

INTRODUCTION

1. INTRODUCTION

1.1 HISTORY AND DEFINITION OF LECTIN

Lectinology, science of lectin, started more than a century ago when Stillmark (1888) made the startling observation that extracts of the castor bean (*Rinucis communis*) agglutinated erythrocytes (see table 1 for brief history of lectin research). The term 'lectin' which means to pick-up or choose (derived from the Latin word *legere*) was proposed by Boyd in 1954 subsequent from the discovery that certain plant agglutinins (or phytagglutinins, as they were referred to at that time) are blood-type specific. This term was generalised in 1972 to include all sugar-binding and cell-agglutinating proteins, from plants, animals and microorganisms, whether blood-specific or not. And only in 1988, with the exclusion of the carbohydrate-binding protein from immune origin (antibodies) and enzyme, the most satisfying, least-restrictive definition of lectin was born (Barondes, 1988). Thus, lectin is now defined as 'a carbohydrate-binding protein other than an enzyme or an antibody'.

1.2 OCCURRENCE AND ISOLATION

Hundreds of lectins are now well characterised and the number is growing fast. The spectrum of lectin sources is impressive. They occurred in the simplest life form (viruses) to most complex (mammalian tissues). Plant is the major source and more than a thousand plant species have been reported to possess lectins. They have been found in the seed, root, sap, fruit, flower, bark, stem, and leave. In bacteria, lectins may occur on the cell surface or may be found in the periplasm (transport proteins) or

Table 1. A brief history of lectin research

- 1888 description of toxic, cell-agglutinating protein in seed extract of *Ricinus communis*, termed ricin (Stillmark, P. H.)
- 1891 application of toxic plant agglutinins as model antigens (Ehrlich, P.)
- 1898 introduction of the term 'hemagglutinin' for plant proteins that agglutinate erythrocytes (Elfstrand, M.)
- 1902 detection of bacterial agglutinins (Kraus, R.)
- 1907 discovery of non-toxic plant agglutinins (Landsteiner, K., Raubitschek, H.)
- 1913 application of cells for lectin isolation and desorption by acid (Kobert, R.)
- 1919 crystallization of concanavalin A (Summer, J.B.)
- 1936 definition of a carbohydrate group as ligand for concanavalin A (Summer, J.B., Howell, S.F.)
- 1941 detection of viral agglutinins (Hirst, G.K.)
- 1947 description of blood group specificity of certain agglutinins (Boyd, W.C., Renkonen, K. O.)
- 1954 introduction of the term 'lectin' for antibody-like-proteins (Boyd, W.C.)
- 1960 detection of elicitation of a mitogenic response of lymphocytes by binding of a lectin to the cell surface (Nowell, P.C.)
- 1963 introduction of affinity chromatography to lectin isolation (Goldstein, I. J.)
- 1972 sequencing and analysis of the three-dimensional structure of the first lectin (Edelman, G. M., Hardman, K.D., Ainsworth, C.F.)
- 1974 purification of the first mammalian lectin from liver (Ashwell, G.)
- 1980 definition as carbohydrate-binding protein of non-immune origin that agglutinates cells (Goldstein, I. J. et al.)
- 1988 definition as carbohydrate-binding protein other than an antibody or an enzyme (Barondeś, S.H.)

cytoplasm. In animal, lectins have been isolated from tissue, serum, egg, venom, and body fluid of some invertebrates.

There are no rapid-screening methods for all lectins. Because most lectin tend to be multivalent, they generally have the ability to aggregate cells, such as erythrocytes. In examining biological specimen or their extracts for lectins, it must be considered that lectin frequently exhibit a narrow specificity. A lectin may agglutinate one kind of red cell, or only the red cells of one or a few species may be susceptible to aggregation. This is because different red cells have unique glycoconjugate compositions and unique distributions of lectin receptors. Nevertheless, hemagglutination is the most reliable and direct means for screening for lectins. Furthermore, the specificity of the lectin can be determined by hapten-inhibition experiments, once a lectin has been shown to aggregate a particular cell. Knowledge of the specificity then leads to affinity purification methods for the isolation of this particular lectin.

