

Purification, crystallization and preliminary X-ray structure analysis of the banana lectin from *Musa paradisiaca*

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The banana lectin from *Musa paradisiaca*, MW 29.4 kDa, has been isolated, purified and crystallized. The trigonal crystals contain one dimeric molecule in the asymmetric unit. The structure has been solved using molecular replacement to a resolution of 3 Å. The structure of the subunit is similar to that of jacalin-like lectins.

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1. Introduction

Lectins, a well known class of carbohydrate-binding proteins, mediate a variety of biological processes through their ability to specifically bind different sugar structures (Lis & Sharon, 1998; Vijayan & Chandra, 1999). They are found in all forms of life, but those from plants constitute the most thoroughly studied group (Loris *et al.*, 1998; Rudiger & Gabius, 2001; Barre *et al.*, 2001). Plant lectins have been classified into five structural families (<http://www.cermav.cnrs.fr/lectines>) in terms of their polypeptide folds. Of these, the β -prism I fold in lectins was first characterized in this laboratory in jacalin from jackfruit seeds (Sankaranarayanan *et al.*, 1996). The other lectin from jackfruit seeds, artocarpin, also exhibits the same fold (Pratap *et al.*, 2002), despite the differing physicochemical properties and carbohydrate specificities of the two lectins (Jeyaprakash *et al.*, 2002, 2003, 2004; Pratap *et al.*, 2002). Originally, the β -prism I fold was thought to be characteristic of *Moraceae* lectins. However, lectins from other families were subsequently shown *via* X-ray analysis to have the same fold: *e.g.* calsepa from *Calystegia sepium* (family Convolvulaceae; Bourne *et al.*, 2004) and heltuba from *Helianthus tuberosus* (family Asteraceae; Bourne *et al.*, 1999). On the basis of sequence similarity, it has been suggested that banana lectin also has the same fold (Peumans *et al.*, 2000).

The banana lectin was isolated from *Musa paradisiaca* and characterized in 1990 (Koshte *et al.*, 1990). The dimeric lectin was shown to be mannose-specific. Subsequently, detailed studies were carried out on the lectin from *M. acuminata* (Peumans *et al.*, 2000). The studies confirmed the banana lectin to be a dimer, with each subunit containing 141 amino-acid residues and having a molecular weight of about 15 kDa. The lectin was shown to be a powerful murine T-cell mitogen. Taking advantage of its sequence similarity to other jacalin-like lectins, Peumans and coworkers proposed a molecular model of banana lectin.

It was subsequently shown that banana lectin is mannose/glucose-specific, with a preference for α -anomeric forms. It was also demonstrated that unlike other mannose/glucose-binding lectins, banana lectin recognizes internal α -1-3-linked glucosyl residues, reducing glucosyl groups of β -1-3-linked glucosyl oligosaccharides and β -1-6-linked glucosyl end-groups (Goldstein *et al.*, 2001; Mo *et al.*, 2001). In view of its interesting sugar-binding properties and its similarity to and differences from other jacalin-like lectins, the X-ray analysis of banana lectin was undertaken.

2. Materials and methods

2.1. Purification and characterization

A variant of the reported procedure (Peumans *et al.*, 2000) was used to isolate and purify the lectin from *M. paradisiaca*. 750 g of ripe banana was mashed and soaked in 1 l of 150 mM acetic acid solution overnight. It was homogenized for 6 h after dilution of the pulp to 2 l. The extract was centrifuged at 9184g for 10 min at 277 K and the supernatant was clarified through muslin cloth. 65% ammonium sulfate was added to the supernatant and stirred overnight. It was then centrifuged at 9184g for 45 min at 277 K. The pellet was reconstituted in 10 mM phosphate-buffered saline solution (PBS) pH 7.4 and dialysed against the same buffer for 48 h with six changes. The clear supernatant was loaded onto a mannose-Sepharose 4B column. After loading, the column was washed with 10 mM PBS pH 7.4 at 277 K until the A_{280} of the flowthrough was less than 0.01. The column was then equilibrated at room temperature and the protein was eluted with 0.3 M methyl- α -D-mannopyranoside. The eluant was concentrated using a lyophiliser and dialysed against double-distilled water for 36 h with six changes. The dialysate was concentrated using 10 kDa cutoff Centricons. The purity of the protein, as indicated by 15% SDS-PAGE, appeared to be better than 99%. The molecular mass was

measured using a Bruker Ultraflex MALDI-TOF mass spectrometer (Bruker Daltonics).

