Chapter 4

Functional characterization of Glucose/mannose specific lectin and Galactose specific lectin


**Introduction**

The major function of lectins appears to be in cell recognition. This is due to the complementarity shown between lectins and the structures present on the cell surfaces. This concept represents the "Lock and Key" hypothesis introduced by Emil Fischer to represent the specificity between enzymes and substrates.

Legume lectins associate symbiotically with the soil bacteria of *Rhizobia* family, thus making them independent from soil nitrogen supplies. When *Rhizobia* encounter root hairs in the soil, its attachment to the root hairs occurs by interaction between the rhizobial surface carbohydrates and the lectins present in the roots of the legume plants. This is known as the "lectin recognition hypothesis". The toxicity of various plant lectins for animals and their growth inhibitory effect on fungi (Mirelman *et al.*, 1975) are the basis for the assumption that they function in the defense of plants against phytopathogenic fungi, predatory animals, insects and bacteria (Keen, 1992; Ayoubo, 1994).

The immature lymphocytes of the mouse and human cortical lymphocytes differ markedly from the niedullary mature lymphocytes in their surface carbohydrates, as evidenced from the fact that the former are bound and agglutinated by pea nut agglutinin (PNA+ cells) whereas the latter are not (PNA-) (Sharon, 1983). Separation with peanut agglutinin provides facile access to the individual thymocyte sub-populations and makes it possible to examine *invitro* their developmental and functional relationship. Selective agglutination by SBA permits separation of B and T mouse splenocytes. The main application of this lectin is for purging human bone
marrow for transplantation (Aversa et al, 1994). It is employed routinely for the transplantation into children born with severe combined immune deficiency. It is also used for leukemic patients as an alternative for T cell depletion such as monoclonal antibodies. The lectin from *Dolichos bijlorus* is used to distinguish between A1 and A2 subgroups.

Certain lectins are potent mitogens, activating lymphocytes and inducing them to divide. PHA and Con A for example, stimulate T lymphocytes, while pokeweed mitogen stimulates both the T and the B cells (Di Sabato et al, 1987; Borrebaeck et al, 1989). The mitogenic lectins are polyclonal activators in that they activate lymphocytes irrespective of their antigenic specificity. Prior to the advent of monoclonal antibodies to cell surface antigens, lectins were the major tools for studies of the mechanism of cell activation. Mitogenic stimulation of lymphocytes by plant lectins is routinely made use in cell culture techniques. It also provides an easy and simple means to assess the immunocompetence of patients suffering from diseases such as AIDS and to monitor the effects of various immunosuppressive and immunotherapeutic manipulations. It has also been used to examine the effect of stress, both physical and psychological, on the immune system. It is employed for the preparation of chromosome maps for different purposes, such as karyotyping, sex determination, and detection of chromosome defects since chromosomes are easily visualised in the stimulated cells.

The mitogenic lectins interact with unique cell surface sugars that may act as "triggering receptors" present on the membranes (Lis and Sharon, 1977). The earliest detectable changes are seen in the membrane. These include increased permeability for a variety of metabolites such as glucose, amino acids, \( \nabla C \) and \( Ca^{2+} \) ions and an
accelerated turnover of membrane phospholipids. This leads to the stimulation of acetylation of histones, phosphorylation of nuclear proteins and modification of lipid and carbohydrate metabolism. After a day, RNA and protein synthesis accelerate and morphological changes become apparent. At about 48 hr, DNA synthesis starts and the cells enter mitosis. This causes reversion of the postmitotic cells to small lymphocytes. The stimulated lymphocytes release biologically active lymphokines (Cohen et al., 1979) like the IL-2 (Robb, 1984) and y interferon (Epstein, 1981). 76 hours after stimulation, other differentiated functions of the activated lymphocytes, such as immunoglobulin production by B cells and cytotoxicity of T cells are detected (O'Brien et al., 1978).