The purification of lectins has never been so direct until the introduction of affinity chromatography in 1965 by Agrawal and Goldstein. Taking advantage of the fact that lectins react specifically only with certain carbohydrate, tailor-made affinity adsorbents prepared by immobilising monosaccharides, oligosaccharides, glycopeptides or glycoproteins to hydrophilic and water-insoluble matrices have always been the choice as the first chromatographic step. Standard procedures for chromatographic protein purification such as ion exchange, chromatofocusing and hydrophobic chromatography are not recommended to start with because it may resolve isolectin before other contaminating proteins have been removed. This will complicate the purification procedure because after these chromatography separation,

every impure but lectin-containing fraction will have to be subjected to affinity chromatography separately. Figure 1 presents a general scheme for the isolation of lectins primarily from plants. The procedures are, however, applicable to lectins from other sources as well.

1.3 CLASSIFICATION OF LECTIN

1.3.1 By Structural Homology

As the number of purified lectins and our knowledge about their properties expanded, it became apparent that many of them could be grouped into families with sequence homologies and common structural properties (Table 2). The largest and best-characterised family is that of the Leguminosae lectins. In leguminous plants, lectin have been detected in more than 600 species and varieties, and have been purified from close to 70 of these (Rudiger 1988). Even though they differ markedly in their specificity, they resemble each other in their physico-chemical properties. They usually consist of two or four subunits of 25-30 kDa, each with a single carbohydrate-binding site. Interaction with carbohydrates requires tightly bound Ca^{2+} and Mn^{2+} (or another transition metal ion). In terms of secondary structures, they are devoid of or low in α -helice structure, and contain an abundance of antiparallel β -sheets; differences between the structures are confined primarily to the loops regions (Sharon and Lis, 1990).

The cereal lectins form a second, much smaller family. WGA, wheat germ agglutinin, is the only member of this family which was characterised in molecular detail (Wright, 1989). Other lectins similar to WGA in their specificity for N-acetylglucosamine and N-acetylneuraminic acid, as well as by immunological and

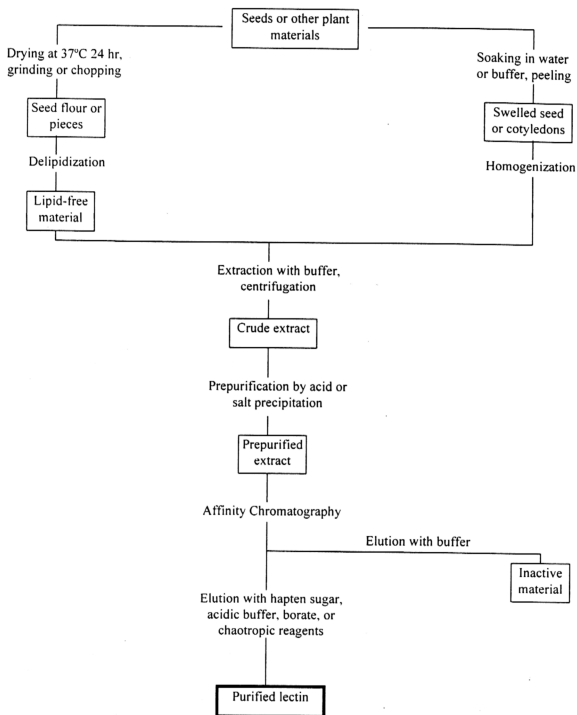


Figure 1. General scheme for the isolation of lectins

Table 2. Lectin families by structural homology

<i>Lectin type</i>	<i>Specificity</i>	<i>Subunits molecular mass (kDa)</i>	<i>No. of subunits</i>	<i>Binding sites per subunit</i>	<i>Disulphide bonds</i>	<i>Metal ion requirement</i>
Plant						
Legume	Diverse	25 - 30	2 or 4	1	-	Ca ²⁺ , Mn ²⁺
Cereals	GlcNAc, NeuAc	18	2	2	++	-
Animals						
C-Type	Diverse	>> 15	Variable	1 - 8	+	Ca ²⁺
S-Type	Gal	14 - 35	?	1	-	-

biochemical criteria, including sequence homologies, are also present in rye, barley, rice and a few grasses (Raikhel *et al* 1991). WGA is different from leguminous lectins in that it contains at least two independent, non-cooperative binding site per subunit and is without any regular secondary structure (α -helix or β -strand).

Among animal lectins, two distinct families have been identified: the C-type (Ca^{2+} -dependent), containing both membrane-bound and soluble lectins with diverse carbohydrate specificity; and S-type (thiol-dependent), consisting of lectins that share a specificity for β -galactosides. As pointed out by Drickamer in 1995, the primary structures of the two animal lectins appear to be unrelated, and there is no clear relationship between them and the families of plant lectins.

