Chapter 2

Purification and characterization of *Ganoderma lucidum* lectin
SUMMARY
A lectin from the fruiting body of *Ganoderma lucidum* was purified to homogeneity by 0-30% ammonium sulphate precipitation and hydrophobic chromatography on Phenyl-Sepharose column with an overall yield of 35%. The molecular mass of the lectin determined by gel filtration is 114 kDa and it is made up of six identical subunits of 18.6 kDa. It is a basic protein with a pI of 9.7. The purified lectin is a glycoprotein and contains 9.3% carbohydrate. Lectin is stable in the pH range 5-9, and temperature up to 50°C. The partial N-terminal sequence did not show similarity with any known lectin. Purified lectin agglutinated pronase treated human erythrocytes (A, B and O), with very low titre values. The hemagglutinating activity was inhibited by glycoproteins containing N-linked and O-linked glycans.

INTRODUCTION
Lectins are multivalent proteins or glycoproteins of non-immunogenic origin, which recognize and bind reversibly to diverse sugar structures in a highly stereo-specific and non-catalytic manner (1,2). Lectins have been isolated from various organisms such as- animals, plants, bacteria, viruses and fungi; though lectins from plant and animal sources have been extensively studied (3,4), very little information is available on lectins from fungal sources (5,6). In last few years mushroom-lectins have attracted increased attention due to their antitumor, antiproliferative and immunomodulatory activities (7-9). Lectins from several pathogenic fungi have also been studied in great detail but their physiological role has not been ascertained or remains uncertain (10-14).

The role of lectins in fungi seems to be more diverse than that in plants. In higher fungi, lectins probably play differential roles in different circumstances. Various roles have been postulated for fungal lectins which do not appear to be mutually exclusive - some of these seem to concern fungal metabolism itself, while other activities are implicated in symbiotic or parasitic relationships with other organisms (5).
Lectins from mushrooms viz *Agaricus bisporus, Boletus satanus, Flammulina velutipes, Ganoderma lucidum, Grifola frondosa, Tricholoma mongolicum, and Volvariella volvacea* show immunomodulatory and/or antitumor/ cytotoxic activities (6). *Ganoderma lucidum* is a well-known medicinal mushroom and many health-promoting and therapeutic effects have been attributed to this mushroom. An immunomodulatory protein with a molecular mass of 12.4 kDa, designated LZ-8, has been isolated from *G. lucidum* mycelia. Its amino acid sequence was homologous to that of a known immunomodulatory protein from the straw mushroom and it also showed hemagglutinating and mitogenic activities (15). An 18 kDa lectin was isolated from *G. lucidum* mycelia and another 16 kDa lectin was purified from *G. lucidum* fruiting bodies (16), both of which were distinct from LZ-8 as judged from the molecular mass and sugar binding activity.

**MATERIALS**

N-Acetylimidazole, 2,4,6-trinitrobenzenesulphonic acid, phenylglyoxal, diethylpyrocarbonate, phenylmethylsulfonyl fluoride, N-bromosuccinimide, 5,5' dithiobis-(2-nitrobenzoic acid), sodium borohydride, hydroxylamine hydrochloride, galactose, galactosamine, 2-deoxygalactose, L-fucose, glucose, mannose, methyl-α-D-galactose, methyl-β-D-galactose, mellibiose, lactose, Phenyl-Sepharose CL-4B, pronase-E, all glycoproteins, gums and the molecular weight markers (Sigma Chemical Co. St. Louis, U.S.A); Sephadex G-25 and Sephacryl S-200 (Amersham Bioscience, Uppsala, Sweden); trypsin and neuraminidase (Sisco Research Laboratories, Mumbai, India) and dextrose, peptone, malt extract, agar and yeast extract (HiMedia Laboratories Pvt. Ltd, Mumbai, India), were used. All other reagents were of analytical grade.
METHODS

Identification of *Ganoderma lucidum* fruiting bodies

Identification of *Ganoderma lucidum* was based upon the following characteristic features: Basidiocarps stipitate, stipe central, excentric or lateral, upper surface red, laccate, shiny fruiting bodies, leathery to tough woody, clamp connection present, spores broadly ellipsoid, with an apical thickening, truncate, brown, 8.3 – 10 x 5.8 – 6 µm, minutely vernucase, context brown, 2-10 mm, thick, hymenial surface whitish or creamish, later turning brown (17-22).

