Crystallization and preliminary X-ray diffraction studies of frutalin, an α -D-galactose-specific lectin from *Artocarpus incisa* seeds

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Received 7 November 2014

Accepted 14 August 2015

Edited by B. Hazes, University of Alberta, Canada

Keywords: frutalin; jacalin lectin-related family; *Artocarpus incisa*.

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Frutalin is an α -D-galactose-specific carbohydrate-binding glycoprotein with antitumour properties and is a powerful tool for tumour biomarker discovery. The crystallization and preliminary X-ray diffraction analysis of this lectin, which was isolated from *Artocarpus incisa* seeds, are reported here. Frutalin was purified and submitted to mass-spectrometric analysis. Diverse masses at approximately 16 kDa were observed in the deconvoluted spectra, which support the presence of isoforms. The best frutalin crystals were grown within a week in 0.1 M citric acid pH 3.5 which contained 25% PEG 3350 as a precipitant at 293 K, and diffracted to a maximum resolution of 1.81 Å. The monoclinic crystals belonged to space group *I*2, with unit-cell parameters $a = 76.17$, $b = 74.56$, $c = 118.98$ Å, $\beta = 96.56^\circ$. A molecular-replacement solution was obtained which indicated the presence of four monomers per asymmetric unit. Crystallographic refinement of the structure is in progress.

1. Introduction

Lectins are proteins that contain at least one noncatalytic domain that reversibly binds to specific glycans, which are present either in the free form or as parts of glycoproteins and glycolipids (Van Damme *et al.*, 2008, 2011). Lectins have the unique ability to discriminate complex glycoconjugate structures without altering their molecular structure (Hirabayashi *et al.*, 2011). Several lectins have been characterized in almost all groups of organisms, including viruses, but they are particularly prominent in plant seeds, particularly leguminous seeds.

Artocarpus incisa (breadfruit) is widespread in different habitats in subtropical and tropical regions. The lectin from *A. incisa*, which is named frutalin (FTL), has been isolated and characterized (Moreira *et al.*, 1997), and its refolding process has been studied using thermal and chemical denaturation (Campana *et al.*, 2002). It belongs to the jacalin lectin-related family (Moraceae family) and has an affinity for α -D-galactose monosaccharides and complex carbohydrates that contain Gal α 1–3 glycans (Moreira *et al.*, 1997; Oliveira, Teixeira *et al.*, 2009; Nobre *et al.*, 2010). FTL is homologous to jacalin and has an identical electrophoresis pattern, with two bands between 20 and 14 kDa which correspond to different glycosylation forms. However, distinct biological activities have been

observed between the lectins, such as a higher haemagglutination activity of FTL in comparison to jacalin (Nobre *et al.*, 2010).

FTL forms homotetramers in which each monomer contains two chains, an α -chain (~16 kDa) and a β -chain (2 kDa), which probably result from co-translational and post-translational processing in a similar way as in jacalin from *A. integrifolia*, which is synthesized *in vivo* as an unusual preproprotein (Yang & Czaplá, 1993). FTL has similar properties to jacalin and jacalin-like lectins. The following features can be highlighted: FTL is partially glycosylated with approximately 2.1% glycans and is expressed in different glyco-isoforms. In jacalin one-third of the α -chain, termed the α' -chain, is glycosylated. Marshall (1972) proposed that the α' -chain has three possible sites of *N*-glycosylation at positions 16, 35 and 74 based on the consensus sequence Asn-X-Thr/Ser. FTL also shows similar conserved consensus sequences, which suggests that three sites of *N*-glycosylation may be present.

FTL has been tested for biomedical applications, particularly because of its notable antitumour properties (Oliveira *et al.*, 2011), potential as a tumour biomarker (Oliveira, Costa *et al.*, 2009), immunomodulatory effects (Brando-Lima *et al.*, 2005, 2006), gastroprotective effects (de Vasconcelos Abdon *et al.*, 2012), cytotoxicity (Oliveira *et al.*, 2011), glycomembrane interaction (Nobre *et al.*, 2010) and chemotaxis (Brando-Lima *et al.*, 2005). We have performed the crystallization and X-ray diffraction analysis of native FTL in order to solve its three-dimensional structure. This work is a prerequisite for understanding and providing insights for further biological and chemical studies on the interactions between FTL and glycosylated ligands.

2. Materials and methods

2.1. Purification of FTL, SDS-PAGE and ESI mass-spectrometric analysis

A. incisa seeds were obtained from Ceará State, Brazil. The seeds were cut into small pieces and dehydrated using propanone for 48 h. The seeds were then ground into a fine powder and the soluble proteins were initially extracted by resuspension in 0.15 M NaCl solution at a 1:10(w:v) ratio and maintaining the suspension under agitation for 1 h at room temperature. The sample was then centrifuged for 10 min at 10 000g and the supernatant was filtered through filter paper and applied onto a D-galactose agarose column which had previously been equilibrated with 0.15 M NaCl. After washing away the unbound molecules with 0.15 M NaCl, the retained protein was eluted by adding 0.2 M D-galactose to the buffer. The eluted peak was exhaustively dialysed against distilled water and subsequently lyophilized (Moreira *et al.*, 1997).

