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Crystallization and preliminary crystallographic analysis of winged bean acidic lectin

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The acidic lectin (WBAII) from the winged bean (*Psophocarpus tetragonolobus*) binds to the H-antigenic determinant on human erythrocytes and to the T-antigenic disaccharide Gal-β1,3-GalNAc. Two crystal forms of WBAII were obtained in the presence of methyl-α-D-galactose. Form I belongs to space group $R3$ with unit-cell dimensions $a = b = 182.11$, $c = 44.99$ Å and has one dimer in the asymmetric unit. Form II belongs to space group $C2$ with unit-cell dimensions $a = 135.36$, $b = 127.25$, $c = 139.98$ Å, $\beta = 95.9^\circ$ and has four dimers in the asymmetric unit. Intensity data were collected to 3.0 Å and to 3.5 Å from crystals of form I and II, respectively. The structures were solved by the molecular-replacement method using the coordinates of the basic form of winged bean lectin.

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1. Introduction

Lectins are multivalent carbohydrate binding proteins of non-immune origin with a high degree of specificity for cell-surface carbohydrates. They are present in a variety of organisms, performing diverse biological functions involving cellular recognition and interaction (Sharon & Lis, 1989). Legume lectins, the most abundant of all classes of lectins, are widely studied in terms of their structural and biological characterization. Here, we report the crystallization and preliminary X-ray crystallographic analysis of a legume lectin, WBAII, isolated from the seeds of winged bean (*Psophocarpus tetragonolobus*) at an acidic pI of 5.5. We recently reported the structure of the basic form of winged bean lectin, WBAI (Prabu *et al.*, 1998), with pI > 9.0. Both of these lectins are dimeric proteins (M_r = 55 kDa), with about 60% sequence identity. They show a high affinity for methyl-α-D-galactose at the monosaccharide level (Kortt, 1984, 1985), but have entirely different affinities for oligosaccharides. WBAI binds to A and B antigenic determinants of erythrocytes but fails to recognize O-type antigens (Kortt, 1984; Khan *et al.*, 1986; Matsuda *et al.*, 1989). WBAII, on the other hand, shows no affinity for A and B types but binds strongly to O-group erythrocytes. In addition, WBAII can also bind to T-antigen, the tumour-associated disaccharide Gal-β1,3-GalNAc (Patanjali *et al.*, 1988). Thermodynamic studies on ligand binding to WBAII revealed its stronger affinity towards terminally monofucosylated sugars compared with difucosylated or internally fucosylated oligosaccharides (Acharya *et al.*, 1990). The present analysis of WBAII, taken up as part of a programme on structural investigations of lectins (Banerjee *et al.*, 1994, 1996; Sankaranarayanan *et al.*, 1996; Chandra *et al.*, 1997; Prabu *et al.*, 1998), should provide the structural basis for the unique specificity of WBAII as well as reasons for the differences in the carbohydrate-binding affinities of WBAI and WBAII.

2. Materials and methods

WBAII was isolated and purified as described previously (Kortt, 1985; Patanjali *et al.*, 1988). Crystals were grown in the presence of methyl-α-D-galactose. Crystal form I was grown by the batch method by mixing 35 µl of a solution containing 25 mg ml^{-1} protein and a 20-fold molar excess of the sugar in phosphate buffer pH 7.2 with 23 µl of 30%(w/v) PEG 4000. Form II crystals were grown by the hanging-drop method. The drops contained 5 µl of 15 mg ml^{-1} protein, a 20-fold molar excess of the sugar in phosphate buffer pH 7.2 and 2 µl of 35%(w/v) PEG 4000. 1 ml of 35%(w/v) PEG 4000 was used as the reservoir solution. Both the crystal forms grew as rectangular plates of size 0.8 × 0.5 × 0.1 mm within a week. Intensity data were collected using a MAR Research imaging-plate system and processed using *MAR-XDS* (Kabsch, 1988) and *DENZO* (Otwinowski, 1993).

3. Results and discussion

Details of data collection and crystallographic characterization of both the forms of WBAII are given in Table 1. The solvent contents of both forms are within the range normally observed for proteins (Matthews, 1968). Molecular-replacement studies were carried out for both crystal forms using *AMoRe*

Table 1
Data for crystals of WBAII.

	Form I	Form II
Space group	*R*3	*C*2
Cell parameters		
a (Å)	182.11	135.36
b (Å)	182.11	127.25
c (Å)	44.99	139.98
β (°)	—	95.9
Number of dimers per asymmetric unit	1	4
Solvent content (%)	53.8	55.7
Data collection		
Resolution (Å)	3.0	3.5
Completeness (%)	99	81
$R_{\rm merge}$† (%)	8.8	9.4

† $R_{\rm merge} = \sum I_i - |\langle I\rangle|/\sum\langle I\rangle$, where I_i is the intensity of an individual reflection and $\langle I\rangle$ is the mean intensity of that reflection.

(Navaza, 1994), and unique solutions were obtained (Table 2) using the coordinates of the dimeric basic lectin from winged bean (Prabu *et al.*, 1998; Protein Data Bank code 1WBL) as the search model. Detailed analysis of form I has been initiated as the resolution and quality of the intensity data of this form are much superior to those of form II. Electron-density maps calculated after a few cycles of rigid-body refinement followed by positional refinement using *X-PLOR* (Brünger, 1992) showed clear density for many side chains that were absent in the initial model and also for the bound carbohydrate. Further refinement and analysis are in progress.

Table 2
Results of molecular replacement for WBAII.

Gradual improvement of the correlation coefficient and the *R* factor by adding the contribution of each dimer to the already positioned models is shown for form II.

	α (°)	β (°)	γ (°)	*x*	*y*	*z*	Correlation coefficient (%)	*R* factor (%)
Form I	63.4	159.4	313.1	0.021	0.444	0.000	58.2	40.7
Form II	111.6	112.2	136.5	0.349	0.000	0.010	23.4	49.9
	110.8	21.6	318.1	0.374	0.379	0.189	31.2	47.6
	105.1	65.2	137.5	0.091	0.409	0.630	39.3	44.9
	114.5	158.1	323.8	0.621	0.530	0.321	47.9	41.9

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