CHAPTER 3

DETECTION, OCCURENCE AND ISOLATION

Lectins are ubiquitous in nature, and are found in all classes of organism. They are easy to detect and often to isolate. In addition, many are available from commercial suppliers. They are now obtainable also by recombinant techniques.

3.1 DETECTION

The classic, and still simplest, way to detect the presence of a lectin in a biological material is to prepare an extract from the material and examine its ability to agglutinate erythrocytes (Fig. 3.1) (Rüdiger, 1993). A more refined screening procedure is based on the ability of these proteins to precipitate polysaccharides (Goldstein, 1976) (Fig. 3.2) or glycoproteins. If a positive result is obtained, it is essential to show that the agglutination or precipitation is specifically inhibited by mono- or oligosaccharides, i.e., it is sugar specific (Fig. 3.1). Hemagglutination is commonly assayed by the serial dilution technique using erythrocytes from humans or rabbits. Occasionally erythrocytes that have been treated with trypsin or sialidase are employed, since such cells are often more sensitive to agglutination than the untreated cells (Fig. 3.3). Hemagglutination also serves to monitor and quantify the activity of lectins in the course of purification.

Because of the wide use of the agglutination reaction, it deserves some comments (Lis & Sharon, 1986). For agglutination to occur, the lectin must bind to the cells and form cross-bridges between them. There is however no simple relation between the amount of lectin bound and agglutination. Cases are even known where considerable amounts of lectin are bound to cells, without causing agglutination. This is because agglutination is affected by many factors, among them accessibility of receptor sites, membrane fluidity and metabolic state of the cells. It is also influenced by external conditions of the assay, such as temperature, cell concentration, mixing and so on. The relative contribution of the different factors depends on both the lectin and the cells examined. When agglutination does occur and it is inhibited by mono- or oligosaccharides, it serves as an indication that carbohydrate structures for

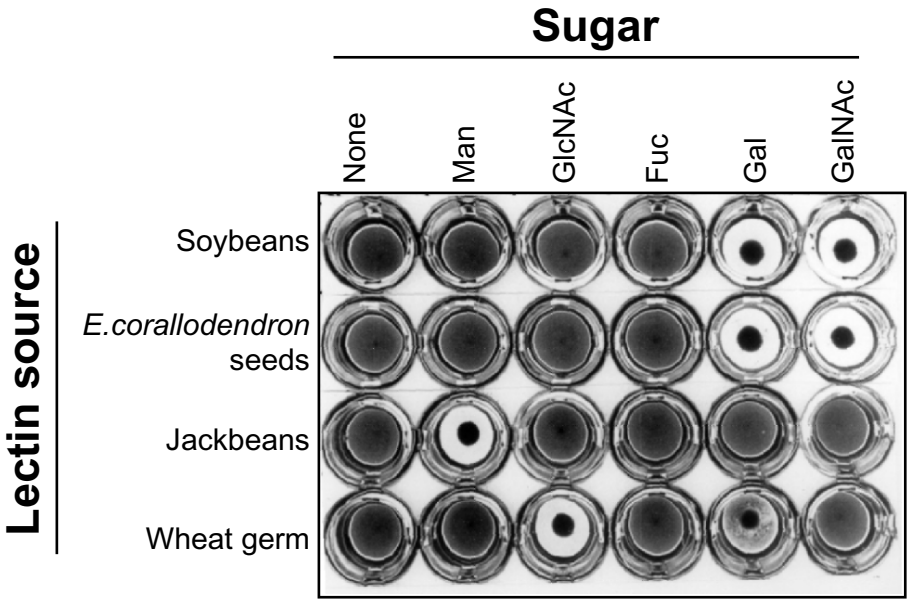

Fig. 3.1 Hemagglutination and its inhibition as a means to demonstrate the presence of lectins in seed extracts. Ground seeds (or wheat germ) were extracted with ten times their weight of phosphate buffered saline, pH 7.4. In the case of soybeans, the oil was removed prior to extraction. Each well of the microtiter plate contained 50 µl of seed extract, 50 µl of a 4% suspension of rabbit erythrocytes and 50 µl of 0.2 M sugar solution in phosphate-buffered saline, pH 7.4. The agglutinated erythrocytes form a carpet that covers the whole well; where no agglutination occurs, the cells form a button at the bottom of the well. Picture taken after 2 hours at room temperature.

which the lectin is specific are present on the surface of the cell. Additional information on the nature of the receptors may be obtained with erythrocytes pretreated with enzymes, in particular glycosidases, or with sugar-modifying reagents, such as periodate. Agglutination with lectins is also of use in following changes on cell surfaces during physiological and pathological processes.

Currently, a number of other methods for lectin detection is available. Thus, microarrays of different carbohydrates coupled to wells of a microtiter plate have been developed, with a range and complexity such as found in naturally occurring glycans (see fig. 4.1) (Bryan et al., 2002; Gargir, 2001). Such glycochips should greatly facilitate the screening for lectins in biological materials, as well as the definition of their specificity. By a completely different method, lectins can be detected in situ, in tissue sections and on cells, by staining with suitably derivatized glycoproteins or

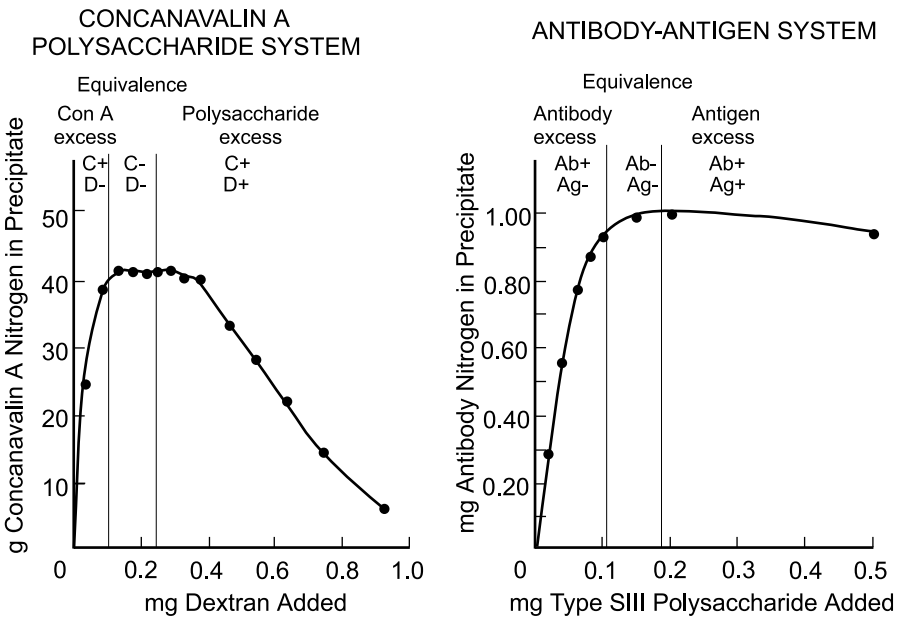

Fig. 3.2 Precipitin reaction between concanavalin A and dextran is similar to that between an anti-Type SIII polysaccharide antibody and the polysaccharide. Courtesy Dr.Irwin J. Goldstein, University of Michigan, Ann Arbor.

