Acta Cryst. (1998). D**54**, 90–92

Crystallization and preliminary structural studies of *Scilla campanulata* lectin complexed with α1–6 mannobiose

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(Received 18 February 1997; accepted 12 May 1997)

Abstract

Recent work has shown that *Scilla campanulata* agglutinin from bluebell bulbs has a strong affinity for $\alpha(1,3)$- and $\alpha(1,6)$-linked mannosyl residues and possesses moderate antiretroviral activity. This lectin has been crystallized by the hanging-drop method of vapour diffusion complexed with the disaccharide mannose-α1,6-D-mannose. The crystals are in the space group $P2_12_12$ with unit-cell dimensions a = 70.63, b = 92.79 and c = 47.25 Å, and with a dimer in the asymmetric unit. The crystals diffract X-rays to beyond 1.5 Å resolution at 277 K and are stable in an X-ray beam. Data to 1.6 Å resolution have been collected using a MAR image-plate system at a synchrotron source and the structure of the complex has been solved by the molecular replacement method.

1. Introduction

Lectins are a heterogeneous group of proteins which have the ability to bind carbohydrates specifically and reversibly (Sharon & Lis, 1989). They are oligomeric proteins with one or more saccharide-binding sites per subunit and may occur in all types of living organisms but are especially ubiquitous within the plant kingdom. The lectins from plant sources have been most widely investigated and based on a substantial body of biochemical and molecular biological knowledge they have been classified into four main families of structurally related proteins, although new families are beginning to emerge *e.g.* the lectins from *Artocarpus integrifolia* seeds (Sankaranarayanan *et al.*, 1996) and *Calystogia sepium* rhizomes (Van Damme, Barre, Verhaert, Rougé & Peumans, 1996) appear to form a fifth family. Examples from three of these families: the chitin-binding cereal and grass lectins (Raikhel, Lee & Broekaert, 1993), the legume lectins (Van Driessche, 1988; Sharon & Lis, 1990) and the type II ribosome-inactivating proteins (Barbieri, Batelli & Stirpe, 1993) have been extensively characterized over the last 25 years. More recently the fourth well defined family, the monocot mannose-specific lectins from the bulbs, corms, rhizomes and tubers of perennial plants, have been studied in some detail with respect to their biochemical properties, carbohydrate-binding specificity and chemical structure (Cammue, Peeters & Peumans, 1986; Van Damme, Allen & Peumans, 1987; Kaku, Goldstein & Oscarson, 1991; Kaku & Goldstein, 1992; Van Damme, Smeets & Peumans, 1995).

The crystal structures of *Galanthus nivalis* agglutinin (GNA) from snowdrop bulbs complexed with Me-α-D-mannose (Hester, Kaku, Goldstein & Wright, 1995; Hester & Wright, 1996), with mannose-α1,3-D-mannose-α-*O*Me (Hester & Wright, 1996) and with Man1,6(α1,3-Man) Man-α1,6-(α1,3-Man) Man (Wright & Hester, 1996) have been reported. The X-ray structure of a second monocot lectin *Hippeastrum hybrid* agglutinin (HHA) from amaryllis bulbs has been solved in complex with α-D-mannose (Wood, 1995; Chantalat, Wood, Rizkallah & Reynolds, 1996). The only native monocot lectin structure known to date is that of *Scilla campanulata* agglutinin (SCA) from bluebell bulbs (Wright *et al.*, 1996). All three of these proteins have a similar tertiary structure which is completely different from that found in the other plant lectin families and contain three potential saccharide binding sites (on subdomains I, II and III) per subunit (Hester *et al.*, 1995; Hester & Wright, 1996; Chantalat *et al.*, 1996). In this paper we report the crystallization and preliminary X-ray crystallographic studies of SCA complexed with α1–6 mannobiose at 1.6 Å spacing, the highest resolution of a lectin–saccharide complex published to date.

