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# *Scilla campanulata* agglutinin crystallized in complex with the trimannoside α-D-Man-(1→6)-[α-D-Man-(1→3)]-α-D-Man

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**Abstract**

The monocot mannose-specific lectin, *Scilla campanulata* agglutinin (SCA), from bluebell bulbs has a strong affinity for α1,3- and α1,6-linked mannosyl residues. SCA has been co-crystallized with the trisaccharide α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranoside {α-D-Man-(1→6)-[α-D-Man-(1→3)]-α-D-Man}, the core structure of biantennary *N*-linked oligosaccharides. Crystals of the complex were obtained by the hanging-drop vapour-diffusion technique. A complete data set to 2.5 Å resolution has been collected at 100 K, using a MAR image-plate system at a synchrotron source, from crystals which belong to the space group *C*2 with unit-cell dimensions $a$ = 99.38, $b$ = 119.86, $c$ = 77.10 Å and $\beta$ = 105.56°. Use of a CCD detector with cryo-cooled crystals improved the resolution to 2.3 Å. A molecular replacement solution, with the 2.5 Å data set, using the native SCA as a search model was obtained, with six subunits per asymmetric unit.

## 1. Introduction

Lectins are a structurally diverse class of oligomeric proteins which have the capability to serve as recognition molecules, binding specifically and reversibly to carbohydrates. These proteins are found in all types of living organisms but the plant lectins have been most widely studied (Sharon & Lis, 1989). The majority of plant lectins can be classified into four main families: (*a*) legume lectins (Sharon & Lis, 1990), (*b*) cereal and grass lectins (Raikhel *et al.*, 1993), (*c*) type 2 ribosome-inactivating proteins (Barbieri *et al.*, 1993), and (*d*) monocot bulb lectins (Van Damme *et al.*, 1995). Until quite recently most structural studies on plant lectins have been focused on the dicot legume lectins and the monocot cereal lectins (Rini, 1995). Since 1995, crystallographic studies on a number of monocot mannose-binding lectins and their carbohydrate complexes have been undertaken. The structure of the Amaryllidaceae lectin *Galanthus nivalis* agglutinin (GNA) complexed with methyl-α-D-mannose was the first bulb lectin to be reported (Hester *et al.*, 1995). Subsequently, the crystal structure of GNA in complex with a dimannoside, α-D-Man-(1→3)-D-Man-α-OMe (Hester & Wright, 1996) and a branched mannopentose, α-D-Man–(1→6)-[α-D-Man-(1→3)]-α-D-Man-(1→6)-[α-D-Man-(1→3)]-α-D-Man (Wright & Hester, 1996) have been determined. The X-ray structure of *Hippeastrum* hybrid agglutinin (HHA), a second bulb lectin from the Amaryllidaceae family, has been solved in complex with α-D-mannose (Wood, 1995; Chantalat *et al.*, 1996). More recently, a third lectin from the Amaryllidaceae family, *Narcissus pseudonarcissus* agglutinin (NPA), in complex with α-D-Man-(1→3)-α-D-Man has been determined (Rizkallah *et al.*, unpublished work). We have recently solved and refined the native structure of the Lilliaceae lectin *S. campanulata* agglutinin (SCA; Wright *et al.*, 1996; Wood, *et al.*, unpublished work) as well as two complexes: with α-D-mannose (Wright *et al.*, 1997) and with α-D-Man-(1→6)-α-D-Man (Wright *et al.*, 1998). SCA has a strong affinity for α1,3- and α1,6-mannosyl sugars (Wood *et al.*, 1996). We report here the crystallization and preliminary X-ray diffraction analysis of a complex of SCA with the trimannoside α-D-Man-(1→6)-[α-D-Man-(1→3)]-α-D-Man. The results of our study and those of others on the monocot bulb lectins, used in combination with the results from complementary techniques such as microtitration calorimetry, NMR spectroscopy and modelling, may help to provide a framework for understanding the molecular basis of protein–carbohydrate recognition.

## 2. Experimental

### 2.1. *Protein purification*

The lectin was isolated from *S. campanulata* bulbs using affinity chromatography on a mannose–Sepharose 4B column as described by Wright *et al.* (1996).

### 2.2. *Protein characterization*

Analysis of the purified SCA using SDS–PAGE and gel filtration on a Superose 12 column has shown that the lectin is a tetrameric protein composed of four identical 14 kDa subunits (Wright *et al.*, 1996).