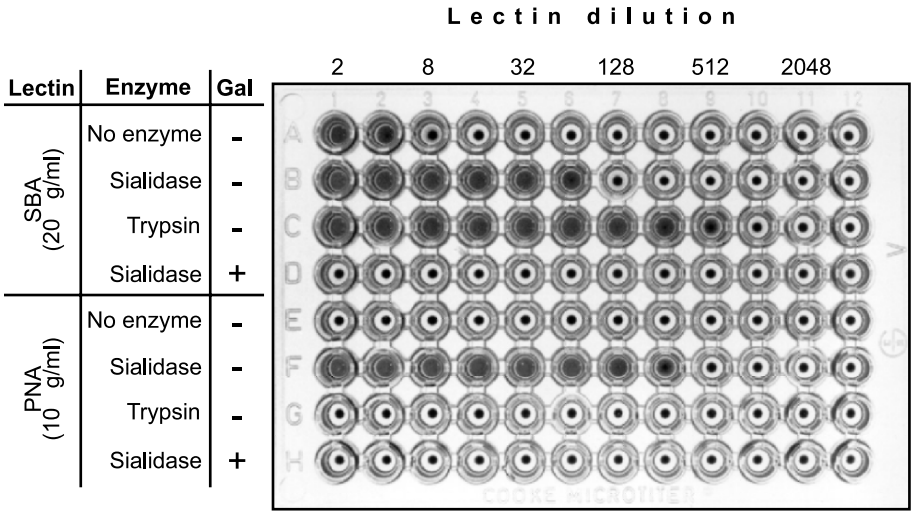

Fig. 3.3 Effect of enzyme treatment of human erythrocytes on their agglutination by SBA and PNA in the absence or presence of galactose.

neoglycoproteins (Gabius, H. J. et al., 1994). A third method is based on sequence similarities of newly discovered proteins to known lectins, by homology searches in data bases at the protein or cDNA level. It has had a pronounced impact in the field of animal lectins, where it led to the identification of many new proteins of this class. It also resulted in the discovery of large numbers of diverse lectin-like proteins, not all of which however possess carbohydrate-binding activity (Drickamer & Dodd, 1999).

3.2 OCCURRENCE

Lectins occur in all classes and families of organisms examined, although not necessarily in every genus or species {For an extensive survey of the early literature on the subject, see (Gold, 1975). Their tissue and cellular distribution is variable, and it may be affected by miscellaneous factors, such as developmental stage, age and pathological conditions. In the following we focus on the distribution of lectins in nature, touching only briefly on their specificity, which is the subject of Chapter 4.

3.2.1 Higher plants

Lectins have been detected in over a thousand species of plants and several hundreds have been isolated (Goldstein & Poretz, 1986; Rüdiger, 1988; Van Damme et al., 1998c). Some of the better characterized plant lectins and their specificity are listed in Table 3.1. The majority of these have been obtained from the seeds, especially those of the dicotyledonous legumes, where they accumulate during maturation and disappear upon germination. They may constitute as much as 10% of the total seed protein, although the quantities isolated are usually lower, between 0.1-1%. Their location within the seeds differs among various plant families (Rüdiger, 1998).

Table 3.1 Plant lectins[a]

Family and species	*Name/ abbrevi-ation*	*Location in plant*	*Specificity*	[b]*Ref.*
Monocotyledons				
Amaryllidaceae				
Galanthus nivalis (snowdrop)	GNA	Bulb	Man	
Narcissus pseudonarcissus (daffodil)	NPL	Bulb	Man	
Gramineae				

Table 3.1 Plant lectins[a]

Family and species	*Name/ abbrevi-ation*	*Location in plant*	*Specificity*	[b] *Ref.*
Monocotyledons				
Amaryllidaceae				
Galanthus nivalis (snow drop)	GNA	Bulb	Man	
Narcissus pseudonarcissus (daffodil)	NPL	Bulb	Man	
Gramineae				
Oryza sativa (rice)		Seed	GlcNAc	
Salt-stressed *Oryza sativa*[c] (rice)		Seed	Man	(1)
Triticum aestivum[d] (bread wheat)	WGA	Germ	GlcNAc & Neu5Ac	
Iridaceae				
Iris hollandica (Dutch iris)		Bulb	Gal/GalNAc & Man	(2)
Liliaceae				
Allium sativum[f] (garlic)	ASA	Bulb	Man	
Scilla campanulata	SCA	Bulb Bulb	Man; Fetuin	(3)
Orchidaceae				
Listera ovata (twayblade)	LOA	Leaves	Man	
Dicotyledons				
Caprifoliaceae				
Sambucus nigra (elderberry)	SNA	Bark	Neu5Ac-OS	
Compositae				
Helianthus tuberosus[c] (Jerusalem artichoke)	Heltuba (HTL)	Tuber	Man	(4)
Convolvulaceae				
Calystegia sepium[c] (hedge bindweed)	Calsepa	Rhizome	Man & maltose	
Cucurbitaceae				
Momordica charantia (bitter pear lemon; bitter gourd)		Seed	Gal/GalNAc	
Euphorbiaceae				

Table 3.1 Plant lectins[a]

Family and species	*Name/ abbrevi-ation*	*Location in plant*	*Specificity*	[b] *Ref.*
Ricinus communis (castor bean)	RCA	Seed	Gal/GalNAc	
	Ricin	Seed	Gal/GalNAc	
Hura crepitans (sand-box tree)		Seed; latex	Gal/GalNAc	
Labiatae				
Moluccella laevis (bells of Ireland)	MLL	Seed	Gal/GalNAc	
Salvia sclarea (clary sage)		Seed	Gal/GalNAc	
Leguminosae				
Abrus precatorius[e] (jequirity bean)	Abrin	Seed	Gal/GalNAc	
	APA	Seed	Gal/GalNAc	
Arachis hypogaea (peanut)	PNA	Seed	Gal/GalNAc	
Bauhinia purpurea	BPA	Seed	Gal/GalNAc	
Bowringia mildbraedi	BMA	Seed	Man/Glc	
Canavalia ensiformis (jack bean)	Con A	Seed	Man/Glc	
Dioclea grandiflora	DGL	Seed	Man/Glc	(5)
Dolichos biflorus (horse gram)	DBL	Seed	Gal/GalNAc	
	DB58	Leaf	Gal/GalNAc	
	LNP	Root	$(GlcNAc)_{2-5}$	
Dolichos lablab (*Lablab purpureum*) (hyacinth bean)	FRIL	Seed	Man	(6)
Erythrina corallodendron[g]	ECorL	Seed	Gal/GalNAc	
Erythrina cristagalli	ECL	Seed	Gal/GalNAc	
Glycine max (soybean)	SBA	Seed	Gal/GalNAc	
Griffonia simplicifolia	GSL-I	Seed	Gal/GalNAc	
	GSL-II	Seed	GlcNAc	
	GSL-IV	Seed	Fuc-OS[h]	
Lathyrus ochrus[i]	LOL	Seed	Man/Glc	
Lens culinaris (lentil)	LCL	Seed	Man/Glc	
Lotus tetragonolobus (asparagus pea)	LTA	Seed	Fuc	
Maackia amurensis	MAL	Seed	Neu5Ac-OS[j]	(7)
	MAH	Seed	Neu5Ac-OS	(8)

