

## Crystallization and initial X-ray diffraction analysis of a mannose-binding lectin from champedak

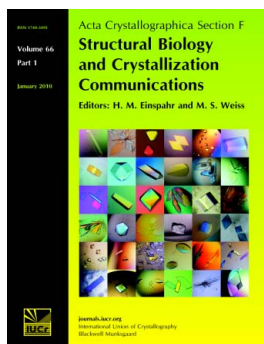
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## Crystallization and initial X-ray diffraction analysis of a mannose-binding lectin from champedak

Mannose-binding lectin from champedak (*Artocarpus integer*) is a homotetramer with a single-monomer molecular weight of 16 800 Da. Previous work has shown it to bind IgE and IgM, as well as being a mitogen of T cells in humans. Champedak mannose-binding lectin has successfully been used to detect altered glycosylation states of serum proteins. The protein was crystallized at 293 K in space group  $P2_12_12_1$  (unit-cell parameters  $a = 76.89$ ,  $b = 86.22$ ,  $c = 95.37$  Å) and the crystals diffracted to 2.0 Å resolution.

### 1. Introduction

Champedak (*Artocarpus integer*) is a fruit tree found in the south-east of Asia. The seeds of this fruit have been shown to be rich in lectins binding galactose (champedak galactose-binding lectin; CGB; Hashim *et al.*, 1991) or mannose (champedak mannose-binding lectin; CMB; Lim *et al.*, 1997). CMB recognizes and binds strongly to IgE and IgM and weakly to IgA2, with no binding to IgG or IgA1 (Lim *et al.*, 1997). CMB has been shown to be a potent mitogen of T cells in humans (Lim *et al.*, 1998) and work performed by Hashim *et al.* (2001) and Yong *et al.* (2006) has shown that CMB can be used as a tool to detect alterations in the glycosylation states of serum proteins.

Although the sequence of CMB is not known, it is likely to be similar to that of the lectin artocarpin found in jackfruit. Like artocarpin, CMB comprises a single polypeptide chain with a molecular weight of 16 800 Da. The biologically active form is a homotetramer in which, in contrast to artocarpin, at least some of the subunits are covalently linked *via* disulfide bonds (Lim *et al.*, 1997). The crystal structure of artocarpin has been solved (Pratap *et al.*, 2002).

Here, we describe the crystallization of CMB and the characterization of the crystals.

### 2. Material and methods

#### 2.1. Protein preparation and fractionation

The purification of CMB has been described elsewhere (Lim *et al.*, 1997). Briefly, 80 g powdered champedak seeds was suspended in phosphate-buffered saline (PBS) pH 7.4 at 277 K for 24 h and the resulting supernatant was subjected to precipitation with 60% ammonium sulfate. The pellet containing the crude extract was collected, dissolved in cold PBS and dialysed against several changes of PBS for 48 h. The crude champedak seed extract was then subjected to galactose affinity chromatography to remove CGB (Gabrielsen *et al.*, 2009), which is also present in the champedak seeds. The flowthrough fractions were pooled, applied onto a mannose Sepharose 4B affinity column and washed extensively with PBS until the absorbance reached baseline. CMB was eluted with 0.8 M D-mannose in PBS. Fractions with high  $A_{280}$  absorbance were pooled, dialysed against distilled water and freeze-dried prior to storage at 253 K. The lyophilized lectin was redissolved in 20 mM Tris-HCl pH 7.5 before use.

Mannose and galactose affinity columns were prepared using the method of Hermanson *et al.* (1992). Divinyl sulfone (DVS) was used to introduce reactive vinyl groups into Sepharose 4B to allow the immobilization of the sugars *via* their hydroxyl groups. DVS-activated Sepharose gel was resuspended in equal volumes (50 ml) of



