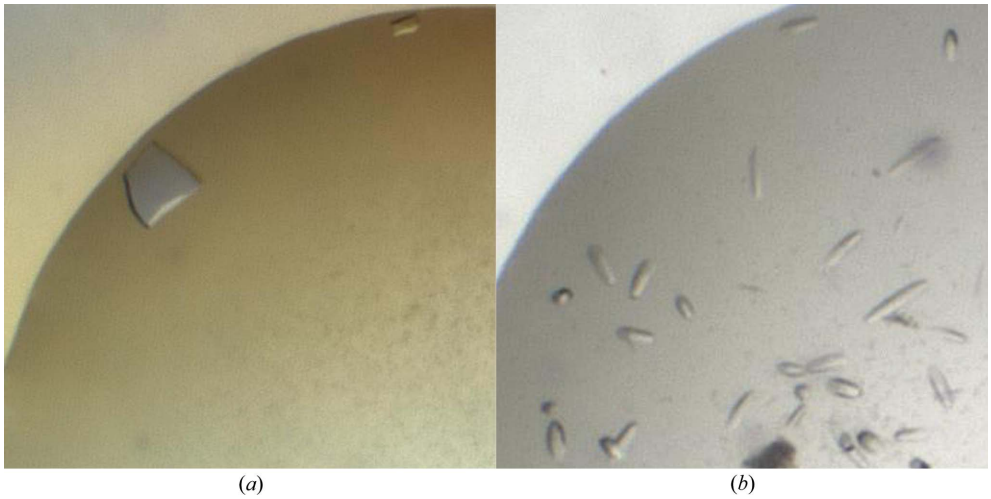
Space group	$P2_1$	$P2_12_12$
Unit-cell parameters (Å, °)	a = 76.17, b = 121.72, c = 77.74, β = 90.60	a = 76.19, b = 120.98, c = 77.48
Resolution (Å)	76.25–1.65 (1.74–1.65)	121.3–2.60 (2.74–2.60)
Observed/unique reflections	630031/163935	128426/22228
R_{merge}	0.123 (0.614)	0.119 (0.27)
$R_{p.i.m.}$	0.053 (0.384)	0.049 (0.162)
$\langle I/\sigma(I)\rangle$	13.4 (1.9)	11.2 (6.1)
Wilson B (Å^2)	20	50
Completeness (%)	96.5 (80.2)	96.6 (77.1)

3. Results

Despite being obtained from similar conditions, the two crystals belonged to different space groups. The in-house data set belonged to space group $P2_12_12$ (unit-cell parameters a = 76.19, b = 120.98, c = 77.48 Å), whereas the data set collected at Diamond Light Source station I02 belonged to space group $P2_1$ (unit-cell parameters a = 76.17, b = 121.72, c = 77.74 Å, β = 90.60°). Assuming a protein molecular weight of approximately 18 kDa (based on the molecular weight of jacalin), the calculated Matthews coefficient of 2.5 Å^3 Da^{-1} (Matthews, 1968) and solvent content of 53% suggested the presence of eight monomers in the asymmetric unit in $P2_1$ and four in $P2_12_12$.

The space-group difference between two crystals with close to identical morphology and highly isomorphous unit cells is interesting and can be explained by a crystallographic twofold from the orthorhombic crystal being readjusted in the monoclinic cell to become a pseudo-crystallographic twofold.

The output log files of the self rotation for the $P2_1$ cell (*MOLREP*; Vagin & Teplyakov, 1997) show a higher peak along the b axis compared with the other NCS peaks on the κ = 180° section, whilst the $P2_12_12$ cell has peaks of similar heights along all three axes. The differences in crystallographic symmetry may have been caused by accidental annealing during crystal transfer.



(*a*) (*b*)

Figure 1
(*a*) Crystals grown using the sitting-drop vapour-diffusion method. The crystals appeared after 1–2 weeks and were grown against a reservoir consisting of 40% PEG 600, 100 m*M* phosphate–citrate pH 4.2. (*b*) The second crystal form. Poor diffraction led to these crystals not being followed up.

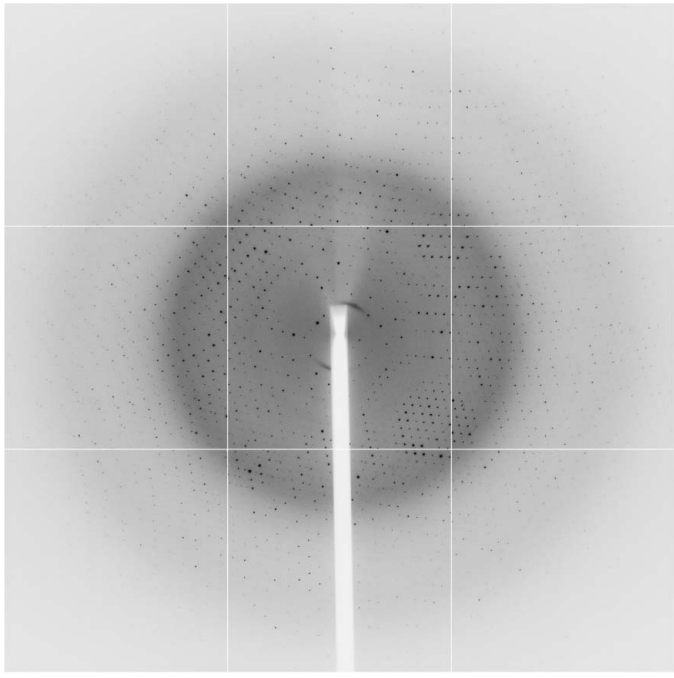


Figure 2
Diffraction pattern of a CGB crystal collected at Diamond Light Source station I02 to a resolution of 1.3 Å (at the corners).

The crystal structure of jacalin has been shown to be tetrameric, with a fourfold rotational symmetry that is either crystallographic (PDB code 1ugx) or arises from NCS (*e.g.* PDB code 1ws4), and it is expected that CGB will behave in a similar manner considering the predicted high sequence similarity between the two.

We have crystallized CGB, a galactose-binding lectin from the Malaysian fruit champedak, in two different space groups; the crystals diffracted to 1.65 and 2.6 Å resolution, respectively. Heavy-atom phasing experiments, as well as molecular-replacement trials using models of the homologue jacalin, are currently under way.

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