Table 3.1 Plant lectins[a]

Family and species	*Name/ abbrevi-ation*	*Location in plant*	*Specificity*	*[b]Ref.*
		Bark	Neu5Ac-OS	
Onobrychis viciifolia (sainfoin)		Seed	Man/Glc	
Phaseolus lunatus (*P. limensis)* (lima bean)	LBA	Seed	Gal/GalNAc	
Phaseolus vulgaris (red kidney bean)	PHA	Seed	Gal/ GalNAc-OS[k]	
	PvFRIL	Seed	Man	(9)
Pisum sativum (pea)	PSL	Seed	Man/Glc	
Psophocarpus tetragono-lobus (winged bean)	WBA-I WBA-II	Seed Seed	Gal/GalNAc Gal/GalNAc	
Robinia pseudoacacia		Bark	Gal/GalNAc	
Sophora japonica (Japanese pagoda tree)	SJL	Seed Bark	Gal/GalNAc Man/Glc	
Ulex europaeus (furze or gorse)	UEA-I UEA-II	Seed Seed	Fuc $(GlcNAc)_2$	
Vicia faba[l] (fava bean)	Favin	Seed	Man/Glc	
Loranthaceae				
Viscum album[e] (mistletoe)	ML-I/ Viscumin	Green tissue	Gal/GalNAc	
Moraceae				
Artocarpus intergrifolia (jackfruit)	Jacalin KM+ Artocarpin	Seed Seed Seed	Gal/GalNAc Man/Glc Man/Glc	(10
Maclura pomifera[c](osage orange)	MPA	Seed	Gal/GalNAc	
Musaceae				
Musa acuminata[c] (banana)		Fruit	Man/Glc	(11
Passifloraceae				
Adenia (Modecca) digitata[e] (modecca flower)	Modeccin	Root	Gal/GalNAc	
Phytolaccaceae				
Phytolacca americana (pokeweed)	PWM	Root	$(GlcNAc)_{2-4}$	
Solanaceae				

Table 3.1 Plant lectins[a]

Family and species	*Name/ abbrevi-ation*	*Location in plant*	*Specificity*	[b]*Ref.*
Datura stramonium (Jimson weed or thorn apple)	DSA	Seed	$(GlcNAc)_{2-4}$	
Lycopersicon esculentum (tomato)		Fruit	$(GlcNAc)_{2-4}$	
Solanum tuberosum (potato)	STL	Tuber	$(GlcNAc)_{2-4}$	
Urticaceae				
Urtica dioica (stinging nettle)	UDA	Rhizome	$(GlcNAc)_{2-4}$	

[a]For more exhaustive lists, see Goldstein & Poretz, 1986; Goldstein et al., 1997; Van Damme et al., 1998b; [b]references given only to publications not appearing under (a): (1) Zhang, W. et al., 2000; (2) Hao et al., 2001; (3) Wright, L. M. et al., 2000; (4) Bourne et al., 1999; (5) Dam et al., 1998a; (6) Colucci et al., 1999; (7) Knibbs et al., 1991; (8) Konami et al., 1994; (9) Moore et al., 2000; (10) Rosa et al., 1999; (11) Goldstein et al., 2001; [c]structurally related to jacalin; [d]formerly *Triticum vulgaris* or *T. vulgare*; [e]structurally related to ricin; [f]and closely related lectins from other Allium species; [g]and closely related lectins from some 20 other Erythrina species (Perez-Gomez, 1993); [h]Fuc-terminated oligosaccharides; [i]and closely related lectins from seven other Lathyrus species; [j]Neu5Ac-terminated oligosaccharides; [k]Gal/GalNAc-terminated oligosaccharides; [l]and closely related lectins from other Viciae species.

In seeds of the legumes, most of the lectin is localized in the cotyledons in protein bodies, subcellular organelles related to lysosomes, (Fig. 3.4) where it may be in complex with endogenous proteins, named "lectin binders" (Rüdiger, 1998). In those of the Euphorbiaceae (e.g. castor bean), the endosperm is the major site where the lectins occur and here, too, they are confined mainly to protein bodies. In rhizomes of the hedge bindweed (*Calystegia sepium*) the lectin is in the cytoplasm (Peumans et al., 2000a) whereas in cereals, it is all in the seed embryo.

Besides seeds, lectins have been found in all kinds of vegetative tissue (Table 3.1). The level of the lectins in these tissues is variable, and exhibits seasonal changes. It is usually lower than in seeds, but can be as high as 30% of the total tissue protein, e.g., the bulb lectins of garlic and ransom, or as low as 0.01% (e.g. in leaves of the leek) (Peumans et al., 2000a).

Most plant tissues contain a single lectin, although occasionally two (or more) lectins that differ in their sugar specificities and other properties are found in the same tissue (Peumans, W. J. et al., 2000a). Thus, two distinct

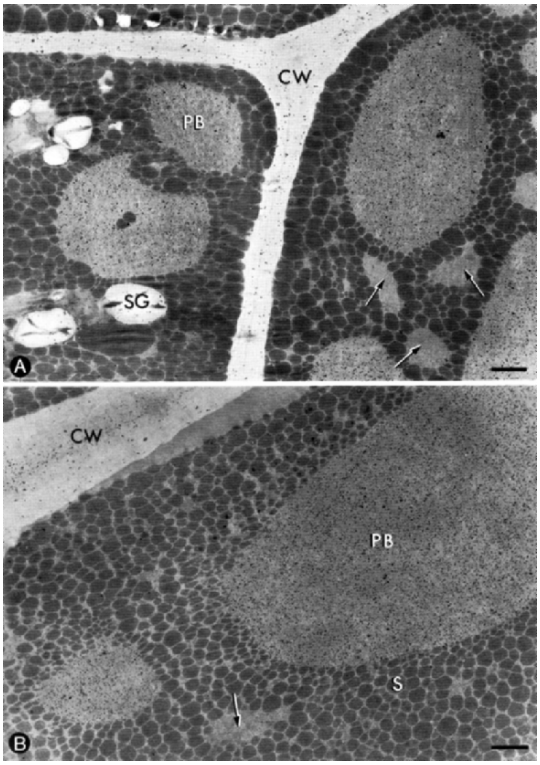

Fig. 3.4 (A) Localization of SBA in a thin section of *Glycine max* var. Altona. The anti-SBA antibodies labeled with gold particles were found in most of the protein bodies (PB). Some protein bodies (arrow) were not labeled. The spherosomes (S) were free of SBA. The starch granules (SG), cell walls (CW) and space between the spherosomes was weakly stained in a non-specific manner. The thin section was obtained in the middle of the flat part of the cotyledon. (B). At a higher magnification, labeling was very uniform within the protein bodies (PB). The thin section was obtained at the periphery of the cotyledon, opposite to the embryonic axis. Again some protein bodies were not labeled (arrow). Reproduced with permission from Horisberger & Volanthen, 1980; copyright 1980 Springer Verlag.