1.3.2 By Sugar Specificity

Lectin could also be categorised into six small groups according to their carbohydrate binding specificity: N-acetylgalactosamine, galactose, mannose and/or glucose, N-acetylglucosamine, L-fucose and sialic acid. The carbohydrate specificity of lectins is customarily examined by the hapten inhibition technique, in which different monosaccharides, oligosaccharides or glycopeptides are tested for their ability to inhibit either hemagglutination, polysaccharides (or glycoproteins) precipitation by lectin or by competitive binding assay. A scheme of the classification is shown in Table 3.

1.4 BIOLOGICAL ROLES OF LECTINS

The three dimensional structure of about a dozen lectins have been determined by X-ray crystallography at high resolution (Rini 1995). Extensive homologies have

Table 3. Lectins families by sugar specificity

Sugar specificity	Species	Common name	Preferred Abbreviation
I. GalNAc	<i>Amphicarpaea bracteata</i>	Hog-peanut	ABrA
	<i>Bauhinia purpurea alba</i>	Camel's foot tree	BPA
	<i>Caragana arborescens</i>	Siberian pea tree	CGA
	<i>Codium fragile</i>	Green algae	CDF
	<i>Dolichos biflorus</i>	Horse gram	DBA
	<i>Griffonia simplicifolia A4</i>	African legume	GSI-A ₄
	<i>Glycine max</i>	Soy bean	SBA
	<i>Helix pomatia</i>	Edible snail	HPA
	<i>Helix aspersa</i>	Garden snail	HAA
	<i>Maclura pomifera</i>	Osage orange tree	MPA
	<i>Momordica charantia</i>	Bitter pear melon	MDC
	<i>Phaseolus lunatus</i>	Lima bean	LBA
	<i>Salvia sclarea</i>	Clary shrub	SSA
	<i>Sophora japonica</i>	Japanese pagoda tree	SJA
	<i>Vicia villosa</i>	Hairy vetch	VVA
	<i>Viscum album</i>	Mistletoe	VAA
	<i>Wisteria floribunda</i>	Japanese wisteria	WFA
II. Gal	<i>Abrus precatorius</i>	Jequirity bean	APA
	<i>Agaricus bis porus</i>	Mushroom	ABiA
	<i>Agropyrum repens</i>	Couch grass	ARA
	<i>Allomyrina dichotoma</i>	Japanese beetle	AlloA
	<i>Arachis hypogaea</i>	Peanut	PNA
	<i>Artocarpus integrifolia</i>	Jackfruit (jacalin)	AIA
	<i>Artocarpus lakoocha</i>	Jackfruit	ALA
	<i>Bauhinia purpurea alba</i>	Camel's foot tree	BPA
	<i>Cytisus scoparius</i>	Scotch broom	CSA
	<i>Datura stramonium</i>	Thorn apple	TAA
	<i>Electrophorus electricus</i>	Electric Eel	EEL
	<i>Erythrina cristagalli</i>	Coral tree	ECA
	<i>Eranthis hyemalis</i>	Winter-aconite root	EHA
	<i>Euronymus europaeus</i>	Spindle tree	EUE
	<i>Geodia cydonium</i>	Sponge	GEC
	<i>Griffonia simplicifolia I-B4</i>	African legume	GSA-B ₄
	<i>Maclura pomifera</i>	Osage orange tree	MPA
	<i>Momordica charantia</i>	Bitter pear melon	MDC
	<i>Phaseolus vulgaris</i>	Red kidney bean	PHA-E, PHA-L
	<i>Phytolacca americana</i>	Pokeweed mitogen	PAA
	<i>Pseudomonas aeruginosa</i>	Bacterium	PA-I
	<i>Psophocarpus tetragonolobus</i>	Winged bean	PTA
	<i>Ptilota plumosa</i>	Red marine algae	PPL
	<i>Ricinus communis</i>	Castor bean	RCA-I
	<i>Sambucus nigra</i>	Elderberry	SNA
	<i>Sophora japonica</i>	Japanese pagoda tree	SJA
	<i>Tridacna crocea</i>	Molluse	TCA
	<i>Trichosanthes kinlouii</i>	Chinese herb	TKA
	<i>Urtica dioica</i>	Stinging nettle	UDA
	<i>Vigna radiata</i>	Mung bean	VGR
	<i>Vicia graminea</i>	Herb	VGA
III. Man and/or Glc	<i>Allium cepa</i>	Onion	ALCE
	<i>Allium porrum</i>	Leek	ALPO
	<i>Canavalia ensiformis</i>	Jack bean	ConA
	<i>Calendula officinalis</i>	Artichoke	CAO

