Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

A legume lectin from the bark of *Robinia pseudoacacia* crystallizes in two crystal forms: preliminary diffraction analyses

A. Rabijns,[a] C. Verboven,[a] E. J. M. Van Damme,[b] W. J. Peumans[b] and C. J. De Ranter[a]*

[a]Laboratorium voor Analytische Chemie en Medicinale Fysicochemie, Faculteit Farmaceutische Wetenschappen, K. U. Leuven, Van Evenstraat 4, B-3000 Leuven, Belgium, and [b]Laboratorium voor Fytopathologie en Plantenbescherming, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, K. U. Leuven, W. de Croylaan 42, 3001 Leuven, Belgium

Correspondence e-mail: camiel.deranter@farm.kuleuven.ac.be

Received 8 June 2000
Accepted 5 September 2000

The A_4 isoform of the bark lectin RPbAI from *Robinia pseudoacacia* has been crystallized in two different crystal forms. Crystal form I grows in the $P2_1$ space group with two tetramers in the asymmetric unit, whereas crystal form II grows in the $I222$ space group with a monomer in the asymmetric unit. Data sets were collected for both crystal forms to resolution limits of 2.55 and 1.81 Å, respectively, which will allow successful structure determinations.

1. Introduction

The legume lectins form a large family of carbohydrate-binding proteins occurring in seeds and various vegetative tissues of plants belonging to the *Leguminosae* family (Sharon & Lis, 1990; Van Damme *et al.*, 1998). Despite the strong similarity in their amino-acid sequences and tertiary structures, legume lectins exhibit marked differences in carbohydrate-binding specificity and quaternary structure (Loris *et al.*, 1998; Bouckaert *et al.*, 1999). Structural analysis pinpoints these differences to be in the binding sites and at the interfaces between the monomers. Though numerous legume lectins have already been studied by X-ray crystallography (Sharma & Surolia, 1997), many other members of this lectin family with interesting carbohydrate-binding properties and/or biological activities still await structural analysis at the atomic level.

An interesting group of poorly studied legume lectins are the carbohydrate-binding proteins from the bark of *R. pseudoacacia* (black locust). Biochemical and molecular studies demonstrated that black locust bark expresses three genes encoding three different lectin polypeptides. One of these genes encodes lectin polypeptide C (26 kDa) that associates exclusively into homotetramers (called *R. pseudoacacia* bark agglutinin II or RPbAII). Two other genes encode lectin polypeptides A and B (31.5 and 29 kDa, respectively), which associate in all possible combinations to produce five different tetrameric isolectins (A_4, A_3B, A_2B_2, AB_3 and B_4; Van Damme *et al.*, 1995). The mixture of these five isoforms is called *R. pseudoacacia* bark agglutinin I (RPbAI; Van Damme *et al.*, 1995). It is worth mentioning here that the isolectin composition of RPbAI is reminiscent of that of the *Phaseolus vulgaris* agglutinin, which is also a mixture of five isolectins that find their origin in the association of E and L subunits in tetramers (Feldsted *et al.*, 1977; Leavitt *et al.*, 1977). RPbAI exhibits interesting biological activities. When orally administered to rats, the lectin binds to the sugar moieties on the epithelial cells of the small intestine and induces hyperplastic growth by stimulating crypt-cell proliferation (Pusztai *et al.*, 1993). Most probably, this particular activity contributes to the presumed defensive role of the bark lectin against herbivorous animals (Peumans & Van Damme, 1995). To corroborate both the mitogenic activity and defensive role of the black locust bark lectin it would be helpful to determine the carbohydrate-binding specificity and three-dimensional structure of the binding sites. Therefore, crystallographic studies were undertaken to determine the structure of RPbAI. Since the complex mixture of isoforms was not suited for the envisaged structural studies, we focused on the homotetramer consisting of four 31.5 kDa subunits (A_4).

2. Experiments and results

2.1. Purification

RPbAI was purified from the bark of *R. pseudoacacia* by affinity chromatography on immobilized fetuin as described elsewhere (Van Damme *et al.*, 1995). Isoform A_4 was isolated from a total preparation of RPbAI by ion-exchange chromatography using a Mono-S column (Amersham Pharmacia Biotech, Uppsala, Sweden) as described previously (Van Damme *et al.*, 1995).

2.2. Crystallization

To determine initial crystallization conditions, Hampton Crystal Screen I (Jancarik & Kim, 1991) was applied to a sample of RPbAI dissolved at a concentration of 100 mg ml^{-1} in

Table 1
Data collection and reduction statistics.

Values in parentheses are for data in the highest resolution shell.

