CHAPTER I

*Introduction and Review of Literature*
INTRODUCTION

The social behavior of the living cells including intercellular communication, the regulation of cell growth and differentiation, the immune response and perhaps malignancy is primarily mediated by the surface of the cells or more particularly by the branching sugar molecules that stud the cell surface. Our present knowledge about the architecture and function of the cell surface are due in large part to a class of proteins called "lectins" (1). Lectins (from the Latin word legere, to pick or choose) are sugar binding proteins or glycoproteins of nonimmune origin, which agglutinate cells and/or precipitate glycoconjugates (i.e. carbohydrate rich macromolecules) having saccharide of appropriate complementarity (2). They bear at least two binding or recognition sites which bind non-covalently to specific carbohydrate residues without modifying these residues chemically (3). The important properties of these lectins on the cells include blood group specific hemagglutination (4-6), preferential agglutination of malignant cells (7-9), and mitogenicity (10-13). Because of their specificity towards specific binding of sugar molecules on the cell surface, they have found widespread application in cell biology as macromolecular probes for delineating the architecture of cell surface glycoconjugates (14-17). These lectins have become
fashionable tools used in diverse research fields such as hematology (18), immunology (19), oncology (20), virology (21), electron microscopy (22) and other areas of biochemistry (19, 23).

HISTORY

The first description of a lectin or hemagglutinin was reported by Stillmark in 1888 (24). While investigating the toxic effects of extracts of castor bean (Ricinus communis) on blood, he observed that the red blood cells from human and animals were being agglutinated. He obtained evidence that the material responsible for the agglutination was a protein and gave it the name as 'ricin'. Shortly afterward, Hellin in 1891 discovered that the toxic extracts of the seeds of Abrus precatorius also caused the red cells to clump or agglutinate together and he named it as 'abrin' (25). In 1897, Elfstrand reported the presence of one more toxic factor called 'crotin' from Croton triglium (26) and in 1915, Harris similarly showed that extracts of Glycine max (soybean) seeds exhibit the property of agglutinating erythrocytes (27).

Lectins have played an important role in the development of immunology. As early as 1897, Ehrlich (28) showed that specific immunity to the toxic lectins 'ricin' (from Ricinus communis) and 'abrin' (from Abrus precatorius) could
be achieved by repeated subcutaneous injections of small amounts of these antigens into white mice. In the same year, he also carried out the first quantitative determination of an antibody in vitro (29), this immune serum inhibited 'ricin' - red blood cell agglutination. Furthermore, 'abrin' was employed by Landsteiner (30) in an early demonstration of the reversibility of the antigen-antibody reaction; 'abrin' was displaced from agglutinated RBC's by incubating at 50°C and then centrifuging the cells.

Later in 1908, Landsteiner and Raubitschek (31) reported for the first time the specificity of hemagglutinins for RBC's of different animal species and non-toxicity of these hemagglutinins. In 1948, Renkonen (32) and Boyd and Reguera in 1949 (33) independently reported that extracts of some seeds contain agglutinins specific for blood group antigens. As a result of these observations, Boyd and Shapleigh (34) in 1954 proposed the term 'Lectins' for agglutinins. Usually 'lectin' has been generally accepted; 'phytoagglutinins' and 'phytohemagglutinins' are still used for lectins from plant sources.

Two discoveries in the early 1960s by Nowell and others (11) contributed significantly to the realisation of the enormous potential of lectins in biological research. First one is the transformation of lymphocytes from small
resting cells into large blast like cells by phytohemagglutinin (PHA), which ultimately may undergo mitotic division (11). The second discovery by Aub and his coworkers in 1963 (35) was that *Triticum vulgare* (wheat germ agglutinin) agglutinated malignant cells at concentrations much lower than that required for the agglutination of normal ones. Since then, mitogenic activity was detected in 16 species out of 166 species, belonging to the family Leguminosae. Waxdal in 1974, isolated the most potent plant mitogen from crude extracts of pokeweed, (*Phytolacca americana*) (36). Certain agglutinins have also been shown to agglutinate malignant cells preferentially and inhibit the growth of such cells *in vitro* and *in vivo* (37-40).

Recently, many reports have shown the correlation between the lectin-binding activities of nitrogen-fixing bacteria which nodulate leguminous plants (41,42), and the involvement of lectins in the inhibition of fungal growth (43,44). Both these functions indicate, that these proteins play an important role in host-parasite relationships in plants.

**DEFINITION AND NOMENCLATURE**

The definition of lectins is an operational one, which has undergone several modifications depending upon the accumulation of information on them. By 1972 the
original definition of lectins as plant agglutinins was expanded to include 'agglutinins' from sources other than plants, and particular emphasis was placed on the carbohydrate-binding properties of these molecules (45). Goldstein and his coworkers (2) in the year 1980, have proposed the definition of lectin as "a sugar-binding protein or glycoprotein of non-immune origin which agglutinates cells and/or precipitates glycoconjugates". This definition deletes those carbohydrate-binding proteins like carbohydrate-specific enzymes, transport proteins, hormones, and toxins that contain only one carbohydrate-binding site and was adapted in 1981 by the Nomenclature Committee of IUB and IUB-IUPAC Joint Commission on Biochemical Nomenclature (46).

Since the above definition has eliminated carbohydrate-binding proteins that have not been shown to agglutinate cells, yet are structurally related to lectins from the same plant, another revised definition has been proposed. Recently in 1983, Kocourek and Horejsi (47) have formulated a new definition that almost overcomes the above drawbacks; hence, the lectins can be defined as 'proteins of non-immunoglobulin nature capable of specific recognition and reversible binding to carbohydrate moieties of complex carbohydrates without altering the covalent structure of any of the recognised glycosyl ligands'.
Lectins are known by their trivial names (e.g., concanavalin A, PHA, PNA) or according to their source, either by its common name (e.g., soybean agglutinin, phytohaemagglutinin) or botanical name (e.g., Glycine max lectin, Dolichos biflorus lectin, Arachis hypogea lectin). Most of the new lectins are named by the latter system. Lectins from plant sources are usually named by their common names. The latter naming system will be used in this thesis.

REVIEW OF LITERATURE

The characteristic biological properties of lectins and their physiological role has led to intense research studies over the years and the subject matter has been reviewed extensively. The reviews and monographs by Sharon and Lis (45), Goldstein and Hayes (2), Sharon (1), Gold and Balding (48), Brown and Hunt (3) and Bog Hansen and coworkers (48a) deal with the general aspects of lectins from different sources. Lis and Sharon (49), Liener (50), Etzler (51) and Markov and Kharkin (52) have reviewed various aspects of lectins, particularly from plant kingdom. The lectins have been reviewed with respect to their toxicity effects (53, 54), applications in the study of cell surface architecture (55, 56), as tools in cancer research (57, 58), in immunological studies (59, 60) and in the isolation of glycoproteins and glycoconjugates (61-63). Caron and his coworkers in 1984,
have reviewed the biological functions of lectins in plants, animals and man (64). Recently, Kolb-Bachofen has reviewed mammalian lectins and their functions (64a). Barondes has reviewed animal lectins (65). A monograph on Con A by Bittiger and Schnebli (66), emphasises the many applications of this lectin, but the procedures described may be used with other lectins too. Different purification procedures for lectins, their characterisation and applications have been described briefly in several volumes of *Methods in Enzymology* (67-69). Lis and Sharon in 1986, have reviewed the biological roles and applications of plant, animal and microbial lectins (69a).

The survey of literature on lectins is mainly confined to plant lectins, especially those from leguminosae family.

**DISTRIBUTION AND OCCURRENCE OF LECTINS**

Lectins are widely distributed in nature and are particularly rich in plants. Lectin containing plants have been found in many botanical groups including mono- and dicotyledons, but most frequently they have been detected in leguminosae and Euphorbiaceae families (53,70). According to Toms and Western (71), 95 percent of the lectins reported since 1948 are derived from the seeds belonging to the family Leguminosae. In a survey of 2663 varieties of plant extracts, hemagglutinating activity was found in over 800 varieties (51)
and in the family Leguminosae alone, over 600 species and
varieties have been shown to contain such activity (71).
Leguminosae, Euphorbiaceae, Malvaceae, Verbenaceae, Polygonaceae, Solanaceae and Acanthaceae are the dicotyledonous families which contain hemagglutinin positive species, whereas the important monocotyledonous families containing hemagglutinin positive species are Araceae, Zingiberaceae, Palmae and Graminaceae.

Plant seeds are the richest source of lectins, where
they may comprise up to several percent of the dry matter,
usually 2-10 percent of the total protein (51,72).

Besides seeds, lectins are also present in other
vegetative tissues such as tubers, roots, rhizomes, stem,
leaves, bark, phloem exudate etc. The distribution of lectins
is not confined only to plants, but they are now being isolated in increasing numbers from animal tissues (48). Similarly there are several reports about the presence of lectins in fish (73), snails (74), eels (75) and even in mammalian tissue (76-79). They are also detected in bacteria (80), fungi (81) and lichens (82).