lectins occur in the seeds of gorse, jackfruit and *Vicia cracca,* while in seeds of *Griffonia simplicifolia* three different lectins (in addition to a number of isolectins, see below) are present. Lectins with dissimilar specificities are also found in the bark of the elderberry and the Japanese pagoda tree. In the same plant, lectins are not necessarily confined to a single tissue. Cases are known when lectins found in vegetative parts of the plant are identical with those in the seeds (e.g., the three lectins of G*riffonia simplicifolia* seeds are also present in leaves of the same plant) but this is not always so. For example, in

Dolichos biflorus, the most extensively studied plant with respect to the distribution of lectins in various tissues, the leaves contain a lectin (DB58) homologous to that of the seed lectin (DBL), but with some differences in its fine specificity (Etzler, 1997). In addition, a root lectin (LNP) has been found in the same plant that is distinct from the seed lectin both in amino acid composition, molecular weight, isoelectric point and specificity (Etzler et al., 1999).

Single lectins, in particular from legumes, occur occasionally as a mixture of isoforms referred to as isolectins. Typically, isolectins have a similar molecular structure, although they may differ slightly in their specificity or some physical property such as electric charge. They can, therefore, be separated by ion exchange chromatography, as found for WGA (Fig. 3.5), or by affinity chromatography on immobilized sugars.

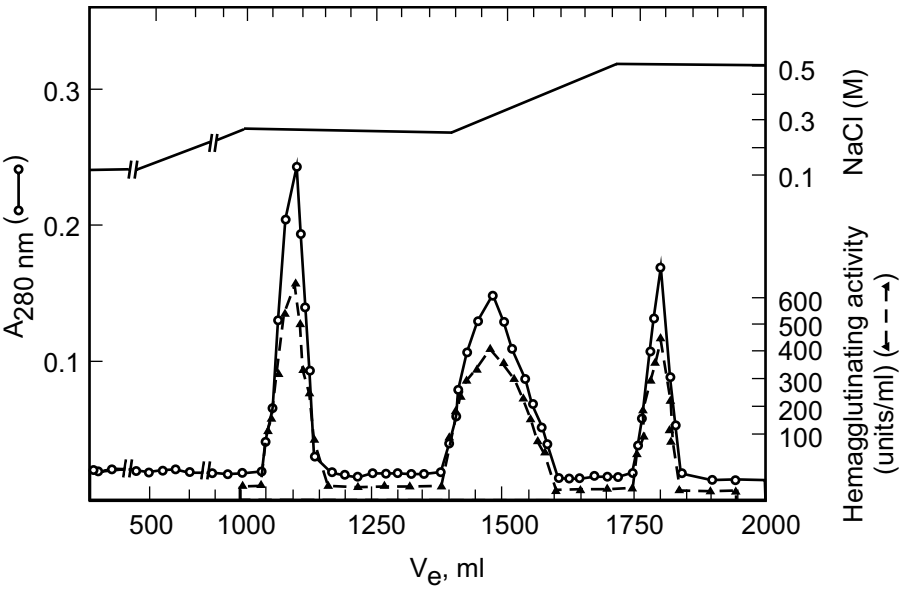

Fig. 3.5 Separation of WGA isolectins I, II and III by ion exchange chromatography on a column of SP-Sephadex C-25. Elution was performed by increasing sodium chloride concentrations as indicated in the upper part of the figure. o-o, protein; ▲-▲, hemagglutinating activity; V_e, elution volume. Courtesy Dr. Reuben Lotan, Rehovot.

Some isolectins originate from distinct genes, as is the case of those of WGA (cf. Chapter 7). Others result from the differential posttranslational modifications of a single lectin gene product (Young et al., 1995b) or of the different assembly of closely related subunits. The two winged bean seed

isolectins WBA-I and WBA-II differ mainly in their isoelectric points, one being acidic and the other basic, whereas the *Maackia amurensis* seed isolectins differ in their cell specificity, one (MAH) being a hemagglutinin and the other (MAL) having leukoagglutinating activity (i.e. the ability to agglutinate preferentially white blood cells such as lymphocytes) (Van Damme et al., 1998b). GSL-I consists of a family of five isolectins, each a tetramer of one or two types of subunit, A and B (Fig. 3.6) that are very

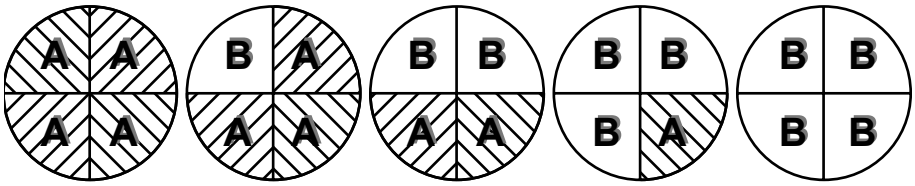

Fig. 3.6 Tetrameric structure of the five isoforms of GSL-I. A and B denote the two types of subunit of the lectin.

similar in molecular size and amino acid composition, but differ in specificity (see 4.2.1.b). The PHA isolectins also represent a family of five tetrameric proteins with varying proportions of two classes of subunit, E and L. They differ in carbohydrate and cell specificity, as well as in biological properties: E_4 (E-PHA) is a potent hemagglutinin, L_4 (L-PHA) has leukoagglutinating activity and is a potent mitogen; the intermediate forms (E_1L_3, E_2L_2 or E_3L) possess lower levels of the above activities. Similar mixtures of isoforms are found in the seeds of *Vicia villosa* and in the bark of *Maackia amurensis, Robinia pseudoacacia* and *Sophora japonica* (Van Damme et al., 1998c). In *Datura stramonium* seeds three isolectins are present, two of which are homodimers composed of either A or B subunits, whereas the third is a heterodimer comprised of both subunits. The two types of lectin (toxin and agglutinin) found, among others, in the seeds of *Ricinus communis* and of *Abrus precatorius* may be considered as a special case of isolectins.

3.2.2 Lower plants

Hemagglutinating activity has been detected in many species of marine algae, particularly red ones (Hori et al., 2000; Rogers, D. J. & Fish, 1991). However, only a few algal lectins have been purified and characterized, such as the *N*-acetylgalactosamine-specific lectin from the green alga *Codium fragile* subspecies *tomentosoides* (Wu, A. M. et al., 1997), the galactose-specific one from the red alga *Ptilota filicina* (Sampaio et al., 1998).