Erythrocyte preparation

Human erythrocytes of A, B and O blood groups were washed 5 to 6 times with 20 mM Tris-HCl buffer pH 7.2 containing 150 mM NaCl. A 3% (v/v) suspension of the erythrocytes in the above buffer was treated with different enzymes (pronase 0.05%, trypsin 0.05% and neuraminidase 0.1 U/ml) at 37 ºC for 1 h, washed 3 times with the same buffer and used for further studies.

Hemagglutination assays

Human erythrocytes of A, B and O blood groups were prepared as above, treated with 0.05% (w/v) pronase at 37 ºC for 1 h, and washed 4 times with the same buffer. Hemagglutination assays were performed in standard microtitre plates by the two-fold serial dilution method. A 50 µl aliquot of the erythrocyte suspension was mixed with 50 µl of serially diluted lectin and agglutination was examined visually after incubation for one hour. The unit of hemagglutination activity (U) was expressed as the reciprocal of the highest dilution (titre) of the lectin that showed complete agglutination. The specific activity of the lectin was defined as the number of hemagglutination units per milligram of the protein (U/mg).
**Hemagglutination inhibition assays**

Hemagglutination inhibition assays were performed similarly, except that serial dilutions of the sugar solutions (25 µl) were pre-incubated for 15 minutes at 27 ºC with 25 µl of the lectin (8 U). Erythrocyte suspension (50µl) was added, mixed and the plates read after one hour. The glycoproteins were desialated by incubating in 0.1M H₂SO₄ at 60 ºC for 1 h followed by dialysis against distilled water.

**Protein determination**

Protein concentrations were determined according to Bradford (23) using BSA as standard.

**Extraction and purification of lectin**

All the steps involved in purification of the lectin were carried out at 4–8 ºC. *Ganoderma lucidum* fruiting body was homogenized in liquid N₂. The homogenized powder was suspended (10% w/v) and crushed in 10 mM MOPS, 0.1% Tween20 (pH 6.5) for 5-6 h. Supernatant was collected by centrifugation at 10000g (20 min). The extract was then precipitated with ammonium sulphate (0-30% saturation). The precipitate was redissolved in 10 mM MOPS, 200 mM NaCl (pH 6.5).

The partially purified extract thus obtained was dialyzed against 10 mM MOPS, 200 mM NaCl (pH 6.5) and loaded on Phenyl Sepharose column pre-equilibrated with the same buffer at a constant flow rate of 12 ml/h. The column was washed with the same buffer and washing collected as fraction of 5 ml until $OD_{280}$ was <0.05. The elution was done by 10 mM MOPS (pH 6.5), 10% v/v ethylene glycol at constant flow rate (12 ml/h) and 3mL fractions were collected, checked for $A_{280}$ and hemagglutination activity.
Electrophoresis

Native PAGE of the purified lectin was carried out in 10% (w/v) polyacrylamide gel, pH 8.8, and the gels were stained with Coomassie Brilliant Blue R-250. SDS-PAGE was performed in 10% (w/v) polyacrylamide gel at pH 8.8 as described by Laemmli (24) and the protein visualized by staining with silver nitrate. Isoelectric focusing (IEF) in polyacrylamide gels was done according to Vesterberg (25) over the pH range 3-10.

Carbohydrate content

Purified lectin (400µg in 400 µl water) was incubated with 400 µl of 5 % (w/v) phenol for 10 min at room temperature. Two ml of sulphuric acid was then added and the mixture was allowed to cool for 20 min at room temperature. The color developed was then measured spectrophotometrically, at 490 nm by using galactose-mannose (4:3) as standard (26).

Molecular mass determination

Gel filtration: The molecular mass of the purified lectin was determined by HPLC (Waters Corporation) on a gel permeation column (Protein-PAK SW300, 300x7.8 mm) at pH 6.0 and 27 °C using β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome C (12.4 kDa) as reference proteins.

SDS-PAGE: SDS-PAGE was performed in 10% (w/v) polyacrylamide gel at pH 8.8 as described by Laemmli (24) using glutamate dehydrogenase (55 kDa), ovalbumin (45 kDa), glyceraldehyde 3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20 kDa), α-lactalbumin (14.2 kDa).
**MALDI-ToF**: Molecular mass of the purified lectin was determined by matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectrometry using a Voyager DE-STR (Applied Biosystems) equipped with a 337-nm nitrogen laser.

