Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic analysis of *Sclerotium rolfsii* lectin

Demetres D. Leonidas,[a]* Bale M. Swamy,[b] Anuradha G. Bhat,[b] Shashikala R. Inamdar,[b] Magda N. Kosmopoulou,[a] Evangelia D. Chrysina[a] and Nikos G. Oikonomakos[a]

[a]Institute of Biological Research and Biotechnology, The National Hellenic Research Foundation, 48 Vas. Constantinou Ave, 11635 Athens, Greece, and [b]Department of Biochemistry, Karnatak University, Dharwad 580003, India

Correspondence e-mail: ddl@eie.gr

Received 30 August 2002
Accepted 18 November 2002

Sclerotium rolfsii lectin (SRL), from the soil-borne phytopathogenic fungus *S. rolfsii*, has been crystallized. SRL crystals were grown by the hanging-drop vapour-diffusion method using an MPD–ammonium acetate mixture in Tris–HCl buffer pH 8.5. A complete data set from a single crystal at 100 K was collected to 1.1 Å resolution using synchrotron radiation. Preliminary crystallographic analysis showed that the crystals belong to the tetragonal space group $P4_22_12$, with unit-cell parameters $a = b = 99.81$, $c = 63.99$ Å and two molecules per asymmetric unit.

1. Introduction

Lectins are non-enzymatic carbohydrate-binding proteins present in plants, bacteria, fungi and animals which preferentially bind reversibly to specific carbohydrate structures, either free in solution or on cell surfaces, and play an important role in cell recognition. *Sclerotium rolfsii* lectin (SRL) was purified from the sclerotial bodies of *S. rolfsii*, a soil-borne phytopathogenic fungus (Swamy *et al.*, 2000). SRL is a monomer under acidic conditions (pH 4.3) with a molecular weight of 17 kDa and forms a dimer at neutral or basic pHs. SRL has been shown to recognize the Galβ1$\rightarrow$3GalNAc-α1$\rightarrow$Ser/Thr residues in glycoproteins, but has a diminished reactivity towards sialylated glycotopes (Swamy *et al.*, 2000; Wu *et al.*, 2001). This carbohydrate sequence occurs as the mucin type I core structure of O-linked oligosaccharide chains and its role as a T-antigenic substance has been established (Springer *et al.*, 1979). SRL binds to this glucosyl moiety; considering that the T antigen is an oncodevelopmental cancer-associated antigen that predominantly occurs in several carcinoma cell surfaces (Spinger *et al.*, 1975; Itzkowitz *et al.*, 1989; Shamsuddin *et al.*, 1995), SRL and similar lectins may have greater applications (Wu, 1984). In this regard, peanut agglutinin and a lectin from *Agaricus bisporous* have been widely employed as special reagents for cell markers and as fine tools for elucidating changes on mammalian cell-surface structures during their differentiation. It has also been shown that subtle differences in carbohydrate reactivity of such T-antigen-binding lectins have marked effects on their interactions with human cancer cells and melanoma cell lines (Yu *et al.*, 1993; Lorea *et al.*, 1997; Ryder *et al.*, 1998).

Microbial lectins, particularly bacterial lectins called adhesins, have gathered significance over the last two decades, as they play a pivotal role in the initiation of the infection process by mediating adhesion to host cells (Sharon & Ofek, 2000). Findings with bacterial adhesins have attracted many to assign a similar role to phytopathogenic fungal lectins in host–parasite interaction (Rudiger, 1998). In the past, only a few fungal lectins, mostly from higher fungi (mushrooms), have been successfully crystallized. Notable examples are those from *Flammulina velutipes* (Hirano *et al.*, 1987), *Aleuria aurentia* (Nagata *et al.*, 1991) and *Pleurotus ostreatus* (Chattopadhyay *et al.*, 1999). Partial structural characterization of two lectins from *Coprinus cinereus* (Cooper *et al.*, 1997) and *Rhizoctonia solani* (Candy *et al.*, 2001) has been reported based on their primary sequence; to date, no crystal structure is known of a fungal lectin. The structure determination of SRL will provide the first crystal structure of a fungal lectin and may also help in defining the structure–function relationships of other fungal lectins. Furthermore, structural analysis of SRL will allow the elucidation of the structural basis of its fine sugar specificity and will shed light on its biological role.