2.2. Crystallization and data collection

A 5 mg ml⁻¹ protein sample in double-distilled water containing 100 mM methyl- α -D-mannopyranoside was used for crystallization using the hanging-drop method. The initial crystallization conditions were screened using Hampton kits. In the final experiments, the drop, made up of 10 μ l protein solution and 1 μ l reservoir buffer, was equilibrated against 800 μ l reservoir buffer containing 0.01 M zinc acetate dihydrate, 0.1 M sodium cacodylate pH 7.5, 10% (w/v) PEG 8000. The crystallization trays were stored at 298 K. The diffraction data were collected at room temperature (298 K) on the X9B beamline, NSLS, Brookhaven National Laboratory using an ADSC Quantum 4 CCD detector. Cryo-cooling of crystals led to poorer data. The data were processed and scaled using the *HKL* package (Otwinowski & Minor, 1997). Intensities were converted to structure factors using *TRUNCATE* (Collaborative Computational Project, Number 4, 1994). Solvent content was estimated using the method of Matthews (1968). Structure solution was obtained by the molecular-replacement method using *PHASER* (Storoni *et al.*, 2004). The structure was refined using *CNS* v.1.1 (Brünger *et al.*, 1998) involving iterations of rigid-body and positional refinement and simulated annealing. Model building was carried out using the program *FRODO* (Jones, 1978).

3. Results and discussion

3.1. Molecular weight and oligomeric state

SDS-PAGE of the protein gave a single band at about 14 kDa. The mass spectrum yielded two major peaks, one at 29 400 and the other at 14 700. The results indicate that

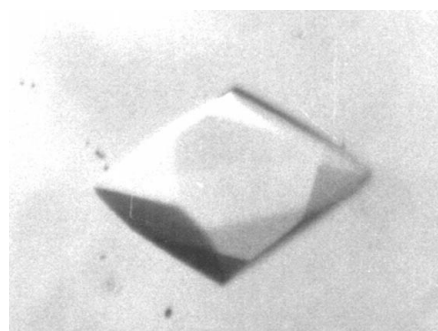


Figure 1
A crystal of banana lectin. The maximum dimension is about 0.2 mm.

the *M. paradisiaca* lectin is a dimer with a molecular weight similar to that of the lectin from *M. acuminata*.

3.2. X-ray analysis and gross structural features

The crystals (Fig. 1) grew to approximate dimensions of 0.20 \times 0.15 \times 0.05 mm. Crystal data and data-collection statistics are given in Table 1. The crystals have a relatively high solvent content of 75%, with two subunits of the lectin in the asymmetric unit. An ensemble of three structures, namely *heltuba* (PDB code 1c3m), *calsepa* (PDB code 1ouw) and *Mpa* (PDB code 1jot), was used as the search model in molecular-replacement calculations using *PHASER* (Storoni *et al.*, 2004). Two monomers, which make up the dimer with twofold symmetry, were located. The structure of *heltuba* was used as the initial model for refinement. The current *R* and *R*_{free} values are 29 and 34%, respectively, in the 30.0–3.0 Å resolution range. Further refinement is in progress.

At the present stage of refinement, it is clear that the topology of the fold is the same as that in *artocarpin* (Pratap *et al.*, 2002), *heltuba* (Bourne *et al.*, 1999) and *calsepa* (Bourne *et al.*, 2004). The asymmetric unit of the crystals contains two monomers, which share an interface that has

Table 1
Crystal data and data-collection statistics.

