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Immunolocalization and functional role of Sclerotium rolfsii lectin in development of fungus by interaction with its endogenous receptor

Bale M. Swamy1,2, Anuradha G. Bhat2, Ganapatil V. Hegde2, Ramachandra S. Naik2, Srikanth Kulkarni2, and Shashikala R. Inamdar2

1Department of Biochemistry, Karnatak University, Dharwad 580 003, India, and 2Department of Plant Pathology, University of Agricultural Sciences, Dharwad 580 005, India

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Fungal lectins are ubiquitous and are being intensively studied in plants, animals, and bacteria because they specifically recognize and bind to carbohydrates present on cell surfaces. Although occurrence of lectins in fungi has been known for a while (Gold and Balding, 1975), they did not receive much attention compared to bacterial lectins. It has been shown that lectins are common in fungi, and in the recent past several lectins were purified and characterized (Guillot and Konska, 1997; Kawagishi, 1995; Kellens et al., 1989; Pemberton, 1994; Wang et al., 1998a). Majority of the fungal lectins isolated were from the fruiting bodies and rarely from the vegetative mycelia (Giollant et al., 1993; Kellens et al., 1992; Wang et al., 1998b; Oda et al., 2002). Contrary to established roles of bacterial lectins in host-parasite interactions, functional roles assigned for fungal lectins are speculative. Although many believe that fungal lectins do mediate host-parasite interactions (Rudiger, 1998) similar to bacterial adhesins, several other roles are also put forth. Often physiological roles for fungal lectins were attributed based on the location and selection of the source for isolation (Barak and Chet, 1990; Elad et al., 1983; Inbar and Chet, 1994; Kellens and Peumans, 1990). Some of the roles assigned to fungal lectins are storage proteins (Kellens and Peumans, 1990), fungal-fungal interactions (mycoparasitism), and host-parasite interactions (Fukazawa and Kagaya, 1997; Hostetter, 1994; Rudiger, 1998). Another argued function gaining greater attention is their involvement in morphogenesis and development of the fungi (Cooper et al., 1997; Yatohgo et al., 1988). However, none of these assigned roles are established.

Similar uncertainty also exists for the functional roles of membrane-bound fungal sphingolipids referred to as phytosphingosines. Inositolphosphoryleramides (IPCs), a family of membrane lipids with characteristic glycosyl moieties, occur exclusively in fungi (Dickson, 1998). These molecules, once believed to be simply structural components of membranes, are now implicated for their diversified roles as second messengers that activate intracellular signal transduction pathways leading to regulation of cell cycle and growth (Dickson, 1998). Our findings with Sclerotium rolfsii lectin (SRL) and its putative endogenous receptor suggest that the cell wall-associated lectin and the sphingolipids in fungi with obscure functions collude to carry out important biological function in the development of the fungus.

Earlier we reported the purification and the fine sugar specificity of a Thomsen-Friedenreich antigen–specific lectin from S. rolfsii (Swamy et al., 2001; Wu et al., 2001); its...
preliminary X-ray crystallographic data has been reported recently (Leonidas et al., 2003). Interesting sugar specificity exhibited by SRL toward Galβ1→3GalNAc-ser/thr, an oncofetal antigen, prompted us to study its functional role. The present article describes the localization and expression of the lectin and identification of its putative endogenous glycosphingolipid receptor, to explain the functional role of the lectin and the receptor in the development and morphogenesis of *S. rolfsii*.

**Results**

**Localization of SRL**

Immunolocalization of SRL in vegetative mycelia and sclerotial bodies using fluorescein isothiocyanate (FITC) anti-SRL demonstrated that the lectin is detected in small amounts on the mycelia and large amounts in sclerotial bodies. Binding of the FITC anti-SRL to vegetative mycelia and sclerotial bodies of *S. rolfsii* as seen under fluorescence microscope are presented in Figure 1. The interaction of FITC anti-SRL with the lectin occurring on the mycelia was observed as discrete intense fluorescence spots at the branching points of the mycelium (Figure 1a). However, uniform weak fluorescence was observed all along the hyphal surface (Figure 1a, inset). These observations indicate that although the lectin is distributed all along the hyphae but occur more densely at the branching points. Immature sclerotial bodies in the final stage of formation still associated with highly branched mycelia around showed dense mass of congregated lectin sites arising due to aggregation of mycelium (Figure 1b) in addition to weaker fluorescence on nonaggregated regions. In the completely matured sclerotial bodies, intense uniform fluorescent label (Figure 1c) was seen over the entire surface of the mature sclerotial bodies, revealing uniform distribution of lectin at very high levels compared to vegetative mycelia.