2. Crystallization

Purification of SRL from the fungus *S. rolfsii* has been described previously (Swamy *et al.*, 2000). The freeze-dried lectin was diluted in 5 m*M* Tris–HCl buffer pH 8.0 prior to crystallization trials. Commercially available screens were used to determine the initial crystallization conditions (Molecular Dimensions Ltd, UK) using the hanging-drop vapour-

diffusion method at a temperature of 289 K. Initial crystallization trials produced small needle-like crystals on mixing 2 µl of protein solution (20 mg ml^{-1}) with an equal volume of a reservoir solution containing 0.2 *M* ammonium acetate, 30%(*v*/*v*) 2-propanol, 0.1 *M* Tris–HCl buffer pH 8.5. However, these crystals (Fig. 1*a*) were extremely thin, while the drops contained a large amount of precipitate. The crystals diffracted to 4.0 Å resolution using an R-AXIS IV image plate mounted on a Rigaku RU-H3RHB rotating-anode X-ray source with Cu $K\alpha$ radiation (λ = 1.5418 Å). As these SRL crystals grew in the presence of 2-propanol, various concentrations of 2-propanol, MPD and PEGs of various molecular weights were used to improve the crystal quality and size. Single crystals of identical morphology but of considerably better quality and size were grown within two weeks at 289 K using a reservoir solution containing 0.2 *M* ammonium acetate, 30% MPD, 0.1 *M* Tris–HCl buffer pH 8.5 and a protein concentration in the drop of 8 mg ml^{-1} (Fig. 1*b*). Although these conditions produced better crystals, they did not help to eliminate protein precipitation in the drop. This optimization procedure is a good example of the influence that different non-polar precipitants can have on the quality and size of protein crystals.

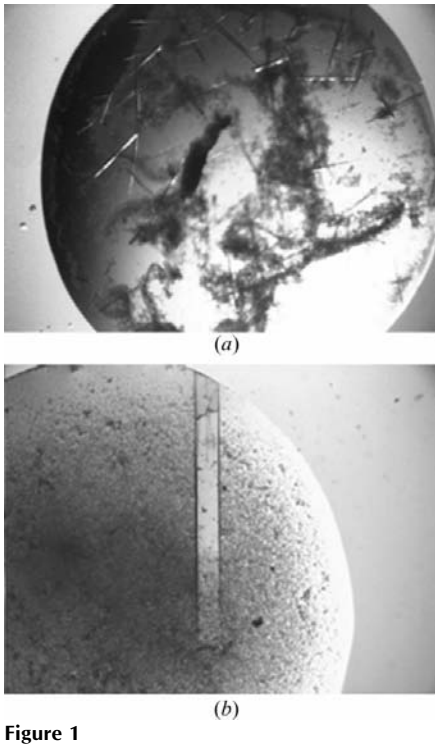

Figure 1
Photograph of the SRL crystals (*a*) grown from the initial screening and (*b*) after optimization of the crystallization conditions. The dimensions of the crystal in (*b*) are approximately 1.0 × 0.1 × 0.1 mm.

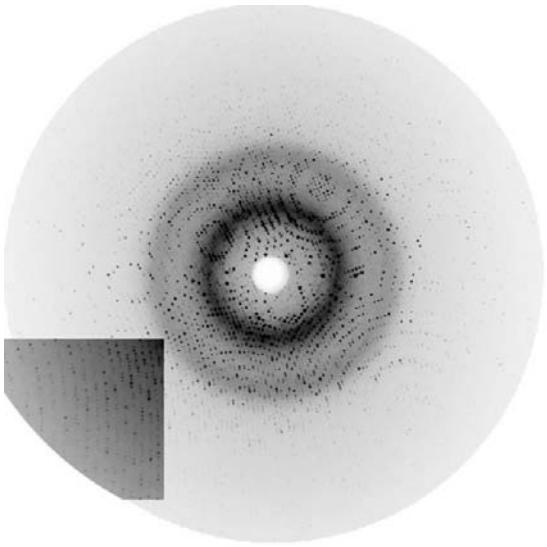

Figure 2
An SRL crystal X-ray diffraction oscillation image. Inset: a magnification of a section of the diffraction pattern, where it can be seen that the SRL crystal diffracts beyond 1.1 Å (the edge).

