SHORT COMMUNICATIONS

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Crystallization and preliminary X-ray studies on the *N*-acetylgalactosamine-specific lectin from tulip bulbs.

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Abstract

Affinity-purified tulip lectin was used to grow single crystals under a variety of conditions. Several morphologically different crystal forms could be obtained, some of which diffract to a resolution of 3.3 Å. The crystals were analysed by rotation photography on beamline 7.2 of the Daresbury Synchrotron Source and were shown to be orthorhombic with unit-cell dimensions $a = 51.8$ (1), $b = 64.4$ (1), $c = 244.9$ (3) Å or monoclinic with unit-cell dimensions $a = 49$ (1), $b = 82$ (1), $c = 161$ (2) Å, $\beta = 104$ (2)°.

Introduction

Lectins are proteins of non-immune origin specifically recognizing complex carbohydrate cell determinants (Goldstein, Hughes, Monsigny, Osawa & Sharon, 1980). Although their function remains mostly obscure, plant lectins have been well studied and are used in a wide variety of immunological and biochemical applications. Most of this work has focused on the seed lectins from legume plants (Van Driessche, 1988), but it has become apparent that large amounts of lectins, often with unusual properties, can be isolated from species of other plant families (Rüdiger, 1988).

In recent years a variety of lectins has been purified from monocotyledonous plants, most of them having unusual polysaccharide affinities. Among these are lectins from the families of Amaryllidaceae, Lilliaceae and Orchidaceae. These proteins are different from the dicotyledonous lectins and exhibit some unique properties that make them promising tools for the analysis and purification of glycoproteins. Some of them are selective inhibitors of retroviruses (Cammue, Peeters & Peumans, 1986; Van Damme, Allen & Peumans, 1987*a*,*b*, 1988).

Three unrelated lectins have been purified from tulip species (Cammue, Peeters & Peumans, 1986; Oda & Minami, 1986; Oda, Minami, Ichida & Aonuma, 1987). The *N*-acetylgalactosamine-specific lectin found in the bulbs of tulips is a dimer or tetramer composed of identical subunits with a molecular mass of 30 000 dalton and is not related to any other known plant lectin. The lectin has a specificity towards *N*-acetylgalactosamine and can be purified by affinity chromatography on a fetuin agarose matrix. Its accumulation in bulbs is developmentally regulated (Van Damme & Peumans, 1989).

Until now only two monocotyledonous lectins have been crystallized: wheat-germ agglutinin and the mannose-specific lectin from snowdrop bulbs (Wright, 1977; Wright, Kaku & Goldstein, 1990), both totally unrelated to the tulip lectin. Only in the case of wheat-germ agglutinin has the three-dimensional structure been elucidated. We thus present here the first report on the crystallization of a lectin from a species of the Lilliaceae family.

Experimental

Materials

Tulip bulbs (*Tulipa* cv. Darwin Hybrid - Golden Apeldoorn) were obtained from a local purchaser. This cultivar is a hybrid resulting from crosses between *Tulipa gesneriana*, *T. snaveolens* and *T. kaufmanniana*. Fetuin agarose was purchased from Sigma Chemical Co. (St Louis, Missouri, USA). Diethylaminoethyl (DEAE)-Bio-Gel was a product of Bio-Rad Laboratories (Richmond, California, USA).

Purification of tulip lectin

The bulb lectin from *Tulipa* was purified following a modification of the procedure of Cammue, Peeters & Peumans (1986). Briefly, tulip bulbs, depleted of their outer scleroid layer, were homogenized with a blender in 1.2% NaCl and extracted overnight at room temperature. After centrifugation and filtration over glasswool, solid $(NH_4)_2SO_4$ was added to a final concentration of 0.3 *M*. The clear solution was applied to a fetuin agarose column equilibrated with 0.3 *M* $(NH_4)_2SO_4$. Unbound proteins were washed off with 0.3 *M* $(NH_4)_2SO_4$ and the lectin was desorbed with 20 m*M* unbuffered 2,3-diaminopropane. The resulting solution was dialyzed against 10 m*M* TRIS pH 8.8 and applied to a DEAE column equilibrated with the same buffer. The lectin was eluted using a linear gradient of 0–500 m*M* NaCl in 10 m*M* TRIS pH 8.8. The

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Table 1. *Crystallization conditions and diffraction characteristics of the tulip lectin crystals*