**SRL expression during development**

To ascertain the expression of lectin quantitatively during the course of growth and development of *S. rolfsii*, lectin content in developing mycelia and sclerotial bodies was determined by hemagglutination assay. Results of the expression of lectin in the mycelium and sclerotial bodies on different days of growth are shown in Figure 2a and b, respectively. The dry weight of mycelial mass increases progressively after fourth day with concomitant increase in protein and total sugar contents until the ninth day. However, the mycelial lectin content increases slowly, but not as a function of mycelial growth; instead a sudden spurt in lectin content was observed between 13th and 15th days (Figure 2a). Subsequently mycelial mass decreases rapidly, as did the protein and sugar content, resulting in the aggregation of mycelium to the onset of sclerotial bodies. As the formation of sclerotial bodies continued, the mycelial mass decreased rapidly (Figure 2a). In contrast, lectin, protein, and total sugar contents increased linearly in the sclerotial bodies with the increase in their dry weight (Figure 2b).

At any given stage of growth, the specific activity of lectin in the sclerotial bodies is ~1000-fold higher compared to mycelium. These results reflect the previous observations of immunolocalization, and it becomes apparent that much of the lectin was secreted at once during the aggregation of mycelia to form sclerotial bodies. Probably the lectin secreted will help the mycelial filaments cross-link and form sclerotial bodies because of its specific sugar-binding property.

**Inhibition of germination by neutralizing antibody**

To further investigate the involvement of lectin in growth of the fungus, we examined the germination of sclerotial bodies after capping the lectin sites by anti-SRL. Interestingly, sclerotial bodies treated with anti-SRL did not germinate (Figure 3b) even after 7 days, but the sclerotial bodies treated with normal rabbit serum germinated normally and lavish growth was observed (Figure 3a). Similarly, the mycelia treated with anti-SRL also failed to grow. Not only anti-SRL but also SRL-binding glycoproteins fetuin and mucin inhibited the germination similarly (data not shown). Observations of inhibition of germination by extraneous lectin-binding molecules suggests that
Fig. 2. Expression of SRL at different stages of development. Lectin content in (a) vegetative mycelium and (b) sclerotial bodies, expressed as total hemagglutination activity (diamonds) on different days of growth. Growth was expressed in terms of dry weight (open circles), carbohydrate (closed circles) and protein content (squares). Mean values of triplicate experimental sets are presented.

Fig. 3. Effect of capping the lectin by anti-SRL on germination of sclerotial body. (a) Sclerotial body treated with normal rabbit serum showing normal growth. (b) Binding of anti-SRL strongly inhibits the germination after 3 days of incubation.

the lectin expressed in response to nutrient stress interacts with the endogenous receptor, apart from facilitating the mycelial aggregation, to form sclerotial bodies, which also play key role in germination process.

Endogenous receptor of SRL

Specific binding of biotinylated SRL to the lipid components of sclerotial bodies separated on thin-layer chromatography (TLC) plates, as demonstrated by avidin peroxidase reaction, lead to the identification of endogenous glycolipid receptors. After TLC, the chromatogram was treated with periodate-treated bovine serum albumin (p-BSA) to abolish nonspecific binding and allowed to interact with biotinylated SRL. Developed blue color revealed the presence of two SRL binding bands and were assigned as receptor bands RI and RII (Figure 4, 1). To confirm the specificity of the SRL binding, another parallel chromatogram was treated with biotinylated peanut agglutinin (PNA) (Figure 4, 2). Both the SRL binding lipid bands (RI and RII) coincided with the PNA binding bands, and because SRL and PNA have common sugar specificity, this confirmed the specificity of lectin binding.