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	<i>Citrullus vulgaris</i> <i>Galanthus nivalis</i> <i>Galega officinalis</i> <i>Ipomoea rubrocoerulea</i> <i>Lathyrus odoratus</i> <i>Lens culinaris</i> <i>Leucojum vernum</i> <i>Medicago sativa</i> <i>Perca flaviatilis</i> <i>Phytolacca americana Pa-2</i> <i>Pisum sativum</i> <i>Vicia faba</i>	Watermelon Snow drop Goat's rue Morning glory Sweet pea Lentil White snowflower Alfalfa Perch Pokeweed Pea Fava bean	CIV GNA GAO IPR LAO LCA LEV MES PEF PAA PSA VFA
IV. GlcNAc	<i>Aaptos papillata</i> <i>Allomyrina dichotoma</i> <i>Cucurbita maxima</i> <i>Cucurbita pepo</i> <i>Datura stramonium</i> <i>Griffonia simplicifolia II</i> <i>Hordeum vulgare</i> <i>Lycopersicon esculentum</i> <i>Luffa acutangula</i> <i>Oryza sativa</i> <i>Phytolacca americana Pa-2</i> <i>Psathyrella velutina</i> <i>Solanum tuberosum</i> <i>Triticum vulgare</i> <i>Urtica dioica</i> <i>Ulex europaeus</i>	Sponge Japanese beetle Pumpkin Squash Thorn apple African legume Barley Tomato Ridge gourd Rice Pokeweed Mushroom Potato Wheat germ Stinging nettle Gorse or Furz seed	APA-I, APA-II AlloA CMA CPA DSA GSA-II HRV LEA LAA ORS PWM PSV STA WGA UDA UEA-II
V. L-Fuc	<i>Anguilla anguilla</i> <i>Cytisus sessilifolia</i> <i>Euonymus europeus</i> <i>Griffonia simplicifolia IV</i> <i>Laburnum alpinum</i> <i>Lotus tetragonolobus</i> <i>Photoloba squarrosa</i> <i>Ulex europaeus</i> <i>Ulva lactuca</i>	Eel serum Shrub Spindle tree African legume Scotch laburnum Asparagus pea Broad-leaf tree Gorse or Furz seed Green marine algae	AAnA CSA EEA GSA-IV LAA LTA PHS UEA-I, UEA-II ULL
VI. Sialic acid	<i>Achatina granulata</i> <i>Biomphalaria glabrata</i> <i>Birgus latro</i> <i>Boltonia ovipera</i> <i>Carcinoscorpius rotunda cauda</i> <i>Cancer antennarius</i> <i>Cepaea hortensis</i> <i>Homarus americanus</i> <i>Limax flavus</i> <i>Sanbucus nigra</i> <i>Triticum vulgare</i>	Snail Saharan scorpion Coconut crab Tunicate Indian horseshoe crab California crab Snail Lobster Slug Elderberry Wheat	ACG BGA BLA BOO CRCA CCA CHT HAA LFA SNA WGA

Data derived from Wu, 1988 and Doyle, 1994

been observed between the primary sequences of lectins from taxonomically related sources, demonstrating that these proteins may have been conserved throughout evolution and arguing that they must play important roles in nature.

Two observations have provided insights into the roles of lectins in nature. One was the demonstration of the involvement of the hepatic binding protein specific for D-galactose in the clearance of glycoproteins from the circulatory system (Ashwell *et al.*, 1974 & Stockert *et al.*, 1974). Additional animal lectins have subsequently been discovered. Some of these function similarly to the hepatic binding protein, while others, such as the D-mannose 6-phosphate binding protein, are responsible for the intracellular routing of glycoproteins (Ashwell *et al.*, 1982). They may also be involved in the clearance of bacteria from blood (Perry *et al.*, 1984).