### 2.3. *Crystallization*

Since the protein binds to the polysaccharide-based membranes used in commercial concentrators, *e.g.* Amicon YM10 membrane filters, resulting in the loss of up to 50% of purified material, SCA was concentrated to 11 mg ml$^{-1}$ using a mannose–Sepharose 4B affinity column with a 5 ml void volume and eluted with the minimum amount of 20 m*M* unbuffered 2,3-diaminopropane and then neutralized to pH 7.0. The protein solution was screened for crystallization conditions using the hanging-drop vapour-diffusion method (Ducruix & Giegé, 1992) on siliconized cover slips in Linbro trays at 293 K. The reservoir volume was 1 ml for all crystallization trays. Using 10 µl drops of protein solution and adding 10 µl of 600 m*M* phosphate-buffered saline containing 20 m*M* α-D-Man-(1→6)-[α-D-Man-(1→3)]-α-D-Man (Dextra Laboratories, Reading, UK), crystals grew from several drops of the initial screen with ammonium sulfate of varying concentrations (55–75%) as the reservoir solution and the pH in the range 3–7.

2.4. *Data collection*

X-ray diffraction data were collected at 100 K on station PX7.2 at the SRS Daresbury Laboratory using a MAR Research image-plate detector system ($\lambda$ = 1 448 Å, 2 GeV, 200 mA). A total of 101 images, each covering 1 or 2°, were recorded with exposure times of 5 min per image from two crystals. The images were processed with the *MOSFLM* package (Leslie, 1992) and the data scaled and merged using the *CCP*4 (Collaborative Computational Project, Number 4, 1994) package of programs for protein crystallography.

2.5. *Molecular replacement*

The *AMoRe* program (Navaza, 1994) from the *CCP*4 suite was used for the molecular replacement solution. The native SCA structure (Wright *et al.*, 1996; Wood *et al.*, unpublished work) was used to conduct the rotation and translation search, including all residues with their side chains. The top 20 rotation solutions with the highest correlation coefficients were used in the translation function with ten cycles of rigid-body refinement.

## 3. Results and discussion

The best quality crystals of the SCA–trisaccharide complex for X-ray studies were obtained using 70% saturated ammonium sulfate in the reservoir at pH 4.8. Crystals usually appeared after approximately 30 d and grew up to 0.3 × 0.3 × 0.4 mm in size with an unusual morphology, as shown in Fig. 1. A full data set was collected to 2.5 Å resolution at 100 K from two crystals; statistics of this data set are given in Table 1. In a pilot study on station 9.6 of the SRS, using a newly developed CCD detector in combination with cryocooling to 100 K, crystals of the complex were shown to diffract at 2.3 Å spacing, see Fig. 2(*a*).

The SCA–trimannoside complex crystallized in space group *C*2 with cell dimensions $a$ = 99.38, $b$ = 119.86, $c$ = 77.10 Å and $\beta$ 105.56°. For four, six or eight subunits per asymmetric unit the corresponding $V_m$ values are 3.95, 2.63 and 1.98 Å$^3$ Da$^{-1}$, respectively. The limits for the volume per unit molecular weight for protein crystals determined by Matthews (1968) are 1.7–3.5 Å$^3$ Da$^{-1}$. Thus, crystals of the complex contain either a tetramer plus a dimer (with 53.2% solvent) or two tetramers (with 37.7% solvent) in the asymmetric unit.

