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Crystallization and preliminary X-ray diffraction analysis of the lectin from *Dioclea rostrata* Benth seeds

Lectins from the Diocleinae subtribe (Leguminosae) are highly similar proteins that promote various biological activities with distinctly differing potencies. The structural basis for this experimental data is not yet fully understood. *Dioclea rostrata* lectin was purified and crystallized by hanging-drop vapour diffusion at 293 K. The crystal belongs to the orthorhombic space group *I*222, with unit-cell parameters $a = 61.51$, $b = 88.22$, $c = 87.76$ Å. Assuming the presence of one monomer per asymmetric unit, the solvent content was estimated to be about 47.9%. A complete data set was collected at 1.87 Å resolution.

1. Introduction

Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origin found in all types of living organisms that decipher the glycodes encoded in the structure of glycans attached to soluble and integral cell-membrane glycoconjugates (Gabius & Gabius, 1997). Plant lectins, especially those purified from species of the Leguminosae family, represent the best studied group of carbohydrate-binding proteins (Van Damme *et al.*, 1998).

Although the Diocleinae subtribe lectins sequenced to date show a high degree of similarity, they present significant differences in the potency/efficacy of their many biological activities (Alencar *et al.*, 1999; Andrade *et al.*, 1999; Assreuy *et al.*, 1999; Havt *et al.*, 2003; Lopes *et al.*, 2005). For instance, *Dioclea rostrata* lectin (DRL) has been found to be more efficient in promoting the synthesis of IFN- γ and histamine secretion than other lectins from the same subtribe (e.g. *D. grandiflora* and *D. guianensis*). On the other hand, DRL is less efficient than other Diocleinae lectins in stimulating the synthesis of TNF- α and IL-10 (Cavada *et al.*, 2001). Several factors contribute to these differences in the activities of Diocleinae lectins: for example, the pH-dependent oligomerization that some of these lectins present and the relative position of the carbohydrate-binding site (Wah *et al.*, 2001). The distinct biological activities presented by ConA and ConBr have been shown to be a consequence of a non-conservative substitution that affects the carbohydrate binding site, making it more open in ConBr than it is in ConA (Sanz-Aparicio *et al.*, 1997). *Canavalia maritima* lectin presents decreased nitric oxide release compared with ConA, an effect that was explained by a difference in only one amino-acid residue (Gadelha *et al.*, 2005). The present study reports the crystallization and X-ray diffraction of the *D. rostrata* lectin.

2. Material and methods

2.1. Purification and crystallization

D. rostrata seeds were collected in 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 000g at 277 K for 20 min. The supernatant was applied onto a Sephadex G-50 column (10 \times 50 cm) previously equilibrated with 0.15 M NaCl containing 5 mM CaCl₂ and 5 mM MnCl₂ 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 fraction was eluted with 0.1 M glycine

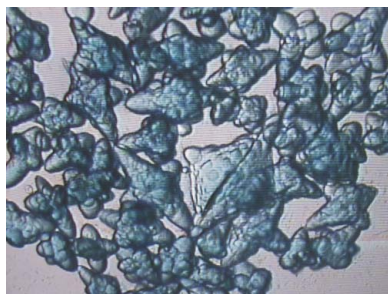


Table 1

Statistics of data collection.

Values in parentheses are for the highest resolution shell.

R_{merge}	6.7 (34.1)
Resolution limit (Å)	33.150 (2.05)
$I/\sigma(I)$	9.7 (2.1)
Completeness (%)	99.8 (99.8)
Redundancy	9.4
Unit-cell parameters (Å)	$a = 61.51, b = 88.21, c = 87.76$
Space group	$I222$
Wavelength (Å)	1.431
Total No. of reflections	144142
Total No. of unique observations	15297

pH 2.6 containing 0.15 M NaCl and dialyzed exhaustively against Milli-Q water. This fraction was freeze-dried, resuspended in 0.075 M Tris pH 9.3 (5 mg ml⁻¹) and loaded onto a MonoP pre-packed column connected to an HPLC/FPLC AKTA system (GE Healthcare). The lectin (DRL) was eluted with Polybuffer 96 pH 6.0. The purity of all DRL preparations was monitored by SDS-PAGE (Laemmli, 1970). The purified lectin was diluted in Tris pH 7.5 containing 5 mM CaCl₂ and 5 mM MnCl₂ to perform the crystallization experiments. The crystallization trials were carried out by the hanging-drop vapour-diffusion method at 293 K. 1 µl lectin solution was mixed with 1 µl reservoir solution and equilibrated against 300 µl reservoir solution. Initial screening was performed using a sparse-matrix method (Jancarik & Kim, 1991) with Crystal Screens I and II (Hampton Research, Riverside, CA, USA).