Considering the sugar specificity of SRL and PNA, it may be concluded that the receptors RI and RII are glycolipids containing Galβ1→3GalNAc-α-glycoconjugate moiety. To get an insight into the chemical nature of the lectin binding receptors, parallel chromatograms were sprayed with specific detection reagents: orcinol to detect glycolipids (Figure 4, 3), ninhydrin to detect free amino groups (Figure 4, 4), and ammonium molybdate to detect phospholipids (Figure 4, 5). Staining for glycolipid with orcinol showed an intense
Identification of lectin-binding lipid components extracted from the sclerotial bodies on thin-layer chromatograms. Lectin binding receptors from the lipids (2 μl) separated were identified by treating the chromatograms with biotinylated SRL (1), biotinylated PNA (2) incubated with avidin-horseradish peroxidase and the color developed with tetramethylbenzidine/H2O2 substrate. Analytical identification of lectin-binding lipid components, chromatograms after development sprayed with orcinol reagent to detect glycolipids (3) ninhydrin to detect free amino groups (4) and ammonium molybdate reagent to detect phospholipid (5) components. Fivefold excess lipid (10 μl) loaded to TLC plates to confirm the glycolipid staining by orcinol for RI and RII (6), without alteration in the mobility of SRL binding bands (7).

Identification of free amino groups in the receptors on TLC by spraying with ninhydrin indicated positive reaction only for RI but not for RII (Figure 4, 4), even with excess amounts of lipid. However positive indication for the presence of phosphate moiety in both RI and RII was demonstrated by detection with ammonium molybdate (Figure 4, 5). From these analytical findings, we concluded that both the receptors (RI and RII) identified are ceramide-type glycosphingophospholipids. Also, orcinol and ninhydrin staining revealed that RI is different than RII by having higher content of carbohydrate and with free amino groups. These differences suggest that RII could probably be a modified form of RI. Heterogeneity associated with the receptor bands leading to such discrepancies could not be ruled out. Indeed both the receptors contain Galβ1-3GalNAc, an essential criterion for binding by SRL and PNA.

Discussion

For establishing the physiological role of lectins, it would be significant to have detailed information on their distribution and cellular localization. The distinction between intracellular and extracellular localization is of paramount importance for understanding its function. The results of immunolocalization and the expression studies revealed that SRL is formed initially on the young hyphae in small amounts, and very high levels accumulated rapidly at the time of sclerotial body formation.

Similar observations were made in *Rhizoctonia solani*, where in the lectin occurring in small amounts on young hyphae increased dramatically at the time of maturation and accumulated in mature sclerotina (Kellens and Peumans, 1990). Lectin accumulated in the sclerotina, represented as high as 40% of the total sclerotel protein, hence they concluded that the lectin serve as reserve storage protein as in plant seeds. In contrast Cooper et al. (1997) suggested that the small, saline-soluble galactose-binding lectins (fungal galectins) secreted by many fungi are developmentally regulated with high expression in fruiting bodies. A fucose-specific lectin secreted by *Rhizopus stolonifer* only under spore-forming conditions reported recently (Oda et al., 2003) supports this latter view.

Our results of localization and expression of SRL revealed that the SRL is developmental-stage specific lectin secreted in response to stress, playing key role in the formation of sclerotial bodies rather than simply serving as reserve storage protein. Germination of sclerotial bodies is another event in the development of the fungus, which also involves the role of the lectin as shown by lectin capping studies. Capping of the lectin sites by anti-SRL strongly inhibits germination of the sclerotial bodies. Similar inhibition was also found by treating these bodies with mucin or fetuin, with which SRL strongly binds (Swamy et al., 2001). These observations suggest that the lectin in a bound form with any extraneous lectin-binding molecules, such as antibodies, fetuin, or mucin will result in inhibition of sclerotial body germination. For the onset of sclerotial body germination, lectin-receptor complex could be mediating as critical signaling molecule, probably this signaling event is interrupted when the lectin is complexed with anti-SRL or a hapten.