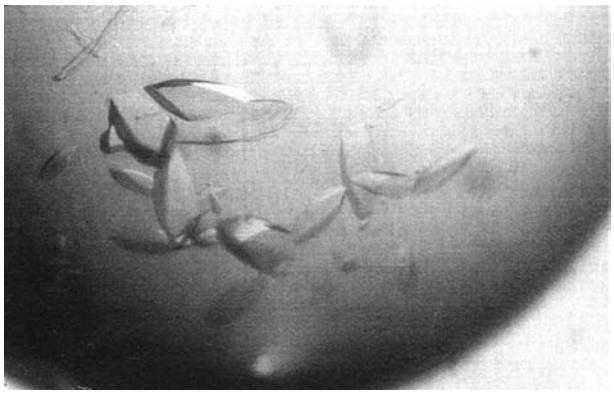
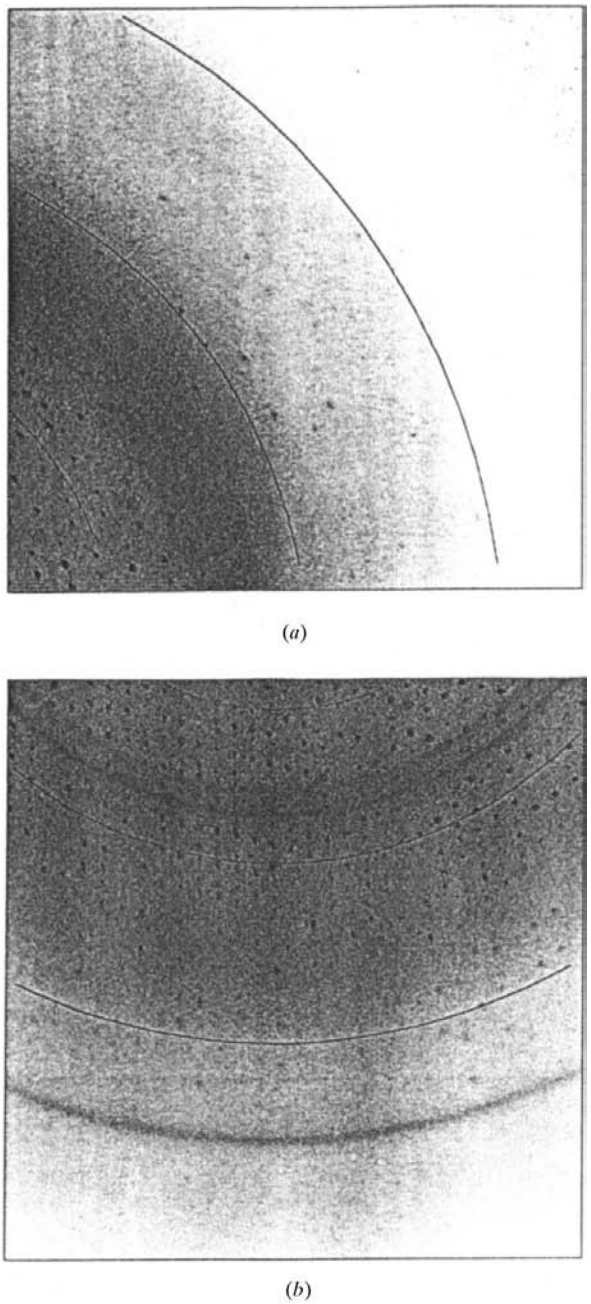


Fig. 1. Crystals of the lectin from bluebell (*Scilla campanulata*) bulbs complexed with a trimannoside. The largest crystals are approximately 0.3 × 0.3 × 0.4 mm.

The native SCA structure (Wright *et al.*, 1996; Wood *et al.*, unpublished work) was used as a search model in the molecular replacement studies and showed that the asymmetric unit contains six subunits. Six clear solutions for both the rotation and translation function were obtained, with correlation coefficients and $R$ factors of 37.0–40.3% and 49.6–52.4%, respectively. After ten cycles of rigid-body refinement, using



(*a*)

(*b*)

Fig. 2. Sections of a 2° oscillation image from a crystal of the SCA–trimannoside complex. (*a*) Image recorded at the SRS, Daresbury Laboratory on a MAR CCD detector. The outer resolution ring is at 2.3 Å. (*b*) Image recorded at the SRS, Daresbury Laboratory on a large MAR image plate. The outer resolution ring is at 2.5 Å.

Table 1. *Data-collection statistics of the Scilla lectin–trimannoside complex*

| Resolution | $R_{merge}$† | $N_{meas}$ | $N_{unique}$ | % Complete | Multiplicity | % > 3$\sigma$ |
|---|---|---|---|---|---|---|
| 20.00–8.77 | 0.083 | 1239 | 556 | 85.6 | 2.2 | 99.6 |
| 8.77–6.52 | 0.089 | 2026 | 884 | 88 | 2.3 | 98.3 |
| 6.52–5.42 | 0.088 | 2585 | 1141 | 90.7 | 2.3 | 98.9 |
| 5.42–4.74 | 0.087 | 2980 | 1349 | 91.8 | 2.2 | 99 |
| 4.74–4.26 | 0.091 | 3377 | 1571 | 95.2 | 2.1 | 99.4 |
| 4.26–3.91 | 0.098 | 3654 | 1716 | 94.5 | 2.1 | 97.7 |
| 3.91–3.63 | 0.105 | 3952 | 1877 | 95.5 | 2.1 | 96.3 |
| 3.63–3.40 | 0.111 | 4255 | 2011 | 95.5 | 2.1 | 95.1 |
| 3.40–3.21 | 0.115 | 4593 | 2147 | 96 | 2.1 | 95.9 |
| 3.21–3.05 | 0.134 | 5041 | 2320 | 98.3 | 2.2 | 91.9 |
| 3.05–2.91 | 0.186 | 5141 | 2369 | 95.8 | 2.2 | 91.7 |
| 2.91–2.79 | 0.162 | 5575 | 2517 | 97.3 | 2.2 | 87.4 |
| 2.79–2.68 | 0.202 | 5831 | 2593 | 96.1 | 2.2 | 81.2 |
| 2.68–2.59 | 0.228 | 6214 | 2727 | 97.5 | 2.3 | 73.8 |
| 2.59–2.50 | 0.259 | 6176 | 2712 | 93.7 | 2.3 | 68.8 |
| Overall | 11.5 | 62639 | 28490 | 94.9 | 2.2 | 89.1 |