The sample purity was assessed by SDS-PAGE according to Laemmli (1970). Electrospray ionization mass spectrometry (ESI-MS) was performed using a Synapt HDMS mass spectrometer (Waters, Manchester, England) which was coupled to a nano UPLC system. 1 μ l of a sample at 1 mg ml⁻¹ in

Table 1
Crystallization.

Method	Hanging drop
Plate type	Linbro
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	6.0
Buffer composition of protein solution	20 mM Tris-HCl pH 7.5
Composition of reservoir solution	0.1 M citric acid pH 3.5, 25% PEG 3350
Volume and ratio of drop	1.5 μ l (1:1)
Volume of reservoir (μ l)	400

0.1%(v/v) formic acid was fractionated by reverse-phase chromatography using a gradient from 3 to 70%(v/v) acetonitrile with 0.1%(v/v) formic acid on a BEH C4 column (1.7 μ m, 100 $\times$ 100 mm). The acquired MS data were processed using a maximum-entropy technique (MaxEnt) to obtain a deconvoluted spectrum (Ferrige *et al.*, 1991).

2.2. Crystallization, data collection and initial processing

Purified FTL was dissolved to approximately 6.0 mg ml⁻¹ in 20 mM Tris-HCl pH 7.5. The solution was centrifuged at 10 000g for 10 min and the supernatant was used in hanging-drop crystallization trials (Jancarik & Kim, 1991) in Linbro plates at 293 K. Initially, 146 crystallization conditions were set up manually (Crystal Screen, Crystal Screen 2 and Index; Hampton Research, California, USA), in which the drops were composed of equal volumes of protein and reservoir solutions (1.5 μ l) and were equilibrated against 400 μ l reservoir solution. Two 96-well plates were also prepared by a Honeybee 961 robot using the Morpheus and Index HT crystallization kits. Crystallization information is given in Table 1. X-ray diffraction data were collected at a wavelength of 1.5418 Å using a Rigaku MicroMax-007 HF X-ray source, which was coupled to a Rigaku R-Axis IV⁺⁺ image-plate detector. The X-ray data were collected from the FTL crystal at 100 K; to avoid freezing, the crystals were soaked in a cryoprotectant solution that consisted of 50% reservoir

Figure 1
Intact deconvoluted mass spectrum of FTL showing masses around 16 kDa. Inset, SDS-PAGE showing two bands between 20 and 14 kDa. Lane M, molecular-mass markers (labelled in kDa); lane 2, FTL.

solution and 50% PEG 3350. The data were indexed, integrated and scaled using *iMosflm* (Battye *et al.*, 2011) and *AIMLESS* (Evans & Murshudov, 2013).

2.3. Molecular replacement

Molecular replacement was performed using *Phaser* (McCoy *et al.*, 2007).

3. Results and discussion

3.1. Mass spectrometry and optimization of FTL crystals

FTL is expressed in different isoforms, which mainly reflect differences in post-translational glycosylation. The SDS-PAGE showed a typical jacalin-like lectin mass pattern with two bands between 20 and 14 kDa (Fig. 1), which correspond

Figure 2
Crystals of FTL grown in citric acid pH 3.5 containing PEG 3350 as a precipitant.

Figure 3
Diffraction pattern of an FTL crystal to 1.81 Å resolution.

Table 2

Data-collection statistics.

Space group	<i>I</i> 2
Unit-cell parameters (Å, °)	$a = 76.17$, $b = 74.56$, $c = 118.98$, $\alpha = 90.0$, $\beta = 96.56$, $\gamma = 90.0$
Detector	Rigaku R-Axis IV ⁺⁺
X-ray source	Rigaku MicroMax-007 HF
Wavelength (Å)	1.5418
No. of images	400
Oscillation range (°)	0.5
Resolution range (Å)	27.02–1.81 (1.85–1.81)
Multiplicity	4.0 (3.7)
R_{meas} (%)	10.0 (53.3)
$R_{\text{p.i.m.}}$ (%)	4.9 (27.1)
$CC_{1/2}$	99.6 (78.4)
Completeness (%)	99.8 (97.0)
Total reflections	240259 (12493)
Unique reflections	59769 (3386)
$\langle I/\sigma(I) \rangle$	10.3 (2.6)
Asymmetric unit content	4 monomers

to a glycosylated fraction and a slightly or nonglycosylated fraction, respectively (Oliveira *et al.*, 2008). The deconvoluted FTL mass spectrum showed different masses at approximately 16.5 kDa, which is consistent with the presence of isoforms of identical monomers.