3.2.3 Fungi (including mushrooms and yeasts)

The first lectins to be purified from these sources were from the fruiting bodies of the meadow mushroom, *Agaricus campestris*, and the common (commercial) mushroom, *Agaricus bisporus* (Goldstein & Poretz, 1986). By now, many other fungal lectins became known (Guillot, J. & Konska, 1997). Lectins have also been found in phytopathogenic fungi, such as *Botrytis cinerea* (Kellens et al., 1992), *Pleurotus ostreatus* (Chattopadhyay et al., 1999; Wang, H. et al., 2000), *Rhizoctonia solani* (Candy et al., 2001), in different members of the *Sclerotiniaceae* (Goldstein, 1990; Inbar, J. & Chet, 1994) and in the nematode-trapping fungus *Arthrobotrys oligospora* (Rosen, S. et al., 1996). Recently, a lectin with unique carbohydrate-binding properties, including blood group-B-specificity, and high affinity for Galα3Gal and Galα3Galβ4GlcNAc, but no reactivity with methyl α-galactoside, has been isolated from the mushroom *Marasmius oreades* (Winter et al., 2002). A galectin has been isolated from the fruiting bodies of *Coprinus cinereus*, the first of this lectin family found outside the animal kingdom (Cooper et al., 1997). It is possible that when additional galactose-specific fungal lectins are sequenced, at least some of them, too, will turn out to be galectins. The above and other fungal lectins are listed in Table 3.2.

Table 3.2 Fungal lectins

Source	*Specificity*	*Ref*[a]
Agaricus bisporus	Galβ3GalNAc-Ser/Thr	(1)
Agrocybe cilindracea	Neu5Ac-OS	(2)
Aleuria aurantia	Fuc	(3)
Arthrobotrys oligospora	Galβ3GalNAc-Ser/Thr; sulfated glycoconjugates	(4)
Botrytis cinerea	Gal	(5)
Coprinus cinereus	Gal	(6)
Hericium erinaceum	Neu5Gc	(7)
Hygrophorus hypothejus	Gal	(8)
Ischnoderma resinosum	Gal	(9)
Lactarius deliciosus	Gal/GalNAc	(10)
Lactarius deterrimus	Gal/GalNAc	(11)
Marasmius oreades	Galα3Gal	(12)
Melastiza chateri	Fuc	(13)
Pleurotus cornucopiae	Gal/GalNAc	(14)
Pleurotus ostreatus	Gal/GalNAc	(15)
Polyporus squamosus	Neu5Ac-OS[b]	(16)
Psathyrella velutina	GlcNAc, Neu5Ac-OS[b]	(17)

Table 3.2 Fungal lectins

Source	*Specificity*	*Ref*[a]
Rhizoctonia solani	Gal/GalNAc	(21)
Sclerotium rolfsii	[c]	(22)

[a] For a more exhaustive lists, see Guillot and Konska, 1997; (Wang, H., Ng, T.B. & Ooi, 1998); (1) (Presant & Kornfeld, 1972); (2) (Yagi et al., 1997); (3) (Nagata et al., 1991); (4) (Rosen, S. et al., 1996); (5) (Kellens et al., 1992); (6) (Cooper et al., 1997); (7) (Kawagishi et al., 1994); (8) (Veau et al., 1999); (9) Kawagishi, 1991 #1030}; (10) (Guillot, J. et al., 1991); (11) (Giollant et al., 1993); (12) (Winter et al., 2002); (13) (Ogawa et al., 2001); (14) (Oguri et al., 1996); (15) (Chattopadhyay et al., 1999; Wang, H. et al., 2000); (16) (Mo et al., 2000; Zhang, B. et al., 2001); (17) (Kochibe & Matta, 1989); (Ueda et al., 1999); (18) (Candy et al., 2001); (19) (Inbar, J. & Chet, 1994); [b]Neu5Ac-terminated oligosaccharides; [c]inhibited only by glycoproteins

Lectins have ben isolated from a few yeast species, namely a galactose-specific one from a fatty acid auxotroph of *Saccharomyces cerevisiae* (Kundu et al., 1987) and two from the culture medium of *Kluyveromyces bulgaricus,* one specific for galactose and the other for *N*-acetylglucosamine (al-Mahmood et al., 1991).

3.2.4 Animals

Until the late 1980's, the major source of animal lectins was invertebrates. During the last decade, numerous lectins have been isolated from higher animals, and their number is fast growing. Unlike plant lectins, which can be grouped in families along taxonomic lines, animal lectins often exhibit structural similarities even when derived from diverse phyla. These lectins are therefore classified largely on the basis of shared sequence characteristics of their carbohydrate recognition domains (CRDs) (Drickamer, 1988; Dodd & Drickamer, 2001) as discussed in detail in section 5.2. According to a recent count, at least 12 structural families of animal lectins are known to exist (Kilpatrick, 2002a), the major ones of which are the C-type lectins (a superfamily), galectins and siglecs. However, not all animal lectins fall into any of the known families.

3.2.4.a. Vertebrates

The most widely occurring family of animal lectins is that of the galectins, (originally S-lectins), so called because they are galactose-specific (Table 3.3). Twelve mammalian galectins have been described, as well as many additional ones from other species, including birds, lower vertebrates, worms and sponges (Leffler, 2001; Rabinovich et al., 2002; Vasta et al., 1997). They occur in nearly all cell types, both inside and outside cells, but each galectin

tends to be enriched in a few cell types. Thus, galectin-4 and 6 are present almost exclusively in epithelial cells of the gastrointestinal tract, galectin-5 is expressed in erythrocytes and galectin-7 in keratinocytes. The intracellular galectins are located in the cytosol as well as in the nucleus; the extracellular ones are either attached to the cell surface or present in the intercellular space between closely packed cells. They are also found in connective tissues, where they are usually not free but bound to *N*-acetyllactosamine-containing carbohydrate units of glycoproteins.

C-Type lectins (so called because they require Ca^{2+} for binding of sugars) have been identified in a wide range of animals (Drickamer, 1999). They consist of three major classes - endocytic lectins, collectins and selectins - and a minor one - lecticans, and are confined to particular species, organs, or tissues (Table 3.3). Many are associated both with the plasma membrane and with intracellular membranes. The prototype endocytic lectin is the galactose-specific mammalian hepatic asialoglycoprotein receptor (ASOGR), or hepatic binding protein (HBP), originally isolated from rabbit liver (Ashwell & Harford, 1982; Ashwell & Morell, 1974); similar lectins are also present in liver of man, rat and other mammals. In birds, the corresponding lectin is specific for *N*-acetylgalactosamine and not for galactose. The prototype of the avian lectins is that of chicken, known as chicken hepatic lectin (CHL). In all cases examined, the HBPs are located exclusively on parenchymal hepatocytes. Other endocytic C-type lectins are the fucose- and the galactose-specific receptor found on liver macrophages (Kupffer cells), and the mannose receptor of macrophages (MMR) and of hepatic endothelial cells. The collectins, so called because of their collagenous domains, are present predominantly in mammals and are the only family of C-type lectins that are soluble and not membrane bound. Examples are the serum and liver mannose binding lectins (MBLs), bovine serum conglutinin, and the pulmonary surfactant proteins A and D (SP-A and SP-D, respectively), components of the surfactant that line alveoli in the lung.