Recently it was shown that the disruption of the glucosyl ceramide synthesis using inhibitors of UDP-Glc, ceramide glucosyltransferase, leads to inhibition of spore germination, cell cycle, and hyphal growth in *Aspergillus nidulans* and *Aspergillus fumigatus* (Levery et al., 2002). Membrane-bound glucosyl ceramides are reported to be widely distributed in many fungi, and during their syntheses, sugar moieties are added directly to ceramides, which are referred to as glycosylinositol phosphorylceramides (GIPCs). This class of glycosyl ceramides occurring in fungi (Lester and Dickson, 1993; Dickson, 1998) are mostly glycosylceramides. However there are also reports of galactosylceramides occurring in some fungi (Levery et al., 2000; Toledo...
et al., 1999, 2000). GPCs are believed to act as signaling molecules and are implicated for their diversified roles in viability (Zhong et al., 2000) and cell growth (Chung et al., 2001). We believe that the inhibition of spore germination by disrupting the ceramide synthesis using inhibitors of glycosyltransferases in A. nidulans and A. fumigatus (Levery et al., 2002) is analogous to our observed inhibition of sclerotial body germination by capping the lectin sites on sclerotial bodies.

Identification of lectin binding receptors in the lipid extracts of sclerotial bodies by specific lectin labeling method substantiates the significance of the lectin–receptor interaction in the development of the fungus. Considering the sugar specificity of SRL, which binds specifically to Gal residues in the procedure (Swamy et al., 2001), it becomes evident that lectin-binding receptor could essentially contain this moiety. Preliminary characterization studies of the lectin-binding receptors after TLC using specific detection reagents indicate that the receptor R1 and RII resemble phytoceramides reported in fungi (Dickson, 1998), in particular IPCs. The lectin receptor identified in S. rolfsii appears to be galactosyl ceramide. Based on our findings, we propose that the lectin expressed abundantly on the mycelia at the time of sclerotial body formation facilitates the aggregation of the mycelium by interacting with endogenous glycosyl ceramide receptor(s) having specific carbohydrate moiety. Furthermore, it appears that the lectin–receptor complex formed at the time of sclerotial body formation will remain intact during the dormant stage, and the complex would undergo changes to facilitate germination. In the near future these findings would shed light on the importance of fungal lectins as well the IPC receptors.

Materials and methods

Stock cultures of S. rolfsii were maintained on potato dextrose agar slants containing 5% dextrose. Cultures grown on Byrde's liquid synthetic media (Byrde et al., 1956) were used for studying the expression of lectin at different stages of development and growth of the fungus. SRL was purified from sclerotial bodies as described earlier (Swamy et al., 2001). Antibodies for SRL (anti-SRL) were obtained by immunizing rabbits (New Zealand breed) with purified SRL, further fractionating serum by 50% ammonium sulfate precipitation (Livingston, 1974) and chromatography on Sephadex G-50 column. Specificity of anti-SRL was confirmed by Ouchterlony immunoprecipitation method. FITC-labeled SRL antibodies were prepared as described by Goldman (1968). p-BSA for blocking nonspecific sites was prepared in twice glass-distilled water.

Immunolocalization of lectin

Localization of lectin in vegetative mycelia and immature and mature sclerotial bodies of S. rolfsii during development was carried out by immunolabeling the lectin sites with FITC anti-SRL. Vegetative mycelial mass from the S. rolfsii culture broth of 10 days was washed repeatedly by centrifugation at 8,000 g. Washed culture broth was suspended in phosphate buffered saline (PBS) containing p-BSA (3%) and incubated for 30 min at 37°C to block nonspecific binding by lectin antibodies, followed by extensive washing. Subsequently the culture broth was incubated with FITC anti-SRL in PBS (50 µg/ml) for 30 min with gentle shaking. Excess unbound antibodies were removed by washing with PBS on centrifugation. Essentially the same procedure was adopted for lectin localization on sclerotial bodies. Interaction of FITC anti-SRL on vegetative mycelia and sclerotial bodies was observed and photographed under fluorescence microscope (Carl Zeiss Jenalumar, model Fluooval 2) using excitation filter G-247 in the path of excitation light and barrier filter B 430 in the path of emitted light.