Information about the cellular and subcellular loca-
tion of lectins is limited. High levels of lectin are
found in the cotyledon and embryo axis of the lentil (83,84),
pea (85) and red kidney bean (86) but little in the seed
costs. High levels of lectin activity were also detected in the extracts of inner mitochondrial membranes of the endosperm, golgi bodies, endoplasmic reticulum and plasma membrane fractions of mung bean hypocotyls (87-89).

DETECTION AND ASSAY OF LECTINS

The presence of a lectin in a plant is readily detected by testing whether an extract of the plant agglutinates, erythrocytes from human or animal sources, or both (90-92). Figure 1.1 shows the agglutination of cells by lectins.

Blood group specific lectins are identified with the aid of a panel of typed human erythrocytes. Incubation of erythrocytes with trypsin, papain, neuraminidase and some other proteolytic enzymes renders them more sensitive to agglutination (5,6,92). The cells may also be cross-linked by glutaraldehyde or formaldehyde to stabilise them and to provide a standard cell suspension that can be used for long periods of time (93).

The hemagglutinating activity of lectins is generally carried out at room temperature by adding 2 to 3% erythrocyte suspension to tubes or concave wells in microtiter assay plates containing serially diluted lectin solution. After incubation for 1-2 hours, the agglutination is observed by the naked eye or photometrically or microscopically (55).
FIGURE 1.1
AGGLUTINATION OF RABBIT RBCS BY LECTIN(WBTL).

FIGURE 1.1a
SCHEMATIC REPRESENTATION OF AGGLUTINATION OF CELLS.
(Figure taken from reference 1).

- Lectin
- Glycoconjugate receptors
The visual determination is accurate only within a factor of 2 (72), while the photometric method is laborious and requires larger amounts of material. Agglutination may also be monitored automatically using a fragiligraph (94,95) or aggregometer (96). Lectin activity is expressed in terms of 'titers', the reciprocal of the maximal dilution of lectin that gives visible agglutination. Under suitable conditions, purified lectins agglutinate erythorocytes at concentrations as low as 0.1-1 μg/ml and occasionally even lower (72).

Lectins may also be detected by their ability to form precipitates with naturally occurring glycoproteins (97-99), synthetic carbohydrate-protein conjugates (100-103) and polysaccharides (104-107), either in liquid (capillary tubes) or semisolid (agar gel) media (108). Such interactions also provide information regarding lectin specificity, as well as on the constituent sugars and glycosidic linkages of the glycoprotein or polysaccharide precipitated.

A new procedure for the detection of lectins termed "affinity electrophoresis" which combines the principles of affinity chromatography and electrophoresis has been developed recently by Horejsi and his coworkers (109,110). In this technique, proteins are subjected to electrophoresis on a matrix formed by copolymerization of alkenyl glycosides with acrylamide. Proteins having combining sites complementary
to the ligand are retarded, whereas other proteins undergo a normal separation. By determining the electrophoretic mobility of a lectin in the presence of several concentrations of its specific sugar ligand, one can determine the dissociation constant of the lectin-sugar complex. Affinity electrophoresis has also been used to study sugar-binding heterogeneity of lectins and their chemically modified derivatives (111).

**ISOLATION AND PURIFICATION OF LECTINS**

Isolation of lectins generally begins with a saline (0.9% NaCl) or buffer extraction of the finely ground meal. Pre-extraction with organic solvents like acetone or methanol or diethyl ether is often employed to remove lipid or other interfering substances (112-115). Ammonium sulphate fractionation, centrifugation, and dissolution of the precipitate yields a supernatant containing the lectin(s). Plant agglutinins may be isolated from the saline extracts by conventional protein-purification techniques, affinity chromatography, or a combination of both. At present, virtually all lectin purification schemes employ affinity chromatography, based on the ability of lectins to bind saccharides specifically and reversibly (116,117). Knowledge of the sugar specificity of a lectin, which can be obtained from hapten-inhibition studies using simple sugars and crude lectin
preparations, permits the design of a suitable purification procedure. The potent inhibitors of lectin like mono- or oligosaccharides, or glycoproteins such as hog gastric mucin or desialylated fetuin are coupled to insoluble matrices; naturally occurring or chemically modified substances such as Chitin (118), Sephadex (119-124) and Agarose or Sepharose (125-128) have also been employed as affinity matrices.

Application of affinity chromatography to lectin purification have been adapted to purify different lectins from a variety of different sources (117). A few examples are: Horejsi and Kocourek (128) copolymerised a series of alkenyl glycosides with acrylamide, whereas Matsumoto and Osawa (129) incorporated a variety of sugar residues into starch; both materials have been used as affinity matrices in the isolation of lectins (130). Reaction of epoxy-activated Sepharose-63 with 2-acetamido-2-deoxy-D-glucose and D-galactose afforded affinity columns used for the purification of wheat germ agglutinin (WGA) and soybean agglutinin (SBA), respectively (130). Affinity chromatography on aminoethyl polyacrylamide gel containing reductively aminated disaccharide residues like lactose, melibiose, maltose and N,N'-diacetyl chitobiose was used by Baues and Gray (131) to isolate lectins from Bandeiraea simplicifolia seeds, castor beans, jack beans, lentil and wheat germ. Different purification schemes have been employed in Sephadex affinity
chromatography; \( \alpha\)-D-glucopyranosyl and \( \alpha\)-D-mannopyranosyl
binding lectins from the seeds of *Canavalia ensiformis*
(Cor*\( ^{23} \)*A) (119-121), *Vicia cracca* (132), *Vicia faba* (122).
*Pisum sativum* (PNA) (123) and *Lens culinaris* (Lentil) (120,
124) were isolated in this way. Similarly, \( \beta\)-D-galacto-
pyranosyl binding proteins such as 'ricin' (*Ricinus communis*)
(126) and 'abrin' (*Abrus precatorius*) (133) have been purified
on Agarose. Lectins from soybean, *Wisteria floribunda,
Bauhinia purpurea alba* and *Sophora japonica* have been isolat-
ed by affinity chromatography on acid treated Sepharose-6B,
but not on untreated Sepharose (134). Cross-linked guaran
was used for the isolation of lectins from *Helix pomatia,
Glycine max*, *Ricinus communis* and *Echinocystis lobata* (Wild
cucumber) and the \( \alpha\)-D-galactopyranosyl binding protein from
*Bandeiraea simplicifolia*; however, the lectins from *Dolichos
biflorus*, *Phaseolus lunatus* and *Sophora japonica* failed to
bind to this adsorbent (135). In 1978, Majumdar and Surolia
(136) have reported a general method for the isolation of
\( \alpha\)-galactose specific and \( \beta\)-galactose specific lectins by
using cross-linked arabinogalactan. The lectin from *Dolichos
biflorus* which is N-acetyl-D-galactosamine specific has been
purified by adsorbing on copolymerised hog blood group A+H
substance followed by elution with N-acetyl-D-galactosamine
(137).
A variation on chemical coupling of saccharides to solid supporting substances has been described by Matsumoto and Osawa (129). They used epichlorohydrin to couple L-fucose to starch and utilised this to purify eel and *Ulex europeus* specific lectins. The eel serum lectin has been shown to bind to the column and was eluted by lowering the pH, while the *U. europeus* lectins were retarded but did not bind to the column. But when tri-N-acetyl chitotriose was coupled to starch, *U. europeus* and *Cytisus sessilifolius* lectins were purified, showing their specificity towards diacetyl chitobiose. Different methods have been used for the incorporation of the sugar ligand to the matrix and also used in the purification of lectins from *U. europeus*, *B. simplicifolia*, *P. sativum* etc. (128,129,138-142).

Some of the lectin binding glycoproteins coupled to the insoluble matrix have been used in the purification of different lectins. An affinity system for the isolation of the lectin from red kidney bean (*Phaseolus vulgaris*) (129,143) involved thyroglobulin-Sepharose, and for the purification of *Limulus polyphemus*, bovine submaxillary mucin-Sepharose (144). Fetuin-Sepharose was employed for the isolation of the agglutinins from wheat germ (*Triticum vulgaris*), horse-shoe crab (*L. polyphemus*), jack bean (*C. ensiformis*) and several other sources (145). Wheat germ agglutinin has
also been isolated by using insolubilised ovomucoid (146).

Reitherman et al. and others have used erythrocytes treated with formaldehyde and glutaraldehyde as affinity adsorbents for lectin isolation (147-149). Trypsinised erythrocytes suspension have also been used as affinity adsorbent for the purification of a lectin from tomato fruit juice (150).

CARBOHYDRATE-BINDING SPECIFICITY OF LECTINS

The carbohydrate-binding specificities of lectins were determined by measuring the ability of free monosaccharides or their alkyl glycosides to inhibit lectin induced agglutination of cells or glycoconjugates. This can be usually established by the hapten-inhibition technique of Landsteiner (151). Sugar-lectin complementarity is established by comparing sugar on the basis of the minimal concentration required to inhibit:

i) The precipitation reaction between the lectin and a specific glycoconjugate (152-154),

ii) The hemagglutination reaction (155).

