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Crystallization and preliminary X-ray studies on the mannose-specific lectin from Amaryllis bulbs.

By STEPHEN D. WOOD, COLIN D. REYNOLDS, STANLEY LAMBERT and PAUL A. D. MCMICHAEL, *School of Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, England,* ANTHONY K. ALLEN, *Department of Biochemistry, Charing Cross and Westminster Hospital Medical School, Fulham Palace Road, London W6 8RF, England,* and PIERRE J. RIZKALLAH, *SERC Daresbury Laboratory, Keckwick Lane, Daresbury, Warrington WA4 4AD, England*

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Abstract

Affinity-purified amaryllis lectin was used to grow single crystals using the hanging-drop method. The space group was found to be *C*2 with unit-cell dimensions a = 73.4 (1), b = 100.3 (1), c = 62.2 (1) Å and β = 137.3 (2)°. Data to 2.25 Å resolution have been recorded and solution of the structure is currently underway by means of molecular-replacement techniques.

Introduction

A number of lectins have been isolated and characterized from plants (Goldstein & Poretz, 1986). Many of those that have been intensively studied and subjected to X-ray crystallographic studies are from the seeds of leguminous plants (dicotyledonous). Recently, the storage organs of monocotyledonous plants have been shown to be a good source of lectins with unique sugar specificities (Van Damme & Peumans, 1991). The family Amaryllidaceae contains a number of highly mannose-specific lectins (Van Damme, Goldstein & Peumans, 1991) which have different specificities for manno-oligosaccharides (Kaku, Van Damme, Peumans & Goldstein, 1990). Lectins from six members of this family have been purified including snowdrop and daffodil.

Experimental

Materials

Amaryllis bulbs (Hippeastrum hybrid) were bought locally. Phenyl-agarose was from Sigma Chemical Co. Mannose-agarose was produced by the method of Uy & Wold (1977). This involved activating 25 g of Sepharose 4B in 0.6 *M* NaOH with 10 ml 1,4-butanediol diglycidyl ether and 40 mg $NaBH_4$. This was then reacted with 8 g of mannose.

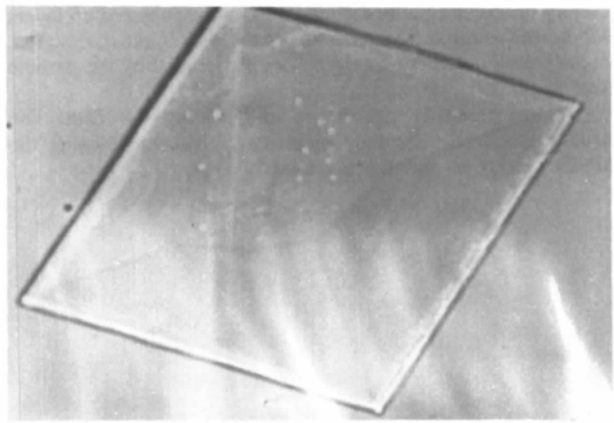
Purification of amaryllis lectin

The procedure was that used for the snowdrop lectin (Van Damme, Allen & Peumans, 1987). The purified lectin was dialysed against water and freeze dried. Amino-acid analysis (LKB amino-acid analyzer after 6 *M* HCl hydrolysis) shows the composition was in good agreement with the published results of Van Damme, Goldstein & Peumans (1991). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (Pharmacia Phast System using 10–15% gradient gels) gave a band of about 14 000 Da. Under non-reducing conditions SDS-PAGE gave a band at 56 000 Da indicating a tetrameric arrangement of the protein subunits. Capillary electrophoresis in SDS under reducing conditions (Applied Biosystems ProSort) gave only one component of molecular weight of 14 300 ± 200 Da which is also in agreement with the above reference.

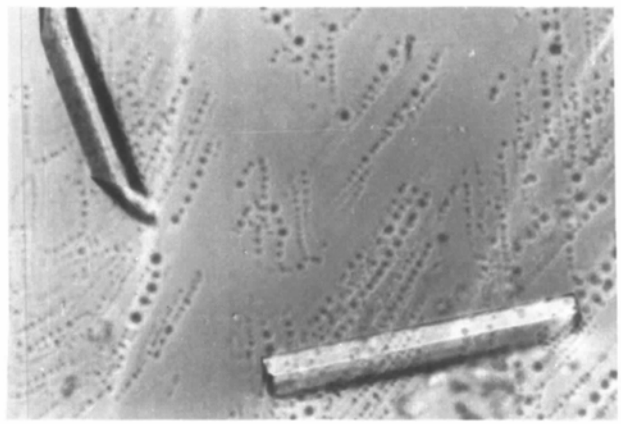
Crystallization and diffraction analysis