**Amino acid analysis**

The amino acid content of the purified lectin was analyzed using a commercial kit (AccQ-Fluor, Waters Corporation). Salt free lyophilized lectin (50 µg) was hydrolyzed under 6N constant boiling HCl, in vacuum sealed hydrolysing tubes for 24 h at 110 °C. The sample was then derivatized by 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) in borate buffer at pH 9 and 10 picomoles of the hydrolysate was loaded on AccQ-Ta9 column equipped with a fluorescent detector. Total cysteine was determined according to Cavallini et al. (27) and total tryptophan according to Spande and Witkop (28).

**N-terminal sequence analysis**

N-terminal sequence was determined by automatic Edman degradation by use of a Procise™ protein sequencer (Applied Biosystem) at the protein sequencing facility of IIT, Mumbai, India. The sample (700 pmol) was applied to a glass-fiber filter and then cycled through a conditioning process of fifteen repetitions of Edman chemistry.

**Effect of metal ions**

The activity of the purified lectin was determined in the presence of 10 mM each of Ca++, Mn++, Mg++ and also after dialysis against 10 mM of EDTA.

**Temperature and pH stability**

Effect of temperature on lectin stability was monitored in the range of 10 °C to 80 °C by incubating 25 µg of the lectin for 15 minutes at the
respective temperature, rapidly cooling in ice and assaying for hemagglutinating activity.

The pH stability of the lectin was determined by incubating 25 µg of lectin at different pH (pH 2-12) for 24 h. Buffers used – glycine-HCl (pH 2-3), acetate (pH 4-5), citrate-phosphate (pH 5), phosphate (6-7), Tris-HCl (pH 8-9) and glycine-NaOH (pH 10-12). The hemagglutinating activity was checked after 6, 12 and 24 h.

**Preparation of plant polysaccharides (gums)**

A number of plant gums were tested for hemagglutination inhibition of the lectin. The suspension of plant gums, gum arabic, gum tragacanth, gum guar, gum ghatti, gum karaya, gum locust, gum arabinogalactan, gum rosin and gum pontianac 0.5 g each in 10 ml of distilled water and gum mastic, gum elemi and gum storax 0.5 g each in 10 ml of 0.1 M NaOH were boiled for 2 h, centrifuged at 7,800 g for 30 min at 25 °C and the supernatants were subjected to alcohol precipitation (1:2 v/v). The precipitate formed was re-dissolved in 10 ml of hot water and reprecipitated with alcohol (1:2 v/v). The precipitate obtained was dissolved in distilled water and dialysed against the same. The concentration of sugar solution were determined in terms of neutral sugar by phenol sulphuric acid method (26).
RESULTS AND DISCUSSION

Identification of *Ganoderma lucidum* fruiting bodies

*Ganoderma lucidum* fruiting bodies were identified based on the features described in materials and methods. The mushroom *Ganoderma lucidum* has been said to be a complex species and suggestions have been made to identify and designate its strains separately. In our study fruiting bodies of *Ganoderma lucidum* have been obtained repeatedly from the same site and the same tree to maintain uniformity of the samples.

Lectin characterization

The results of a typical procedure for the purification of *G. lucidum* sp. lectin is given in Table 2.1. *G. lucidum* lectin eluted as single large peak of OD$_{280}$ and coincident single hemagglutination activity peak from Phenyl Sepharose column (Fig. 2.1). It was purified approximately 13-fold with final recovery of 35% (Table 2.1).

![Figure 2.1: Elution profile of *G. lucidum* lectin on Phenyl Sepharose column.](image-url)
The purified lectin migrated as a single band in native- as well as SDS-polyacrylamide gels indicating homogeneity (Fig. 2.2). The molecular mass of the lectin as determined by gel filtration in the presence and absence of dithiothreitol (DTT) was 114 kDa (Fig. 2.3), whereas that determined by SDS-PAGE was 18.5 kDa (Fig. 3).

The molecular mass of lectin was also determined by MALDI-ToF, which showed a peak corresponding to $m/z$ 18.6 kDa (Fig. 2.4). The results of SDS-PAGE, gel filtration, and MALDI-ToF mass analysis indicated that this lectin is a homo hexamer of 18.6 kDa subunits which are held together by non-covalent bonds. The lectin showed resemblance in being polymeric with other mushroom lectins reported from *Grifolia frondosa* and *Pholiota aurivella* (6).

