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Crystallization and preliminary X-ray diffraction analysis of a lectin from *Canavalia maritima* seeds

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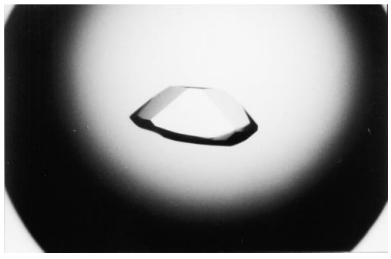

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A lectin from *Canavalia maritima* seeds (ConM) was purified and submitted to crystallization experiments. The best crystals were obtained using the vapour-diffusion method at a constant temperature of 293 K and grew in 7 d. A complete structural data set was collected to 2.1 Å resolution using a synchrotron-radiation source. The ConM crystal belongs to the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 67.15$, $b = 70.90$, $c = 97.37$ Å. A molecular-replacement search found a solution with a correlation coefficient of 69.2% and an R factor of 42.5%. Crystallographic refinement is under way.

1. Introduction

Many plants contain sugar-binding proteins commonly known as lectins, designated as carbohydrate-binding proteins of non-immune origin that specifically recognize diverse sugar structures and mediate a variety of biological process (Vijayan & Chandra, 1999).

Plant lectins (Peumans & Van Damme, 1995), especially those purified from species of the Leguminosae family, represent the most well studied group of carbohydrate-binding proteins (Van Damme *et al.*, 1998). Lectins from the Diocleinae subtribe demonstrate a high degree of similarity. Despite being highly analogous, they present significant differences in many biological activities, such as induction of rat paw oedema (Bento *et al.*, 1993), peritoneal macrophage spreading in mouse (Rodriguez *et al.*, 1992), pro- and anti-inflammatory effects (Alencar *et al.*, 1999; Assreuy *et al.*, 1999), capacity for induction of histamine release (Gomes *et al.*, 1994; Ferreira *et al.*, 1996), induction of apoptosis (Barbosa *et al.*, 2001), induction of NO production (Andrade *et al.*, 1999), various renal effects (Havt *et al.*, 2003), mitogenicity (Barral-Neto *et al.*, 1992) and induction of *in vitro* and *in vivo* cytokine production (Cavada *et al.*, 2001).

Despite some minor differences in their primary and three-dimensional structures, it remains clear that this group of proteins diverge considerably in many biological properties, which makes them an excellent model for the study of structure–function relationships (Cavada *et al.*, 2001; Moreno *et al.*, 2004).

The lectin ConM was obtained from *Canavalia maritima*, commonly known as the bay bean, sand bean, beach bean or MacKenzie bean. ConM is a 25.5 kDa protein with 237 residues per monomer. Like other legume lectins, ConM posseses a high amino-acid sequence similarity to the well known concanavalin A (ConA) from *C. ensiformis*, reaching up to 90% identity (Perez *et al.*, 1991).

The present work reports the crystallization and preliminary X-ray diffraction analysis of a lectin from *C. maritima* seeds, a protein that has previously been purified (Perez *et al.*, 1991), tested for histamine-releasing properties in rat peritoneal mast cells (Gomes *et al.*, 1994) and has had its affinity for several monosaccharides determined (Ramos *et al.*, 1996).

2. Materials and methods

2.1. Purification of *C. maritima* seed lectin

Wild mature *C. maritima* seeds were collected in the Ceará state in northeast Brazil. The seeds were ground to a fine powder in a coffee

mill and the soluble proteins were extracted at 298 K by continuous stirring with 0.15 *M* NaCl [1:10(*w*/*v*)] for 1 h, followed by centrifugation at 10 000*g* at 277 K for 20 min. The supernatant was applied onto a Sephadex G-50 column (10 × 50 cm) previously equilibrated with 0.15 *M* NaCl containing 5 m*M* $CaCl_2$ and $MnCl_2$, as described by Cavada *et al.* (1996). The unbound material was eluted with 0.15 *M* NaCl at a flow rate of 45 ml h^{-1} until the absorbance at 280 nm of the effluent stabilized at 0.05. The retained material (a lectin, called ConM) was eluted with 0.1 *M* glycine pH 2.6 containing 0.15 *M* NaCl, dialyzed exhaustively against Milli-Q water and lyophilized. The purity of all ConM preparations was monitored by SDS–PAGE (Laemmli, 1970).