Other different procedures used for the carbohydrate specificities of lectins include equilibrium dialysis (156), ultraviolet spectroscopy (157,158), elution from solid phase
adsorbents (159), precipitation studies with natural (160-162) and model carbohydrate-protein conjugates (163), fluorimetric, NMR and temperature jump relaxation (156, 157, 164-168) etc. and have provided very valuable information.

In general, lectins recognise D-pyranose sugars and in most instances they exhibit rather stringent configurational and structural requirements in the interacting constituents (169). For example, at C-1 a strong preference of sugars in the α-anomeric configuration is shown by lectins which bind to erythrocyte 'ABO' - blood group antigens specified by α-linked N-acetyl galactosamine, galactose and fucose respectively, while Con A and RCA show specificity for α-mannosides which are unrelated to known blood group determinants (170, 171). Other lectins however, have little or no anomeric preference. Lectins accept minimal variation at C-3 and C-4 with the result that they may effectively discriminate between galactose and glucose or N-acetyl galactosamine and N-acetyl glucosamine. Some lectins can accommodate variation at C-2 as illustrated by the relatively weak inhibition of Con A by glucose, which has about one-tenth the activity of mannose (172). An N-acetamido group at C-2 is important for the sugar binding properties of many lectins such as SBA (GalNAc-specific) and WGA (specific for GlcNAc sequence) but it inhibits sugar recognition by PNA and RCA which exhibit a primary specificity for galactose and show
little interaction with GalNAc (127,173). A small group of lectins, for example Ulex europaeus - I and the eel serum agglutinin, exhibit strong specificity for the L-sugar, L-fucose and are not inhibited by most of the unsubstituted D-sugars (174,175) or their methyl glycosides. Sialic acid binding lectins have been described in the haemolymph of marine invertebrates. A lectin from L. polyphemus, which preferentially recognises the N-glycosyl derivative of sialic acid, the acyclic region of the sugar molecule does not influence lectin binding (176).

In 1957, Makela (177) studied the inhibition by sugars of hemagglutination of over 50 plant lectins. According to his observation and also of others, he divided aldo-pyranoses into four inhibitory groups based on their configuration at C-3 and C-4 of the pyranose form. Some examples of sugars of these classes are:

Group I : D-Arabinose, L-Fucose and L-Galactose

Group II : D-Galactose, D-Fucose, L-Arabinose and Lactose

Group III : D-Glucose, D-Manose, Fructose, Sucrose and Maltose.

Group IV : D-Iodose, D-Gulose, L-Rhamnose, L-Gulose and L-Xylose.
According to this classification, lectins from pea (Pisum sativum) (178), lentil (179,180) and Con A preferentially bind Makela's group III sugars and are inhibited by D-glucose and D-mannose. Whereas lectins from the seeds of Sophora japonica, R. communis, Z. simplicifolia (181) were inhibited by group II sugars and the lectins from Lotus tetragonolobus and U. europeus (182) belong to group I, being inhibited by L-fucose. As yet, no lectin has been isolated that interacts with Makela's IV group sugars.

For some lectins, no efficient monosaccharide inhibitors have been found and they are inhibited only by some oligosaccharides. These lectins include PHA which is inhibited by very low concentration (~10 nM mol/ml) of a glycopeptide released from human erythrocytes by trypsin (183,184). The best inhibitor of potato (S. tuberosum) lectin is the (1→4) linked pentasaccharide of N-acetyl glucosamine which is more active than the corresponding tetramer, trimer and dimer (185); GlcNAc by itself is not inhibitory at concentrations up to 0.2M. The lectin from Datura stramonium is also inhibited by oligomers of GlcNAc but not by the monomer (186,187). On the other hand, wheat germ agglutinin, which is specific to oligosaccharides of GlcNAc, is also inhibited by monosaccharide (112). WGA also interacts with sialic acid residues (7,188) by electrostatic interactions because
of its high pI value (8.7±0.3). In addition, the lectin also binds GalNAc. The binding of sialic acid and GalNAc is possible because of the configurational similarity of these sugars with GalNAc at positions C-2 (N-acetamido group) and C-3 (hydroxyl-) of the pyranose ring.

The use of alkyl αC- and β-glycosides as hapten inhibitors in addition to free sugars yields valuable information on the anomeric specificity of a lectin. Glycosides with aromatic aglycons are often strong inhibitors because they interact with hydrophobic sites on the lectin (78). Moreover, lectins may bind strongly to hydrophobic molecules that do not contain saccharide structures.

Lectins differ markedly with respect to their anomeric specificity. Some are specific for only one anomeric configuration, whereas others interact equally well with both anomeric configurations. The lectins from Con A (189), B. simplicifolia (115) and L. tetragonolobus (175) exhibit pronounced specificity for the α-anomers of mannose, glucose, galactose and L-fucose respectively. On the other hand, SBA (190,191) and R. communis lectin (126) are completely devoid of anomeric specificity. There are no cases of lectins with clear cut specificity for aliphatic β-glycosides. The specificity of the 'B-lectins' found in many plants (192) is based on studies with complex aromatic glycosides.
From the specificity studies, it is possible to draw conclusions about the size and shape of the saccharide-binding sites of lectins (193). Obviously, lectins which interact more strongly with oligosaccharides than with monosaccharides have more extended sites. The combining site of the lectin from Euonymus europeus (194) is unusual in that it is complementary to the two blood group-B tetrasaccharides which differ in the linkage of the subterminal galactose to N-acetyl galactosamine.

Generally, the specificity of lectin varies widely. A few lectins exhibit narrow specificity, while some have a broader specificity. According to the sugar specificities, the lectins can be classified into nine groups (116). These are as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Sugar specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L-Fucose</td>
</tr>
<tr>
<td>2</td>
<td>N-acetyl-D-glucosamine and N-acetylated chitodextrins</td>
</tr>
<tr>
<td>3</td>
<td>N-acetyl-D-galactosamine</td>
</tr>
<tr>
<td>4</td>
<td>D-Galactose</td>
</tr>
<tr>
<td>5</td>
<td>N-acetyl-D-galactosamine and D-galactose</td>
</tr>
<tr>
<td>6</td>
<td>D-Glucose</td>
</tr>
<tr>
<td>7</td>
<td>oC-Glycosides and oC-N-acetyl-glucosaminides</td>
</tr>
</tbody>
</table>
When lectins bind to carbohydrates in glycoproteins or other glycoconjugates they interact with specific domains of relatively complex oligosaccharides. Based upon their sensitivity to different carbohydrate inhibitors, recently Gallagher (169) has classified lectins into two main groups or classes.

Class I: Lectins with simpler binding mode

Class II: Lectins with complex binding mode

All lectins in class I recognise the complementary monosaccharides located at the non-reducing end of carbohydrate chains (195). They may be defined as lectins that can be inhibited by 50 percent or more during quantitative precipitation or agglutination binding assays in the presence of monosaccharide concentrations of 10 mM or less than that. Most of the GlcNAc-specific (196,197) and GalNAc-specific (191,198) lectins fall into this category. Others, such as Con A and RCA, will bind to monosaccharides found either as internal or external constituents (170,199). The binding
energy of many of these lectins may be profoundly influenced by sugars in the immediate vicinity of the complementary sugar and this may result in marked differences in oligosaccharide recognition by lectins with identical monosaccharide specificities.

Class II lectins constitute an important and striking group of recognition molecules which may be effectively inhibited only by linear or branched carbohydrate sequences. They exhibit little or no affinity for the component monosaccharide units of the interacting glycan chains. Two of the examples are the isolectins from *P. vulgaris*, erythroagglutinin (E-PHA) and leucoagglutinin (L-PHA), so called because of their ability to selectively agglutinate red cells and lymphocytes. Both these lectins bind to complex, N-linked glycans but there are conspicuous differences in the fine structural features of their complementary oligosaccharides. Several of the class-II lectins bind to the 'ABO' - blood group substances and also bind to carbohydrate sequences consisting of two or more different monosaccharides.

The class-II lectins are further divided into two groups:

i) homotypic sequences

ii) heterotypic sequences.
Homotypic sequence groups are those, which bind most strongly to sequences of identical sugar units, whereas, heterotypic sequence groups are those which bind to carbohydrate sequences consisting of two or more different monosaccharides.

The well known examples for class-II category are potato lectin (185) and wheat germ agglutinin (112).

**PHYSICO-CHEMICAL PROPERTIES OF LECTINS**

**a) Molecular properties**

Lectins show considerable variation in their composition, molecular weight, subunit structure and number of sugar binding sites etc. (Table 1.1 and 1.2). Some lectins are very unusual with respect to composition and structure: these are lectins from potato, tomato and *Datura stramonium* (200,201) which belong to Solanaceae family. These lectins are glycoproteins containing a high percentage of L-arabinose and hydroxy-proline, a rarely occurring amino acid.