Values in parentheses are for the highest resolution shell.	
Space group	<i>P</i> 3 ₂ 21
Unit-cell parameters	
<i>a</i> (Å)	82.1
<i>b</i> (Å)	82.1
<i>c</i> (Å)	149.1
Packing density <i>V</i> _M (Å ³ Da ⁻¹)	4.9
Solvent content (%)	75
No. subunits in AU	2
Resolution range (Å)	30–3.00 (3.11–3.00)
Observed reflections	43259
Unique reflections	12102 (1189)
Completeness (%)	99.3 (99.7)
<i>R</i> _{merge} † (%)	8.1 (45.6)

† $R_{\text{merge}} = \frac{\sum_h \sum_i |I(h,i) - \langle I(h) \rangle|}{\sum_h \sum_i I(h,i)}$, where $I(h,i)$ is the intensity of the *i*th measurement of reflection *h* and $\langle I(h) \rangle$ is the average value over multiple measurements.

an approximate total buried surface area of 1500 Å² (Fig. 2). None of the other protein–protein interfaces resulting from crystal packing have such a large buried surface area, suggesting that the subunits in the asymmetric unit of banana lectin crystals represent the dimeric form of the protein. Also, these two subunits are related by a rotation of 178°. The pseudo-twofold rotation axis which relates the two subunits is illustrated in Fig. 2. The tetramer of *artocarpin* and the octamer of *heltuba* contain two distinct dimeric interfaces. One of the two interfaces is common to *artocarpin* and *heltuba*. This interface, called the AB-type

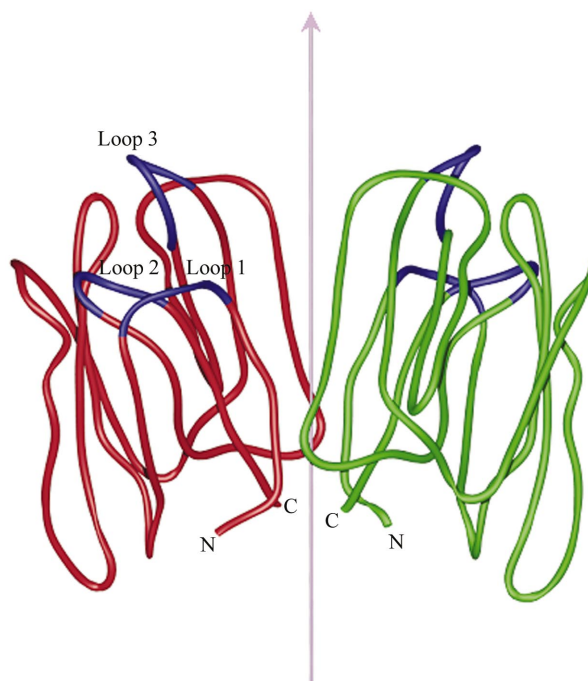


Figure 2
Overall fold of the banana (*M. paradisiaca*) lectin dimer at the current stage of refinement. Also shown is the pseudo-twofold axis that relates the two subunits of the dimer. The three loops that interact with the sugar are coloured identically (blue) in the two subunits.

interface by Pratap *et al.* (2002) in the case of artocarpin and the 1–2 interface by Bourne *et al.* (1999) in the case of heltuba, is similar to the interface in banana lectin. However, the banana-lectin dimers fail to oligomerize further into a higher order quaternary structure. This situation is reminiscent of a situation documented in the family of bulb lectins. Snowdrop lectin and garlic lectin, despite having very similar tertiary structures, exist as a tetramer and a dimer, respectively (Chandra *et al.*, 1999).

In artocarpin and heltuba, the sugar-binding site is made up of three loops. It has been shown that the first two loops are involved in interactions with monomeric mannoside. These two loops have similar length and composition in the two lectins and consequently have a comparable affinity for mannose. The third loop determines the specificity at the oligosaccharide level. This loop in artocarpin is longer than in heltuba. This leads to profound differences in the oligosaccharide specificity of the two lectins (Jeyaprakash *et al.*, 2004). At the present stage of refinement, the loops in banana lectin appear to be similar in length and conformation to those in heltuba.

While continuing refinement using the available room-temperature data set, efforts are also currently under way to modify the crystallization conditions to produce crystals that diffract better and to optimize conditions for cryocooling.

X-ray intensity data were collected on the X9B beamline, NSLS, Brookhaven National Laboratory, USA and at the X-ray Facility for Structural Biology at the Indian Institute of Science, supported by the Department of Science and Technology (DST) and Biotechnology (DBT), Government of India. Computations were performed at the Supercomputer Education and Research Centre at the Institute and the Bioinformatics Centre and the Interactive Graphics Facility, both funded by the DBT.

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