For numerous purposes lectin derivatives are required. Lectins conjugated to fluorescent dyes, gold particles or enzymes are employed as histochemical and cytochemical reagents for detection of glycoconjugates in tissue sections, on cells and subcellular organelles, and in investigations of intracellular pathways of protein glycosylation (Rhodes et al., 1998). The binding of the lectins to the membrane receptors of hormones, growth factors, neurotransmitters and toxins indicate that they are glycoconjugates. Immobilized lectins that are covalently bound to Sepharose are indispensable for the isolation and purification by affinity chromatography, glycoproteins, glycopeptides and oligosaccharides (Debray, 1991; Hasselback et al., 1993; Yamamoto, et al., 1993; Carlson, 1994).

In the present study it was envisaged to see whether the glucose/mannose specific lectin and the galactose specific lectin purified from Indian lablab beans can act as antifungal
agents, as mitogens and whether they could be used in the purification of some
glycoproteins.
Materials

All the fungi used in the present study were a kind gift from Prof. Appa Rao Podile, Department of Plant Sciences, University of Hyderabad. Blood samples were collected from healthy donors. $^3$H Thymidine was obtained from BRIT, BARC, India. Mannose 6-phosphate receptor deficient cells MPR minus (-) cells were a kind gift from Prof. Dr. Dr. h.c. Kurt von Figura, Göttingen, Germany. All the chemicals and the reagents used in the present study were of high purity and obtained from reputed firms.

Methods

Preparation of biospecific adsorbents

The following ligands have also been coupled to Sepharose that has been activated with divinyl sulfone as described in chapter 2: N- acetylglucosamine and Lactose

Purification of lectins

All operations were carried out at 4 °C. Centrifugations were done at 12,000 rpm using a Sorvall refrigerated centrifuge. The following lectins were purified using published procedures and the purity of the lectins confirmed by SDS-PAGE and then these were used in the studies.

Purification of Concanavalin A lectin from Jack Bean Meal (legume)

100 g of Jack Bean Meal (Canavalia ensiformis) was suspended in 1 M sodium chloride containing 5 mM calcium chloride and 5 mM manganese chloride (extraction buffer). This was stirred overnight at 4 °C and the crude extract obtained after centrifugation was subjected to 80% ammonium Sulfate saturation. The pellet was dissolved in extraction buffer and extensively dialyzed against the same buffer. The
suspension was centrifuged and the supernatant was applied on G-100 gel equilibrated with the same buffer. After extensively washing the gel with buffer, bound protein was eluted with 0.1 M glucose and the absorbance was measured at 280 nm. Peak fractions were pooled and dialyzed against water, lyophilized and stored at -20 °C.

**Purification of Wheat germ agglutinin (non-legume)**

100 g of wheat germ defatted seed powder was stirred overnight with 0.05% sodium acetate buffer at pH 4.5 at 4 °C. To the crude extract obtained after centrifugation, 40% ammonium Sulfate was added and the protein pellet obtained was dissolved and dialyzed against phosphate buffered saline (PBS, pH 7.4). The protein was applied on an affinity gel Sepharose-DVS-N-acetylglucosamine. The gel washed with buffer and eluted using 0.5 M N-acetyl glucosamine in PBS. Absorbance was measured at 280 nm. Peak fractions were pooled, dialyzed against water, lyophilized and stored at -20 °C.

**Purification of Unio lectin from whole animal tissue (animal lectin)**

200 g of *Unio* tissue was homogenized in 25 mM Tris-HCl buffer pH 8.0 containing 50 mM sodium chloride (TBS) and the extract clarified by centrifugation in cold. To the clear supernatant solid ammonium Sulfate was added to 80% saturation. The suspension was clarified by centrifugation and the pellet so formed was dialyzed against TBS and applied on Sepharose-lactose gel equilibrated with TBS. After extensively washing the gel with TBS, bound lectin was eluted with 0.2 M lactose in buffer. Protein containing fractions were pooled, dialyzed against water and stored at -20 °C.
Purification of Mannose specific lectin from Dolichos lablab seeds