The selectins (E-, L- and P-) are membrane lectins found on vascular endothelium, and on leukocytes. L-Selectin is present on essentially all blood

Table 3.3 Vertebrate lectins[a]

Lectin	*Occurrence*	*Specificity*	*Refs*[a]
Galectins[b]	Widespread	Gal	(1)
C-Type			
Endocytic Lectins			
Mammalian hepatic lectin	Parenchymal hepatocytes	Gal/GalNAc	
Avian hepatic lectin	Parenchymal hepatocytes	GlcNAc	

Table 3.3 Vertebrate lectins[a]

Lectin	*Occurrence*	*Specificity*	*Refs*[a]
Fucose- and galactose-specific receptor	Mammalian liver macrophages	Fuc, Gal	
Galactose receptor	Mammalian peritoneal macrophages	Gal	
Langerin	Langerhans cells	Man	(2)
Mannose/GalNAc-4-sulfate receptor	Mammalian macrophages	Man	
	Hepatic endothelial cells	GalNAc-4-sulfate	(3)
Collectins			
Mannose-binding protein A	Mammalian serum	Man	
Mannose-binding protein C	Mammalian liver	Man	
Conglutinin	Bovine serum	GlcNAc/Man	
Collectin CL-43	Bovine serum	Man	
Surfactant protein A	Mammalian pulmonary alveoli	ManNAc	
Surfactant protein D	Mammalian pulmonary alveoli	Maltose	
Lecticans			
Aggrecan	Cartilage	Fuc, Gal	
Brevican	Neural tissue	Sulfated glycolipids	
Neurocan	Neural tissue	Sulfated glycolipids	
Versican	Fibroblats	Fuc, GlcNAc	
Selectins			
L-selectin	Leukocytes	Neu5Acα2,3-(6-sulfate)Galβ4-(Fucα3)-GlcNAc	
E-selectin	Stimulated endothelial cells	sLe^x; sLe^a	
P-selectin	Secretory granules of platelets and endothelial cells	sLe^x ; sLe^a	
P-type			

Table 3.3 Vertebrate lectins[a]

Lectin	*Occurrence*	*Specificity*	*Refs*[a]
Mannose-6-phosphate receptors	Widespread	Man6*P*	
Siglecs	Hemopoietic system; nervous system[b]	Neu5Ac-OS[c]	
Pentraxins			
C-reactive protein	Serum	Gal-6-P	
Serum amyloid P component	Serum	4,6-*O*-[(*R*)-carboxyethylidene] galactose	
Others			
AAA	Eel serum	Fuc	
Calreticulin	ER[d]	Glcα3Man	
Calnexin	ER	Glcα3Man	
DC-SIGN[e]	Dendritic cells	Man-OS[f]	(4)
DC-SIGNR[g]	Dendritic cells	Man-OS	(4)
Dectin	Macrophages; dendritic cells	β3 and β6 glucans	(5)
EDEM[h]	ER	Glcα3Man	(6)
ERGIC-53[i]	ER-Golgi intermediate compartment	Man	
Ficolins[j]	Various tissues	GlcNAc	(7)
Intelectin	Placenta	Gal*f*	(8)
SP 56	Mouse sperm	Galβ3GalNAc	(9)
Spermadhesin	Boar sperm	αGal	(10)
VIP36[f]	Golgi compartment	Man	
Ym1	Macrophages	GlcN	(11)

Table 3.3 Vertebrate lectins[a]

Lectin	*Occurrence*	*Specificity*	*Refs*[a]

[a]For references see Kilpatrick, D.C., 2000, unless otherwise stated. (1) Hirabayashi et al., 2002); (2) Valladeau, 2000; (3) Roseman & Baenziger, 2000; (5) Mitchell, D. A. et al., 2001; (6) Brown & Gordon, 2001; (6) Braakman, 2001; (7) (Lu et al., 2002; Matsushita & Fujita, 2001); (8) Tsuji et al., 2001; (9) Bookbinder et al., 1995; (10) Töpfer-Petersen et al., 1998; (11) Chang et al., 2001; [b]Found in all kinds of organism, from nematodes to mammals; [c]Neu5Ac-OS, Neu5Ac-terminated oligosaccharides; [d]ER, endoplasmic reticulum; [e]DC-SIGN, dentritic cell-specific ICAM-3-grabbing nonintegrin; [f]Man-OS, Man-terminated oligoccharides; [g]DC-SIGNR, DC-SIGN-related protein; [h]EDEM, ER degradation-enhancing α-mannosidase-like protein; [i]ERGIC, endoplasmic reticulum Golgi intermediate compartment; [j]found also in lower animals, e.g. ascidians (Kenjo et al., 2001); [k]VIP, vesicular integral membrane protein.

monocytes and neutrophils, on the majority of blood borne T-and B- cells, and on a subset of natural killer (NK) cells. Its expression is however variable and depends on different factors, among them the developmental stage of the cells. Thus, on B-cells it occurs relatively late during development, well after Ig gene rearrangement, and just before the mature, virgin, immunocompetent B cells migrate out of the bone marrow (Kansas, 1996).

E-Selectin is confined to endothelial cells, its expression being stimulated at the level of transcription, principally in response to inflammatory stimuli such as interleukin-1 (IL-1) or tumor necrosis factor-α (TNF-α). Cytokine induction of the E-selectin gene requires activation and nuclear translocation of the transcription factor NF-κB, that controls many genes involved in immune and inflammatory responses. Within 6 to 9 hours after induction, transcription of the E-selectin gene is sharply down regulated. In addition, the lectin is rapidly internalized and degraded in the lysosomes. These are the major factors ensuring that the expression of E-selectin on cytokine-simulated cells is transient.

In addition to C-type lectins proper, domains homologous to the C-type CRD, referred to as C-type lectin-like domains or CTLDs, have been identified in a variety of proteins that do not appear to have carbohydrate-binding activity (Drickamer & Dodd, 1999). Many such CTLDs are found, for instance, in surface receptors of natural killer (NK) cells (Kogelberg & Feizi, 2001; Weis, W. I., Taylor, M.E. & Drickamer, 1998), e.g., the murine Ly-49 family and the NKR-P1 series that is polymorphic in rodents, but has only one known homologue in humans, and in anti-freeze proteins from arctic fish (Davies & Sykes, 1997).

In contrast to the large family of C-type lectins that is highly diverse, both with respect to structure and to carbohydrate specificity, the P-type lectin

family consists of only two homologous members. It owes its name to the fact that both are specific for mannose-6-phosphate (Man6*P*). These lectins, known as Man6*P*-receptors, are widespread and occur on most cell types.