2.1. Data collection

Diffraction data were collected from a single crystal at 100 K at station BW7B (λ = 0.8441 Å), EMBL Hamburg c/o DESY. As SRL crystals were grown in a medium containing 30% MPD, no other cryoprotectant media was used and the crystal was simply transferred to a nitrogen-gas cold stream (Oxford Cryosystems Cryostream Cooler). Diffraction data to 1.1 Å resolution were recorded on a MAR Research 345 mm image-plate detector (Fig. 2). The exposure time was 5–10 s per image (dose mode), the oscillation range was 0.6° and a total of 160 images were collected. Additional data to medium (2.45 Å) and low (3.45 Å) resolution were collected using oscillation ranges of 1.4 and 2°, respectively. A total of 237 images were collected, producing 1 560 478 measurements. Raw data images were indexed, integrated, corrected for Lorentz polarization effects, scaled and merged together using the *HKL* program suite (Otwinowski & Minor, 1997). Complete data-collection statistics are presented in Table 1.

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Space group	$P4_22_12$
Unit-cell parameters (Å)	$a = b = 99.81$, $c = 63.99$
No. of crystals	1
Temperature (K)	100
Resolution range (Å)	30.0–1.1 (1.14–1.10)
No. of measurements	1560478 (89797)
No. of unique reflections	128064 (10622)
Mosaicity (°)	0.15
Multiplicity	12.2 (8.5)
$R_{\rm merge}$†	0.034 (0.129)
Completeness (%)	98.1 (94.8)
$I/\sigma(I)$	27.4 (13.1)
B factor from Wilson plot (Å^2)	6.2

† $R_{\rm merge} = \sum(|I_j - \langle I\rangle|)/\sum\langle I\rangle$, where I_j is the observed intensity and $\langle I\rangle$ is the statistically weighted average intensity of multiple observations of symmetry-related reflections.

3. Preliminary X-ray diffraction analysis

The systematic absences and symmetry were consistent with the space group $P4_22_12$, with unit-cell parameters $a = b = 99.81$, $c = 63.99$ Å. The real-space self-rotation function with origin removal as implemented in the program *CNS* (Brünger *et al.*, 1998) showed a single strong peak at $\kappa = 180°$, indicating twofold non-crystallographic symmetry. Thus, assuming two SRL molecules per crystallographic asymmetric unit, the Matthews coefficient (Matthews, 1968) is 2.3 Å^3 Da^{-1} and approximately 47% of the crystal volume is occupied by solvent. The presence of a dimer in the asymmetric unit is consistent with biochemical data indicating that SRL exists as a dimer at neutral or basic pH values (Swamy *et al.*, 2000).

Attempts to solve the structure by molecular-replacement methods using as a starting model structures of various plant lectins [*e.g.* pea lectin (Prasthofer *et al.*, 1989), peanut lectin (Banerjee *et al.*, 1996), *Maackia amurensis* leukoagglutinin (Imberty *et al.*, 2000)] and animal lectins [galectins 1 (Liao *et al.*, 1994), 7 (Leonidas *et al.*, 1998) and 10 (Leonidas *et al.*, 1995)] have so far failed. These failures might be attributed to the high internal symmetry of the lectin-fold motif (β-sandwich; 12–14 strands in two sheets). We are presently searching for suitable heavy-atom derivatives in order to apply the isomorphous replacement method to the SRL crystal structure determination.

We thank the staff at EMBL Hamburg Outstation for their help during data collection. Access to the EMBL Hamburg Outstation (EU Contract No. HPRI-CT-1999-00017) is greatly acknowledged. This work was supported in part by Greek GSRT through PENED-204/2001 and ENTER-EP6/2001.