100 g of defatted bean powder was suspended in TBS and stirred overnight at 4 °C. The crude extract obtained after centrifugation was subjected to 60% ammonium Sulfate saturation. The suspension was clarified by centrifugation and the pellet was dissolved and dialyzed against TBS. This was applied on Sepharose-mannose gel equilibrated with TBS. After extensively washing the gel with TBS, bound lectin was eluted with 0.25M glucose in TBS. The lectin was dialyzed against water and lyophilized and stored at -20 °C.

The Galactose specific lectin from these seeds was purified as described in chapter 2.

Preparation of lectin-Affigel

Affinity purified lectins obtained above were immobilized to Affigel (Bio-Rad labs) following the instructions of the manufacturer. Coupling of purified lectins to affigel was carried out at 4 °C. For each lectin 4 mL of affigel -10 was washed successively with ice cold isopropanol, water and then with 0.1 M Hepes buffer.

Purified lectins (10 mg/mL) were dialyzed against 0.1 M Hepes and the corresponding ligand (0.1M) that is inhibitory to the lectin was added. The lectins were separately coupled overnight to affigel-10. The unbound fraction was then collected and stored. To the gel, 0.2 mL of 0.1 M ethanolamine–HCl pH 8.0 was added, followed by Hepes buffer to attain a final concentration of 0.1 M. The gel was allowed to rotate for 1 hour at 4 °C. The gel was finally washed with phosphate buffered saline and stored at 4 °C until use. The amount of lectin bound to each of the affigel was calculated by measuring the protein concentration in the unbound fraction according to manufacturer's instructions.
Inhibition of fungal growth by the lectins

Petri plates containing potato dextrose agar were inoculated with *Trichodenna viridae*, *Aspergillus flavus* and *Aspergillus niger*. Simultaneously, wells were drilled in the plates about 3 cm from the centre and filled with 100 µg of Glucose/mannose specific lectin, Galactose specific lectin. Wheat germ lectin, Concanavalin A and the *imio* lectin. The plates were kept in dark at 25 °C. The inhibition of fungal growth was assessed by the growth of the fungal colony on the plates.

Mitogenic stimulation of lymphocytes by the lectins

**Cell preparation:** Lymphocytes were isolated from heparinised human peripheral blood by centrifugation over histopaque solution (specific gravity 1.077) (Parish *et al*., 1974). 8 mL of venous blood was collected and was put into a 30 mL tube containing 6 units of heparin per mL of blood and was mixed gently. The blood sample was kept under refrigeration until use. The blood sample was diluted with an equal volume of normal saline. 8 mL of diluted blood was layered over 3 mL of histopaque solution. Care should be taken to prevent the mixing of the two solutions. The tubes were centrifuged for 25 minutes at 2000 rpm. Supernatant containing the plasma was carefully removed with a pasteur pipette without disturbing the interface which contains the lymphocytes and platelets. Then, the interface was collected. The cell suspension was then transferred to RPMI-1640 containing 5% FCS. The cells were sedimented by centrifugation at 2000 rpm for 10 minutes and washed once more with the medium and then stored in ice until use.

**Lymphocyte count:** Lymphocyte suspension was diluted appropriately in Turk's solution. Using a micropipette a small quantity of the diluted cell suspension was
loaded into the counting chamber. The cells were allowed to settle for a couple of minutes and then counting was done under 10X magnification.