Determination of SRL activity during growth and development of S. rolfsii

In mycelia. For the determination of lectin activity in developing mycelia, S. rolfsii cultures were grown in Byrde's mineral medium (100 ml) in different flasks by inoculating one sclerotial body for each flask. On different days from the date of inoculation, the mycelial mass formed was collected from three flasks separately (triplicate) after filtration on a sintered funnel. Individual mycelial lots of each day were separately washed with distilled water and freeze-dried. Dried mycelium (10 mg/ml) was homogenized with PBS (50 mM, pH 7.2) using pestle and mortar, briefly sonicated, and kept for extraction on a rotary shaker (150 rpm) for 1 h. The homogenate was subjected to centrifugation at 10,000 x g for 30 min, at 4°C (Kubota refrigerated centrifuge, RA 300F angle rotor). Clear supernatant after ultrafiltration on 0.2 µ membrane was used for the determination of lectin activity. In sclerotial bodies. Sclerotial bodies forming during the course of development were harvested from each flask (in triplicate) by picking up manually from the culture broth on different days, washed thoroughly with distilled water, and powdered after freeze drying. Dry powder of the sclerotial bodies was suspended in PBS (5 mg/ml), sonicated to obtain homogeneous suspension and extracted for 1 h on a rotary shaker. Clear supernatant obtained on centrifugation at 8000 x g for 30 min was used for the determination of lectin activity. Lectin activity in the extracts of mycelia and sclerotial bodies harvested on different days was determined by hemagglutination assay. Total carbohydrate, protein content, and dry weight were also determined. The results of the lectin activity, total sugar, protein content, and dry weight of mycelium/sclerotial bodies were plotted against the age of culture.

Hemagglutination assay

The hemagglutinating activity of the lectin was routinely assayed by serial twofold dilution technique of Liener and
Hill (1953) in microtiter assay plates using trypsinized human (O group) erythrocytes. The highest dilution of the extract causing visible hemagglutination was regarded as the titer and the protein content in the highest dilution causing visible agglutination as 1 unit of hemagglutination activity (minimum concentration of protein required for agglutination). Total activity in the extracts was determined from the total protein content.

**Estimation of protein and sugar**

Protein concentration was estimated by the method of Lowry et al. (1951) using BSA as standard. The total sugar content was estimated by the phenol-sulfuric acid method of Dubois et al. (1956) using glucose as standard sugar.

**Inhibition of sclerotial body germination by anti-SRL**

Sclerotial bodies from a fresh culture were incubated for 10 min at 37°C with anti-SRL in a screw-capped vial. Treated sclerotial bodies were allowed to germinate in a petri dish containing Byrne’s media with 1.5% agar at 28°C. As control, a sclerotial body that was treated with normal rabbit serum was inserted in another well of the plate. Germination of the sclerotial body in the plate was photographed after fourth day of inoculation.

**Identification of putative endogenous lectin receptor**

**Biotinylated lectin labeling assay**

Lectin-binding lipid components on TLC plates were identified by enzyme-linked lectinosorbent detection using biotinylated SRL. In principle, the method we adopted is similar to solid-phase methods, used to demonstrate binding of viruses and bacteria and to characterize receptor carbohydrates based on their specific binding to glycolipids separated on thin-layer chromatograms (Karlsson, 1989). In this detection assay, the TLC plates with fractionated lipid components were immersed in PBS containing polyvinyl pyrrolidone (1%) for 5 min. Subsequently the plates were blocked with p-BSA (3%) in PBS for 1 h to prevent non-specific binding and washed gently three times with PBS and incubated overnight at 4°C in screw-capped tubes containing biotinylated SRL or biotinylated PNA in PBS (20 μg/ml). The plates were gently washed with PBS to remove unbound lectin and treated with streptavidin horseradish peroxidase. The plates were finally washed with distilled water and incubated in tetramethyl benzidine/H2O2 for 5 min; the blue-colored bands developed were photographed immediately.

**Analytical characterization of lectin binding receptors**

Analytical characterization of lectin-binding receptors resolved on TLC plates was carried out using specific sprayin reagents. The chromatograms were dried and sprayed separately for detection of glycolipids by orcinol, free amino groups by ninhydrin, and phospholipids by ammonium molybdate reagents. Reagents and the methods used were essentially as described by Kates (1972). After the development of the color the plates were photographed.