2.2. Crystallization, data collection and processing

ConM was diluted homogeneously to a concentration of 10.0 mg ml^{-1} in 50 m*M* Tris–HCl pH 7.5 contaning 5 m*M* $CaCl_2$ and $MnCl_2$ for all crystallization experiments. Crystallization conditions for ConM were screened using the hanging-drop vapour-diffusion method with Hampton Research Crystal Screens I and II (Hampton Research, Riverside, CA, USA; Jancarik & Kim, 1991) at room temperature (293 K). Microcrystals were obtained using crystallization condition No. 4 of screen I (0.1 *M* Tris–HCl pH 8.5 and 2.0 *M* ammonium sulfate). Improvement of this crystallization condition was obtained by raising the pH and the salt concentration. The best crystals were obtained from drops containing equal volumes of protein (3 µl) and 0.1 *M* Tris–HCl pH 9.0 with 2.2 *M* ammonium sulfate. Crystals grew within a week to maximum dimensions of approximately 0.8 × 0.4 × 0.4 mm (Fig. 1).

X-ray data were collected from a single crystal cooled to a temperature of 100 K. To avoid ice formation, crystals were soaked in a cryoprotectant solution containing 75% 0.1 *M* Tris–HCl pH 9.0 and 25% glycerol and submitted to data collection at a wavelength of 1.4270 Å using a synchrotron-radiation source (CPr station, Laboratório Nacional de Luz Síncrotron-LNLS, Campinas, Brazil). A complete data set was obtained using a CCD (MAR Research) in 120 frames with an oscillation range of 1°. The data set was indexed, integrated and scaled using *MOSFLM* and *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

Several lectins have been crystallized and their structures solved. More than 50 different entries for lectins from the Diocleinae subtribe can be accessed in the Protein Data Bank (Berman *et al.*, 2000); the well known plant lectin ConA represents approximately 90% of these data.

The crystal data were scaled in the range 39.52–2.10 Å and Table 1 shows the data-collection statistics. Assuming the presence of two molecules (474 residues, 25.5 kDa each) in the asymmetric unit, the calculated Matthews coefficient (V_M; Matthews, 1968) was 2.3 $Å^3$ Da^{-1}, indicating a solvent content of 46.5%.

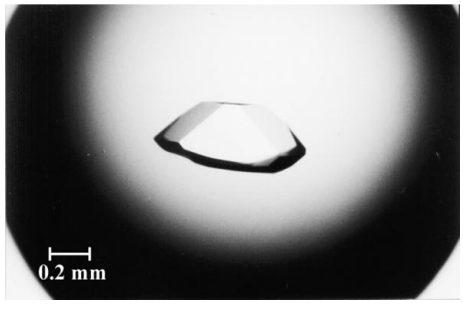

Figure 1
Native crystal of the lectin from *C. maritima* seeds.

Table 1
Summary of data-collection statistics for ConM.

Values in parentheses are for the highest resolution shell.

X-ray wavelength (Å)	1.4270
Unit-cell parameters	
a (Å)	67.15
b (Å)	70.90
c (Å)	97.37
Space group	$P2_12_12$
Resolution (Å)	39.5–2.1 (2.21–2.10)
No. measurements with $I > 2\sigma(I)$	246192
No. independent reflections	25202
Completeness (%)	91.3 (91.3)
$\langle I/\sigma(I)\rangle$	5.4 (3.0)
R_{sym}†	7.6 (23.7)

† $R_{sym} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection h.

The preliminary crystal structure of ConM was determined by molecular-replacement methods using the program *AMoRe* (Navaza, 1994). The atomic coordinates of several lectins were used in the search for a structural model. The best result was obtained with the lectin isolated from *C. ensiformis* (PDB code 3enr; Bouckaert *et al.*, 2000), which presented a final correlation coefficient of 69.2% and an *R* factor of 42.5%. Refinement of the structure is in progress.

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