Number of cells/mL = average number of cells/large square \times 10^4

Dilution

**Determination of cell viability:** Four parts of 0.2% Trypan blue is mixed with one part of 5 X saline. To one part of the Trypan blue - saline solution, one part of cell suspension was added (1:2 dilution). The cells were loaded on a hemocytometer and the number of unstained and stained cells was counted separately. The viable cells were counted using the formula

$$\text{Dilution} = \frac{\text{Average number of viable cells in large square} \times 10^4}{\text{mL}}$$

% of viability was counted as $\frac{\text{number of viable cells} \times 100}{\text{number of viable cells} + \text{dead cells}}$

**Mitogenic stimulation:** This was done following the method of Bradley (1979). Peripheral blood lymphocytes ($2 \times 10^5/0.2$ mL) were cultured in triplicate in RPMI-1640 medium supplemented with 5% fetal calf serum. 5 (ig of the lectins (the galactose specific lectin and the glucose / mannose specific lectin) were added to these cells. In addition, wheat germ agglutinin was also taken as the control. The cultures were incubated in an atmosphere of 5% CO$_2$ for 72 hours. For the last 24 hours of culture, the cells were pulsed with 1 uCi of $^3$H-thymidine. The cells were harvested onto glass-fiber filters using Skatron automatic cell harvester. The incorporation of radioactivity
into DNA was determined in a Beckman scintillation counter. The values are expressed as cpm per 10⁶ cells.

In order to determine the sugar specificity of the mitogenic stimulation by the glucose / mannose specific lectin and the galactose specific lectin, they were pre-incubated in the presence of specific sugars, that is 0.2 M mannose and 0.3 M galactose, respectively, for 24 hours. Then, the lectins were assessed for mitogenic activity as described above.

Glucose consumption during mitogenic stimulation: Mitogenic stimulation on the lymphocytes by the lectins was also determined by a modification of the glucose consumption method described by Hori el al., (1987). Similar cultures were set up as described above. At 24 hour intervals, the glucose concentration in the supernatant in each well was estimated by the Phenol - sulphuric acid assay. The glucose consumption by the lymphocytes is determined by subtracting the concentrations of glucose obtained at different intervals of time from that obtained initially.

Interaction of the galactose specific lectin with normal human peripheral blood lymphocytes and multiple myeloma cells

Lymphocytes have been prepared as described as above.

Preparation of FITC labeled lectin: This was prepared according to Clark and Shepard (1963). About 1% of the galactose specific lectin in 0.025 M Na₂CO₃ and 0.025 M NaHCO₃ was dialyzed against fluorescein isothiocyanate (0.1 mg/mL) in the same buffer. After 24 hours the conjugated galactose specific lectin was dialyzed against phosphate buffered saline, pH 7.3 until fluorescence was no longer detected in the dialysate. The conjugate was then ready for use.
**FITC - lectin binding assay:** Human peripheral multiple myeloma cells (U266 cells) and normal human peripheral blood lymphocytes (0.1 mL, 3x 10⁶) were incubated with FITC conjugated to galactose specific lectin (0.1 mL) for 15 minutes at room temperature (Reisner *et al.*, 1979). The cells were then washed with PBS and finally suspended in 0.1 mL of the same buffer. The percentage of fluorescent cells was determined for the two sets of lymphocytes under a fluorescent microscope.

**Interaction of the lysosomal enzymes secreted from cultured MPR (-) cells with the lectins coupled to affigels**

MPR (-) cells were cultured in Dulbecco's modified eagle medium, DMEM, containing 10% fetal calf serum. Cells were grown in standard flasks, as well as in 3 cm petri plates in an incubator, at 37 °C, in an atmosphere containing 5% CO₂ as described by Matzner *et al.*, (1996). Later, the cell secretions were concentrated by ammonium sulphate and the protein pellet dialyzed extensively against TBS, pH 7.4. This protein (2 mg/mL) was loaded on the different lectin affigels pre-equilibrated with TBS, pH 7.4. The gels were then washed with the same buffer and the bound protein was eluted using specific sugars that is: 0.3 M mannose on glucose/mannose lectin affigel; 0.3 M galactose on galactose lectin affigel column; and 0.3 M N acetyl glucosamine on wheat germ lectin column.