The other advance came with the demonstration that *Escherichia coli* and related organisms produced D-mannose specific surface lectins in the form of appendages known as fimbriae or pili (Ofek *et al.*, 1977). Bacterial surface lectins with specificities for other sugars have subsequently been identified (Mirelman 1986). Direct evidence for the involvement of these lectins in the initiation of infection was first obtained in 1979, when Aronson *et al.* showed that urinary tract infection in mice by the mannose-specific *E.coli* could be prevented by methyl- α -D-mannoside. These studies have raised the possibility that sugar inhibitors of lectin-mediated bacterial adherence may prove to be useful in protection against natural infection.

More recent study has shown that the mannose-specific bacterial surface lectins may also mediate attachment of the bacteria to phagocytic cells in the absence of antibodies and complement (collectively known as opsonins), leading to engulfment and killing of the bacteria (Sharon 1987). This process, designated by

them as 'lectinophagocytosis' may be of importance in the clearance of bacteria from non-immune patients, or from areas poor in opsonins.

Although scientists have some indications on the biological roles of lectins in bacteria and vertebrates, little is known with certainty on the role of lectins in plants where they are so abundant. The major hypothesis is they serve as mediators of the symbiosis between nitrogen-fixing microorganisms (primarily) and leguminous plants (Dazzo *et al.*, 1986). This is based mainly on the finding that a lectin from a particular legume binds in a specific manner to the corresponding rhizobial species and not to bacteria that are symbionts of other plants. Other attractive proposals are: lectins may play a role in the defence of plants against fungal, bacterial and viral pathogens during germination and early growth of seedlings (Broekaert *et al.*, 1989); may be important in sugar transport or carbohydrate storage (Judd, 1980) and/or may serve as a storage protein (Peumans and Van Damme, 1993).

1.5 APPLICATIONS OF LECTIN

In addition to being of interest in their own right, lectins have become indispensable tools in biological and medical research.

1.5.1 Blood Typing and Erythrocytes Polyagglutinate Studies

Many lectins display blood-group specificity and provide an alternative to human sera as a source of blood typing reagent. This is the earliest application of lectins and still in wide use today. The first blood-group specific lectin was discovered by Renkonen (1948) when he found that crude extracts of winged peas (*Lotus tetragonolobus*) agglutinated only type O erythrocytes. Subsequently, extracts from

lima beans (*Phaseolus limensis*) was reported to agglutinate only blood type A erythrocytes (Boyd, 1949). Since then, lectins with other blood-type specificity have also been isolated. Among the most widely used are: lectins from *Dolichos biflorus* (anti-A1; Bird, 1951) and *Ulex europaeus* (anti-H; Cazal and Laularie, 1952).

Lectins are frequently used together with other parameters like red cell electrophoretic mobility studies in the attempt to recognise and classify polyagglutinable red cells (Judd, 1980). Polyagglutination is defined as that condition where the red blood cells are agglutinated by a high-proportion of ABO blood group compatible, normal adult human sera. *In vitro*, contamination of blood bank reagents or blood samples may result in polyagglutination. To date, nine forms of polyagglutination have been described. The principle lectins employed for this purpose are *Arachis hypogaea* (anti-T/Tk), *Dolichos biflorus* (anti-A1/Tn/Cad), *Salvia sclarea* (anti-Tn), *Salvia horminum* (anti-Tn + Cad), *Glycine max* (syn, soja) and BS II lectin, from *Bandeiraea simplicifolia* seeds (anti-Tk).

Because of their specific carbohydrate-binding properties, lectins have also been used as probes in studies on the topography of the red cell surface (Judd, 1980). This application of lectin has provided much information on the structure of the MN, T, and Tn red cell surface receptors and has aided in defining the red cell membrane abnormalities associated with certain uncommon phenotypes within the MN blood-group system.

1.5.2 Fractionation of Oligosaccharide or Glycoproteins

Glycoproteins are macromolecules consisting of a polypeptide structure with covalently-linked oligosaccharide side-chains or glycan units. They are classified

according to the type of glycoside linkage. There are two main types: the N-glycosidic type which involves a linkage between N-acetylglucosamine and the amide group of asparagine; and the O-glycosidic type that involves a linkage between N-acetylglucosamine and the hydroxyl group of serine or threonine. Most proteins in human serum contain one or more N-linked glycan; notable exceptions being albumin and C-reactive protein which are not glycosylated. O-Glycans are found frequently in mucin, but are rare in serum glycoproteins.