	RPbAI form I	RPbAI form II
Space group	$P2_1$	$I222$
Wavelength used (Å)	1.018	0.9091
Resolution limit (Å)	2.55 (2.59–2.55)	1.81 (1.84–1.81)
Total observations	143727	97833
Unique reflections	59072 (3138)	26646 (1330)
Redundancy	2.43	3.67
Completeness of all data (%)	90.1 (95.8)	99.4 (100.0)
Completeness of data (%) ($I > 3\sigma$)	75.8 (50.8)	82.5 (52.9)
Mean I/σ	20.1 (5.2)	22.4 (4.4)
R_{sym} value (%)	3.5 (11.9)	4.9 (25.8)

0.1 *M* Tris buffer pH 8.0. Crystallization was carried out by the hanging-drop vapour-diffusion technique at 277 K using Linbro multiwell tissue-culture plates. Each well was filled with 700 µl of reservoir solution; drops consisting of 1.5 µl protein solution and 1.5 µl reservoir solution were placed on cover slips and set to equilibrate against these reservoir solutions. Several crystallization conditions from this screening yielded crystals, but only the two crystal forms from the following conditions were further investigated. Crystal form I (Fig. 1*a*) was grown in 0.1 *M* sodium cacodylate buffer pH 6.5 containing 0.2 *M* magnesium acetate and 20%(*w*/*v*) polyethylene glycol 8000, whereas crystal form II (Fig. 1*b*) was grown in 0.2 *M* ammonium sulfate containing 30%(*w*/*v*) polyethylene glycol 4000 pH 4.8.

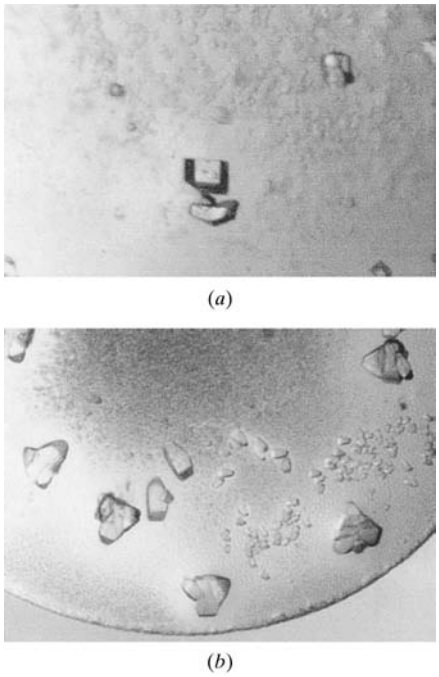

Figure 1
Photographs showing crystals of RPbAI A_4 form I (*a*) and form II (*b*). The typical crystal size for both crystal forms is approximately 0.2 × 0.2 × 0.2 mm.

2.3. Data collection and processing

2.3.1. Crystal form I. Diffraction data were collected at 100 K using synchrotron radiation (I711 beamline, Lund, Sweden) after soaking the crystal for 2 min in a solution of the mother liquor with 25% sucrose. The crystals grow in space group $P2_1$ and diffract to a resolution of 2.55 Å, with unit-cell parameters a = 99.04, b = 108.87, c = 101.06 Å, β = 113.8°. All data processing was performed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The final data set is 90.1% complete and is characterized by an R_{sym} of 3.5%. Further statistics are summarized in Table 1. According to Matthews coefficient calculations (Matthews, 1974), the asymmetric unit should consist of two tetramers. However, the self-rotation function calculated in *X-PLOR* (Brünger *et al.*, 1987) shows only a single peak, suggesting that both tetramers are positioned in the same orientation. This is in good accordance with the native Patterson function calculated in the program *PHASES* (Furey & Swaminathan, 1990), which displayed a peak at position u = 0.5, v = 0.27, w = 0.0 (Fig. 2), indicating a translational relationship between the two tetramers. A clear molecular-replacement solution was found with the structure of the tetrameric legume lectin phytohaemagglutinin-L (PDB code 1fat; Hamelryck *et al.* (1996) as the search model. A translation function using two molecules with an identical orientation confirmed the results from the native Patterson function. A translation of 0.5, 0.27, 0.0 was indeed found between the two tetramers. Refinement of the structure is now under way.

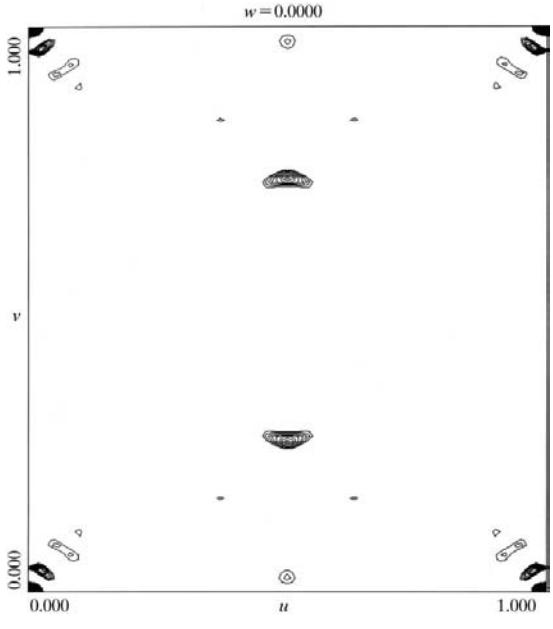

Figure 2
w = 0 section of the native Patterson map from RPbAI form I crystals shows a clear peak at u = 0.5, v = 0.27, w = 0.0 indicating translational symmetry between the two NCS-related RPbAI molecules.

