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Crystallization and preliminary X-ray studies of the basic lectin from the seeds of *Artocarpus hirsuta*

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The basic lectin from *Artocarpus hirsuta* specific towards methyl α-galactose has been purified and crystallized using the hanging-drop vapour-diffusion method with ammonium sulfate as precipitant. Three different crystal forms, orthorhombic I, orthorhombic II and hexagonal, were grown under the same crystallization conditions. The orthorhombic forms belonged to space group $P2_12_12_1$ with unit-cell dimensions a = 92.9, b = 99.8, c = 166.2 Å and a = 89.9, b = 121.9, c = 131.6 Å, respectively. The unit-cell dimensions of the hexagonal form were $a = b$ = 84.1 and c = 271.7 Å and the space group was $P6_122$.

1. Introduction

Lectins are multivalent carbohydrate-binding proteins of non-immune origin showing haemagglutination activity (Goldstein *et al.*, 1980). They are widely distributed in nature. Lectins play an important role in several biological processes such as infection, cell differentiation, host–bacteria symbiosis, organ formation and metastasis (Sharon & Lis, 1989). Lectins are an ideal tool for investigating carbohydrate structures and for studying protein–carbohydrate interactions (Sharon, 1993). The three-dimensional structures of lectins and their saccharide complexes have contributed to the understanding of the molecular mechanisms involved in cell recognition (Weis & Drickamer, 1996). Lectins are used in biological research and in biomedical applications such as blood typing, cancer-cell staining and lymphocyte agglutination (Lis & Sharon, 1986).

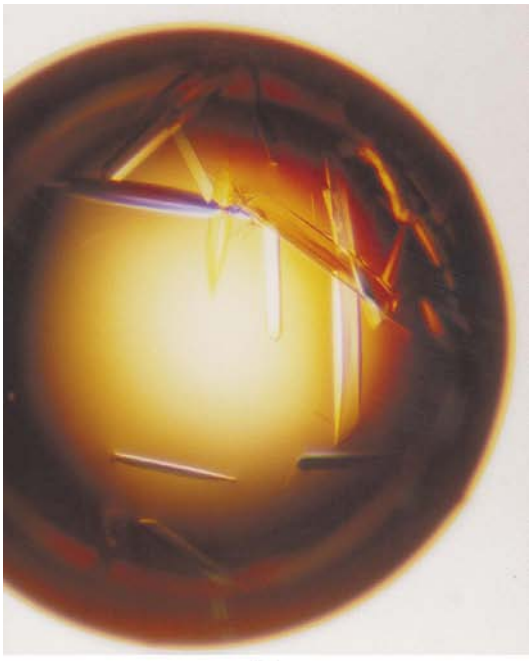
The legume lectins are the most widely studied and structurally characterized (Loris *et al.*, 1998). Three-dimensional structures have also been reported for non-seed plant lectins (Wright, 1997) and animal and viral lectins (Weis, 1997). *Artocarpus hirsuta* is a tropical plant belonging to the *Moraceae* family. Seed lectins from around half a dozen species of *Artocarpus* have been isolated. Jacalin, one of the lectins present in *A. integrifolia*, has received considerable attention in recent years because of the realization of its potential biomedical applications (Kabir & Daar, 1994). The crystal structure of jacalin, which is specific towards α-linked oligosaccharides (Mahanta *et al.*, 1990), has been reported recently and this structure revealed a new lectin fold (Sankaranarayanan *et al.*, 1996).

Here, we report the crystallization and characterization of a basic lectin from the seeds of *A. hirsuta* which binds the monomer methyl α-galactose (Me-α-gal) with high specificity, but has no affinity for α- or β-oligomers of galactose. Isolation, purification and biochemical characterization of this lectin have previously been reported (Gurjar *et al.*, 1998). Its sugar specificity has been studied using fluorescence spectroscopy (Gaikwad *et al.*, 1998). Many lectins show higher binding capacity towards the oligosaccharides when compared with their constituent monomers (Elgavish & Shaanan, 1997). This is also the case for jacalin, the other *Artocarpus* lectin whose structure is known (Mahanta *et al.*, 1990). This is not, however, true of the lectin reported here. Also unlike jacalin, the *A. hirsuta* lectin did not show any T-antigen specificity. Therefore, an investigation of the structure and the sugar-binding site of *A. hirsuta* lectin is of considerable interest.

