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Crystallization and preliminary X-ray analysis of a novel plant lectin from *Calystegia sepium*

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Abstract

A mannose-specific lectin from *Calystegia sepium* has been crystallized by the hanging-drop vapour-diffusion method using ammonium sulfate as precipitant. The needle-shaped crystals are orthorhombic, space group $C222_1$ with cell dimensions $a = 55.2$ (1), $b = 55.9$ (1), $c = 196.1$ (1) Å. Fresh crystals diffract to 1.9 Å resolution on a synchrotron radiation source. The asymmetric unit contains a dimer of two identical 16 kDa subunits with a packing density of 2.36 Å^3 Da^{-1}. Intensity data have been observed beyond 2.0 Å, but reasonable statistics restricted the usable range to 2.0 Å.

1. Introduction

Lectins are proteins that bind reversibly to specific carbohydrates. They are found in plants and other types of living organisms and function as recognition molecules in biological systems. Lectins are widely used as models for probing the functions of glycans attached to cells, glycoproteins and glycolipids. Although the physiological role of lectins in plants is not fully understood, there is growing evidence that it is related to the plant's defense against pathogens and predators (Peumans & Van Damme, 1995).

The best structurally characterized group of lectins are those from the seeds of the leguminous plants, which all contain essentially the same polypeptide fold consisting of a flat six-stranded β-sheet and a curved seven-stranded β-sheet interconnected by loops of various lengths (Rougé, Cambillau & Bourne, 1991). More recently, the structures of lectins from snowdrop and amaryllis bulbs were determined (Hester, Kaku, Goldstein & Wright, 1995; Chantalat, Wood, Rizkallah & Reynolds, 1996) and shown to contain a new class of protein fold consisting of three antiparallel four-stranded β-sheets arranged as a 12-stranded β-barrel.

We report here the crystallization of a new lectin (Calsepa) from *Calystegia sepium*, a member of the Convolvulaceae family, which exhibits potent mitogenic properties, strongly inducing lymphocyte activation and proliferation. Calsepa may, therefore, have important applications in immunology, for example, in the study of primary and secondary immune deficiency. Calsepa is a dimeric protein with 16 kDa subunits. It has recently been sequenced (Van Damme & Peumans, unpublished results) and shown to contain a single chain of approximately 160 amino-acid residues. The amino-acid sequence of Calsepa has no significant homology with other lectins or with other proteins in the sequence databases and may well have a novel tertiary fold.

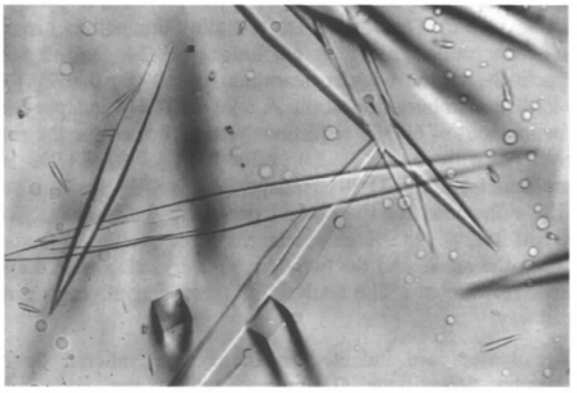
2. Materials and methods

2.1. *Protein purification*

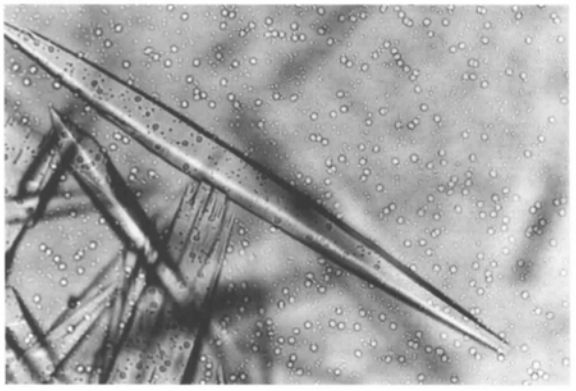
The lectin was isolated from the rhizomes of *Calystegia sepium* using affinity chromatography on a column of mannose Sepharose 4B (Peumans *et al.*, 1997).

2.2. *Protein characterization*

Analysis of the purified lectin using SDS–PAGE and gel filtration has shown that Calsepa is a dimeric protein composed of two identical 16 kDa lectin polypeptides (Peumans *et al.*, 1997).



(*a*)



(*b*)

Fig. 1. Photographs of needle-shaped crystals of Calsepa lectin from *Calystegia sepium*, obtained using 70% saturated ammonium sulfate at pH 7.0. The approximate dimensions of these crystals are 0.1 × 0.1 × 0.8 mm.