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**Abbreviations**

BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; IFCs, inositolphosphorylceramides; GIPC, glycosylinositol phosphorylceramides; PBS, phosphate buffered saline; PNA, peanut agglutinin; SRL, Sclerotium rolfsii lectin; TLC, thin-layer chromatography.

**References**


1. Introduction

Lectins are non-enzymatic carbohydrate-binding proteins present in plants, bacteria, fungi and animals which preferentially bind reversibly to specific carbohydrate structures, either free in solution or on cell surfaces, and play an important role in cell recognition. *Sclerotium rolfsii* lectin (SRL) was purified from the sclerotial bodies of *S. rolfsii*, a soil-borne phytopathogenic fungus (Swamy et al., 2000). SRL is a monomer under acidic conditions (pH 4.3) with a molecular weight of 17 kDa and forms a dimer at neutral or basic pHs. SRL has been shown to recognize the Galβ1→3GalNAc-α1→Ser/Thr residues in glycoproteins, but has a diminished reactivity towards sialylated glycotopes (Swamy et al., 2000; Wu et al., 2001). This carbohydrate sequence occurs as the mucin type I core structure of O-linked oligosaccharide chains and its role as a T-antigenic substance has been established (Springer et al., 1979). SRL binds to this glucosyl moiety; considering that the T antigen is an oncodevelopmental cancer-associated antigen that predominately occurs in several carcinoma cell surfaces (Springer et al., 1975; Itzkowitz et al., 1989; Shamuddin et al., 1995), SRL and similar lectins may have greater applications (Wu, 1984). In this regard, peanut agglutinin and a lectin from *Agaricus bisporus* have been widely employed as special reagents for cell markers and as fine tools for elucidating changes on mammalian cell-surface structures during their differentiation. It has also been shown that subtle differences in carbohydrate reactivity of such T-antigen-binding lectins have marked effects on their interactions with human cancer cells and melanoma cell lines (Yu et al., 1993; Lorenz et al., 1997; Ryder et al., 1998).

Microbial lectins, particularly bacterial lectins called adhesins, have gathered significance over the last two decades, as they play a pivotal role in the initiation of the infection process by mediating adhesion to host cells (Sharon & Ofer, 2000). Findings with bacterial adhesins have attracted many to assign a similar role to phytopathogenic fungal lectins in host-parasite interaction (Radiger, 1998). In the past, only a few fungal lectins, mostly from higher fungi (mushrooms), have been successfully crystallized. Notable examples are those from *Phialophora verrucipes* (Ferrando et al., 1987), *Aturia aureusia* (Nagato et al., 1991) and *Pleurotus ostreatus* (Chattopadhyay et al., 1999). Partial structural characterization of two lectins from *Coprinus cinereus* (Cooper et al., 1997) and *Rhizoctonia solani* (Candy et al., 2001) has been reported based on their primary sequence; to date, no crystal structure is known of a fungal lectin. The structure determination of SRL will provide the first crystal structure of a fungal lectin and may also help in defining the structure-function relationships of other fungal lectins. Furthermore, structural analysis of SRL will allow the elucidation of the structural basis of its fine sugar specificity and will shed light on its biological role.

2. Crystallization

Purification of SRL from the fungus *S. rolfsii* has been described previously (Swamy et al., 2000). The freeze-dried lectin was diluted in 5 mM Tris-HCl buffer pH 8.0 prior to crystallization trials. Commercially available screens were used to determine the initial crystallization conditions (Molecular Dimensions Ltd, UK) using the hanging-drop vapour-
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diffusion method at a temperature of 289 K. Initial crystallization trials produced small needle-like crystals on mixing 2 µl of protein solution (20 mg ml⁻¹) with an equal volume of a reservoir solution containing 0.2 M ammonium acetate, 30%(v/v) 2-propanol, 0.1 M Tris-HCl buffer pH 8.5. However, these crystals (Fig. 1a) were extremely thin, while the drops contained a large amount of precipitate. The crystals diffracted to 4.0 Å resolution using an R-AXIS IV image plate mounted on a Rigaku RU-HE3HB rotating-anode X-ray source with Cu Kα radiation (λ = 1.5418 Å). As these SRL crystals grew in the presence of 2-propanol, various concentrations of 2-propanol, MPD and PEGs of various molecular weights were used to improve the crystal quality and size. Single crystals of identical morphology but of considerably better quality and size were grown within two weeks at 289 K using a reservoir solution containing 0.2 M ammonium acetate, 30% MPD, 0.1 M Tris-HCl buffer pH 8.5 and a protein concentration in the drop of 8 mg ml⁻¹ (Fig. 1b). Although these conditions produced better crystals, they did not help to eliminate protein precipitation in the drop. This optimization procedure is a good example of the influence that different non-polar precipitants can have on the quality and size of protein crystals.

