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Crystallization and preliminary crystallographic studies on the mannose-specific lectin from garlic

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Abstract

A mannose-specific agglutinin from garlic (*Allium sativum*) which forms part of a well conserved super-family of bulb lectins has been purified and crystallized by the hanging-drop vapour-diffusion technique, by equilibrating with a 20% solution of PEG 8000 in the presence of α-D-mannose. Crystals of the dimeric form of this protein are monoclinic *C*2 with unit-cell dimensions $a = 203.2$, $b = 43.8$, $c = 79.3$ Å and $\beta = 112.4^\circ$ and have two dimers in the asymmetric unit. Data have been collected to 2.4 Å resolution and the structure solved by molecular replacement using the coordinates of the snowdrop lectin as the search model.

1. Introduction

Lectins are carbohydrate-binding proteins of non-immune origin found abundantly in a wide range of organisms and have important biological roles ranging from cell-surface recognition to defense against pathogens (Sharon, 1993). Recently there has been considerable interest in the non-seed lectins from the monocotyledonous families of Amaryllidaceae, Alliaceae, Liliaceae and Orchidaceae, which are all grouped into a super-family of bulb lectins (Van Damme, Goldstein & Peumans, 1991). The only known structures from this group are those from snowdrop (Hester, Kaku, Goldstein & Wright, 1995) and amaryllis bulbs (Chantalat, Wood, Rizkallah & Reynolds, 1996) and are similar to each other. They represent a new polypeptide fold comprising three anti-parallel four-stranded β-sheets arranged as a 12-stranded β-barrel. The bulb lectins, owing to their high mannose specificity, exhibit unique biological properties such as selective inhibitory activity against HIV and other retroviruses and selective agglutination of rabbit but not human erythrocytes (Balzarani *et al.*, 1991; Pusztai *et al.*, 1993). These properties which may lead to useful applications, are presumably due to their ability to bind envelope glycoproteins which are known to be highly glycosylated with oligomannosides (Hester & Wright, 1996; Kaku & Goldstein, 1991). In this context, the structure of the garlic agglutinin, which we have taken up for X-ray analysis as part of a programme of structural studies on lectins (Banerjee *et al.*, 1994, 1996; Sankaranarayanan *et al.*, 1996, 1993), should provide further insights into the structural requirements for oligomannosaccharide recognition. Lectins, in general, have interesting and diverse subunit associations (Drickamer, 1995; Banerjee *et al.*, 1996) and provide a useful means by which to understand the structural basis for quaternary association in oligomeric proteins. Also in this context, the structure of garlic lectin is expected to reveal interesting features of subunit association, since it is stabilized into a functional dimer as compared with the tetrameric forms of the other two bulb lectins whose structures are known.

2. Materials and methods

The garlic bulbs were obtained locally and the mannose-specific lectin was purified on an affinity matrix containing mannose–Sepharose as reported by Van Damme *et al.* (1992). The lectin has a molecular weight of 25 kDa and runs as two bands very close to each other on a sodium dodecyl sulfate gel indicating that it is a hetero-dimer. Crystals were grown at 294 K using the hanging-drop vapour-diffusion method with a drop that contained 8 µl of 5.5 mg ml^{-1} protein in 20 m*M* phosphate-buffered saline at pH 7.0 and 2 µl of the reservoir solution made up of 20%(*w*/*v*) polyethylene gycol 8000 in the same buffer. Good bipyramidal crystals were noticed within a week with average dimensions of 0.8 × 0.45 × 0.2 mm. X-ray data were collected on a MAR Research imaging-plate detector and processed using the *MAR-XDS* suite of programs (Kabsch, 1988).

3. Results and discussion

Data extended up to a resolution of 2.4 Å with about 98% completeness. The crystals belong to the monoclinic *C*2 space group with unit-cell dimensions $a = 203.2$, $b = 43.8$, $c = 79.3$ Å and $\beta = 112.4^\circ$. The solvent content of these crystals, assuming two dimers in the asymmetric unit was calculated to be 62%, which lies in the range expected for globular proteins (Matthews, 1968). The sequence of the garlic lectin is highly homologous, with 48% identity, to that of the snowdrop lectin, which was therefore used as a search model in molecular replacement (PDB entry 1MSA, Hester *et al.*, 1995; Bernstein *et al.*, 1977). The snowdrop lectin is a tetramer with two kinds (*A*–*B* and *A*–*D*) of subunit association. Molecular replacement carried out using *AMoRe* (Navaza, 1994) resulted in a unique solution with a correlation coefficient of 51% and an initial *R* factor of 46% (Table 1) when the *A*–*D* type of the dimer of the snowdrop lectin was used as the search model but not with the *A*–*B* type of dimer. Packing considerations indicated the solution to be acceptable. The same solution was also obtained when only a monomer of the snowdrop lectin was used as a search model confirming the dimerization in the garlic lectin to be of the *A*–*D* type of the snowdrop lectin. The electron-density maps calculated after a rigid-body refinement followed by a few cycles of positional refinement using *X-PLOR* (Brünger, 1993) clearly show the location of one mannose molecule in every monomer and also the differences in length at the C termini of the two monomers in each dimer. Further refinement of the structure is in progress.

The data were collected on an image-plate facility supported by the Departments of Science & Technology and Biotechnology, Government of India, and the computations were performed at the Supercomputer Education and Research

Table 1. *Molecular replacement results for the two dimers in the asymmetric unit*

CC and Rf in the first line are for the first dimer only while those on the second line are for both the dimers.

	α (°)	β (°)	γ (°)	x	y	z	CC (%)	Rf (%)
(i)	78.3	5.2	132.9	0.418	0.005	0.223	27.1	52.0
(ii)	2.8	152.5	13.5	0.702	0.282	0.562	50.8	46.1

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