The N-glycan units of serum proteins can be divided into three groups which are classified according to the structure of the oligosaccharide chain. These are called high mannose-, hybrid- and complex-type. These different forms share a common pentasaccharide chain consisting of two N-acetylglucosamine and three mannose residue. Typical examples of these sugar chains are shown in Figure 2.

The microheterogeneity of carbohydrate moieties in glycoproteins have created difficulties in their purification by the conventional methods. Recently, lectin immobilised column chromatography has been used not only for purification of the glycoproteins but also for separating and determining structure of glycoprotein-derived oligosaccharides and glycopeptides. In contrast to other fractionation techniques such as gel permeation, ion-exchange, partition, reversed-phase and normal-phase chromatography, lectin column chromatography is basically different because the principle of its fractionation is based on the carbohydrate-binding specificities of lectins.

Different lectins have distinct sugar specificity and thus can be successfully used for separation of closely related glycoproteins, glycopeptides or oligosaccharides that differ in their structure to a small extent only. For instance, immobilised Con A

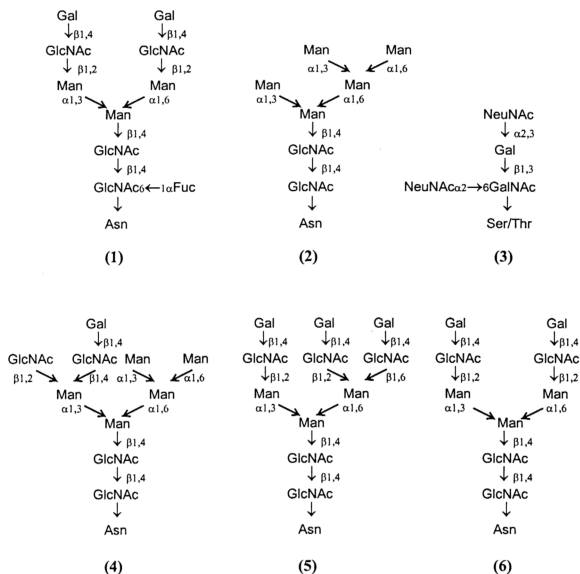


Figure 2. Structural diversity of oligosaccharide of glycoproteins.

Structures of oligosaccharides of glycoproteins are illustrated. Unless specifically stated, all structures are linked to asparagine residues (N-linked) of glycoproteins.

- (1) Fucosylated biantennary complex-type
- (2) High mannose type
- (3) Mucin type (O-linked)
- (4) Hybrid-type
- (5) Triantennary complex-type
- (6) Biantennary complex-type

can bind to high mannose-type, hybrid-type and biantennary complex-type oligosaccharides but not tri or tetraantennary complex-type glycans (Ogata *et al.*, 1975); *Aleuria aurantia* lectin (AAL) is specific to oligosaccharides with a fucosylated core regardless of the structures of the outer-chain moieties (Yamashita *et al.*, 1985); *Macckia amurensis* lectin (MAL) only detected sialylated oligosaccharides containing the terminal sequence NeuNAc α 2 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc structure (Wang and Cummings, 1988) and chromatography on lentil or pea lectin serves to demonstrate the presence of L-fucose residue attached to the innermost GlcNAc residue in the core (Kornfeld *et al.*, 1981).

1.5.3 Mitogenic Stimulation of Lymphocytes

Plant lectins have been shown to have the ability to bind to cells surface receptors and in the case of some lymphocytes, trigger the cells to undergo mitosis. The classical mitogens are Con A and PHA, which selectively stimulate the proliferation of T lymphocytes subpopulation (Powell and Leon, 1970). Pokeweed mitogen on the other hand induces both the proliferation of T and B cells (Janossy *et al.*, 1976).

The mitogenic properties of lectins have made it possible to analyse the biochemical events that occur during lymphocyte stimulation *in vitro*. In addition, mitogenic stimulation by lectins has provided a ready, simple means to assess the immunocompetence of patients suffering from various diseases including cases of AIDS (Favero *et al.*, 1993). It has also been employed to test the lymphocytes efficiency of astronauts and cosmonauts after space flights since 1973 (Cogoli and Tshopp, 1985). No less important is the fact that in the stimulated cells the

chromosomes are easily visualised, so that they can serve for facile karyotyping, sex determination and detection of chromosomal defects (Sharon and Lis, 1987).

Occasionally, mitogenic lectin may also serve as polyclonal activator and is thus useful in the synthesis of large amount of immunoglobulins for structural or biological studies.