The eleven siglecs (sialic acid-binding, Ig-like lectins) identified to date constitute a distinct subset of the Ig superfamily (see 5.2.5). Apart from siglec-4a and -4b, that are expressed exclusively in the nervous system, on oligodentrocytes and Schwann cells, respectively, all other members of this family are confined to discrete subpopulations of leukocytes (Fig. 3.7).

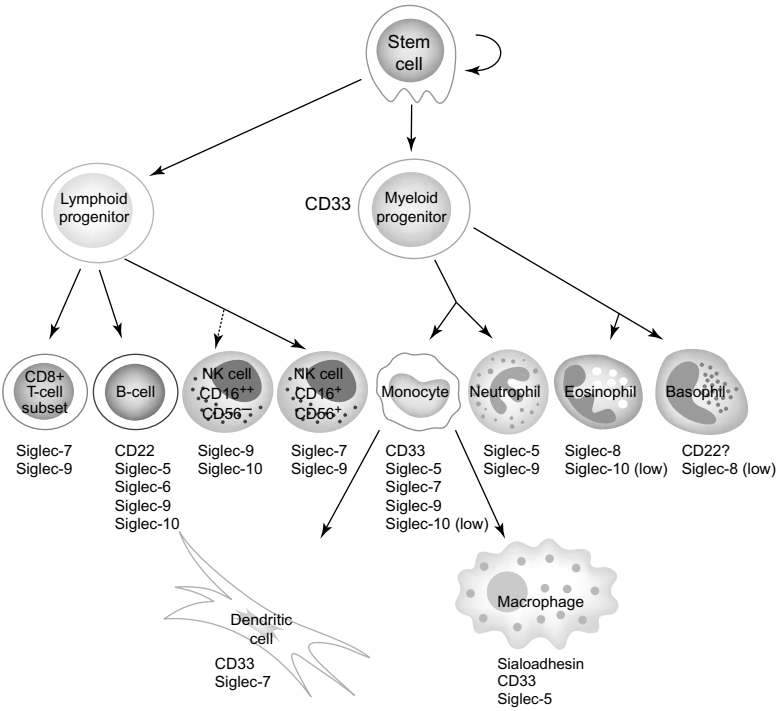

Fig. 3.7 Expression pattern of siglecs within the hematopoietic system. Apart from CD33 and CD22, little is known about the expression patterns of siglecs on stem cells and progenitors. To date, CD33 and siglec-7 are the only CD33-related siglecs reported to be expressed on monocyte-derived dendritic cells. Sialoadhesin, CD33 and siglec-5 are expressed by subsets of tissue macrophages, but nothing is known about the expression of other CD33-related siglecs on macrophages. NK, natural killer. Reproduced from Crocker & Varki, 2001b; copyright 2001, with permission from Elsevier Science. The recently discovered siglec-11 was not found on peripheral blood leukocytes but was present on macrophages (Angata et al., 2002).

The pentraxins, so named for the arrangement of their subunits into discs with cyclic pentameric symmetry, are serum proteins. The intracellular animal lectins include calnexin, calreticulin and EDEM present in the endoplasmic reticulum, ERGIC-53 in the ER-Golgi intermediate compartment and VIP36 in the Golgi. The newly discovered lectins DC-SIGN and dectin are constituents of the membranes of dendritic cells and of macrophages, respectively. Nuclei also contain lectins, as indicated by the specific binding of neoglycoproteins to these organelles, but only one such lectin (galectin-3, previously known as CBP35) has been isolated and characterized (Gaudin et al., 1995; Wang, L. et al., 1995).

Some other proteins, such as the different interleukins and tumor necrosis factor exhibit carbohydrate binding activities (Cebo et al., 2002).

3.2.4.b. Invertebrates.

Practically all classes and subclasses of invertebrate examined have lectins (Table 3.4). These include crabs, snails, worms (helminths) (Greenhalgh et al., 1999; Hirabayashi et al., 1998), insects (Ingram & Molyneux, 1991; Kubo et al., 2001), mollusks and sponges (Müller et al., 1997). The lectins are present mainly in the hemolymph and sexual organs, e.g. albumin glands and eggs, and occur also on membranes of hemocytes, cells that function in innate immunity (Vasta, 1992). Perhaps the best known invertebrate lectins are from the garden snail, *Helix pomatia*, from the body wall of the slug, *Limax flavus*, and from the serum of the horseshoe crab, *Limulus polyphemus*. These and other well-characterized invertebrate lectins are listed in Table 3.4. More exhaustive listings are found in (Kilpatrick, 2000).

Table 3.4 Invertebrate lectins

Phylum/species	*Occurrence in tissue/organ*	*Specificity*
Annelida		
Haemopis marmorata[b] (mud leech)	Membranes	Gal
Lumbricus terrestris[c] (earthworm)	Coelomic fluid	Gal
Arthropoda		
Allomyrina dichotoma (beetle)	Larvae	Neu5Ac-OS
Cancer antenarius (marine crab)	Hemolymph	Neu5,9Ac$_2$
Carcinoscopus rotunda (Indian horseshoe crab)	Hemolymph	Neu5Ac/ Neu5Gc
Homarus americanus (American lobster)[d]	Hemolymph	Neu5Ac; GalNAc

Table 3.4 Invertebrate lectins

Phylum/species	*Occurrence in tissue/organ*	*Specificity*
Limulus polyphemus (horseshoe crab)	Amebocytes; plasma	Neu5Ac
Megabalanus rosa (acorn barnacle)	Coelomic fluid	Neu5Ac
Periplaneta americana[e] (cockroach)	Hemolymph	Fuc; L-Rha
Sarcophaga peregrina (flesh fly)	Fat body, plasma	Gal
Scylla serrata (marine crab)	Hemolymph	Neu5Gc
Selenocosmia huvena (spider)f	Venom	ManN
Tachypleus tridentatus[g] (Japanese horseshoe crab)	Hemolymph	GlcNAc; Neu5Ac-OS
Chordata		
Clavelina picta (tunicate)[h]	Plasma	Fuc
Didemnum candidum (tunicate)	Plasma	Gal
Polyandrocarpa misakiensis[i](tunicate)		Gal; sucrose
Echinodermata		
Anthocidaris crassispina (sea urchin)	Coelomic fluid; eggs	Gal
Achatina fulica (snail)	Hemolyph	Neu5,9Ac_2
Aplysia depilans (sea hare)	Gonads	GalU
Cucumaria echinata[j](sea cucumber)	Not known	GalNAc
Helix pomatia (garden snail)	Albumin gland	GalNAc
Limax flavus (slug)	Body wall	Neu5Ac;NeuGc
Tridacna maxima (clam)	Hemolymph	GalNAc
Nematoda		
Caenorhabditis elegans[k]	Cuticle	Gal
Porifera		
Axinella polypoides[l](sponge)	Cytoplasm	Gal
Geodia cynodium (sponge)	Cytoplasm; cell surface	Gal

Table 3.4 Invertebrate lectins

Phylum/species	*Occurrence in tissue/organ*	*Specificity*

[a]For references see (Kilpatrick, D.C., 2000), unless otherwise stated; [b](Cole & Zipser, 1994); [c](Hirabayashi, J. et al., 1998); [d]Neu5Ac-OS, Neu5Ac-terminated oligosacharides; [e]the hemolymph contains a mixture of lectins, two of which have been isolated; [f](Lü et al., 1999); [g]four lectins with different specificities have been isolated from the hemolymph; two of them (regenectin and the 26kDa lectin) appear transiently in leg homogenates during regeneration; [h]contains at least five different lectins; [i]contains four lectins; [j]detectable only during budding; [k]the two galactose-specific lectins isolated from this organism were shown to be galectins (Hirabayashi, J. et al., 2001); [l]contains 5 lectins, one of which is specific for hexuronic acids.