2.2. Data collection and processing

X-ray data were collected from a single crystal cooled to a temperature of 100 K. Crystals were soaked in a cryoprotectant solution consisting of the reservoir solution containing 30% glycerol and submitted to data collection at a wavelength of 1.43 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 59 frames with an oscillation range of 1.5°. The data set was indexed, integrated and scaled

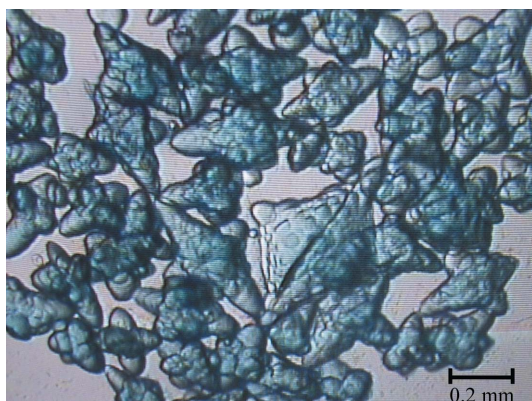


Figure 1
Crystals of *D. rostrata* lectin.

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DRL      SIADANSLHFTFNQFSQNPKDLIL
Dgui     SIADANSLHFSFNQFSQNPKDLIL
DGL      SIADANSLHFSFHQFSQNPKDLIL
***** : * : *****
    
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Figure 2
Sequence alignment of the lectin segment 119–142 from *D. rostrata* (DRL), *D. guianensis* (Dgui) and *D. grandiflora* (DGL). Asterisks (*) related to conserved residues and colons (:) to semi-conserved residues.

using *MOSFLM* (Leslie, 1992) and the *CCP4* program *SCALA* (Collaborative Computational Project, Number 4, 1994). Sequence alignment of the lectins was performed using *ClustalW* (Thompson *et al.*, 1994).

3. Results and discussion

Crystals were obtained after a week using condition No. 29 (0.1 M Na HEPES pH 7.5, 0.8 M sodium potassium tartrate tetrahydrate) of Hampton Crystal Screen I. To optimize the crystallization conditions, the concentration of tartrate was modified and different buffers in various pH ranges were tested. Crystals of good size for diffraction (Fig. 1) were obtained under the conditions 0.1 M Na HEPES pH 7.5–8.5, 0.4–1.2 M sodium potassium tartrate.

Surprisingly, the crystals obtained (Fig. 1) provided a data set extending to 1.87 Å resolution which was scaled in the resolution range 33.150–2.05 Å. The *D. rostrata* lectin crystal belongs to the orthorhombic space group $I222$, with unit-cell parameters $a = 61.51, b = 88.22, c = 87.76$ Å. The calculated value for the Matthews coefficient (Matthews, 1968) based on the molecular weight of 25.5 kDa is 2.4 Å³ Da⁻¹, resulting in a solvent content of 47.9%, which corresponds to the presence of a monomer in the asymmetric unit. Data-collection statistics are shown in Table 1. Crystal structure solution is under way.

Sequence alignment of fragment 2 from *D. rostrata* lectin (SWISS-PROT code P58908) with *D. guianensis* lectin (PDB code 1h9p, chain A; Calvete *et al.*, 1999) and *D. grandiflora* lectin (PDB code 1dgl, chain B; Rozwarski *et al.*, 1998) showed that DRL contains an Asn residue in position 131, while *D. grandiflora* lectin contains a His residue in this position (Fig. 2). Comparison of the structures of *D. guianensis* lectin, which presents pH-dependent oligomerization, and *D. grandiflora* lectin, which does not display a dimer–tetramer equilibrium, indicated that substitution of His131 for Asn drastically reduces interdimer contacts and disorders the loop comprising residues 117–123 which, in its ordered conformation, stabilizes the pH-independent tetrameric association of the *D. grandiflora* lectin (Wah *et al.*, 2001). The solution of the DRL crystal structure may be useful to determine whether DRL presents the same features as *D. guianensis* regarding the dimer–tetramer association. Since only the tetravalent form is able to cause cross-linking of receptors on the cell surface, the different ratio of divalent and tetravalent lectin species at a given pH may contribute to the variability of biological functions.