As far as amino acid composition of the lectins is concerned, many of the lectins are relatively rich in aspartic acid, serine and threonine, which may comprise as much as 30 percent of their amino acid content and are low in or completely devoid of sulphur containing amino acids (45).
For example, lectins from *Glycine max* (202), *Lens culinaris* (203), *Canavalia ensiformis* (121), *Phaseolus vulgaris* (204), *Lotus tetragonolobus* (139) and *Dolichos biflorus* (137) are devoid of cysteine. In contrast, lectins from wheat germ, potato and poke weed are rich in cysteine (20, 11.5 and 18 percent of the total amino acid residues respectively). Significant amount of cysteine and methionine (23 and 12 residues per mole respectively) were also detected in the snail lectin. Indeed, the high content of disulphide bonds in WGA endows the protein with stability to heat (7,35), to proteolytic enzymes and to denaturing agents (113,205). Potato tuber lectin contains ornithine besides hydroxy-proline a rare amino acid present in proteins, as reported by Allen *et al.* (206) and Muray and Northcote (207).

The molecular weights ($M_r$) of purified lectins vary from source to source (Table 1.1). It ranges from 36,000 for wheat germ agglutinin (113,208) to 265,000 for lima bean lectin (209). Some lectins exhibit a pronounced tendency to aggregate. Thus, the $M_r$ of Con A at pH below 6 is 51,000 and at physiological pH it is 102,000 (210-212). Upon storage at room temperature, lectins may undergo irreversible self-association to high molecular weight aggregates (213).

Almost all purified lectins consist of subunits, the number of these in plant lectins being two or four.
## Table 1.1
MOLECULAR PROPERTIES OF SOME LECTINS

<table>
<thead>
<tr>
<th>Source</th>
<th>Family</th>
<th>Mol. weight (Mr)</th>
<th>Subunit structure</th>
<th>Sugar binding sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mol. weight</td>
<td>Number</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arachis hypogea</td>
<td>Leguminoseae (P)</td>
<td>110,000</td>
<td>27,500</td>
<td>4</td>
</tr>
<tr>
<td>Canavalia ensiformis</td>
<td>(P)</td>
<td>102,000</td>
<td>25,500</td>
<td>4</td>
</tr>
<tr>
<td>Datura stramonium</td>
<td>Solanaceae</td>
<td>60,000</td>
<td>30,000</td>
<td>2</td>
</tr>
<tr>
<td>Dolichos biflorus</td>
<td>Leguminoseae (P)</td>
<td>109-113,000</td>
<td>26,000</td>
<td>2</td>
</tr>
<tr>
<td>Erythrina cristagalli</td>
<td>(P)</td>
<td>56,000</td>
<td>28,000</td>
<td>1</td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>(P)</td>
<td>120,000</td>
<td>30,000</td>
<td>4</td>
</tr>
<tr>
<td>Griffonia simplicifolia</td>
<td>* (C)</td>
<td>114,000</td>
<td>28,500</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>** (P)</td>
<td>113,000</td>
<td>30,000</td>
<td>4</td>
</tr>
<tr>
<td>Lens culineris</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>Solanaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>Cucurbitaceae</td>
<td>120,000</td>
<td>28,000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaseolus lunatus I</td>
<td>Leguminoseae (P)</td>
<td>247-269,000</td>
<td>31,000</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>124-138,000</td>
<td>31,000</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>----------------</td>
<td>-------</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>Leguminoseae (P)</td>
<td>126-136,000</td>
<td>29-34,000</td>
<td>4</td>
</tr>
<tr>
<td>Pisum sativum</td>
<td>a (P)</td>
<td>49-53,000</td>
<td>5,800</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-18,000</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>Euphorbiaceae</td>
<td>120,000</td>
<td>28-31,000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34-37,000</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>Solanaceae</td>
<td>100,000</td>
<td>50,000</td>
<td>2</td>
</tr>
<tr>
<td>Triticum vulgare</td>
<td>Graminaceae</td>
<td>36,000</td>
<td>18,000</td>
<td>2</td>
</tr>
<tr>
<td>Vicia faba</td>
<td>Leguminoseae (P)</td>
<td>52,500</td>
<td>5,500</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20,700</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Abrus precatorius</td>
<td>a (P)</td>
<td>134,000</td>
<td>32,000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34,000</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Lotus tetragonolobus I</td>
<td>a (P)</td>
<td>120,000</td>
<td>27,800-28,000</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>58,000</td>
<td>27,800-29,000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>117,000</td>
<td>27,000-30,000</td>
<td>4</td>
</tr>
<tr>
<td>Ulex europaeus</td>
<td>a (P)</td>
<td>40,000-65,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vicia gramineae</td>
<td>a</td>
<td>105,000</td>
<td>25,000</td>
<td>4</td>
</tr>
</tbody>
</table>

* Leguminoseae (P), Family Leguminoseae and sub-family Papillaceae;
** Leguminoseae (C), Family Leguminoseae and sub-family Caesalpiniae

The values are taken from the reference numbers 72, 78, and 222.
## Table 1.2

**Molecular Properties of Some Lectins**

<table>
<thead>
<tr>
<th>Source</th>
<th>Monosaccharide specificity</th>
<th>Total carbohydrate % age</th>
<th>Mitogenic activity</th>
<th>Blood group specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arachis hypogea</td>
<td>Galactose</td>
<td>0</td>
<td>+</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Canavalia ensiformis</td>
<td>Mannose/Glucose</td>
<td>0</td>
<td>+</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Datura stramonium</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dolichos biflorus</td>
<td>N-acetyl galactosamine</td>
<td>4.5</td>
<td>+</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Erythrina cristagalli</td>
<td>Galactose</td>
<td>6.2</td>
<td>+</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Glycine max</td>
<td>N-acetyl galactosamine</td>
<td>9</td>
<td>-</td>
<td>A</td>
</tr>
<tr>
<td>Griffonia simplicifolia I</td>
<td>Galactose</td>
<td>4</td>
<td>-</td>
<td>B</td>
</tr>
<tr>
<td>Griffonia simplicifolia II</td>
<td>N-acetyl galactosamine</td>
<td>0</td>
<td>+</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Lens culinaris</td>
<td>Mannose/Glucose</td>
<td>50</td>
<td>-</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>Galactose</td>
<td>12.5</td>
<td>-</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Phaseolus lunatus I</td>
<td>N-acetyl glucosamine</td>
<td>3-5</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Phaseolus lunatus II</td>
<td>N-acetyl glucosamine</td>
<td>3-5</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>Pisum sativum</td>
<td>Mannose/Glucose</td>
<td>0</td>
<td>+</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>Galactose</td>
<td>12</td>
<td>+</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td></td>
<td></td>
<td>-</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Tritium vulgare</td>
<td>N-acetyl glucosamine</td>
<td>0</td>
<td>-</td>
<td>Non-specific</td>
</tr>
<tr>
<td></td>
<td>Vicia faba</td>
<td>Abrus precatorius</td>
<td>Lotus tetragonolobus I</td>
<td>Lotus tetragonolobus II</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>-------------------</td>
<td>------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Mannose/Glucose</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td>9.4</td>
<td>9.8</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl glucosamine</td>
<td>7.2</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values are taken from the reference numbers 72, 78 and 222.
Dissociation into subunits may be irreversible, eg. in the presence of detergents like SDS or reversible eg. in the presence of guanidinium hydrochloride. Different chemical modification studies also affect the subunit structure. For example, Con A is a tetramer at physiological conditions, but the succinylated Con A is a dimer (214). In most lectins, the subunits are identical, but lectins comprising of non-identical subunits are also known. SBA (215) and the lectin from D. biflorus (216, 217) are tetramers, each consisting of two types of slightly different subunits.

Often, purified lectins exist in multiple molecular forms i.e. as 'isolectins' being products of closely related genes (218). The different forms may also be the result of side chain modifications, such as the hydrolysis of the amide group of glutamine or asparagine in the protein during their isolation. If the isolectins are glycoproteins, the difference may be in the carbohydrate side chain or in specificity of the sugar.

Lectins have more than one binding site for the agglutination of cells or for the formation of precipitates with complementary molecules i.e. one sugar binding site per subunit of a lectin. WGA has two such sites per subunit (104, 113). While SBA (103), PNA, PHA (219) and lentil lectin (220) have two binding sites per four subunits.
b) Metal Ion Requirements of Lectins

The metal content and their requirements to lectins have been summarised in Table 1.3. With a few exceptions, all lectins examined so far contain metal ions Mn$^{2+}$ and/or Ca$^{2+}$ and these are essential for their sugar binding and cell agglutinating properties. Certain lectins contain Zn$^{2+}$ along with Ca$^{2+}$ and Mn$^{2+}$ (221). The mechanism by which metals exert their effects on the biological activity of a lectin has been investigated only for Con A (78).