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

all six solutions, the correlation coefficient increased to 78.9% and the *R* factor dropped to 31.0%. Inspection of the crystal packing using the program *O* (Jones & Kjeldgaard, 1993) revealed no bad contacts between the protein subunits in the unit cell. A further 20 cycles of refinement using *REFMAC* (Murshudov *et al*., 1997) reduced the conventional *R* value to 30.2%, with an $R_{free}$ of 34.5%. Difference Fourier maps ($2F_o-F_c$ and $F_o-F_c$) calculated with the partially refined structure clearly showed electron density corresponding to the trimannoside in the neighbourhood of the saccharide binding site. Modelling of $\alpha$-D-Man-(1→6)-[$\alpha$-D-Man-(1→3)z]-$\alpha$-D-Man into the difference density and refinement of the crystal structure of the complex are underway. The refined SCA–trimannoside complex model will provide valuable information on protein–carbohydrate interactions at the molecular level.

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## References

Barbieri, L., Batelli, G. B. & Stirpe, F. (1993). *Biochim. Biophys. Acta*, **1154**, 237–282.

Chantalat, L., Wood, S. D., Rizkallah, P. J. & Reynolds, C. D. (1996). *Acta Cryst.* D**52**, 1146–1152.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 670–673.

Ducruix, A. & Giegé, R. (1992). Editors. *Crystallization of Nucleic Acids and Proteins. A Practical Approach*. Oxford: IRL Press.

Hester, G., Kaku, H., Goldstein, I. J. & Wright, C. S. (1995). *Nature Struct. Biol.* **2**, 472–479.

Hester, G. & Wright, C. S. (1996) *J. Mol. Biol.* **262**, 516–531.

Jones, T. A. & Kjeldgaard, M. (1993). *O Version 5.9*, *The Manual*. Uppsala University, Uppsala, Sweden.

Leslie, A. G. W. (1992). *Joint CCP4 and ESF-EACMB Newsletter on Protein Crystallography*, No. 26. CCLRC Daresbury Laboratory, Warrington, WA4 4AD, England.

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

Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst.* D**53**, 240–255.

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–166.

Raikhel, N. V., Lee, H.-I. & Broekaert, W. F. (1993). *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44**, 591–615.

Rini, J. N. (1995) *Annu. Rev. Biophys. Biomol. Struct.* **24**, 551–577.

Sharon, N. & Lis, H. (1989). *Lectins*, pp. 61–91. London: Chapman and Hall.

Sharon, N. & Lis, H. (1990). *FASEB J.* **4**, 3198–3308.

Van Damme, E. J. M., Smeets, K. & Peumans, W. J. (1995). *Lectins, Biomedical Perspectives*, edited by A. Pusztai & S. Bardocz, pp. 59–80. London: Taylor and Francis.

Wood, S. D. (1995). PhD thesis, Liverpool John Moores University, England.

Wood, S. D., Allen A. K., Wright, L. M. & Reynolds, C. D. (1996). In *Lectins: Biology, Biochemistry and Clinical Biochemistry*, Vol. 11, edited by E. Van Driessche, P. Rougé, S. Beeckmans & T. C. Bøg-Hansen, pp. 86–90. Hellerup, Denmark: Textop.

Wright, C. S. & Hester, G. (1996). *Structure*, **4**, 1339–1352.

Wright, L. M., Wood, S. D., Reynolds, C. D., Rizkallah, P. J., Peumans, W. J., Van Damme, E. J. M. & Allen, A. K. (1996). *Acta Cryst.* D**52**, 1021–1023.

Wright, L. M., Wood, S. D., Reynolds, C. D., Rizkallah, P. J. & Allen, A. K. (1997). *Protein Pept. Lett.* **4**(5), 343–348.

Wright, L. M., Wood, S. D., Reynolds, C. D., Rizkallah, P. J. & Allen, A. K. (1998). *Acta Cryst.* D**54**, 90–92.