1.5.4 Histochemical Studies of Normal and Pathological Conditions.

It is well established that the glycan structures on many serum glycoproteins undergo changes in relation to disease and therefore provide a promising basis for the search of biological markers for specific pathological conditions. Recently lectins have been used as a simple, reproducible, cheap and sensitive method for assessing the changes in the structure and constituents of glycoproteins. Comparisons of the lectin-binding properties of serum glycoproteins have been made largely by adapting some existing techniques, such as Western blotting, affinity chromatography, ELISA, and electrophoresis (Turner, 1992).

Some glycosylation alteration findings that are already being used in diagnostic situations or are associated with pathological manifestation of diseases are: reduced sialylation of transferrin in alcoholics (Gravel *et al.*, 1996); increased fucosylation of haptoglobin and α -foetoprotein in cancer (Thompson *et al.*, 1991; Aoyagi *et al.*, 1985); reduced galactosylation of IgG in rheumatoid arthritis (Parekh *et al.*, 1985); decreased branching of α_1 -acid glycoprotein in infection (Mackiewicz *et al.*, 1987); and desialylation and reduced galactosylation of IgA O-linked oligosaccharide chains in IgA nephropathy (Allen *et al.*, 1995; Shuib *et al.*, 1997).

1.5.5 Cell Separation

Lectins have also been used in the fractionation of cells into biologically distinct subpopulations. The earliest example is the usage of peanut agglutinin in the separation of mouse thymocytes into cortical (immature) and medullary (mature) thymocytes, which allowed the major question relating to the mechanism of lymphocyte maturation in the thymus to be tackled directly (Reisner *et al.*, 1976). More recently, this lectin has been employed to separate germ cells from somatic cells in mouse testis (Maekawa and Nishimune, 1985).

Another impressive example is the ability of soybean agglutinin to effectively remove from human marrow the cells responsible for graft reaction versus-host reaction, the main cause of mortality in patients treated with allogeneic bone marrow. This finding is believed to be the key breakthrough which permitted, eventually bone marrow transplants between genetically non-identical individuals (Reisner, 1990).

1.6 AIMS OF INVESTIGATIONS

Previous studies of this laboratory have shown that the seeds of champedak (*Artocarpus integer*) also contained an IgA1-reactive and D-galactose-binding lectin that was structurally and functionally similar to jacalin (Hashim *et al.*, 1991 & 1992). Jacalin is an IgA1-reactive and D-galactose-binding lectin that was isolated from the seeds of jackfruit (*Artocarpus heterophyllus*; Roque-Barreira *et al.*, 1985). Unlike the heterogeneous jacalins of distinctive origins which were shown to have diverse specificity (Kondoh *et al.*, 1987; Kobayashi *et al.*, 1988; Hashim *et al.*, 1991), champedak D-galactose-binding lectin isolated from several clones of *Artocarpus integer* seeds demonstrated uniform reactivity (Hashim *et al.*, 1993).

Besides jacalin, jackfruit seeds have also been shown to possess a D-mannose-binding lectin termed artocarpin (Miranda-Santos *et al.*, 1991a and 1991b). The lectin was claimed to be the main mitogenic component of the crude extracts of jackfruit seeds. It was shown to stimulate the proliferation of mouse spleen cells and human peripheral blood mononuclear cells and induce a T cell dependent polyclonal activation of human and mouse B cells for the secretion of immunoglobulin (Miranda-Santos *et al.*, 1991b). Structural studies of artocarpin from seeds of jackfruit demonstrated a 65 kDa homotetrameric protein devoid of covalently attached carbohydrates (Misquith *et al.*, 1994). The lectin differed significantly from all previously reported mannose/glucose-binding lectins as it demonstrated strongest affinity to a xylose β 1-2-linked to the core pentasaccharide moiety ($\text{Man}\alpha$ 1-3($\text{Man}\alpha$ 1-6) $\text{Man}\beta$ 1-4GlcNAc β 1-4GlcNAc) of *N*-linked glycans from horseradish peroxidase.

Preliminary work performed in this laboratory has shown that seeds of champedak, like jackfruit, also contains a mannose-binding lectin. The present investigation was made to isolate this lectin, termed champedak lectin M, and characterise it in terms of its physico-chemical property, mitogenic activity and carbohydrate specificity. In addition to that, the potential usage of lectin M in biomedical research was also explored.