Aliquots of the eluted fractions obtained from the different columns were analyzed for (3N -acetyl hexosaminidase and ex-mannosidase activities. 10% SDS - PAGE was carried out for all the peak fractions obtained from the above columns and the protein bands identified by silver staining. In another experiment, the eluted fractions were separated on a 10 % SDS-PAGE, the proteins transferred to a nitrocellulose membrane,
and the membrane blotted using Con A-Biotin. The membrane was developed using Avidin-ALP conjugate and the bands detected using the substrate BC IP/NBT. The eluates from the galactose lectin-affigel and the glucose/mannose lectin affigel were also analyzed on a 10% SDS-PAGE, and the proteins transferred to a nitrocellulose membrane. The membrane was probed separately with the bovine α-mannosidase antibody and the ρ N-acetylhexosaminidase antibody. The blot was developed using standard protocols as described earlier in the thesis for the detection of protein bands.

Interaction of the glycoproteins present in normal human serum with the lectin-affigel

Normal human serum was dialyzed against TBS pH 7.4 and was applied separately on the galactose affigel, glucose/mannose lectin affigel, wheat germ lectin affigel equilibrated with TBS. After washing the gels with TBS, the gels were eluted specifically with 0.3 M galactose and 0.3 M glucose and 0.5 M N-acetylglucosamine respectively. The protein in the eluates from the gels was monitored at 280 nm and aliquots from the peak fractions were separated on 10% SDS-PAGE and identified by silver staining. In a separate experiment the eluted fractions from the galactose lectin affigel and the glucose/mannose lectin affigel were separated by SDS-PAGE and then proteins transferred to nitrocellulose membrane. The membrane was probed with antibodies to bovine α-mannosidase and the ρ N-acetylhexosaminidase to identify if there are any glycoproteins that bind to these lectins specifically.
Results

Table 14 shows the different lectins purified by affinity chromatographic procedures and the concentration of the lectins bound to the affigels. Fig. 23 shows the growth of the fungi, *Trichoderma viridae, Aspergillus niger* and *Aspergillus flavus* in the presence of the lectins - Con A, glucose/mannose specific lectin, galactose specific lectin, wheat germ lectin and *imio* lectin. None of the lectins have exerted any inhibitory effect on the fungi.

From Fig. 24, it is evident that the galactose specific lectin obtained from the seeds of the *Dolichos lablab* did not exhibit any mitogenic activity to the human peripheral blood lymphocytes. In contrast, the glucose / mannose specific lectin from the same seeds exhibits mitogenic stimulation several fold at a concentration of 5 fg. This stimulation by the glucose / mannose lectin is 10,000 fold higher in activity (cpm / 10⁶ cells) when compared to the control mitogenic lectin, wheat germ agglutinin (Greene *et al.*, 1981). In the presence of 0.2 M mannose, the mitogenic stimulation of the glucose / mannose lectin reduced considerably, indicating the specificity of the mitogenicity. Further in the presence of glucose/mannose specific lectin, there was an increase in the glucose consumption with the increase in the time of incubation. On the other hand the galactose specific lectin showed negligible amount of glucose consumption. The control, wheat germ agglutinin showed glucose consumption to the same extent as the glucose/mannose specific lectin. (Fig. 25).

Fig. 26 shows the microscopic view under the fluorescence microscope of the FITC labeled galactose specific lectin that is bound to the normal human peripheral
lymphocytes and to the human peripheral multiple myeloma cells, U266. The galactose specific lectin binds to few normal human peripheral blood lymphocytes (40%) (Fig. 26 A), but it binds to most of the multiple myeloma cells (95%) (Fig. 26 B).

The results of the interaction of the lysosomal enzymes secreted from cultured MPR (-) cells with the lectins coupled to affigel are shown in Fig. 27. (A) shows a 10% SDS-PAGE of the proteins obtained from the MPR (-) cell secretions that were specifically bound to the lectin-affigels and eluted from these gels using the corresponding sugar ligand. Protein bands were detected by silver staining. These proteins in the eluates were also identified by Western blot analysis, using Con A-biotin (Fig. 27 B).