The Mn$^{2+}$ in lectins can be replaced by a variety of transition metal ions without loss of biological activity (222,223). The activity of lectins from pea (224) and lima bean (209) is dependent on metal ions.

c) Carbohydrate Composition of Glycoprotein Lectins

Most of the lectins except Con A (121), WGA (112), PNA (225) and barley lectin (226) are glycoproteins. The percentage of carbohydrate varies from 0.5 to 50 percent from one lectin to another. The potato and tomato lectins contain as high as 50 percent of total sugar (200,201). The carbohydrates (monosaccharides) detected in purified lectins include mannose, glucose, galactose, glucosamine, galactosamine, L-fucose, xylose and in some cases L-arabinose (227). The glycoprotein lectins can be divided into two types.
<table>
<thead>
<tr>
<th>Lectin</th>
<th>Metal content*</th>
<th>Metal requirement</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn$^{+2}$</td>
<td>Ca$^{+2}$</td>
<td>Zn$^{+2}$</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>4.0</td>
<td>4.0</td>
<td>Mn$^{2+}$, Ca$^{2+}$</td>
</tr>
<tr>
<td>Crotolaria juncea</td>
<td>0.2</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Datura stramonium</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td></td>
</tr>
<tr>
<td>Dolichos biflorus</td>
<td>0.7</td>
<td>3.5</td>
<td>0.5 Ca$^{2+}$</td>
</tr>
<tr>
<td>Erythrina cristagalli</td>
<td>1.0</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Euonymus europaeus</td>
<td>8.0</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Griffonia simplicifolia</td>
<td>1.2</td>
<td>2.0</td>
<td>Ca$^{2+}$</td>
</tr>
<tr>
<td>Lentil</td>
<td>0.6</td>
<td>3.8</td>
<td>Mn$^{2+}$</td>
</tr>
<tr>
<td>Lima bean</td>
<td>1.0</td>
<td>4.0</td>
<td>Mn$^{2+}$</td>
</tr>
<tr>
<td>Pea</td>
<td>1.0</td>
<td>2.5</td>
<td>Ca$^{2+}$</td>
</tr>
<tr>
<td>Peanut</td>
<td>Traces</td>
<td>3.9</td>
<td>0.44 Mn$^{2+}$, Ca$^{2+}$</td>
</tr>
<tr>
<td>L-PHA</td>
<td>0.3</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>1-1.7</td>
<td>3.5-4.1</td>
<td>0.28 Mn$^{2+}$</td>
</tr>
<tr>
<td>Ulex europeus I</td>
<td>0.42</td>
<td>2.0</td>
<td>0.82 Mn$^{2+}$</td>
</tr>
<tr>
<td>Vicia cracca</td>
<td>0.9</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Wax bean</td>
<td>0.24</td>
<td>6.2</td>
<td>Mn$^{2+}$</td>
</tr>
</tbody>
</table>

* Expressed as atoms per mole

The values are taken from the reference numbers 72, 78 and 222.
i) Those containing N-acetyl glucosamine and mannose (with or without L-fucose or xylose) and

ii) Those containing L-arabinose or galactose (222).

The structure of carbohydrate moieties of SBA (228), lima bean lectin (229) and tora bean lectin (230') have been determined and they belong to the first group. The second group includes the lectins isolated from the family Solana-ceae, like potato, tomato etc.

d) Primary Structure of Lectins

The primary structure of several lectins have been determined and more work is going on to determine the sequences of the two-chain lectins from the plants belonging to Viciae tribe (221). The amino acid sequences of Con A, favin and lectin from lentil exhibit similar homology (231,232). The homologies between the lectins from pea, lentil and fava bean are more pronounced with respect to those sequences in Con A, which are involved in the binding of metals and in the formation of the hydrophobic cavity (234), as well as those that make up the extended $\beta$-pleated sheets of Con A. Similar homology is also found in the amino acid residues, that constitute the sugar binding site of Con A, but it is less extensive than in the other sequences or regions.
A high degree of sequence similarity of first 25 residues has also been found between the NH$_2$-terminal sequences of single-chain lectins isolated from soybean, peanut, Phaseolus vulgaris (234) and D. biflorus (235) and the NH$_2$-terminal portion of the β-chain of lectins from lentil, pea and faba bean. Such similarity in the amino acid sequences of the lectins from a single plant family (Leguminoseae) implies the common genetic origin for these lectins. However, the cereal lectins, WGA, rye, barley, have also got very similar NH$_2$-terminal sequences. The lectins from the family Solanaceae, potato and Datura stramonium lectins exhibit a high degree of homology in their carbohydrate specificity, carbohydrate composition as well as in amino acid compositions (236).

**e) Secondary and Tertiary Structures of Lectins**

Although a large number of lectins have been isolated and characterised from different sources, only limited information is available on their conformations in solution. Circular dichroism (CD) studies in the far ultra-violet (UV) region of several lectins suggest a β-pleated sheet as the major structural feature of the lectins (238). Saccharide-induced conformational changes have been demonstrated by CD in the near UV region for Con A (239) and for other lectins such as SBA (241), WGA, RCA, D. biflorus lectin, and
The complete three-dimensional structure of Con A based on high resolution crystallographic studies has been determined by Hardman and Goldstein (234). Recently, the partial structure of WGA and other lectins based on high and low resolution maps have been reported.

The Con A molecule has a dome shape, approximately 42Å high, 40 Å wide and 30Å thick. The two-monomers are paired by two-fold symmetry axes to form roughly tetrahedral tetramers. The dominant structural backbone is an arrangement of the polypeptide chain in two extended anti-parallel sheets. The site of the cleavage of the polypeptide chain, that splits the subunit into two fragments is located at the end of a loop extending out into the solvent and is most readily acceptable for the hypothetical enzymatic cleavage. The general distribution of polar and non-polar side chains in Con A is similar to the other proteins, i.e. polar (hydrophilic) side chains on the surface and non-polar (hydrophobic) groups predominantly in the interior. The formation of dimers depends, in addition to hydrogen bonds and vander Waals contacts on salt bridges between lysine residues 114 and 116 in one dimer and a glutamic acid residue in the other.
The X-ray crystallographic study of Con A has shown that the metal ions are bound at two different sites (S_1 and S_2), near the top of the monomer, and each is surrounded by six ligands to form an octahedral coordination shell. For binding of each metal ion, four of the ligands are from the protein and two are water molecules. The carboxyl groups of Asp^{10} and Asp^{19} are involved in the binding of both metal ions and thus constitute bridges between the transition-metal ion of S_1 and Ca^{2+} in S_2. In the demetalized lectin, the distance between the carboxyl groups of these aspartic acid residues is greater than that in the native lectin. This increased distance renders this pair of potential Ca^{2+} ligands ineffective. Besides this, Asp^{19} appears to form a salt bridge with His^{24} and is therefore in an unfavourable position for ligand pairing with Asp^{10}. The metal ion binding at S_1 position deprotonates the histidine residue and consequently breaks the salt bridge with Asp^{19}, so that the carboxyl group of the latter residue is free to assume a position in relation to the carboxyl of Asp^{10} that favours the formation of a Ca^{2+} binding site (242).

In contrast to this, the location of sugar binding site of Con A was in a deep hydrophobic cavity at a distance of about 20Å from the Mn^{2+} binding site (243). The crystallographic examination of crystals of Con A saccharide complex by Becker et al. (244) has shown the distance of
35 Å° from the hydrophobic cavity, 12 Å° from the Mn$^{2+}$ binding site and 7 Å° from the Ca$^{2+}$ binding site. Two tyrosyl and two asparaginyl residues were found in the carbohydrate binding region of Con A. The involvement of carboxyl groups and tyrosine residues in the binding of sugars to the lectin had been postulated by chemical modification and binding studies. The binding of sugars to Con A in solution causes conformational changes in the protein molecule.

The WGA dimer, (40X40X70 Å°) is composed of two closely related subunits centered around a crystallographic two-fold axis at 2.2 Å° resolution (244). Each of these units contains an assembly of four structurally similar and spatially distinct domains. The subunit possesses a large surface area with 80\% of its side chains exposed to the solvent. In contrast, 20-25\% of surface of each subunit is no longer available to the solvent during dimerisation.

Each of the four domains of the WGA subunit consists of 41 amino acid residues and is unique in its architecture when compared to the recurring structures of other proteins. WGA is devoid of commonly occurring secondary structural features like $\beta$-sheet and the $\alpha$-helix. The lectin molecule is folded irregularly; the polypeptide chain of each domain is held in a compact, stable configuration by four disulphide bridges. Fluorescence and chemical modification studies of
WGA, have shown the presence of tryptophan at or near the saccharide binding site of the lectin molecule (245).

Extensive X-ray crystallographic work has been going on for the structural elucidation of several other lectins. Recently, such data have been reported for the following lectins from pea (246), pea nut (247,248) favabean (249), soybean (250), Abrus precatorius (251), wheat germ (252) and lentil (253).

**GENETICS**

Most of the lectins are closely related molecular forms called 'isolectins'. The comparisons of partial amino acid sequence and other structural studies have indicated that these multiple forms of lectins (isolectins) are products of different genes in some cases (36,205,254-256). Recently, a detailed investigation on this aspect is going on.

Evidence of a genetic basis for the isolectins in wheat germ was established by comparing the isolectin contents of the plant differing in their level of ploidy (257,258). These studies showed that each genome in polyploid wheat codes for a single molecular species of lectin subunit and each of these isolectins was correlated to a genome in allopolyploid wheat.