2.1. Data collection

Diffraction data were collected from a single crystal at 100 K at station BW7B (λ = 0.8441 Å), EMBL Hamburg on DESHY. As SRL crystals were grown in a medium containing 30% MPD, no other cryoprotectant media was used and the crystal was simply transferred to a nitrogen-gas cold stream (Oxford Cryosystems Cryostream Cooler). Diffraction data to 1.1 Å resolution were recorded on a MAR Research 345 mm image-plate detector (Fig. 2). The exposure time was 5–10 s per image (dose mode), the oscillation range was 0.0° and a total of 160 images were collected. Additional data to medium (2.45 Å) and low (3.45 Å) resolution were collected using oscillation ranges of 1.4° and 2.0°, respectively. A total of 237 images were collected, producing 1 560 478 raw data images. Raw data images were indexed, integrated, corrected for Lorentz polarization effects, scaled and merged together using the HKL program suite (Otwinowski & Minor, 1997). Complete data-collection statistics are presented in Table 1.

3. Preliminary X-ray diffraction analysis

The systematic absences and symmetry were consistent with the space group P4₂₂₂, with unit-cell parameters a = b = 99.81 Å, c = 63.99 Å. The real-space self-rotation function with origin removal as implemented in the program CNS (Brünger et al., 1998) showed a single strong peak at χ = 180°, indicating twofold non-crystallographic symmetry. Thus, assuming two SRL molecules per crystallographic asymmetric unit, the Matthews coefficient (Matthews, 1968) is 2.3 Å³ Da⁻¹ and approximately 47% of the crystal volume is occupied by solvent. The presence of a dimer in the asymmetric unit is consistent with biochemical data indicating that SRL exists as a dimer at neutral or basic pH values (Swamy et al., 2000).

Attempts to solve the structure by molecular-replacement methods using as a starting model structures of various plant lectins [e.g. pea lectin (Prasthofer et al., 1989), peanut lectin (Banerjee et al., 1990), Maackia amurensis leukoagglutinin (Imberty et al., 2000)] and animal lectins [galectin 1 (Liao et al., 1994), 7 (Leonidas et al., 1998) and 10 (Leonidas et al., 1993)] have so far failed. These failures might be attributed to the high internal symmetry of the lectin-fold motif (β-sandwich: 12–14 strands in two sheets). We are presently searching for suitable heavy-atom derivatives in order to apply the isomorphous replacement method to the SRL crystal structure determination.

We thank the staff at EMBL Hamburg Outstation for their help during data collection. Access to the EMBL Hamburg Outstation (EU Contract No. HPRI-CT-1999-00017) is greatly acknowledged. This work was supported in part by Greek GSRT through PENED-204/2001 and ENTER-EP6/2001.

Table 1. Data-collection statistics.

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<tr>
<th>Space group</th>
<th>Unit-cell parameters (Å)</th>
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<tr>
<td>P4₂₂₂</td>
<td>a = b = 99.81, c = 63.99</td>
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Values in parentheses are for the last resolution shell.

Figure 1
Photograph of the SRL crystals (a) grown from the initial screening and (b) after optimization of the crystallization conditions. The dimensions of the crystal in (b) are approximately 1.0 × 0.1 × 0.1 mm.

Figure 2
An SRL crystal X-ray diffraction oscillation image. Inset: a magnification of a section of the diffraction pattern, where it can be seen that the SRL crystal diffracts beyond 1.1 Å (the edge).

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