A number of crystals suitable for X-ray analysis of amaryllis lectin were obtained using the hanging-drop method. Once crystals had been grown the conditions were refined by systematically testing a range of closely related conditions. 10 µl drops of protein at a concentration of 10 mg ml^{-1} were made up to 20 µl using 10 µl of 50 m*M* phosphate-buffered saline. This drop was equilibrated against a 1 ml reservoir containing ammonium sulfate of varying concentrations and pH in the range 5.0–8.0.

Plate-like crystals with approximate dimensions of 0.50 × 0.50 × 0.03 mm were observed within a few days; Figs. 1(*a*) and 1(*b*) show crystals grown using this method.



(*a*)



(*b*)

Fig. 1. Amaryllis lectin crystal showing plate-like appearance: (*a*) Front view, (*b*) side view.

Results and discussion

Three crystals were sealed in glass capillaries with some of the mother liquor and mounted in random orientations. Data were collected at station 9.6 of the Daresbury Laboratory Synchrotron Radiation source (λ = 0.89 Å) using the MAR-image plate and a single-bunch user beam, the current ranged between 25 and 12 mA and the energy was 2 GeV. Using crystals 1 and 2, data were collected over 90° in steps of 2°, and a further 48° of data were collected from crystal 3 again in steps of 2° (Fig. 2). The amaryllis lectin crystals diffracted to high resolution (2.2 Å) and were stable in the X-ray beam for the duration of the data collection. The crystals belong to the monoclinic space group $C2$ with unit-cell dimensions of a = 73.4 (1), b = 100.3 (1), c = 62.2 (1) Å and β = 137.3 (2)°. Assuming a molecular mass of 28 000 Da for the amaryllis lectin dimer per asymmetric unit, the V_m value is 2.86 Å^3 Da^{-1} and falls in the acceptable range observed for other protein crystals. The solvent content has been estimated to be 56% of the unit-cell volume (Matthews, 1968).

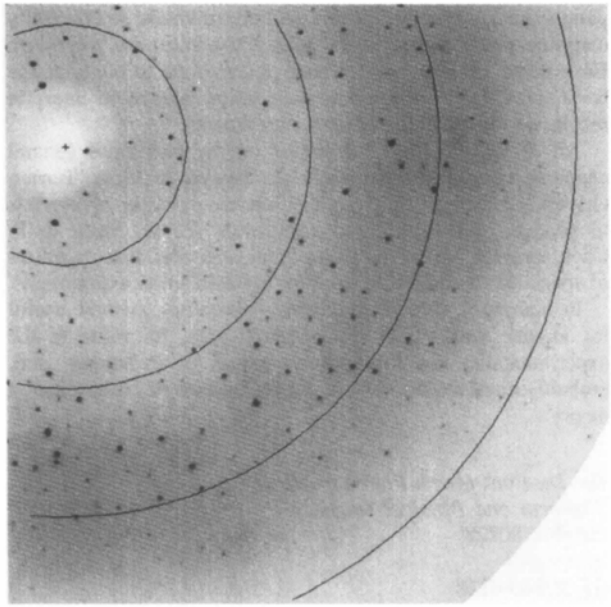


Fig. 2. Randomly orientated rotation photograph. The resolutions of the rings are 10.0, 5.0, 3.3 and 2.5 Å, respectively.

The data were processed with the *MOSFLM* package and merged using the *CCP*4 (SERC Daresbury Laboratory, 1979) package of programs for protein crystallography. For the full data set 14 159 reflections were recorded giving a data set complete to 97% to a resolution of 2.25 Å. This gave an R_{merge} = 10.7% [$R(I) = |I - \langle I \rangle|/I$] with 69% of the data greater than 3 e.s.d.'s. The fraction of data above 3 e.s.d.'s declined rapidly beyond 2.5 Å. In the restricted resolution range to 2.5 Å, there were 10 276 reflections and an R_{merge} = 9.7% with 77% of the data greater than 3 e.s.d.'s.

It is expected with a multi-bunch user beam that data could be collected to a much higher resolution, it is also thought that better crystals could be grown more slowly over a longer period of time.

The structure solution is currently being attempted by molecular replacement using the coordinates of a mannose-binding protein (Weis, Kahn, Fourme, Drickamer, & Hendrickson, 1991) as a starting model.

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