In soybean (Glycine max) plant, the hybridization
studies of leaf DNA with DNA constructed from the mRNA for
the seed lectin showed the presence of two similar lectin
genes, L₁ and L₂ (260). The L₁ gene was identified as the
gene coding for the well characterised N-acetyl galacto-
samine/galactose specific seed lectin and L₂ gene is express-
ed at low levels and its function is not known. The L₁ gene
lectin was present in all but 14 of 2284 soybean lines screen-
ed; and trace amounts have been detected in several of these
lectin-negative lines (261). The presence of the lectin was
found to be under the control of an allele Le at a single
locus and the lectin-negative lines are homozygous for the
recessive allele l; at this locus (262). Both L₁ and L₂
genesis were found in the lectin-negative lines. But, the L₁
gene was modified by the inclusion of insertion sequence in
its coding region. This insertion sequence, which has the
structural features of a transposable element (263), has been
stated to block the transcription of the L₁ gene in these
lectin-negative lines to the extent that the lectin mRNA is
reduced to about 0.01% of the levels as in the lectin positive
lines (259).

Homologies in amino acid sequences of different
isolectins from the same plant indicate that the multiple
lectin genes, may have arisen by gene duplication (256,257).
Similar observation has been made in the case of ricin (264).
In Con A, a similar genetic event followed by deletions or mutations leading to the failure of expression of parts of the gene, may explain the circular permutation of the amino-acid sequence, relative to the sequences of other legume lectins (265-267).

LECTIN SYNTHESIS AND ITS REGULATION

Clones of cDNA from the mRNAs for the seed lectins from plants *Phaseolus vulgaris* (268), *Glycine max* (260), and *Pisum sativum* (269) have been used as probes in measuring the levels of lectin mRNA in plant tissues. The transcripts hybridizing with these cDNAs increase during the midmaturation stage of development of the seeds of these plants and then decrease during the later stages of seed maturation (260, 268, 269). Such increases in lectin mRNA correlates with the time of maximal production of seed lectins in legumes (83, 86). The combined results suggest that, the accumulation of lectin in legumes may be regulated at the transcriptional level.

However, there is evidence for the probable involvement of a plant hormone, abscisic acid in the regulation of lectin synthesis in wheat germ and rice embryos. It has been investigated that this hormone may play a role in shifting developing embryos first into the maturation phase in
which stored reserves accumulate and then into a quiescent state, thereby preventing precocious germination of the seeds (270). The embryos from developing wheat and rice endosperm when cultured in the absence of abscisic acid, stop lectin synthesis and begin to germinate, but the presence of abscisic acid in the medium blocks this germination and stimulates lectin synthesis (265,271). This plant hormone also promotes lectin synthesis in cultures of mature rice embryos and maintains them in a dormant state. Another hormone, gibberellic acid \((\text{GA}_3)\) in the medium without abscisic acid has been shown to promote rapid germination of embryos and suppression of lectin synthesis than control embryos cultured in the absence of both hormones (265,271).

Several investigations on lectin synthesis in plants such as soybean (272), *Vicia faba* (273), pea (269,274), *Phaseolus vulgaris* (275), *Ricinus communis* (278), rice and cereals (277) *in vivo* and *in vitro* have shown that these lectins are all synthesised as precursors with higher molecular weights and subsequently processed at the co- and/or posttranslational levels. Such processing mechanisms have been most intensively studied in some legume lectins from *Vicia faba* (250), pea (269,274), and soybean (272).

The subunit heterogeneity of many lectins may also arise from the biosynthesis and assembly of subunits that
are products of different genes, as indicated for the isol­ectins from *Phaseolus vulgaris* (257) and *Griffonia simplicifolia* I (256). The heterogeneity may also arise by posttranslational proteolytic cleavage of a single gene product into two peptide chains, as observed in fain (273), pea lectin (269), and RCA (276). Such subunit heterogeneity may also be traceable to different degrees of posttranslational modification of common subunits either before or after their oligomerisation.

It is still a mystery whether such interactions between lectin subunits may modulate their individual properties and affect the overall activity of the lectin. However, the possibility of regulation of lectin activity by controlling the relative amounts of subunits by posttranslational modifications, or other factors that may affect lectin subunit composition can not be ignored.

**BIOLOGICAL PROPERTIES OF LECTINS**

Lectins or agglutinins exhibit a number of remarkable biological properties such as, agglutination of different cells, mitogenic stimulation and toxic effects on cells etc. But, all these biological properties are not exhibited by all lectins.
1) **Agglutination of Cells**

To form a clump or agglutination of cells, lectins must bind to their surfaces as shown in Figure 1.1. The surface structures to which lectins bind are the carbohydrate moieties of glycoproteins or glycolipids that protrude from the cell surface. The possible mechanism of agglutination to occur is, that the bound lectin must form multiple cross bridges between apposing cells. In addition to agglutination of intact animal cells, some lectins also agglutinate other cells like, plant cells, bacteria, viruses, fungi, mycoplasma and subcellular particles like nuclei and mitochondria (278). Con A is an example which agglutinates different cells.

The cells which are not agglutinated by low concentrations of a lectin frequently become agglutinable after very mild proteolysis, which does not affect significantly the total number of lectin-binding sites (55). The modification of lectins either by chemicals or enzymes may sometime alters the agglutinability of the lectins. Succinylation of Con A leads to a loss in its hemagglutinating activity by 500-fold as compared to the native Con A (279), but has no effect on WGA (208). Modification of tryptophan residues in winged bean seed basic lectin results in the complete loss of its hemagglutinating activity at acidic pH of 4.0 (280). In contrast, modification of the carbohydrate moieties of
glycoprotein lectins has no effect on their hemagglutinating activity, as is seen with SBA and potato lectins.

The agglutination or clumping of cells by lectins varies from one lectin to the other, as it is influenced by a number of factors such as the properties of lectin, chemical structure of cell surface receptors, cell surface properties, cytoplasmic components besides external conditions like, cell concentration, pH, temperature etc.

2) Blood Group Specificity of Lectins

The specific binding of lectins to sugar moieties imparts them with specificity towards erythrocyte agglutination. Landsteiner and Raubitschek (31) were the first to observe and report the selectivity and also specificity of lectins for different erythrocytes. Renkonen (32), Boyd and Reguera (33) and Makela (177) have extracted, isolated and described the specificities of hemagglutinating activities of many lectins. Several of them are blood group specific and some are non-specific.

Among the blood group specific lectins those from D. biflorus, P. limensis and V. cracca are human 'A' blood group specific. The lectin extracts from L. tetragonolobus, Laburnum alpinum, C. sessilifolius and U. europcus are human 'H (0)' blood group specific. In 1974, Hayes and Goldstein
(115) have isolated a human blood group 'B' specific lectin from *B. simplicifolia*. The agglutinins from the plant *Sophora japonica* and *Celtis aurora* are 'A+B' blood group specific. The lectin from *Iris amara* is 'M' blood group specific and *B. purpurea* and *V. graminea* lectins are human 'N' group specific. However, Irinumura and Osawa (281) have reported that *B. purpurea* lectin is not blood group 'N' specific.

Lectins from *P. vulgaris* (282), *A. hypogea* (227), *C. ensiformis*, *R. communis*, *V. faba* etc. are nonspecific i.e. they agglutinate human erythrocytes regardless of the human 'A', 'B' and 'O' groups.

Some of the lectins are more specific, that they can even distinguish among human blood subgroups. For example, the lectin from *D. biflorus* reacts more specifically with the RBC's of type 'A_1', than those of type 'A_2'.

Recently, a 'Lewis-b' blood group specific lectin and blood group 'B' specific lectin from *G. simplicifolia* (283) and *Plecoglossus altivelis* eggs (284) respectively have been purified.

3) **Mitogenicity of Lectins**

The discovery by Nowell in 1960s (11) that the extracts of red kidney bean stimulates lymphocyte division
in vitro led to the enormous interest in mitogenic lectins as tools for studying the biochemical events accompanying cellular growth, cellular differentiation and cell division (59, 285, 286). These lectins bind to the surface of cells and resulting in the formation of a clump or aggregation of cells. One of the most dramatic effects of the interaction of lectins with cells is the triggering of quiescent, non-dividing lymphocytes into a state of growth and proliferation is called mitogenic stimulation. Lectins from kidney bean (PHA), P. vulgaris, C. ensiformis, W. floribunda, Lens culinaris, Bauhinia carronii, Phytolacca Americana, Pisum sativum, Robina pseudoacacia, Sarathammus scoparius, Maclura pomifera, V. faba and a few others have been shown to be mitogenic in nature (72, 286, 287).

Mitogenic triggering of lymphocytes to proliferate by lectins is accompanied by a variety of morphological and biochemical changes. Morphological changes following mitogenic stimulation of lymphocytes include a progressive increase in cell size, cytoplasmic basophilia and an increase in the number of cell vacuoles. It has been demonstrated that the morphological transformation of lymphocytes is accompanied by biochemical changes such as an increase in intracellular cGMP (288), increased turnover and methylation of membrane lipids (289), increase in the uptake of small
nutrients (290), synthesis of RNA, DNA and proteins (291) and followed by phosphorylation of non-histone acidic nuclear proteins (292). The proposed mechanism for the mitogenic event is that the initial step or 'first signal' involves the binding of the lectin to the cell surface sugar residues. This binding may lead to modification of membrane structure and function, resulting in the generation of a trigger or a 'second signal', the transmission of which to the interior of the same cell initiates a series of biochemical events culminating in cell growth and proliferation (59).