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References

Alencar, N. M. N., Teixeira, E. H., Assreuy, A. M., Cavada, B. S., Flores, C. A. & Ribeiro, R. A. (1999). *Mediators Inflamm.* **8**, 107–113.
 Andrade, J. L., Arruda, S., Barbosa, T., Paim, L., Ramos, M. V., Cavada, B. S. & Barral-Netto, M. (1999). *Cell. Immunol.* **194**, 98–102.
 Assreuy, A. M., Martins, G. J., Moreira, M. E. F., Brito, G. A. C., Cavada, B. S., Ribeiro, R. A. & Flores, C. A. (1999). *J. Urol.* **161**, 1988–1993.
 Calvete, J. J., Thole, H. H., Raida, M., Urbanke, C., Romero, A., Grangeiro, T. B., Ramos, M. V., Almeida da Rocha, I. M., Guimarães, F. N. & Cavada, B. S. (1999). *Biochem. Biophys. Acta*, **1430**, 367–375.

- Cavada, B. S., Barbosa, T., Arruda, S., Grangeiro, T. B. & Barral-Netto, M. (2001). *Curr. Protein Pept. Sci.* **2**, 123–135.
- Cavada, B. S., Grangeiro, T. B., Ramos, M. V., Cordeiro, E. F., Oliveira, J. T. A. & Moreira, R. A. (1996). *Rev. Bras. Fisiol. Veg.* **8**, 31–36.
- Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* **D50**, 760–763.
- Gabius, H. J. & Gabius, S. (1997). Editors. *Glycoscience: Status and Perspectives*. Weinheim: Chapman & Hall.
- Gadelha, A. C. C., Moreno, F. B. M. B., Santi-Gadelha, T., Cajazeiras, J. B., Rocha, B. A. M., Assreuy, A. M. S., Mota, M. R. L., Pinto, N. V., Meireles, A. V. P., Borges, J. C., Freitas, B. T., Canduri, F., Souza, E. P., Delatorre, P., Criddle, D. N., de Azevedo, W. F. Jr & Cavada, B. S. (2005). *J. Struct. Biol.* **152**, 185–194.
- Havt, A., Barbosa, P. S. F., Sousa, T. M., Martins, A. M. C., Nobre, A. C. L., Nascimento, K. S., Teixeira, E. H., Pinto, V. P. T., Sampaio, A. H., Fontales, M. C., Cavada, B. S. & Monteiro, H. S. A. (2003). *Protein Pept. Lett.* **10**, 191–197.
- Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
- Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
- Leslie, A. G. W. (1992). *Jnt CCP4/ESF-EACBM Newsl. Protein Crystallogr.* **26**.
- Lopes, F. C., Cavada, B. S., Pinto, V. P. P., Sampaio, A. H. & Gomes, J. C. (2005). *Braz. J. Med. Biol. Res.* **38**, 935–941.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–487.
- Rozwarski, D. A., Sawmi, B. M., Brewer, C. F. & Sacchettini, J. C. (1998). *J. Biol. Chem.* **273**, 32818–32825.
- Sanz-Aparicio, J., Hermoso, J., Grangeiro, T. B., Calvete, J. J. & Cavada, B. S. (1997). *FEBS Lett.* **405**, 114–118.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). *Nucleic Acids Res.* **22**, 4673–4680.
- Van Damme, E. J. M., Peumans, W. J., Barre, A. J. & Rougé, P. (1998). *Crit. Rev. Plant Sci.* **17**, 575–692.
- Wah, D. A., Romero, A., Sol, F. G., Cavada, B. S., Ramos, M. V., Grangeiro, T. B., Sampaio, A. H. & Calvete, J. J. (2001). *J. Mol. Biol.* **310**, 885–894.