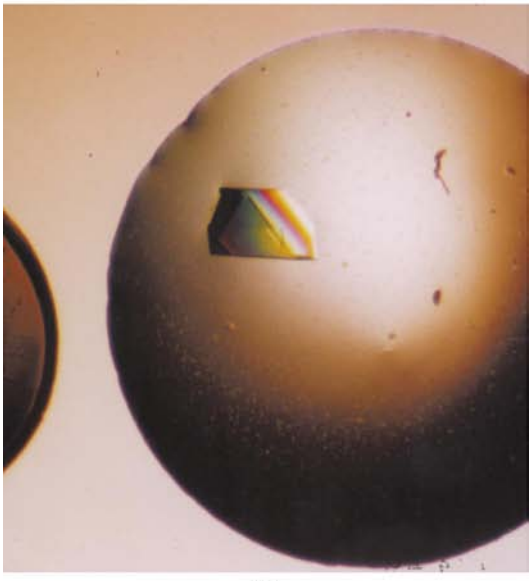
2. Materials and methods

A. hirsuta seeds were collected from central Kerala. The lectin was extracted from crushed seeds and purified using ion-exchange chromatography as reported previously (Gurjar *et al.*, 1998). Electrophoresis on a sodium dodecyl sulfate gel showed two separate bands (15800 and 14130 Da) for the purified lectin, indicating a stable heterodimer. The lectin has an estimated pI of 8.5. Gel filtration on Sephadex G-100 confirmed that the lectin remained a dimer of the above heterodimer in solution. The molecular weight of the tetramer was estimated to be about 60 kDa.

Crystals were grown from solutions of pH range 6.0–7.0 in 0.2 M phosphate buffer at room temperature using the hanging-drop vapour-diffusion method on siliconized cover



(*a*)



(*b*)

Figure 1
(*a*) Orthorhombic crystals, typical size 0.6 × 0.1 × 0.1 mm. (*b*) Hexagonal crystals, typical size 0.50 × 0.25 × 0.25 mm.

slips placed over wells containing 1 ml precipitant solution (35% saturated ammonium sulfate in the same buffer) in multiwell trays. Drops were formed by mixing 2 µl of 60 mg ml^{-1} protein solution and 2 µl of reservoir solution. The protein solution was mixed with 0.5 *M* Me-α-Gal solution in 10:1 proportions prior to setting up crystallization.

Data were collected on a MAR Research image-plate detector mounted on a Rigaku rotating-anode X-ray generator. Crystals were oscillated about an axis perpendicular to the X-ray beam. The frames were indexed using the program *REFIX* and processed using *XDS* (Kabsch, 1988) or *DENZO* (Otwinowski & Minor, 1997). The crystal-to-detector distance was chosen so that the spots were well resolved.

3. Results and discussion

The crystals formed in two orthorhombic forms, which were distinguishable only by unit-cell parameters and not by crystal morphology (Fig. 1*a*), and in a hexagonal form having hexagonal bipyramidal crystals (Fig. 1*b*). Orthorhombic forms I and II belonged to space group $P2_12_12_1$, with unit-cell dimensions a = 92.9, b = 99.8, c = 166.2 Å and a = 89.9, b = 121.9, c = 131.6 Å, respectively. The reflection data of the orthorhombic form I were 99.1% complete to 3.6 Å resolution with an $R_{\rm merge}$ of 0.143. Data for the orthorhombic form II were 98.9% complete to 3.0 Å with an $R_{\rm merge}$ of 0.096. The hexagonal form, space group $P6_122$, had unit-cell dimensions $a = b$ = 84.1 and c = 271.7 Å. Data from this form processed using *DENZO* were 94.3% complete at 3.4 Å resolution and the $R_{\rm merge}$ for equivalent reflections was 0.059. The Matthews number (Matthews, 1968) for the orthorhombic form I, assuming two tetramers in the asymmetric unit, was V_m = 3.5 Å^3 Da^{-1}, which corresponds to 60% solvent. The solvent content is calculated to be 59% in orthorhombic form II, whereas it is only 47% in the case of the hexagonal cell when two tetramers are assumed per asymmetric unit. Structure solution is in progress.

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