Most of the lectins are selective in their ability to stimulate only thymus dependent ('T'-cells) or bone marrow dependent ('B'-cells) lymphocytes (293). Schumann et al. (294) have shown that Con A and lentil lectins are selective in that they stimulate only the 'T'-cells but not the 'B'-cells. However, these lectins can be made to stimulate 'B' cells by chemical cross-linking (295). Waxdal and Bashyam (296) have shown that pokeweed lectin stimulates both 'T' and 'B'-lymphocytes. An alteration of the surface residues of the lymphocytes makes them susceptible to mitogenic stimulation. Similar observations have been made with succinylated Con A and polymerised SBA (297).
4) Interaction of Lectins with Malignant Cells

Recently, lectins are used almost commonly in cancer research (298, 299), because of their unique property to distinguish between normal and malignant cells (55, 223, 300, 301). The alterations in the cell surface have been postulated as the basis for some of the typical characteristics of transformed cells, such as invasive growth and lack of contact inhibition (302). Wheat germ agglutinin preferentially agglutinated tumor cells or malignant cells as compared to normal ones (303). The concentration of native WGA required for the agglutination of transformed or malignant cells is less than that of normal cells. It was proposed that transformation leads to the exposure of saccharide receptors present in a cryptic form on the surface of the normal cells. The increase in the agglutinability of these transformed cells by lectins is due to topological differences of the saccharides receptor sites on the cell surface (304, 305). On the other hand, in normal cells, the lectin molecules are distributed randomly whereas, on the surface of malignant cells, they appear to be aggregated into clusters, which leads to the formation of multiple cross-bridges between the malignant cells and a lectin, and hence increased agglutinability has been observed.

It has been shown that certain lectins inhibit the
growth of tumor cells both in vitro and in vivo. The two toxic proteins, 'ricin' and 'abrin' isolated from *R. communis* and *A. precatorius* respectively have been used to inhibit the growth of Ehrlich ascites tumor cells in vivo (306) but are highly toxic to Yoshida ascites sarcoma (YAS) cells under in vitro conditions (306). The lectin isolated from *Robinia pseudoacacia* strongly increased the rate of growth of human ascites, rat ascites and rat hepatoma cells (307). Con A inhibits ascites tumor growth in vivo and kills cultured marine lymphomas (308,309). Recently, experiments by Fodstad and Pihl (310) on the therapeutic use of 'ricin' with human cancer patients have given encouraging results.

5) **Toxicity of Lectins**

Lectins have been implicated in the toxicity and impaired growth rates as observed in experimental animals. However, not all lectins are toxic or even growth inhibitory. It has been proposed that the toxic effects of lectins may be, due to their ability to bind to specific receptor sites on the surface of the intestinal epithelial cells (53). At the intestinal level, microscopic observations have shown extensive changes caused by PHA (311): duodenal villi were shortened while duodenal and jejunal crypts were significantly lengthened. Villus alterations is induced by different lectins like wheat germ agglutinin and Con A (312). SBA
(313), *Phaseolus acutifolius* lectin (314), winged bean seed lectin (315-317), which react with different regions of the intestine depending upon the specificity of the lectin. Recently, the villi alterations have been discussed using tomato lectin both in rats and the mammalian alimentary canal (318). Such toxic effects of lectins present in plant food stuffs can be generally eliminated by proper heat treatment, although in practical their complete destruction may not be feasible (50).

Some of the bacteria also contain toxic factors (319, 320), which are analogous to the plant toxins, consisting of two domains. One of these binds to sugar moieties on the cell surface, while the other is responsible for the toxic effects. Recently, a hybrid or a chimeric molecule has been prepared, in which the carbohydrate binding fragment of diphtheria toxin is replaced by Con A (321) or by *W. floribunda* lectin, resulting in a highly toxic compound (322).

6) **Inhibition of Fungal Growth by Lectins**

Wheat germ agglutinin specifically binds to the hyphal tips and hyphal septa of *Trichoderma viride* and inhibits hyphal growth as measured by the incorporation of radioactive acetate (323). Similar observations have been made with *S. tuberosum* lectin on *Botrytis cinerea* (324).
Incorporation is not affected by pre-incubating the lectins with their specific inhibitors. The inhibition of incorporation of precursors into the fungal hyphae by lectins of different specificities seems to be the result of inhibition of nutrient uptake caused by coating of the hyphal surfaces with the lectins.

It has been observed and proposed that during inhibition of hyphal growth by lectins spore germination is also inhibited. This inhibition is mainly expressed by prolonging of the latent period which precedes germination.

APPLICATIONS OF LECTINS

The unique biological properties exhibited by lectins make them invaluable tools in chemical and biological research and also in clinical medicine. Hence, lectins find important applications. Recently, McCoy (324a) has described different biochemical applications of lectins.

1) **Isolation of Glycoproteins and Glycopeptides**

Because of the analogy of lectin-saccharide interactions to antigen-antibody reactions, lectins either in solution or in immobilised form have been used for the purification of glycoproteins and glycopeptides. Affinity chromatography on immobilised lectins is also useful for the fractionation and purification of glycoproteins and
glycopeptides which differ only slightly in their carbohydrate composition or in the structure of their oligosaccharide units. The purification of membrane glycoproteins has also been achieved by using some of the immobilised lectins in the presence of detergents. The commonly used affinity column matrices are Con A-Sepharose, RCA-Sepharose, WGA-Sepharose, Lentil-Sepharose, PNA-Sepharose and Lotus tetragonolobus-Sepharose, to purify blood group substances (325), immunoglobulins (326), glycoprotein hormones (327), enzymes (328), intact rod discs (329), rhodopsin (330), interferon (331), Vicia graminea lectin (332), D. biflorus lectin (216), α-fetoprotein from rat amniotic fluid (334), polysaccharides etc.

2) **Cell Surface Characterisation**

The characteristic specificity of lectins towards sugars has made them to be used for the detection, isolation and characterisation of carbohydrate containing (macro) molecules (72,78,116,222). The lectins are also used in the study of cell surface glycoconjugates, to quantify the number of binding sites and their affinity to study the three-dimensional arrangement of cell surface receptors. So far, structures of several glycoconjugates have been identified using different sugar specific lectins (72,78,116). The number of receptor sites for the lectin on the cell surface...
can be determined by using radioactively labelled lectins. The usual number of receptor sites for lectins is in the range of $10^6-10^7$ per cell of lymphocyte regardless of the lectin used and for red blood cell it is $10^5-10^6$ (78). The redistribution and rotation of receptors serves as the powerful evidence for the "fluid mosaic model" of membrane structure. The cell surface architecture can also be determined by using fluorescent labelled lectins.

3) **Cell Separation**

Recently, some lectins have been used for the separation of intact cells, with different carbohydrate residues on the surface. Such cells have been fractionated by differential agglutination with soluble lectins or specific adsorption and elution from immobilised lectins (78,334,335). Peanut agglutinin (PNA) has been used to fractionate immature and mature human and mouse thymocytes (336,337) and also to separate chicken lymphocytes (338). PNA has also been used to fractionate liver cells into subsets of different functions (339) and tetracarcinoma cells into embryonic antigen F9 bearing cells and non-bearing cells. Soybean, peanut and wheat germ agglutinins have been used to separate mouse spleen 'T' and 'B' cells (340,341). SBA has been used to fractionate human bone marrow cells into histocompatible and non-compatible fractions for transplantation across the
histocompatibility barrier (342). WGA, SBA and Con A have also been used to separate axial organ cells of *Astoria rubens* into two cell populations. Sepharose-WGA has been used to separate human peripheral blood 'T'-lymphocytes, into two subpopulations which differ in their response in the mixed lymphocyte reaction and to mitogens. Recently, a new type of affinity column, consisting of lectins specifically bound to a glycoprotein (hog gastric mucin blood group (A+H) substance), that is in turn covalently attached to Sepharose, has been employed for the separation of cells.

4) Clinical Medicine

Mitogenic lectins are being employed in clinical medicine to recognise congenital and acquired immunological deficiencies, to detect sensitization caused by infectious diseases, to monitor the effects of various immunosuppressive and immunotherapeutic manipulations, and in the diagnosis of genetic diseases with chromosomal defects (78,343). Certain lectins are also used in serological laboratories for blood typing and determining the secretor status (344), separating leukocytes from erythrocytes (345) and agglutinating cells from blood in the preparation of plasma. The 'poly agglutination' phenomena can also be studied by using lectins (346,347). Nowadays, some lectins have been used in cancer research study (347a). Recently Caron (347b) has
described potential diagnostic use of phytolectins.

5) **Isolation of Glycosylated Nucleic acids**

An interesting application of lectins is the isolation of tRNA species containing glycosylated bases (348). Con A-Sepharose column has been used for the isolation of trNA Asp from rat liver, rabbit liver and rat ascites hepatoma cells. In addition, RCA-Sepharose affinity column has also been used for the isolation of trNA Tyr from the same source. Recently, Hiroshi et al. (348a) have used lectin affinity HPLC columns for the resolution of nucleotide sugars.