### Table 2.1: Purification of *Ganoderma lucidum* lectin.

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Total Protein (mg)</th>
<th>Total Activity (units)</th>
<th>Specific Activity (units/mg)</th>
<th>Recovery of Activity (%)</th>
<th>Fold Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Extract</td>
<td>44.8</td>
<td>44800</td>
<td>1000</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>0-30% (NH$_4$)$_2$SO$_4$ precipitation</td>
<td>5.67</td>
<td>38400</td>
<td>6772</td>
<td>85.71</td>
<td>6.77</td>
</tr>
<tr>
<td>Hydrophobic interaction Chromatography</td>
<td>1.2</td>
<td>16000</td>
<td>13333</td>
<td>35.71</td>
<td>13.33</td>
</tr>
</tbody>
</table>

$^a$ The reciprocal of the highest dilution (titre) of the lectin that showed complete agglutination was expressed as a unit of hemagglutinating activity.

$^b$ The specific activity of the lectin is defined as units of the hemagglutinating activity per milligram of lectin.
Except for few lectins as from *Lactarius lignyotus* and *Phallus impudicus* the subunits of which are linked by disulphide bridges, the other di-, tetra-, or polymeric lectins studied, namely those of *Agaricus edulis*, *Agaricus campestris*, *Aleuria aurantia*, *Flammulina velutipes*, *Hericium erinaceus*, *Lactarius deliciosus*, *L. deterrimus*, *L. salmonicolor*, *Pholiota aurivella*, *Pleurotus cornucopiae* and *Xerocomus chrysenteron* have subunits held together by non-covalent bonds (5).

Figure 2.2: Electrophoresis of purified lectin.
(A) Native electrophoresis of *G. lucidum* lectin at pH 8.8.
(B) IEF-PAGE of *G. lucidum* lectin. A 50 µg of purified lectin was loaded and carrier ampholines of the range 3-10 were used.
G. lucidum lectin is a glycoprotein containing 9.3% neutral sugar. It is a basic protein with a pI of 9.7 (Fig 2.2). G. lucidum lectin was active between pH 5-9, and temperature up to 50 °C. However, incubation at 60°C and 70°C for 1 hour led to 50% and 100% loss in activity of the lectin, respectively. The lectin also did not require divalent cations for its activity since extensive dialysis against 10 mM EDTA, 20 mM MOPS, 200 mM NaCl (pH 6.5) followed by dialysis against plain buffer (to remove EDTA) did not affect the hemagglutinating activity and there was no increase in hemagglutinating activity on the addition of 1, 5 and 10 mM Ca++, Mn++, Mg++.

Figure 2.3: Molecular mass determination of G. lucidum by gel filtration chromatography on Sephacryl S-200 column. (a) b-amylase (200 kDa), (b) alcohol dehydrogenase (150 kDa), (c) bovine serum albumin (66 kDa), (d) carbonic anhydrase (29 kDa) and (e) cytochrome-C (12.4 kDa). G. lucidum lectin (114 kDa). Inset represents elution profile of G. lucidum lectin on Sephacryl S-200 column.
Figure 2.4: Molecular mass determination of *G. lucidum* lectin by MALDI-ToF showed single sharp peak at 18.6 kDa. Inset represents SDS-PAGE of the *G. lucidum* lectin; Lane 1: Purified *G. lucidum* lectin. Lane 2: (molecular weight markers). Molecular weight of lectin by SDS PAGE. (a)Bovine serum albumin (66,000) (b) Glutamic dehydrogenase (55000), (c) Ovalbumin (45000), (d) Glyceraldehyde 3-phosphate dehydrogenase (36000), (e) Carbonic anhydrase (29000), (f) Trypsinogen bovine pancreas (24000), (g) Trypsin inhibitor (20000), (h) α-Lactalbumin (14200) (i) *Ganoderma* Lectin (18600)

The amino acid composition of *G. lucidum* lectin showed that it contained a large amount of Gly (11.7%), Ala (10.0%), AsX (8.8%), a moderate amount of Thr (7.6%), GlX (7.1%), Ser (7.6%), Leu (6.4%), Tyr (6%), Phe (5.9%), Lys (5.9%), Arg (4.7%), and low amount of Pro (2.4%), Val (2.4%), Ile (3.1%), His (2.4%), Cys (1.77%), Trp (0.6%) (Table 2.2). N-terminal sequence (first twenty residues) of the lectin was QFIYNGKFNWLNYYALNETIT which did not show similarity to any known lectin.
Table 2.2: Amino acid composition of the *G. lucidum* lectin