	Drop	Container	Unit cell and diffraction limit
Form (I)	10 mg ml^{-1} protein 10 m*M* TRIS pH 8.1 1 m*M* $ZnCl_2$ 15% MPD	10 m*M* TRIS pH 8.1 35% MPD	*P*222, 3.3 Å *a* = 51.8 (1) Å *b* = 64.4 (1) Å *c* = 244.9 (3) Å
Form (II)	20 mg ml^{-1} protein 20 m*M* 2,3-diaminopropane 50 m*M* sodium acetate Adjusted to pH 5.5–6.0 with concentrated HCl	50–200 m*M* sodium acetate Adjusted to pH 5.5–6.0 with concentrated HCl	*P*2 or *P*2$_1$, 3.7 Å *a* = 49 (1) Å *b* = 82 (1) Å *c* = 161 (2) Å β = 104 (2)
Form (III)	20 mg ml^{-1} protein 20 m*M* 2,3-diaminopropane 50–200 m*M* sodium acetate Adjusted to pH 4.5–5.5 with concentrated HCl	50–200 m*M* sodium acetate Adjusted to pH 4.5–5.5 with concentrated HCl	Not determined

resulting pure lectin solution was subsequently dialyzed against distilled water and lyophilized.

Crystallization and diffraction analysis

Crystallization conditions were screened using the hanging-drop and sitting-drop methods. Once crystals had grown, crystallization conditions were refined by systematically testing a range of closely related conditions. Crystals were obtained under the conditions given in Table 1. Two 1° rotation photographs for each crystal form were taken 90° apart on the rotation camera of station 7.2 of the SERC Daresbury Laboratory Synchrotron Radiation Source (λ = 1.488 Å). Unit-cell parameters for crystal forms (I) and (II) were measured from these photographs and refined, together with the crystal orientation and crystal-to-film distance, using the *CCP*4 (SERC Daresbury Laboratory, 1986) package of programs for protein crystallography.

Results and discussion

Under the conditions tried, three morphologically different crystal forms were obtained, named (I), (II) and (III) respectively. Although crystals could be grown relatively easily under a variety of experimental conditions and some reached dimensions of over 1 mm, interpretable diffraction patterns could only be obtained from crystal forms (I) and (II) using synchrotron radiation. Typical crystals obtained under the conditions described are shown in Fig. 1. Rotation photographs of crystal forms (I) and (II) are shown in Fig. 2. Crystal form (I) was shown to possess an orthorhombic primitive cell with unit-cell parameters *a* = 51.8 (1), *b* = 64.4 (1), *c* = 244.9 (3) Å. Assuming a molecular mass of 30 000 dalton for the tulip lectin monomer, the asymmetric unit must consist of a dimer, giving a V_m of 3.4 Å^3 dalton^{-1} in accordance with characteristic values for other proteins (Mathews, 1968). Diffraction extended to 3.3 Å resolution. Since the space group cannot be assigned unambiguously from these rotation data alone, low-resolution (8 Å) precession pictures were taken using a conventional sealed-tube X-ray generator operated at 50 kV, 20 mA. No systematic exclusions were observed, indicating space group *P*222.

Crystal form (II) is monoclinic with approximate cell parameters of *a* = 49 (1), *b* = 82 (1), *c* = 161 (2) Å and β = 104 (2)°. The crystals grow as plates with the long *c* axis perpendicular to the plate. Characteristic of this crystal form however is the apparent disorder in the direction of the *c** axis. On precession and rotation photographs, certain rows of spots are smeared out in the *c** direction while other rows give very sharp diffraction spots (Fig. 2*b*). Despite this apparent disorder, the crystals can be grown quite large (0.6 × 0.5 × 0.2 mm) in a reproducible way and diffract to at least 3.7 Å resolution on beamline 7.2 of the Daresbury Synchrotron Source. The disorder pattern can be explained by assuming that in the direction of the *c* axis,