2. Crystallization

SCA was purified according to the method previously described (Wright *et al.*, 1996). For crystallization the protein was concentrated to 11 mg ml^{-1} in 10 mM 2,3-diaminopropane using a mannose–Sepharose 4B affinity column. Crystallization trials were undertaken using the hanging-drop method of vapour diffusion (Ducruix & Giegé, 1992) with Linbro trays. Crystals of the SCA–dimannoside complex were obtained from a 20 µl hanging drop containing 5.5 mg ml^{-1} protein, 10 mM Man-α1,6-D-Man, 5 mM 2,3-diaminopropane, and 600 mM phosphate-buffered saline, equilibrated against 1 ml of 55–75% saturated ammonium sulfate in the reservoir, in the pH range 3–7. Crystals grew as flat diamond plates, up to 0.5 × 0.5

Fig. 1. Crystal of the lectin from bluebell (*Scilla campanulata*) bulbs complexed with α1–6 mannibiose. The crystal is approximately 0.5 × 0.5 × 0.2 mm.

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Table 1. *Data-collection statistics of the Scilla lectin–α1–6 mannobiose complex*

Resolution range (Å)	R_{merge}†	N_{meas}	N_{unique}	% Complete	Multiplicity	%>3 e.s.d.'s
20.00–6.03	0.069	3654	808	92.3	4.5	97.8
6.03–4.32	0.07	6866	1403	99	4.9	99.1
4.32–3.55	0.074	8793	1769	99.3	5	99
3.55–3.08	0.079	10274	2078	99.7	4.9	97.6
3.08–2.76	0.084	11676	2342	99.9	5	96.8
2.76–2.52	0.087	12887	2569	99.9	5	94.9
2.52–2.34	0.093	13974	2794	100	5	93.7
2.34–2.19	0.102	14832	2962	99.6	5	93.2
2.19–2.06	0.109	15764	3163	100	5	91.2
2.06–1.96	0.123	16649	3321	99.8	5	88.8
1.96–1.87	0.148	17788	3514	100	5.1	82
1.87–1.79	0.189	18494	3629	99.6	5.1	75
1.79–1.72	0.247	19437	3786	99.9	5.1	66.9
1.72–1.66	0.27	16291	3652	93.2	4.5	58.8
1.66–1.60	0.326	15455	3684	90.0	4.2	48.9
Overall	0.088	202834	41474	98.2	4.9	81.9

† $R_{merge} = \sum_i \sum_j (I_{ij} - \langle I_j \rangle) / \sum_i I_{ij}$.

× 0.2 mm in size at 293 K after approximately 30 d (Fig. 1). The best crystals of the SCA complex were obtained using 70% saturated ammonium sulfate at pH 4.7.

3. X-ray analysis

Preliminary X-ray images were measured on station PX 9.6 at the CCLRC Daresbury Laboratory Synchrotron Radiation Source (SRS) at 293 K and indicated that the crystals of the SCA–dimannoside complex diffracted to beyond 1.8 Å resolution. Analysis of the data using the cell reduction package *REFIX* (Kabsch, 1988) showed that the crystals have an orthorhombic lattice.