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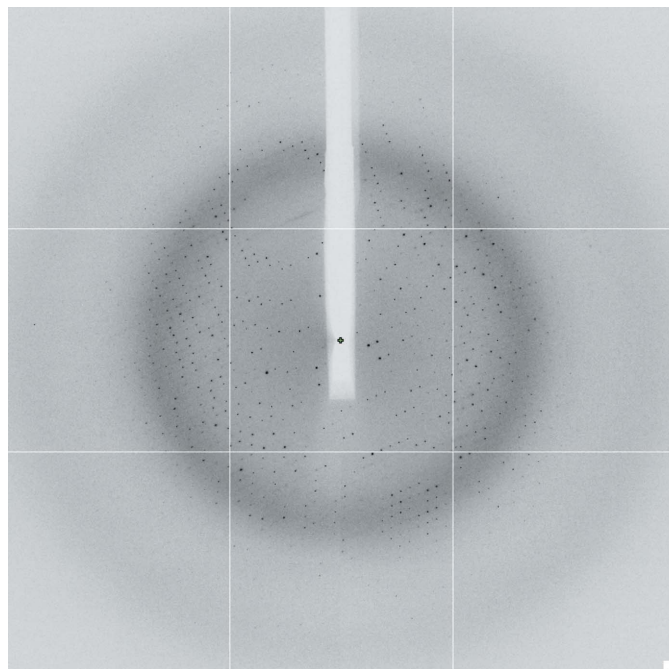
20% (w/v) D-mannose or D-galactose in 0.5 M sodium bicarbonate for 24 h at room temperature (RT) with constant stirring. The sugar-coupled gel was washed successively with 2 l distilled water and 2 l 0.5 M sodium bicarbonate. In order to block excess reactive vinyl groups, the gel was then suspended in 50 ml 0.5 M sodium bicarbonate containing 2.5 ml mercaptoethanol. This step was carried out in a fume cabinet. The suspension was left to stir for 2 h at RT before washing extensively with 2 l each of distilled water and PBS. The resulting gel mixtures were then packed into polypropylene columns of 2.8 cm diameter.

## 2.2. Crystallization

The purified CMB was concentrated to approximately 14 mg ml<sup>-1</sup> based on the absorption at 280 nm, assuming an extinction coefficient of 1 M<sup>-1</sup> cm<sup>-1</sup>. Initial commercially available crystallization screens were set up using sitting-drop vapour diffusion. The drops consisted of 500 nl protein solution and 500 nl reservoir solution and the trays were incubated at 293 K.



**Figure 1**  
Long plate-shaped crystals of champedak mannose-binding lectin (200 × 90 × 20 μm).



**Figure 2**  
Example of a diffraction pattern from champedak mannose-binding lectin crystals.

**Table 1**

Data-collection statistics for the crystal.

Values in parentheses are for the highest resolution bin.

Space group	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit-cell parameters (Å)	<i>a</i> = 76.85, <i>b</i> = 86.32, <i>c</i> = 95.35
Resolution (Å)	49.18–2.00 (2.11–2.00)
Observed reflections	617054
Unique reflections	43581
Multiplicity	14.2 (14.3)
Completeness (%)	100 (100)
<i>R</i> <sub>meas</sub> (%)	13.6 (89.2)
<i>R</i> <sub>p.i.m.</sub> (%)	3.6 (24.3)
<i>I</i> /σ( <i>I</i> )	15.2 (3.5)
Wilson <i>B</i> factor (Å <sup>2</sup> )	26.3

Crystals appeared in Crystal Screen 2 condition 12 (0.1 M cadmium chloride, 0.1 M sodium acetate pH 4.6, 30% polyethylene glycol 400; Hampton Research) within 3 d. The crystal dimensions were approximately 200 × 90 × 20 μm (Fig. 1).

## 2.3. Data collection and processing

Crystals were transferred to a stream of cooled nitrogen gas (110 K; Oxford Cryosystem) without any extra added cryoprotectant. Diffraction data were collected at Diamond Light Source station I03 in 1° oscillations over a total rotation of 360° (Fig. 2). The data were processed in *MOSFLM* (Leslie, 1992) and scaled with *SCALA* (Evans, 1993) and the space group was confirmed by *POINTLESS* (Evans, 2006), all of which are part of the *CCP4* program suite (Collaborative Computational Project, Number 4, 1994).

## 3. Results

Purified CMB was obtained and crystallized. Crystals were obtained and diffraction data were collected; the crystals belonged to space group *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with unit-cell parameters *a* = 76.85, *b* = 86.32, *c* = 95.35 Å. Although the data suggested that the resolution extended to beyond 1.8 Å at the detector corners, the data were truncated to 2.0 Å based on scaling statistics. At this resolution *R*<sub>meas</sub> was 89.2% and *I*/σ(*I*) was 3.5 in the highest resolution bin (2.11–2.0 Å). Further details are presented in Table 1. With a molecular weight of 16 800 Da (Lim *et al.*, 1997), a calculated Matthews coefficient of 2.47 Å<sup>3</sup> Da<sup>-1</sup> and a solvent content of 50%, the asymmetric unit contains a homotetramer.

We have successfully crystallized CMB, a mannose-binding lectin from the Malaysian plant champedak. The crystals diffracted to a resolution of 2.0 Å. An initial solution has been found by molecular replacement (*Phaser*; McCoy *et al.*, 2007) using the known homologous structure of artocarpin (PDB code 1j4u; Pratap *et al.*, 2002). Using a single monomer and searching for four copies, the data yielded a solution comprising a tetramer with 222 symmetry. The solution had a *Z* score of 29.7 and a log-likelihood gain of 807.0, indicating that the model was correct.

Work is being carried out to refine this structure, as well as to determine the actual sequence of CMB by nucleotide sequencing of the gene.

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