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2.3. *Crystallization*

The protein was concentrated with Amicon equipment. Protein concentrations were estimated using the OD_{280}, assuming an OD_{280} of 1.2 for a 1 mg ml^{-1} solution. Crystallization conditions were surveyed using the vapour-diffusion hanging-drop method (Ducruix & Giegé, 1992) over 1 ml reservoirs on siliconized cover slips in Linbro trays at 293 K.

3. Results and discussion

3.1. *Crystallization*

Using 10 µl drops of Calsepa, at a concentration of 7 mg ml^{-1} in 20 m*M* unbuffered 2,3-diaminopropane and adding 10 µl of 150 m*M* phosphate-buffered saline, crystals grew from several drops of the initial screen with ammonium sulfate of varying concentrations as precipitant and the pH in the range 4.5–8.0. The conditions were further refined by systematically testing a range of closely related conditions. The best crystals grew at 70% saturated ammonium sulfate at pH 7.0 within 6 d. These were needle-shaped crystals with approximate dimensions of $0.1 \times 0.1 \times 0.8$ mm. Fig. 1 shows examples of the Calsepa crystals grown in this way.

3.2. *Data collection*

Data were collected on the wiggler beamline station 9.6 at the SRS Daresbury Laboratory, with $\lambda = 0.87$ Å, using the large MAR image plate. Using two crystals, data were collected over 90° in steps of 2°; approximately 45° of data were collected per crystal. The diffraction pattern for fresh crystals extended to 1.9 Å resolution at 277 K (Fig. 2).

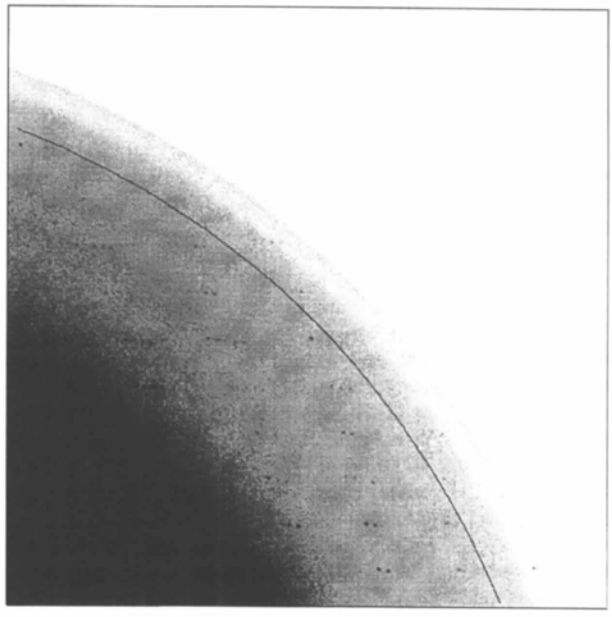

Fig. 2. Section of a 2° oscillation diffraction pattern from a crystal of Calsepa lectin taken at the SRS, Daresbury Laboratory, recorded with a MAR Research image plate. The resolution at the outer ring of the photograph corresponds to 2.0 Å

The data were processed with the *MOSFLM* package (Leslie, 1992) and merged using the *CCP*4 (Collaborative Computational Project, Number 4, 1994) package of programs for protein crystallography. For the full data set 20 743 unique reflections were recorded giving a 99% complete data set to a resolution of 2.0 Å. This gave an $R_{merge} = 10.7\%$ and an overall multiplicity of 4.2 with 81% of the data greater than 3 e.s.d.'s (for the highest resolution shell the data was 96.4% complete with an $R_{merge} = 28.3\%$ and with 57% of the data being greater than 3 e.s.d.'s). The fraction of data above 3 e.s.d.'s declined rapidly beyond 2.0 Å.

The crystals belong to the orthorhombic system, space group $C222_1$, with unit-cell dimensions of $a = 55.2$ (1), $b = 55.9$ (1), $c = 196.1$ (1) Å. Assuming a molecular mass of 32 kDa for the Calsepa dimer per asymmetric unit, the V_m value is 2.36 Å^3 Da^{-1} and falls in the acceptable range observed for other protein crystals (Matthews, 1968), which indicates a solvent content of 48%.

It is expected that with larger crystals or with cryo-cooling techniques that data could be collected to a much higher resolution. We are planning to determine the structure of Calsepa by the multiple isomorphous replacement method since no suitable model is available for molecular replacement studies. The search for suitable heavy-atom derivatives is now in progress.

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