Initially, the crystallization trials yielded several conditions with crystals in both the Index and the Morpheus kits. The FTL crystals mainly grew as plate clusters at pH 8.5 with PEG as the precipitant (12.5% PEG 1000 and 12.5% PEG 2250) and with 20% ethylene glycol as an additive. Although these FTL plates were fragile and not suitable for the collection of X-ray data, the best crystals appeared after a week in 0.1 *M* citric acid pH 3.5 with 25% PEG 3350 as the precipitant and had maximum dimensions of 0.1 × 0.15 × 0.03 mm (Fig. 2).

3.2. Data collection, processing and molecular replacement

The FTL crystals diffracted to a maximum resolution of 1.81 Å using a Rigaku MicroMax-007 HF rotating-anode X-ray source (Fig. 3). We used the coordinates of the jacalin monomer, which shares 91% sequence identity with FTL, as a search model (PDB entry 3p8s; Oliveira *et al.*, 2008; Oliveira, Costa *et al.*, 2009). The best solution was obtained in space group *I*2 with four monomers per asymmetric unit, which yielded an *R* factor, LLG and TFZ of 38.6%, 11 514.0 and 19.9, respectively. The Matthews coefficient (V_M ; Matthews, 1968) for this solution was 2.53 Å³ Da^{−1}, with a solvent content of 51.47%. The data-collection statistics are summarized in Table 2.

Model building/refinement is under way.

Acknowledgements

The Brazilian Council for Research and Development (CNPq) and Fortaleza University (UNIFOR) provided financial support.

References

Battye, T. G. G., Kontogiannis, L., Johnson, O., Powell, H. R. & Leslie, A. G. W. (2011). *Acta Cryst. D* **67**, 271–281.

- Brando-Lima, A. C., Saldanha-Gama, R. F., Henriques, M. G. M. O., Monteiro-Moreira, A. C. O., Moreira, R. A. & Barja-Fidalgo, C. (2005). *Toxicol. Appl. Pharmacol.* **208**, 145–154.
- Brando-Lima, A. C., Saldanha-Gama, R. F., Pereira, C. R., Villela, C. G., Sampaio, A. L., Monteiro-Moreira, A. C., Henriques, M. G. M. O., Moreira, R. A. & Barja-Fidalgo, C. (2006). *Int. Immunopharmacol.* **6**, 465–472.
- Campana, P. T., Moraes, D. I., Monteiro-Moreira, A. C. O. & Beltramini, L. M. (2002). *Eur. J. Biochem.* **269**, 753–758.
- Evans, P. R. & Murshudov, G. N. (2013). *Acta Cryst. D* **69**, 1204–1214.
- Ferrige, A. G., Seddon, M. J., Jarvis, S., Skilling, J. & Aplin, R. (1991). *Rapid Commun. Mass Spectrom.* **5**, 374–377.
- Hirabayashi, J., Kuno, A. & Tateno, H. (2011). *Electrophoresis*, **32**, 1118–1128.
- Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
- Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
- Marshall, R. D. (1972). *Annu. Rev. Biochem.* **41**, 673–702.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
- Moreira, R. A., Monteiro, A. C. O., Horta, A. C. G., Oliveira, J. T. A. & Cavada, B. S. (1997). *Phytochemistry*, **46**, 139–144.
- Nobre, T. M., Pavinatto, F. J., Cominetti, M. R., Selistre de-Araújo, H. S., Zaniquelli, M. E. D. & Beltramini, L. M. (2010). *Biochim. Biophys. Acta*, **1798**, 1547–1555.
- Oliveira, C., Costa, S., Teixeira, J. A. & Domingues, L. (2009). *Mol. Biotechnol.* **43**, 212–220.
- Oliveira, C., Felix, W., Moreira, R. A., Teixeira, J. A. & Domingues, L. (2008). *Protein Expr. Purif.* **60**, 188–193.
- Oliveira, C., Nicolau, A., Teixeira, J. A. & Domingues, L. (2011). *J. Biomed. Biotechnol.* **2011**, 568932.
- Oliveira, C., Teixeira, J. A., Schmitt, F. & Domingues, L. (2009). *BMC Biotechnol.* **9**, 78.
- Van Damme, E. J. M., Fouquaert, E., Lannoo, N., Vandenborre, G., Schouppe, D. & Peumans, W. J. (2011). *The Molecular Immunology of Complex Carbohydrates-3*, edited by A. M. Wu, pp. 271–294. New York: Springer.
- Van Damme, E. J. M., Lannoo, N. & Peumans, W. J. (2008). *Adv. Bot. Res.* **48**, 107–209.
- Vasconcellos Abdon, A. P. de, Coelho de Souza, G., Noronha Coelho de Souza, L. N., Prado Vasconcelos, R., Araújo Castro, C., Moreira Guedes, M., Pereira Lima Júnior, R. C. P., de Azevedo Moreira, R., de Oliveira Monteiro-Moreira, A. C. & Rolim Campos, A. (2012). *Fitoterapia*, **83**, 604–608.
- Yang, H. & Czaplá, T. H. (1993). *J. Biol. Chem.* **68**, 5905–5910.