2.3.2. Crystal form II. A crystal from RpbAI form II was exchanged into a cryoprotectant consisting of 30%(*w*/*v*) polyethylene glycol 4000, 0.2 *M* ammonium sulfate and 20% sucrose. The crystal was flash-frozen in a stream of nitrogen held at 100 K and data were collected at the X11 beamline of the DESY synchrotron (Hamburg, Germany). All data processing was performed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The space group could be assigned to be either $I222$ or $I2_12_12_1$, with unit-cell parameters a = 64.08, b = 75.85, c = 118.09 Å. A 99.4% complete data set was collected to 1.8 Å resolution with an R_{sym} value of 4.9% (Table 1). The calculated Matthews coefficient of 2.27 $Å^3\,Da^{-1}$ suggests that the asymmetric unit consists of a single monomer (Matthews, 1974). A molecular-replacement solution calculated in *X-PLOR* (Brünger *et al.*, 1987) was easily found using the A monomer of phytohemagglutinin-L (PDB code 1fat; Hamelryck *et al.*, 1996) as a search model. A subsequent translation search confirmed that the actual space group is $I222$. Hence, through combination of symmetry-related monomers, tetramers with the same orientation as those in 1fat are formed. Refinement of the structure is now under way.

In conclusion, two different crystal forms of isolectin A_4 of RPbAI were obtained. A quality data set was collected from each crystal form, which will allow us to solve the structures.

AR and EJMVD are Postdoctoral Research Fellows of the Fund for Scientific Research-Flanders (Belgium) and WJP is a Research Director of this fund. CV is a Postdoctoral Research Fellow of the K. U. Leuven Research Fund (OT/98/38). Walter Jottier is acknowledged for technical assistance during in-house data collection. We thank the

beamline scientists at MAX-Lab and DESY for technical support and the European Union for support of the work at EMBL Hamburg through the HCMP to Large Installations Project, contract No. CHGE-CT93-0040. This work was supported in part by grants from the Katholieke Universiteit Leuven (OT/98/17) and the Fund for Scientific Research-Flanders (grant G.0223.97).

References

Bouckaert, J., Hamelryck, T., Wyns, L. & Loris, R. (1999). *Curr. Opin. Struct. Biol.* **9**, 572–577.

Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). *Science*, **235**, 458–460.

Feldsted, R. L., Egorin, M. J., Leavitt, R. D. & Bachur, N. R. (1977). *J. Biol. Chem.* **252**, 2967–2971.

Furey, W. & Swaminathan, S. (1990). Am. Crystallogr. Assoc. Meet. Abstr. 18, Abstract PA33, p. 73.

Hamelryck, T. W., Dao Thi, M. H., Poortmans, F., Chrispeels, M. J., Wyns, L. & Loris, R. (1996). *J. Biol. Chem.* **271**, 20479–20485.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Leavitt, R. D., Feldsted, R. L. & Bachur, N. R. (1977). *J. Biol. Chem.* **252**, 2961–2966.

Loris, R., Hamelryck, T., Bouckaert, J. & Wyns, L. (1998). *Biochim. Biophys. Acta*, **1383**, 9–36.

Matthews, B. W. (1974). *J. Mol. Biol.* **82**, 513–526.

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

Peumans, W. J. & Van Damme, E. J. M. (1995). *Plant Physiol.* **109**, 347–352.

Pusztai, A., Ewen, S. W. B., Grant, G., de Carvalho, A. F. F., Stewart, J. C., Peumans, W. J., Van Damme, E. J. M. & Bardocz, S. (1993). *Lectins: Biology, Biochemistry, Clinical Biochemistry*, Vol. 9, edited by J. Basu, M. Kundu & P. Chakrabarty, pp. 131–138. New Delhi: Wiley Eastern Limited.

Sharma, V. & Surolia, A. (1997). *J. Mol. Biol.* **267**, 433–445.

Sharon, N. & Lis, H. (1990). *FASEB J.* **4**, 3198–3208.

Van Damme, E. J. M., Barre, A., Smeets, K., Torrekens, S., Van Leuven, F., Rougé, P. & Peumans, W. J. (1995). *Plant Physiol.* **107**, 833–843.

Van Damme, E. J. M., Peumans, W. J., Barre, A. & Rougé, P. (1998). *Crit. Rev. Plant Sci.* **17**, 575–692.