The eluates from the galactose lectin affigel and the glucose/mannose lectin affigel were also analyzed by Western blot analysis using the bovine ot-mannosidase antiserum (Fig. 27 C). While no protein band could be detected from the galactose lectin affigel, a single protein band corresponding to Mr 200 kDa was detectable in the glucose/mannose lectin affigel eluates and Fig. 27 D shows the results with p N-acetyl hexosaminidase antiserum. With the galactose lectin affigel eluate, a single protein band at Mr 66 kDa was detectable and no protein band could be detected in the eluates from the glucose/mannose specific lectin affigel.

The results on the interaction of the glycoproteins present in normal human serum with the lectin affigels are shown in Fig 28. (A) shows a 10% SDS-PAGE of the proteins (silver staining) from the normal human serum that bound on the lectin affigels. Two distinct protein bands were detectable in the molecular range of 66 and 55 kDa that were eluted from the galactose lectin affigel and glucose//mannose lectin affigel. However, only a single protein band corresponding to Mr 66 kDa was detectable in the
eluates from wheat germ lectin affigel. Fig. 28 B shows the blot of the eluted fraction from the galactose-affigel and glucose lectin affigel column probed with α-mannosidase antibody. While, three protein bands in the range of 66 kDa, 40 kDa and 35 kDa were detectable in the galactose lectin affigel eluate, no protein bands could be detected in the glucose/mannose lectin-affigel eluates. Fig. 28 C shows the blot of the eluted fraction from the galactose lectin specific affigel and glucose/mannose specific lectin affigel column probed with p-N acetyl hexosaminidase antibody. While a single protein band in the region of 66 kDa was detectable in the galactose lectin affigel, no protein band was detectable in the glucose-mannose lectin affigel eluate.
Table 14: Affinity purified lectins and the concentration of lectins bound to affigel
<table>
<thead>
<tr>
<th>Lectins</th>
<th>Sugar specificity</th>
<th>Native molecular mass and sub unit structure</th>
<th>Affinity matrix</th>
<th>Cone, of protein bound to the affigel (per mL gel)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Canavalia ensifor mis</em></td>
<td>Glucose/mannose</td>
<td>106 kDa $\text{tx}_4$(25)</td>
<td>G-100</td>
<td>6.75 mg</td>
</tr>
<tr>
<td><em>Dolichos lablab</em></td>
<td>Glucose/mannose</td>
<td>60kDa $\text{oc}_2$(15), $\text{oc}_2$(32(12))</td>
<td>Scpharose -Mannose gel</td>
<td>7.75 mg</td>
</tr>
<tr>
<td><em>Dolichos lablab</em></td>
<td>Galactose</td>
<td>120kDa $\text{a}_2$(31), $\text{P}_2$(29)</td>
<td>Scpharose -galactose gel</td>
<td>4.67 mg</td>
</tr>
<tr>
<td>Wheat germ lectin</td>
<td>N-acetyl glucosamine</td>
<td>36 kDa $\text{c}_2$(18)</td>
<td>Scpharose -N-acetyl glucosamine gel</td>
<td>5.9 mg</td>
</tr>
<tr>
<td><em>Unio lectin</em></td>
<td>Lactose</td>
<td>105 kDa $\text{a}_2$(28), $\text{P}_2$(23)</td>
<td>Scpharose-Lactose gel</td>
<td>7.43 mg</td>
</tr>
</tbody>
</table>

Table 14
Figure 23: Effect of lectins on the growth of the fungi

A) *Aspergillus flavus* B) *Aspergillus niger* C) *Trichoderma viridae*  Well A contains Con A, well B contains glucose/mannose specific lectin, well C contains galactose specific lectin, well D contains wheat germ lectin and well E contains *unio* lectin
Figure 23
Figure 24: Mitogenic stimulation of lymphocytes by the lectins

The amount of $^3$H-Thymidine incorporated into the lymphocytes is measured in a scintillation counter. Panel A indicates the response of the lymphocytes in the presence of Galactose specific lectin, B in the presence of Glucose/mannose specific lectin, C in the presence of Wheat germ lectin.

