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Purification, crystallization and preliminary X-ray analysis of a mannose-binding lectin from bluebell (*Scilla campanulata*) bulbs

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Abstract

Crystals have been grown of a mannose-specific lectin from bluebell (*Scilla campanulata*) bulbs in a form suitable for X-ray diffraction studies. The crystals, which diffract to high resolution, grew in hanging drops by vapour diffusion, equilibrating with a solution of 70% saturated ammonium sulfate at pH 4.7–4.8 at 293 K, in the absence of any mannose saccharides. Crystals are orthorhombic, $P2_12_12$, with unit-cell dimensions $a = 70.78$, $b = 93.69$, $c = 46.92$ Å. The functional lectin molecule is organized as a tetramer of four identical 14 kDa subunits, with only two subunits in the asymmetric unit. Data to 1.86 Å resolution have been recorded and the structure determined by the molecular replacement method.

1. Introduction

Lectins are a structurally diverse group of proteins that are found in all types of living organisms and bind specifically and reversibly to saccharides. The most intensely studied group of lectins are those from the seeds of leguminous plants (Van Driessche, 1988). In the last ten years many new lectins have been isolated from the bulbs, corms, rhizomes and tubers of perennial plants and these storage organs have been shown to be a rich source of lectins with biologically interesting properties (Van Damme, Smeets & Peumans, 1995). Recent research on non-seed lectins has been focused on the monocotyledonous families Amaryllidaceae, Alliaceae, Orchidaceae, Araceae and Liliaceae (Van Damme *et al.*, 1996). The only known structures of this group of mannose-specific monocot lectins are those from snowdrop and amaryllis bulbs (Hester, Kaku, Goldstein & Wright, 1995; Wood, 1995; Chantalat, Wood, Rizkallah & Reynolds, 1996). Both of these structures are all β proteins and contain a new class of polypeptide fold which consists of three antiparallel four-stranded β-sheets arranged as a 12-stranded β-barrel.

Lectins from the Liliaceae family have been isolated from meadow saffron tubers (Peumans, Allen & Cammue, 1986) and tulip bulbs (Oda & Minami, 1986; Cammue, Peeters & Peumans, 1986; Oda, Minami, Ichida & Aoruma, 1987), some of which show differences in molecular structure and specificity. Of the two lectins which have been isolated from the bulbs of Tulipa cultivar Apeldoorn TxLMII is dimeric with 12 kDa subunits and belongs to the superfamily of mannose-binding monocot lectins; whereas TxLCI is a chimeric protein with complex specificity, containing 28 kDa subunits which aggregate to form tetramers (Van Damme *et al.*, 1996). Each

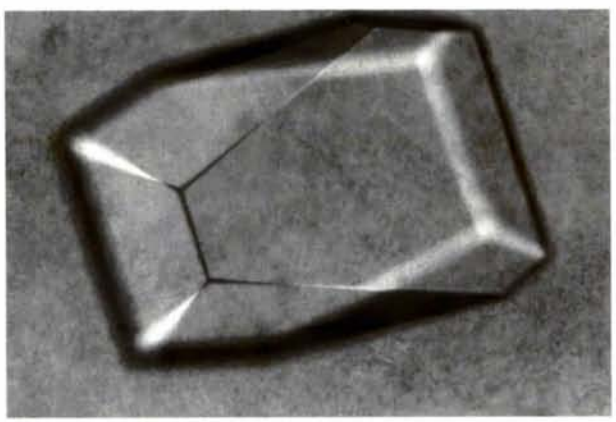

Fig. 1. Photograph of bipyramidal crystals of the mannose-binding protein from bluebell (*Scilla campanulata*) bulbs, obtained using 70% saturated ammonium sulfate at pH 4.7. The longest dimension is approximately 1 mm.

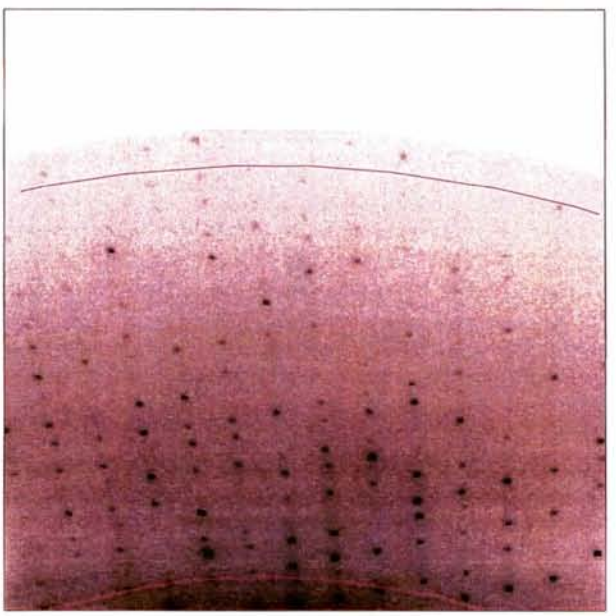

Fig. 2. Section of a 2° oscillation diffraction pattern from a crystal of bluebell lectin taken at the SRS, Daresbury Laboratory, recorded with a MAR Research imaging plate. The resolution at the edge of the photograph corresponds to 1.8 Å.