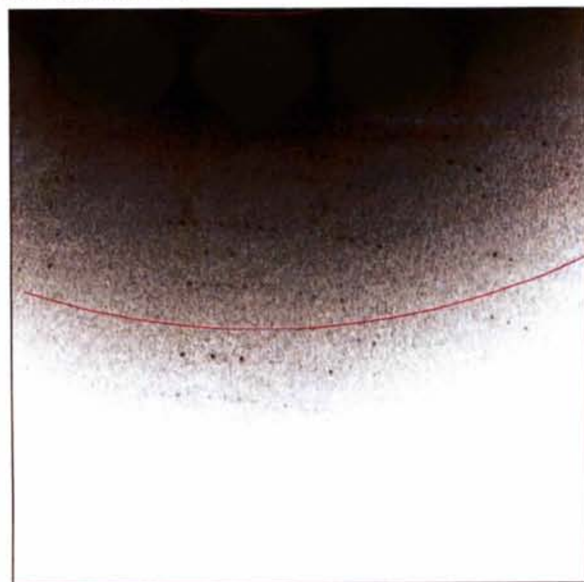

Fig. 2. Section of a 2° oscillation image from a crystal of the *Scilla* lectin–α1–6 mannobiose complex recorded at the SRS, Daresbury Laboratory on a MAR Research imaging plate. The resolution at the edge of the image corresponds to 1.5 Å. The outer resolution ring is at 1.55 Å.

Approximately 90° of data were collected at 277 K from two crystals at station PX 7.2 at the SRS to 1.6 Å resolution, using the rotation method of data collection, with 2° rotations per frame (Fig. 2). Diffraction data were recorded on the MAR Research image plate at an X-ray wavelength of 1.488 Å to give 202 834 observations of which 41 474 were independent. Use of *REFIX* gave cell parameters of $a = 70.63$, $b = 92.79$ and $c = 47.25$ Å, in space group $P2_12_12$. Gel-filtration chromatography of SCA has established that this protein is a tetramer (Wood, Allen, Wright & Reynolds, 1996). The molecular weight, M_r, of an SCA subunit is 14 kDa and the cell volume is 3.097×10^5 Å^3. Assuming that there is a tetramer in the asymmetric unit, the V_m value is 1.383 Å^3 Da^{-1} which is outside the observed range (1.7–3.5 Å^3 Da^{-1}) given by Matthews (1968), whereas the value for a dimer, 2.765 Å^3 Da^{-1}, falls within the normal range observed for other globular protein crystals and gives a predicted solvent content of 56%. Thus, the SCA tetramers must lie on the twofold axis parallel to the c direction of the unit cell.

The 1.6 Å data set was processed using the *MOSFLM* (Leslie, 1992) and *CCP4* (Collaborative Computational Project, Number 4, 1994) software packages and gave an overall merging R factor of 8.8% with 98% completeness from 20 to 1.6 Å resolution. Detailed data-collection statistics are given in Table 1.

A self-rotation function was calculated on data from 8 to 4 Å resolution with an integration radius of 20 Å using the program *POLARRFN* (W. Kabsch, unpublished work; *CCP4*) and gave two strong peaks on the $\kappa = 180°$ section corresponding to the twofold non-crystallographic axes, which formed an orthogonal set with the crystallographic dyad. Thus, the SCA tetramer has approximate 222 symmetry. The *AMoRe* program (Navaza, 1994) from *CCP4* was used for the molecular replacement calculations. The amaryllis lectin coordinates (Chantalat *et al.*, 1996) were used to conduct the rotation and translation searches, using initially a monomer containing all residues with side chains, and later a dimer, as a search model. Rigid-body refinement of the top solution gave an R factor of 34% with a correlation coefficient of 82% and confirmed the presence of a dimer in the asymmetric unit. Inspection of the crystal packing using the program *O* (Jones & Kjeldgaard, 1993) revealed no bad steric contacts between the SCA dimers

in the unit cell. A difference Fourier map computed with the partially refined structure showed clearly defined electron density for the bound disaccharide at the high-affinity binding site on subdomain I (but not II and III) in each of the independent subunits. Further refinement of this structure is under way.

This work was supported by grants from The Leverhulme Trust (F/754/A) and the Mizutani Foundation for Glycoscience. We thank CCLRC Daresbury Laboratory for the provision of beam time and other facilities and Liverpool John Moores for continuing support. We would like to thank the support staff of stations 7.2 and 9.6 at CCLRC Daresbury Laboratory. Professor W. J. Peumans and Dr E. J. M. Van Damme, Catholic University of Leuven, for advice and extensive discussions at the early stages of this work, Professor E. H. Evans and Professor P. F. Lindley for their encouragement.

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