Figure 25: Consumption of glucose by the lymphocytes in the presence of lectins

Circles indicate the glucose consumption by the lymphocytes in the presence of Galactose specific lectin, squares indicate the glucose consumption by the lymphocytes in the presence of Glucose/mannose specific lectin, triangles indicate the glucose consumption by the lymphocytes in the presence of Wheat germ lectin.
Figure 26: Fluorescence microscopy of FITC-galactose specific lectin in sections of A) Normal peripheral blood lymphocytes and B) Human peripheral Multiple myeloma cells (1)266).
Figure 27: Detection of glycoproteins in the secretions of Mannose 6-phosphate deficient cells.

A. 10% SDS-PAGE of the Mannose 6-phosphate deficient [MPR (-)] cell secretions, bound and eluted from the lectin affigels. Lane 1: molecular weight markers, Lane 2 : Galactose lectin affigel eluate, Lane 3 : Glucose/mannose lectin affigel eluate, Lane 4 : Wheat germ lectin affigel eluate.

B. Con A - Biotin blot of the MPR (-) cell secretions eluted from the lectin affigels.
Lane 1: Galactose lectin affigel eluate, Lane 2: Glucose/mannose lectin affigel eluate, Lane 3 : Wheat germ lectin affigel eluate.

Western blot of the MPR (-) cell secretions bound and eluted from the lectin affigels to detect for the presence of

C) a-mannosidase, using bovine mannosidase antibody and D) B N-acetyl hexosaminidase.
Lane 1: Galactose lectin affigel eluate. Lane 2: Glucose/mannose lectin affigel eluate.
Figure 28: Detection of glycomponents in normal human serum.

A. 10% SDS-PAGE of the human serum eluted from the lectin affigel.

Lane 1: molecular weight markers, Lane 2: Galactose lectin affigel eluate, Lane 3: Glucose/mannose lectin affigel eluate. Lane 4: Wheat germ lectin affigel eluate.

Western blot of the normal human serum eluted from the lectin affigel to detect for the presence of B) α-mannosidase and C) 15 N-acetyl hexosaminidase.

Lane 1: Galactose lectin affigel eluate, Lane 2: Glucose/mannose lectin affigel eluate.
Discussion

Mannan, chitin and other saccharides are important components of most of the fungal cell walls. It is believed that most of the antifungal lectins cross-link with these carbohydrates preventing cell expansion at the growing hyphae. This binding could slow hyphal growth as a first line of integrated defense mechanism (Chrispeels and Raikhel, 1991). Some known examples of antifungal lectins are WGA, (Mirelman, 1975; Ciopraga et al., 1999) TEL (Maria das Gracas et al., 2002), GAFP-1, from Gastrodia elata (Xu et al., 1998); UDA from Stinging nettle (Van Parijs et al., 1992). WGA binds to all fungi, except for those that are lacking chitin. Con A, however, binds poorly to these residues which are present on the cell walls. SBA and PNA bind to Pencillia and Aspergilli strongly suggesting the presence of D-galactose on their surfaces. These studies show that the lectins in plants are a part of their protection system helping them to combat attack by fungal pathogens.

Since none of the lectins tested in this study show any antifungal activity towards any of the fungi studied, it is thereby concluded that the lectins tested are poor binders of polysaccharides present on the cell walls of fungi.