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>No. of residues per molecule in <em>Ganoderma lucidum</em> lectin</th>
<th>Mol % in <em>Ganoderma lucidum</em> lectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsX</td>
<td>90</td>
<td>8.8</td>
</tr>
<tr>
<td>Thr</td>
<td>78</td>
<td>7.6</td>
</tr>
<tr>
<td>Ser</td>
<td>78</td>
<td>7.6</td>
</tr>
<tr>
<td>GlX</td>
<td>72</td>
<td>7.1</td>
</tr>
<tr>
<td>Pro</td>
<td>24</td>
<td>2.4</td>
</tr>
<tr>
<td>Gly</td>
<td>120</td>
<td>11.7</td>
</tr>
<tr>
<td>Ala</td>
<td>102</td>
<td>10.0</td>
</tr>
<tr>
<td>Cys(^#)</td>
<td>18</td>
<td>1.77</td>
</tr>
<tr>
<td>Val</td>
<td>24</td>
<td>2.4</td>
</tr>
<tr>
<td>Ile</td>
<td>42</td>
<td>3.1</td>
</tr>
<tr>
<td>Leu</td>
<td>66</td>
<td>6.4</td>
</tr>
<tr>
<td>Tyr</td>
<td>108</td>
<td>6.0</td>
</tr>
<tr>
<td>Phe</td>
<td>60</td>
<td>5.9</td>
</tr>
<tr>
<td>His</td>
<td>24</td>
<td>2.4</td>
</tr>
<tr>
<td>Lys</td>
<td>60</td>
<td>5.9</td>
</tr>
<tr>
<td>Trp(^$)</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>Arg</td>
<td>48</td>
<td>4.7</td>
</tr>
</tbody>
</table>

\(^\#\) Determined according to Cavallini et al. (27)

\(^\$\) Determined according to Spande and Witkop (28)
Two other lectins have been isolated from *Ganoderma lucidum*, GLL-M, an 18 kDa lectin from mycelia and GLL-F, a 16 kDa lectin from the fruiting body (29). GLL-F and GLL-M exist as monomers in native state in contrast to 114 kDa lectin we have isolated which exists as hexamer in its native state. This lectin is different from GLL-M in terms of pI (4.5), carbohydrate content (9.3%). Both 114 kDa lectin from *Ganoderma lucidum* and GLL-M have a higher amount of Gly, Ala, AsX, Thr, GlX, Ser and Leu but remarkable differences can be observed in composition of other amino acids (16). This lectin is also different from LZ-8, an immunomodulatory protein, isolated from *Ganoderma lucidum* which shows hemagglutinating activity but is not characterized as lectin and is a homodimer of 24 kDa (30).

The lectin did not agglutinate normal or trypsinized erythrocytes but did agglutinate only pronase-E treated erythrocytes and showed titre value of 78 ng (Table 2.3). Pronase-E, a cocktail of different proteolytic enzymes, removes all protruding polypeptide from the erythrocyte membrane exposing GPI-anchors, glycoproteins and glycolipids, which may serve as better ligands for *G. lucidum* lectin.

**Table 2.3: Specific activity of *G. lucidum* lectin with different blood group erythrocytes treated with different enzymes.**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Hemagglutinating activity with different erythrocytes (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Untreated</td>
<td>0</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>0</td>
</tr>
<tr>
<td>Pronase</td>
<td>13300</td>
</tr>
<tr>
<td>Trypsin</td>
<td>0</td>
</tr>
</tbody>
</table>
**Hemagglutination inhibition**

Simple sugars glucose, mannose, lactose, galactose, rhamnose, xylose, fucose, raffinose, glucosamine, mannosamine, galactosamine, N-acetylmannosamine, N-acetyl-galactosamine and N-acetyl-glucosamine failed to inhibit the hemagglutinating activity of *G. lucidum* lectin even at 500 mM, whereas the plant polysaccharide gum karaya exhibited inhibitory activity at 7.81 µg (Table 2). The hemagglutinating activity was also inhibited by glycoproteins, *viz.*, fetuin, asialofetuin, fibrinogen, asialo-fibrinogen, thyroglobulin, holotransferrin, ovalbumin and invertase. Among these glycoproteins, asialofetuin was the best inhibitor with a minimum inhibitory concentration of approximately 2.47 µg (Table 2.4).