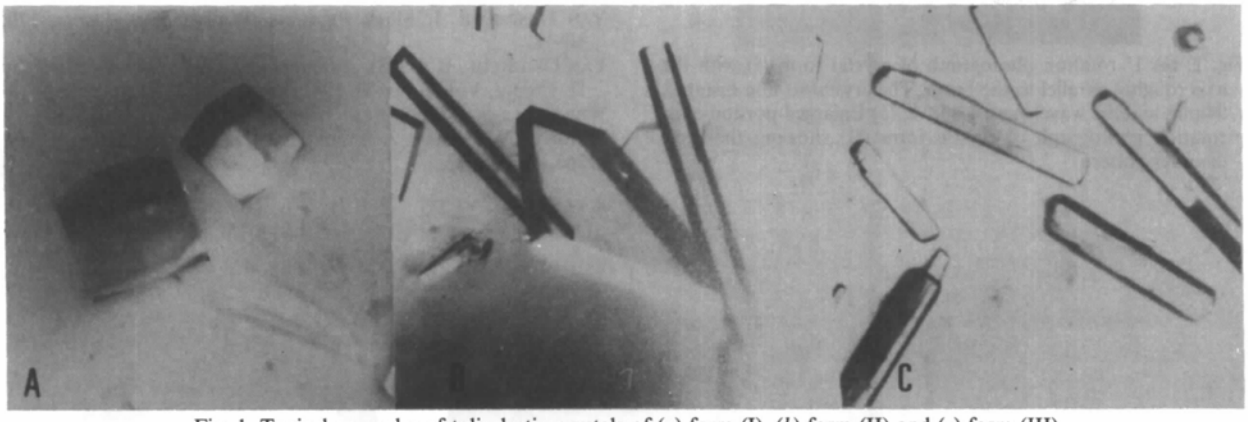

Fig. 1. Typical examples of tulip lectin crystals of (*a*) form (I), (*b*) form (II) and (*c*) form (III).

the intermolecular contacts are very weak and the molecules are allowed to position themselves in two different orientations without disturbing the lattice as a whole. Once the first molecule of a new layer has chosen its orientation, all other molecules in the same layer adopt the same orientation. These crystals are thus statistically monoclinic.

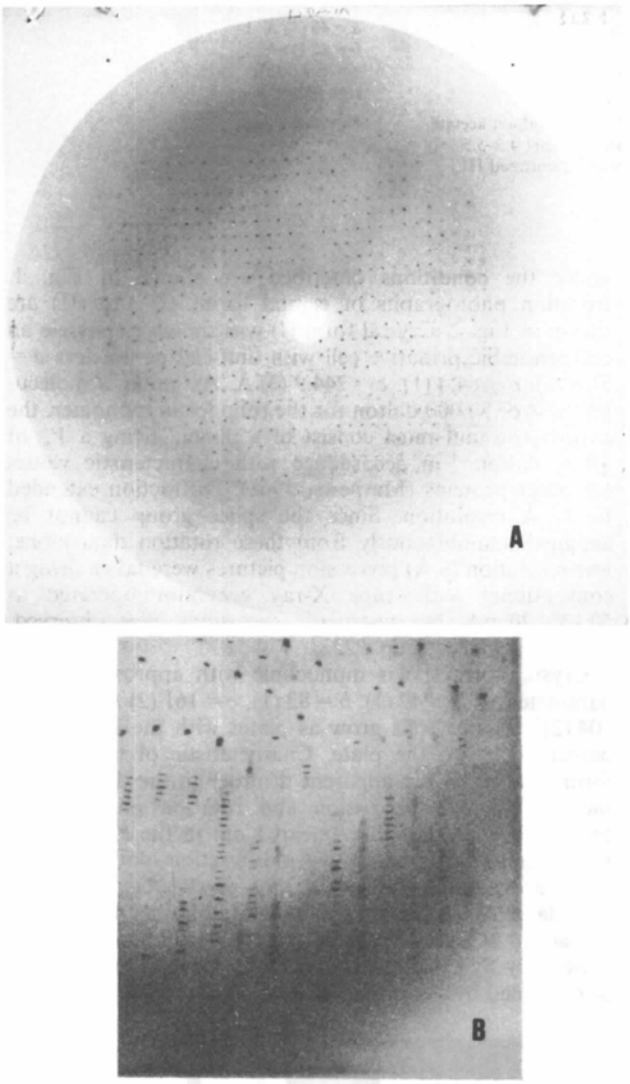

Fig. 2. (*a*) 1° rotation photograph of crystal form (I) with the *c* axis roughly parallel to the beam. The crystal-to-film distance is 94 mm and the wavelength 1.488 Å. (*b*) Enlarged portion of a 1° rotation photograph of crystal form (II) showing the typical disorder pattern.

A similar kind of disorder was observed in the early studies on horse met-haemoglobin (Bragg, 1975). This hypothesis is also in agreement with the apparently high solvent content of these crystals ($V_m = 3.2$ Å^3 dalton^{-1}). Again the lattice is primitive and there is a dimer in the asymmetric unit. Since no diffraction was observed on a conventional source, the existence of a 2_1 screw axis could not be confirmed nor excluded, leaving two possible space groups: $P2$ or $P2_1$.

Although growing to dimensions larger than 1 mm, crystals of form (III) only diffracted to very low resolution and crystals were consistently twinned. Therefore no cell parameters could be obtained. Of the described crystal forms, only form (I) is suitable for data collection. Its structure will be solved by the multiple isomorphous replacement method.

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