subunit of TxLCI possesses a mannose-binding site and an *N*-acetylgalactosamine-binding site, which act independently from each other and are located in separate domains of the polypeptide chain (Van Damme *et al.*, 1996). We have recently isolated two related lectins from bluebell (*Scilla campanulata*) bulbs, which also belong to the Liliaceae family; BLMI is tetrameric with a molecular weight of 14 kDa for the protomer, whereas BLCI has a subunit molecular weight of 30 kDa and forms tetramers. Amino-acid sequencing studies of BLMI and BLCI are currently underway. We have shown that BLMI has a strong affinity for α(1,3)- and α(1,6)-linked mannosyl residues (Wood, Allen, Wright & Reynolds, 1996). BLMI and TxLMII have similar properties and belong to the super family of mannose-binding monocot lectins. A number of mannose-binding monocot lectins, particularly those from the Amaryllidaceae and Orchidaceae families, have been shown to possess potent inhibitory activity against retroviruses, including HIV-1 and HIV-2 (Balzarini *et al.*, 1991; Balzarini *et al.*, 1992). These bulb lectins presumably exert their inhibitory effect *via* binding to the envelope glycoproteins which are known to be highly glycosylated with oligomannosides (Feizi & Larkin, 1990; Ratner, 1992). Preliminary results indicate that BLMI exhibits antiretroviral activity but that it is an order of magnitude lower than the values reported for the lectins from the Amaryllidaceae and Orchidaceae families (Balzarini *et al.*, 1991, 1992). In the current study we report the purification and crystallization of BLMI and our preliminary X-ray crystallographic results.

2. Purification and crystallization

Bluebell (*Scilla campanulata*) bulbs were obtained from a local garden centre. The mannose-specific lectin from bluebell bulbs (BLMI) was purified using the following procedure. Bluebell bulbs (200 g), depleted of their outer sclerotized layer, were washed, chopped up and homogenized in a Waring blender in 1 l of distilled water containing 0.2% ascorbic acid. The homogenate was filtered through cheesecloth and centrifuged (5 min, 3000*g*). Afterwards $CaCl_2$ (2 g l^{-1}) was added to the decanted supernatant and the pH raised to pH 9.0 (with 0.5 *M* NaOH). After standing overnight at 277 K the precipitate was removed by centrifugation (10 min, 3000*g*) and the supernatant was filtered through glass wool. The pH was adjusted to 3.0 (with 1 *M* HCl) and the extract loaded on a Mono-S Fast Flow column (2.6 × 5 cm; 25 ml bed volume). After washing the column with water, the protein was eluted (with 1 *M* NaCl) until the A_{280} fell below 0.01 and the pH of the eluate was increased to 7.0 and centrifuged (5 min, 3000*g*). Solid $(NH_4)_2SO_4$ was added to the supernatant (132 g l^{-1}) and the solution was loaded on a mannose–Sepharose 4B affinity column (2.6 × 5 cm; 25 ml bed volume). After washing the column with 1 *M* $(NH_4)_2SO_4$ until the A_{280} fell below 0.01 the bound lectin was desorbed with 20 m*M* unbuffered 2,3-diaminopropane (DAP). The lectin solution was then neutralized to pH 7.0 and stored at 253 K until required. The lectin preparation was monitored by agglutination assays and by sodium dodecyl sulfate polyacrylamide-gel electrophoresis. The overall yield of BLMI was 0.96 mg per gram of fresh bulb tissue.

The initial crystallization conditions were screened by means of the hanging-drop vapour-diffusion method (Ducruix & Giegé, 1992). 1 ml precipitant solutions were prepared in the wells of standard 24-well Linbro cell-culture plates. Drops containing the protein were made by mixing equal volumes (10 µl) of the protein's stock solution (5.5 mg ml^{-1} of protein,

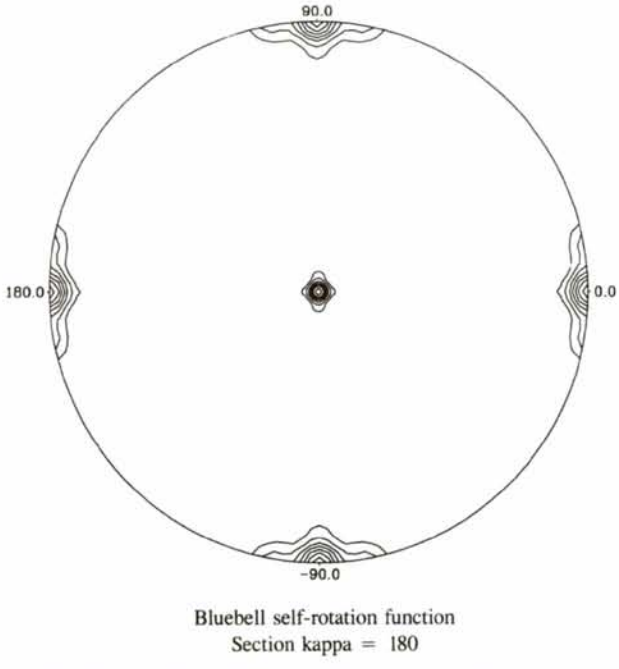

Fig. 3. Stereographic projection of the section $\kappa = 180°$ of the self-rotation function showing twofold local symmetry perpendicular to the crystallographic twofold axis.