Lectins have the ability to trigger quiescent, non-dividing cells into a state of growth and proliferation. Several lectins purified have been shown to be potent mitogens (Lis and Sharon, 1977; Tsuda, 1979). In 1990, lentil seed storage proteins that have been affinity purified on immobilized lentil lectin have been found to be strong stimulators of murine B lymphocyte proliferation (Freier et al., 1990). The galactose specific lectin from Dolichos lablab is found to be unusual as it does not stimulate proliferation of
lymphocytes, unlike other lectins. The mitogenic stimulation by the lectin is also confirmed by the glucose consumption by the lymphocytes, which is a measure of blastogenesis of the cells. In the presence of the galactose specific lectin, the lymphocytes consume negligible amount of glucose, indicating that there was no appreciable mitogenesis. But, in the presence of the glucose / mannose specific lectin, there was an increase in the glucose consumption with the increase in number of days. This increase was almost similar to that observed by the wheat germ agglutinin, a known mitogen. This shows that the glucose / mannose lectin does act as a mitogenic lectin.

FITC conjugated lectins bind more to multiple myeloma cells than to normal human lymphocytes. This indicates that they are more of carbohydrate binding sites in the myeloid series than in the normal lymphocytes. Thus, lectins may serve as targets for drug binding and enable a selective destruction of the pathologic cells. The lectins if bound to a drug may direct and concentrate the drug on the target cells. The use of Duanomycin bound to Con A has been demonstrated in a mouse model system (Kittao and Hattori, 1977). The validity of PNA as a marker of immature blood cells and its potential clinical application have been discussed by Reisner et al., (1979). FITC conjugated PNA binds to exposed carbohydrate surfaces present on the malignant colonic mucin epithelium. (Boland et al., 1982). Thus, from the present study using FITC, the galactose specific lectin can be used as tracers of malignant formations.

Lectins in free form are relatively crude probes for detecting differences in the fine structure of glycoconjugates. Immobilization of lectins covalently bound to affigels are useful tools for the purification of glycoproteins. As mentioned earlier, the laboratory
where this work has been carried out has been studying the evolution of mannose 6 phosphate receptors that mediate the transport of lysosomal enzymes to lysosomes and the cells lacking these receptors [MPR (-) cells] secrete their lysosomal enzymes. So, these cells were cultured in the lab following the standard published protocols. All lysosomal enzymes are known to be glycoproteins and contain N-glycan structures that can be recognized by Con A. In order to analyze if these secretions contain some proteins that can specifically bind to the *Dolichos lablab* lectins, we allowed the secretions containing the proteins to be passed through the lectin affigels prepared. As an additional lectin we chose wheatgerm lectin-affigel. From the results it is apparent that the lectins from the *Dolichos lablab* and the wheatgerm can preferentially bind some proteins that are present in the MPR (-) cell secretions. It is apparent from the results that the proteins eluted can be recognized also in a Con A-biotin blot suggesting them to be glycosylated. On the other hand, the eluted protein from the galactose lectin affigel could be recognized by 3 N-acetylhexosaminidase antibody. The glucose/mannose lectin affigel eluate however could be recognized by the bovine α-mannosidase antibody.

When normal human serum was applied on the galactose lectin affigel, glucose/mannose affigel and wheatgerm agglutinin-affigel, some proteins bound on these gels which could be specifically eluted with the respective sugar ligands. The eluted protein peak fraction from both the gels was analyzed by SDS-PAGE as duplicates, and the proteins transferred to nitrocellulose membrane. One portion of the membrane containing eluates from both the gels, was probed with the bovine α-mannosidase antibody, while the other with the p N-acetyl hexosaminidase antibody.
Interestingly, when the α-mannosidase antibody was used, some protein bands could be detected on the blot. Their molecular sizes are much smaller than the lysosomal enzyme. Since these experiments were performed using human serum, it is likely that these proteins may be the constituents of normal human serum that are being recognized by this antibody. From the results obtained it is difficult to ascertain the nature/function of these proteins now. On the other hand, the P N-acetyl hexosaminidase antibody recognized a single protein species that eluted from the galactose lectin affigel. From these different results, it is logical to conclude that the *Dolichos lablab* lectins can have potential applications in the isolation and characterization of some glycoproteins.