References

Banerjee, R., Das, K., Ravishankar, R., Suguna, K., Surolia, A. & Vijayan, M. (1996). *J. Mol. Biol.* **259**, 281–296.

Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.

Candy, L., Peumans, W. J., Menu-Bouaouiche, L., Astoul, C. H., Van Damme, J., Van Damme, E. J., Erard, M. & Rouge, P. (2001). *Biochem. Biophys. Res. Commun.* **282**, 655–661.

Chattopadhyay, T. K., Lisgarten, J. N., Brechtel, R., Rudiger, H. & Palmer, R. A. (1999). *Acta Cryst.* D**55**, 1589–1590.

Cooper, D. N., Boulianne, R. P., Charlton, S., Farrell, E. M., Sucher, A. & Lu, B. C. (1997). *J. Biol. Chem.* **272**, 1514–1521.

Hirano, S., Matsuura, Y., Kusunoki, M., Kitagawa, Y. & Katsube, Y. (1987). *J. Biochem. (Tokyo)*, **102**, 445–446.

Imberty, A., Gautier, C., Lescar, J., Perez, S., Wyns, L. & Loris, R. (2000). *J. Biol. Chem.* **275**, 17541–17548.

Itzkowitz, S. H., Yuan, M., Montgomery, C. K., Kjeldsen, T., Takahashi, J. K., Bigbee, W. L. & Kim, Y. S. (1989). *Cancer Res.* **49**, 197–204.

Leonidas, D. D., Elbert, B. L., Zhou, Z., Leffler, H., Ackerman, S. J. & Acharya, K. R. (1995). *Structure*, **3**, 1379–1393.

Leonidas, D. D., Vatzaki, E. H., Vorum, H., Celis, J. E., Madsen, P. & Acharya, K. R. (1998). *Biochemistry*, **37**, 13930–13940.

Liao, D. I., Kapadia, G., Ahmed, H., Vasta, G. R. & Herzberg, O. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 1428–1432.

Lorea, P., Goldschmidt, D., Darro, F., Salmon, I., Bovin, N., Gabius, H. J., Kiss, R. & Danguy, A. (1997). *Melanoma Res.* **7**, 353–363.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Nagata, Y., Fukumori, F., Sakai, H., Hagiwara, T., Hiratsuka, Y., Kochibe, N. & Kobata, A. (1991). *Biochim. Biophys. Acta*, **1076**, 187–190.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Prasthofer, T., Phillips, S. R., Suddath, F. L. & Engler, J. A. (1989). *J. Biol. Chem.* **264**, 6793–6796.

Rudiger, H. (1998). *Acta Anat.* **161**, 130–152.

Ryder, S. D., Jacyna, M. R., Levi, A. J., Rizzi, P. M. & Rhodes, J. M. (1998). *Gastroenterology*, **114**, 44–49.

Shamsuddin, A. M., Tyner, G. T. & Yang, G. Y. (1995). *Cancer Res.* **55**, 149–152.

Sharon, N. & Ofek, I. (2000). *Glycoconj. J.* **17**, 659–664.

Springer, G. F., Desai, P. R. & Banatwala, I. (1975). *J. Natl Cancer Inst.* **54**, 335–339.

Springer, G. F., Desai, P. R., Murthy, M. S., Tegtmeyer, H. & Scanlon, E. F. (1979). *Prog. Allergy*, **26**, 42–96.

Swamy, B. L., Hedge, G. V., Naik, R. S. & Inamdar, S. R. (2000). *Lect. Biol. Biochem. Clin. Biochem.* **14**, 45–55.

Wu, A. M. (1984). *Mol. Cell. Biochem.* **61**, 131–141.

Wu, A. M., Wu, J. H., Tsai, M.-S., Hedge, G. V., Inamdar, S. R., Swamy, B. M. & Herp, A. (2001). *Life Sci.* **69**, 2039–2050.

Yu, L., Fernig, D. G., Smith, J. A., Milton, J. D. & Rhodes, J. M. (1993). *Cancer Res.* **53**, 4627–4632.