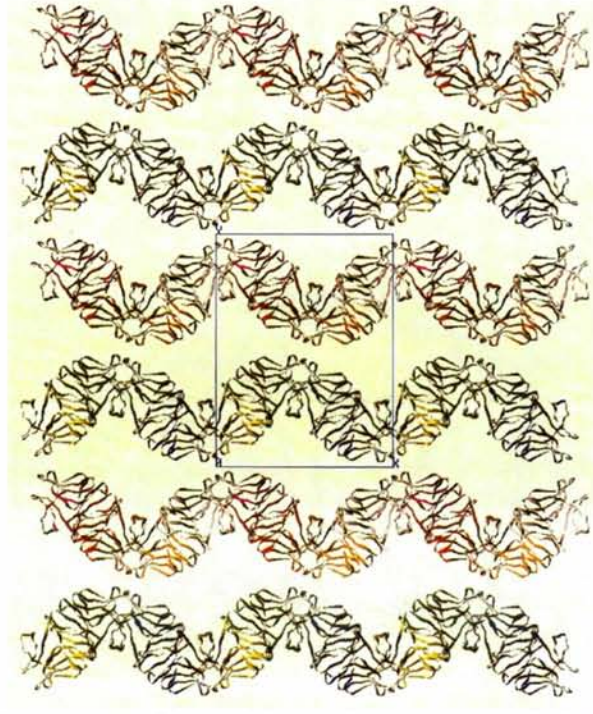

Fig. 4. Packing of the bluebell lectin dimers in the unit cell of the crystal. The view is down the *c* axis, with the *a* axis horizontal and the *b* axis vertical.

10 m*M* DAP and 600 m*M* PBS) and the precipitant solution from the well. The drop was equilibrated against a reservoir containing 55–75% saturated ammonium sulfate and pH in the range 3–7. Bipyramidal crystals, up to 0.5 × 0.5 × 1.0 mm in size, grew after 5 d at 293 K (Fig. 1). The best crystals were obtained using 70% saturated ammonium sulfate at pH 4.7 to 4.8.

3. X-ray analysis

Two crystals were sealed in glass capillaries with a small amount of mother liquor and mounted in random orientations. Data have been collected at the synchrotron radiation source (λ = 1.488 Å) at the CCLRC's Daresbury Laboratory on station 7.2 using the MAR image plate. Data were collected at 293 K over 111.5° in steps of 2.0 or 1.5° to 1.86 Å resolution (Fig. 2). The BLMI crystals diffracted to beyond 1.85 Å and are stable in the X-ray beam. The crystals belong to the orthorhombic space group $P2_12_12$ with unit-cell dimensions of $a = 70.78\,(5)$, $b = 93.69\,(5)$ and $c = 46.92\,(5)$ Å. Assuming a dimer of molecular mass 28 kDa, the V_m value is 2.78 Å^3 Da^{-1} and falls in the normal range observed for other protein crystals (Matthews, 1968) and indicates a solvent concentration of 56%. Diffraction data have been processed by means of *MOSFLM*, yielding, after scaling and averaging by means of *ROTAVATA* and *AGROVATA* (Collaborative Computational Project, Number 4, 1994) a unique data set of 24 583 reflections (99% complete, multiplicity = 3.8) to a resolution of 1.86 Å. This gave an $R_{\rm merge} = 0.060$ with 83% of the data greater than 3 e.s.d.'s.

A self-rotation function (Fig. 3) indicates twofold local symmetry that is perpendicular to the crystallographic twofold axis. Our biochemical studies show that the BLMI molecule associates into tetramers. Molecular replacement calculations were performed with the *AMoRe* package (Navaza, 1994) using the refined coordinates of our amaryllis lectin structure (Chantalat *et al.*, 1996) as a search model and gave a clear solution to both the rotation and translation function. After rigid-body refinement the *R* factor was 47%, correlation coefficient 68% and the packing function indicated very few close packing contacts. One round (40 cycles) of least-squares refinement with *X-PLOR* (Brünger, 1990) reduced the conventional *R* factor to 31% ($R_{\rm free} = 38\%$). Further refinement is in progress.

Packing of the BLMI dimers is shown in Fig. 4. Full details of the MR and refinement studies on BLMI will be published elsewhere.

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