6) **Other Applications**

Lectins have been used with great effectiveness to determine microheterogeneity of glycoproteins (72,78). The microheterogeneity in Thy-1 glycoprotein of mouse fibroblasts (349), human α-fetoprotein (350), ceruloplasmin and fetuin (351) and molecular heterogeneity of variant surface glycoprotein from *Trypanosoma congoense* (352) were determined using purified preparations of lectins.

Con A covalently linked to alcohol and lactate dehydrogenases has been shown to be a useful reagent for the localisation of glycoprotein band in polyacrylamide gels (353). The radio-labelled lectins like PNA and RCA have been used for the identification of surface glycoproteins.
of human skin fibroblasts in polyacrylamide gels (354).

Besides this $^{125}$I - labelled SBA, *G. simplicifolia* I iso-
lectins $A_4$ and $B_4$ and *Helix pomatia* lectin have been used to
detect cell surface glycolipid receptors in human and bovine
erthrocytes after TLC of the glycolipids (355).

**PHYSIOLOGICAL ROLE OF LECTINS IN PLANTS**

The ubiquitous nature of lectins, their wide distribu-
tion in plants particularly in legumes suggests that they
may play a structural role than an enzymatic role. Since
the discovery of lectins, many hypothesis concerning their
possible roles have been postulated but none of them has not
been yet consistently demonstrated. It has been proposed that
lectins function in recognition phenomena, both inter and
intracellular. This implies that lectins play a role in
hostparasite relationships (in plants and animals), and they
serve in the defense mechanisms of plants against patho-
genic microorganisms etc. Recently, Kijne *et al.* (355a) have
described extensively the physiological role of plant lectins.

The high degree of symbiotic relationship between
the legume root hairs and the nitrogen-fixing bacteria has
been well studied and was found to be highly species speci-
fic. Extensive investigations carried out in this area has
been reviewed (19,356,357). The specificity of such
inter-cellular interaction is probably based on the ability of root lectins of the host plant to recognise and bind specifically carbohydrate receptors on the surface of cells of the symbiotic bacteria. The most convincing evidence for the participation of lectins in attachment of symbiotic Rhizobium is available only for the lectin from white clover (358). This lectin is present in the clover seeds as well as roots and only those strains of rhizobia specific for white clover are agglutinated by it (359,360). This agglutination can be specifically inhibited by 2-deoxy glucose. Dazzo et al. (359) have observed the localization of the lectin on the surface of roots and exudates of growing roots in white clover by immunofluorescence.

Pueppeke et al. (361) have concluded that there is no relation between the ability of peanut and soybean rhizobia to nodulate their hosts and the ability to bind to seed lectins of corresponding plants. Some soybean strains have been detected, which are completely devoid of lectin activity, but display normal nodulation by the corresponding rhizobium strains (19). This suggests that lectins are not necessary for infection and nodulation by Rhizobium, since these plants nodulate equally well. A new lectin activity in soybean seeds, which has a specificity towards 4-o-methyl-D-glucurono-L-rhamnan exopolysaccharide produced by some
strains of *Rhizobium japonicum* has been detected recently (362). This lectin activity is found in all the soybean species, including the 'lectin less' varieties.

An unknown protein from stems and leaves of the plant *Dolichos biflorus* has been shown to cross-react with the seed lectin of the same plant immunologically (363). This unknown protein was named as 'cross-reactive material' (CRM). This CRM has properties similar to the seed lectin and its content increases in response to stress conditions of the cell wall such as fungal infection. Later it was confirmed that this CRM is the actual functional lectin in the plant (363). Recently, Etzler and coworkers (364) have identified the subcellular localizations of the *D. biflorus* seed lectin as well as the structurally similar CRM from the stems and leaves of the same plant by immunofluorescence, immunocytochemistry and cell fractionation methods. Similar CRM has also been detected by Lamb *et al.* (365) in *C. simplicifolia* which cross-reacts with the seed lectin.

Lectins also function as storage proteins. Developmental studies indicate that these are rapidly deposited in the cotyledons of the seed during the later stages of development (363). The localization of isolectins in the protein bodies of *Phaseolus vulgaris* supports a role as storage proteins (366,367).
Lectins present in pistils, anthers and pollens of many plants, have been studied and it has been shown that these lectins play a role in pollen germination (52, 368, 369). In a study, lectins were detected in the extracts of female generative organs. The extracts of pistils stimulate the growth of the pollen of short anthers and inhibit the growth of pollen of tall anthers (52). Lectins may also participate in the regulation of plant cell division and differentiation, including regulation achieved through auxin and cytokinin metabolism.

Lectins have been implicated in a variety of roles dealing with the defense mechanisms of plants. It has been proposed that lectins may protect plants against bacterial, fungal, and viral pathogens during imbibition, germination, and early growth of the seedlings. The participation of lectins in defense mechanisms (as in other processes of intercellular recognition) presumes the presence of lectins in the free or associated state with cell walls of surface tissues.

Because of the specific similarity of the binding sites of the lectins to those of glycosidases, lectins could be considered as enzymes which have lost their catalytic sites or enzymes may be considered as lectins that have acquired catalytic sites. It may also be possible that
Lectins possess highly labile enzymatic activity, and that during purification the enzymatic activity of lectins is lost, but the binding activity remains unaltered. Recently, α-Galactose specific lectin with α-galactosidase activity from mung beans have been purified (370), which may serve as an impetus for the search for enzymatic activities in lectins and lectin activities in enzymes.

Recently, another hypothesis concerning glycoprotein lectins found in seeds of A. hypogea has been proposed according to which they may act as allergens (64,371).

Whatever the hypothesis, the physiological role of lectins in plants remain obscure. To get adequate information on this, it is necessary to have a better view on the localization and changes of lectins in the different parts of plants during their life cycle.

**Physiological Role of Lectins in Other Systems**

Lectins are widely distributed not only in plant kingdom but also in animal kingdom, including bacteria, viruses, slime molds etc. These lectins have got specific role in each of the systems. The slime mold amoebae can be made to differentiate from a vegetative to an aggregating and adhesive form. They are useful tools in the studies of the cellular changes that correlate with the development of
cell-cell adhesiveness. In the case of *Dictyostelium discoidium*, the synthesis of two galactose specific lectins are detected on the cell surface and play an important role in cell-cell adhesion. Only the aggregated slime molds formed rosettes with erythrocytes while the vegetative ones did not form; further rosette formation was inhibited by specific sugars (372). The lectin synthesised during the developmental stage has been detected in *Myxococcus xanthus*. These observations are useful for studying the cell-cell interactions as well as the development of specific gene expression (373).

Longback it has been observed by Boyden in 1966, that the lectin from horse shoe crab haemolymph aids in protecting the animal against pathogens (374). In animals, the cell surface lectins also play a role in receptor mediated pinocytosis of potentially degraded glycoproteins before their catabolism. Lectins which are useful during pinocytosis have been detected in birds (375) and in rabbits (376).
SCOPE OF PRESENT INVESTIGATION

The winged bean (Psophocarpus tetragonolobus (L, DC.) is a tropical legume whose potential as a protein rich food source has stimulated in its development as a tropical field crop in many parts of India and other countries (377). Apart from the attention that the agronomic aspects of the crop have received, a number of investigations have been made on the chemical composition and utilization of the mature seeds in particular and also on the immature pods, leaves and tubers. Almost all parts of the plant are edible; the pods, seeds and tubers are the main parts of consumption. Its nutritional advantage lie mainly in the seed and also tubers. The seeds of the title plant contain 36% protein, 42% carbohydrate and 15-18% fat. The nutritive value of winged bean seed protein is similar to that of the soybean.

The National Academy of Sciences (378) has selected the winged bean as a potential food source for both humans and animals along with 35 other legumes, for developing further research.

In addition to a deficiency in sulphur containing amino acids, winged bean seed contains heat labile anti-nutritional factors such as trypsin (379,380) and chymotrypsin (381) inhibitors and hemagglutinins (32). Two different types of acidic and basic lectins have been
isolated, purified and characterised by several authors (382-388). The tubers of the winged bean plant also contain significantly large amounts of protein than other traditional root crops such as cassava, potato and yam. Winged bean tubers are also consumed as human food in almost all parts of India and other countries. The tubers have been shown to contain 20.3% protein, 74.6% carbohydrate and 1.6% fat on the dry weight basis. Like winged bean seeds, tubers of this plant also contain some of the antinutritional factors. The presence of hemagglutinin (lectin) activity in the tubers of this plant has been detected (388a). The isolation, purification, and characterisation of the lectin(s) from the tubers of winged bean plant was undertaken, with a view to make a comparative study with winged bean seed lectin(s). Such comparative investigation would be helpful to find out, whether the lectin(s) present in the tubers of title plant are the same or different as those present in the seeds of the same plant and to exploit the lectin for further structure-function relationships.

Thus, the isolation, purification and characterisation of winged bean tuber lectin and its biological properties have been discussed in successive chapters in this thesis in detail.
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