Glycoproteins possess different N-linked and O-linked glycans, as ligands for lectins to interact. Asialofetuin has three triantennary N-linked core structure with a terminal Galβ1→4GlcNAc and three O-linked structure Galβ1→3GalNAcα1→OSer/Thr (Tα Antigen), while the asialofibrinogen has a biantennary N-linked structure with terminal Galβ1→4GlcNAc residues (31). The lectin activity was also inhibited by plant polysaccharide gum karaya. Gum karaya consists of D-galactose, D-gluconic acid and L-rhamnose but the details of molecular structures are still not known completely (32).

Very few lectins from mushrooms have been reported with hemagglutination activity unaffected by simple sugars and inhibited only by glycoproteins as from *Volvariella volvacea* (7,33) and *Mycoleptodonoids aitchisonii* (34). Other mushroom lectins with hemagglutination activity unaffected by either simple sugars or glycoproteins are from *Flammulina velutipes* (35) and *Lyophyllum shimeiji* (36).

Protein carbohydrate interactions between fungal lectins and carbohydrates could be the basis of various molecular processes such as growth and morphogenesis, molecular recognition and parasitic or symbiotic relationships with other organisms (5).
Table 2.4: Inhibition of hemagglutinating activity of *G. lucidum* lectin with different glycoproteins and polysaccharides.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Minimum inhibitory amount* (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetuin</td>
<td>4.95</td>
</tr>
<tr>
<td>Asialofetuin</td>
<td>2.47</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>39.06</td>
</tr>
<tr>
<td>Asialofibrinogen</td>
<td>19.53</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>9.90</td>
</tr>
<tr>
<td>Holotransferrin</td>
<td>19.53</td>
</tr>
<tr>
<td>Invertase</td>
<td>54.68</td>
</tr>
<tr>
<td>Gum karaya⁵</td>
<td>7.81</td>
</tr>
</tbody>
</table>

Simple sugars, gum elemi, gum arabinogalactan, gum rosin, gum mastic, gum arabic, gum tragacanth, gum locust bean and gum xanthan were not inhibitory.

* Amount of inhibitor that can cause inhibition of 8 U hemagglutinating activity of neuraminidase treated type O human erythrocytes.

⁵ In terms of neutral sugar content.

The lectins, via acting directly on carbohydrates by their specific binding sites, or indirectly by enzyme systems, possibly control depolymerization of stored material and intense movement of sugars, resulting initiation and often explosive growth of the sporomes. Lectin can repress the activity of the glycoprotein enzymes by binding them at their carbohydrate moieties. The release of simple sugars could thus redirect metabolism through competition for lectin binding (37). A lectin may be involved in ensuring cohesion between hyphae during the development of the basidiome as seen in *Pleurotus cornucopiae* (38).
During their growth, many fungi build specialized organs that require mycelial aggregation. These organs, cords, fans of mycelium, strands, rhizoids *etc.* are found particularly in parasitic and wood-rotting species, which use these organs to force their way amongst the host cells. *Rigidoporus lignosus*, a parasite of many tropical trees infects roots by the vegetative mycelium, but propagates *via* the fans of mycelium. A lectin seems to be involved in the building of the latter structures, since it has been found in significant amounts only in the hyphae that are able to aggregate and in the fans of mycelium themselves (5). This lectin seems therefore to be a cell wall lectin involved in a recognition mechanism.

Presence of lectins, with strict specificities, in fungal cells of ectomycorrhizal symbiosis, suggests that lectins might be involved in recognition between the tree and its symbiont. The hypothesis was further exemplified by study of lectins from three fungi *Lacterius deliciosus*, *L. deterrimus* and *L. salmonicolor*, though very close morphologically, are specifically associated with different trees *Pinus*, *Picea* and *Abies*, respectively. Three essential conditions were verified experimentally: (i) In spite of being present in closely related species the three lectin differ significantly in structures and their microspecificities towards oligosaccharides; (ii) It was demonstrated, by shedding from cell walls by enzyme degradation or location by means of polyclonal antibodies, that the lectin is present at the surface of cultured hyphae; (iii) The receptors, bore by the root cell surfaces of each of the conifers, were exclusively for the lectin of their own associated fungus (5).
References


