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Crystallization and preliminary X-ray diffraction analysis of the lectin from *Canavalia gladiata* seeds

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The seed lectin from *Canavalia gladiata* was purified and crystallized. Orthorhombic crystals belonging to space group $C222_1$ grew within three weeks at 293 K using the hanging-drop vapour-diffusion method. Using synchrotron X-ray radiation, a complete structural data set was collected at 2.3 Å resolution. The preliminary crystal structure of the lectin, determined by molecular replacement, had a correlation coefficient of 0.569 and an R factor of 0.412.

1. Introduction

The legume commonly known as the sword bean, *Canavalia gladiata*, belongs to the genus *Canavalia* (family Leguminosae, subfamily Papilionoideae, tribe Diocleinae). This bean represents a cheap source of protein and calories in several areas of Northeast Brazil. Sword beans show favourable agronomic features that make them suitable for cultivation in the tropics and have a high average yield compared with that of soybeans (Ekanayake *et al.*, 2000). Although *C. gladiata* seeds contain a large amount of protein (35%), the protein exhibits a low level of sulfur-containing amino acids (Siddhuraju & Becher, 2001). Anti-nutrient compounds such as phenolics, tannins, saponins, non-proteic amino acids, protease and α-amylase inhibitors and lectins are also present in sword-bean seeds. Lectins are proteins of non-immune origin whose structure includes at least a carbohydrate-binding domain (Peumans *et al.*, 2001). *C. gladiata* seed lectin (CGL) shows sugar-binding specificities similar to those of concanavalin A (ConA), the well characterized lectin from *C. ensiformis* seeds (Kojima *et al.*, 1991). The lectin from *C. gladiata* has been sequenced (Yamauchi & Minamikawa, 1990) and when compared with ConA exhibits 95% amino-acid sequence identity. However, the Diocleinae lectins have been found to express distinct biological properties, as observed for the lectins from *C. brasiliensis* seeds (ConBr) and ConA, which share 99% amino-acid sequence (Sanz-Aparicio *et al.*, 1997). CGL binds to saccharides containing α-D-mannose or α-D-glucose residues and to oligosaccharides or polysaccharides containing these monosaccharides.

Of the leguminous lectins isolated from the tribe Diocleinae, only those purified from the seeds of *C. ensiformis* (Hardman & Ainsworth, 1972), *C. brasiliensis* (Grangeiro *et al.*, 1997), *Dioclea grandiflora* (Rozwarski *et al.*, 1998), *D. guianensis* (Gomes *et al.*, 1994) and *Cratylia mollis* (Souza *et al.*, 2003) have had their three-dimensional structures resolved by X-ray crystallography. These lectins have 99, 98, 83, 88 and 78% identity in their amino-acid sequences with CGL, respectively. They are dimeric or tetrameric structures built up of dome-shaped monomers consisting of two β-sheets of seven and six antiparallel β-strands interconnected by turns and loops (Calvete *et al.*, 1999). Although this group of lectins exhibits a high degree of primary structure similarity, a few amino-acid residue substitutions in key positions endow the homologous lectins with distinct biological effects. Thus, the seed lectins from *C. brasiliensis* (ConBr) and *C. ensiformis* (ConA) differ in just two amino-acid residues in their 237-residue monomer sequences. This minor difference results in lectins that exhibit distinct *in vitro* and *in vivo* biological activities, such as induction of rat paw oedema (Bento *et al.*, 1993), peritoneal macrophage spreading in mouse (Rodriguez *et al.*, 1992) and human lymphocyte stimulation (Barral-Neto *et al.*, 1992). The three-dimensional structures of the lectin monomers of ConA and ConBr can be superimposed with an r.m.s. deviation of 0.65 Å (Sanz-Aparicio *et al.*, 1997). However, structural differences are amplified in the lectin tetramers at 1.62 Å, suggesting that the distinct quaternary structures, which in turn determine the spatial arrangement of the four carbohydrate-binding sites of the lectin tetramers, may account for the differing biological properties of ConA and ConBr (Sanz-Aparicio *et al.*, 1997).

The quaternary structure variation exhibited by lectins that have nearly the same subunit structure precludes the use of a molecular-modelling approach to establish structure–function correlations. Thus, crystallographic studies have been carried out in order to establish the tertiary and quaternary structures of the lectins from the Diocleae subtribe as a first step towards their possible use in nano-

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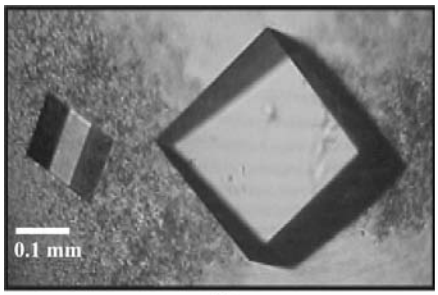
biotechnology and molecular-biology studies. Currently, 54 lectin structures from the Diocleae subtribe have been deposited in the Protein Data Bank (Berman *et al.*, 2000). However, 50 entries correspond to diverse forms of ConA. With the aim of establishing the crystal structure of the lectin from seeds of *C. gladiata*, we report here its purification, crystallization and preliminary X-ray diffraction analysis.

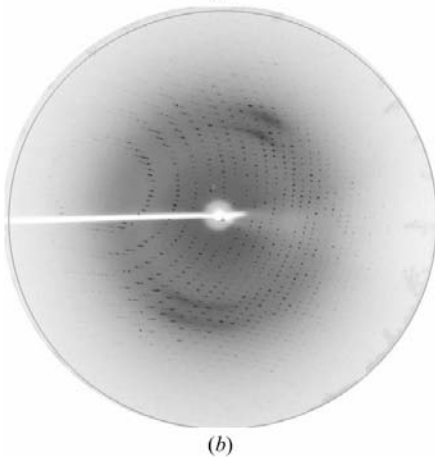
2. Materials and methods

2.1. Purification of *C. gladiata* seed lectin

Mature *C. gladiata* seeds were collected in Ceará state, Northeast Brazil. The seeds were grounded to a fine powder in a coffee mill and then defatted with *n*-hexane. Soluble proteins were extracted at 291 K for 4 h by continuous stirring with 0.15 *M* NaCl [1:10(*w*:*v*)], followed by centrifugation at 10 000*g* at 277 K for 20 min. The supernatant was applied onto a Sephadex G-50 column (5 × 25 cm), which had been equilibrated with 0.15 *M* NaCl containing 5 m*M* $CaCl_2$ and $MnCl_2$, as described by Ceccatto *et al.* (2002). $CaCl_2$ and $MnCl_2$ were added in order to retain carbohydrate-binding activity. The column was then washed with equilibration buffer at a flow rate of 45 ml h^{-1} until the effluent absorbance at 280 nm was below 0.05. The bound lectin was eluted with 0.1 *M* glycine pH 2.6, dialyzed extensively against distilled water and lyophilized. The affinity chromatography fraction was further purified using an Äkta chromatographic system and a Mono-Q column (5 × 0.5 cm) equilibrated with 20 m*M* Tris–HCl pH 7.0 and developed with a linear gradient of 20 m*M* Tris–HCl pH 7.0 containing 1.0 *M* NaCl at a flow rate of 1 ml min^{-1} and a slope of 5% NaCl min^{-1}. The *C. gladiata* lectin recovered in the unbound fraction was exhaustively dialyzed against distilled water and freeze-dried. The purity of CGL was assessed by SDS–PAGE.



(*a*)



(*b*)

Figure 1
(*a*) Native crystal of the lectin from *C. gladiata*. (*b*) The crystal diffraction pattern at 2.0 Å.

2.2. Crystallization, data collection and processing

For crystallization trials, the purified lectin was dissolved at a concentration of 10 mg ml^{-1} in 20 m*M* Tris–HCl pH 7.5 containing 5 m*M* $CaCl_2$ and $MnCl_2$. Crystals of CGL were grown in Linbro plates at 293 K by the vapour-diffusion method (Jancarik & Kim, 1991) in hanging drops, using Crystal Screen from Hampton Research. The drops were composed of equal volumes (3 µl) of protein solution and 0.1 *M* Tris–HCl pH 8.5 with 2.0 *M* ammonium sulfate and were equilibrated against 500 µl reservoir solution. Single crystals were transferred to a cryoprotectant solution consisting of 25% glycerol and 75% 0.1 *M* Tris–HCl pH 8.5 with 2.0 *M* ammonium sulfate and flash-frozen in a stream of nitrogen at 100 K. X-ray diffraction of cryoprotected crystals was performed at a wavelength of 1.4310 Å using Synchrotron Radiation Source (CPr station, Laboratório Nacional de Luz Síncrotron–LNLS, Campinas, Brazil) and a complete data set was collected using a CCD (MAR Research) in 120 frames with an oscillation range of 1°. The data were indexed, integrated and scaled using *MOSFLM* and *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

Small crystals of CGL appeared in 0.1 *M* Tris–HCl pH 8.5 containing 2.0 *M* ammonium sulfate. However, these crystals were not suitable for X-ray diffraction analysis. To optimize the condition of crystallization, changes in the pH range were tested. The best crystals grew in 0.1 *M* Tris–HCl pH 9.0, 2.0 *M* ammonium sulfate within 20 d (Fig. 1*a*) to maximum dimensions of approximately 0.4 × 0.2 × 0.3 mm and diffracted to a maximum resolution of 2.0 Å. The crystals belong to the orthorhombic space group $C222_1$ (unit-cell parameters $a = 101.81$, $b = 117.21$, $c = 243.61$ Å). Data in the 51.99–2.31 Å resolution range were scaled using *SCALA* to an $R_{\rm merge}$ of 0.073 (0.354) with an $\langle I/\sigma(I)\rangle$ of 8.9 (2.4). The final data set to 2.31 Å resolution, with a total of 279 320 observed and 60 699 unique reflections, was 99.1% (99.8%) complete. The values in parentheses are for the highest resolution shell (2.42–2.31 Å). The refined mosaic spread of the crystal was 0.38°. Assuming the presence of four 25.5 kDa lectin monomers in the asymmetric unit, the calculated Matthews coefficient ($V_{\rm M}$; Matthews, 1968) was 3.56 $Å^3\,Da^{-1}$, indicating a solvent content of 65.4%. Like ConA, CGL is a tetramer in the asymmetric unit.

The preliminary crystal structure of CGL was determined by standard molecular-replacement methods using the program *MOLREP* (Vagin & Teplyakov, 1997). The atomic coordinates of various lectin monomers were used in the search for a structural model. The best result was obtained with ConA (PDB code 1gkb; Kantardjieff *et al.*, 2002). The best solution had a final correlation coefficient of 0.569 and an *R* factor of 0.412. After placing the molecule in the unit cell, rigid-body refinement was performed using the *X-PLOR* program (Brünger, 1992). Refinement resulted in a model with an $R_{\rm free}$ of 0.375 and an *R* factor of 0.287. Refinement of the X-ray crystallographic structure of CGL is in progress.

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