Many invertebrates contain multiple lectins, several of which have been purified and characterized. Examples are the four and three lectins, respectively, of the cockroaches *Periplaneta americana* and *Blaberus discoidalis*, the two of sea urchin, and the three of sea cucumber (*Cucumaria echinata*) (Kilpatrick, 2000). A bewildering array of lectins/isolectins has been detected in the Japanese horseshoe crab, some of which are antigenically distinct and exhibit different species specificity for erythrocytes.

Certain invertebrate lectins show significant sequence similarity to vertebrate lectins (Vasta, 1992). Examples are the lectins of the larva of *Sarcophaga peregrina* and of *Anthocidaris crasipina* that are similar to C-type lectins, of *Geodia cynodium* similar to galectins, and of the horseshoe crab and *Didenum candidum* that show sequence similarity to pentraxins. No less than 125 proteins containing CTLDs have been discovered in the genome of *Caenorhabditis elegans* (Drickamer & Dodd, 1999). Since the amino acid sequences of most invertebrate lectins are however not known, the vast majority of these proteins cannot yet be classified into families based on structural similarities.

3.2.5 Microrganisms

3.2.5.a. Protozoa

Among the protozoan lectins (Ward, 1997) the primary examples are the two surface proteins of the pathogenic ameba (*Entamoeba histolytica*), specific for *N*-acetylglucosamine (Mirelman and Ravdin, 1986) and for Gal/GalNAc, respectively (Petri & Schnaar, 1995). Two lectins, one specific for *N*-acetylneuraminic acid, the other for *N*-acetylglucosamine, were isolated from merozoites of the human malarial parasite *Plasmodium falciparum*, and

a lectin specific for heparan sulphate from sporozoites of the same organism (Ward, 1997). A lectins with the latter specificity has been purified from trypomastigotes of *Trypanosoma cruzi*, the parasite causing the Chagas disease (Ward, 1997). *N*-Acetylneuraminic acid-specific lectins were obtained from culture supernatants of the protozoan *Trichomonas mobilensis* (Babál et al., 1994) and of the cattle parasite *Trichomonas foetus* (Babál et al., 1999).

3.2.5.b. Bacteria

Many bacterial species and genera express lectins, frequently of more than one type and with distinct specificities (Ofek & Doyle, 1994; Sharon & Lis, 1997) (Table 3.6). It is not known however whether individual cells co-express multiple lectins or if each lectin is confined to a distinct cell population. In Gram negative bacteria (such as *E. coli, K. pneumoniae* and *Salmonellae* spp.) the lectins often are in the form of submicroscopic hair-like appendages, known as fimbriae (or pili), that protrude from the surface of the cells (Fig. 3.8). During the fimbriated phase, a typical Gram negative bacterium carries 200-500 peritrichously arranged fimbriae.

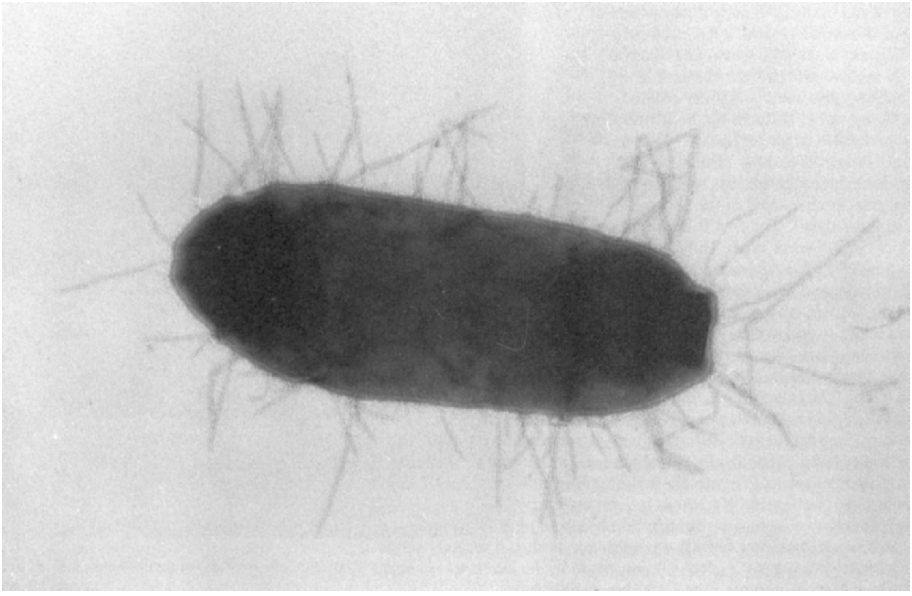

Fig. 3.8 Type I fimbriated *E. coli*. Courtesy Dr. Awni Gbarah, Rehovot.

Fimbrial surface lectins are also produced by Gram positive bacteria, among them the oral *Actinomyces naeslundii* and *Actinomyces viscosus*. Non-fimbrial lectins associated with the bacterial surface have been purified

Table 3.5 Bacterial surface lectins

Organism	*Carbohydrate specificity*	*Form*[a]
Actinomyces naeslundii	Galβ3GalNAc	GP
Campylobacter jejuni[b]	Fucα2Galβ4GlcNAc	GP
Escherichia coli Type 1	Manα3(Manα6)Man	GP
P	Galα4Gal	GSL
S	Neu5Acα2,3Galβ3GalNAc	GSL
CFA/1	Neu5Acα2,8-	GP
K1	GlcNAcβ4GlcNAc	GP
K99	Neu5Acα2,3Galβ4Glc	GSL
Haemophilus influenza	$(Neu5Ac\alpha2,3)_{0,1}$Galβ4GlcNAc-β3Galβ4GlcNAc	GSL
Helicobacter pylori	Neu5Acα2,3Galβ4Glc(NAc); Fucα2Galβ3(Fucα4)Gal	GP
Klebsiella pneumoniae	Man	GP
Mycoplasma pneumoniae	Neu5Acα2,3Galβ4Glc(NAc)	GP
Neisseria gonorrhoeae	Galβ4Glc(NAc)	GSL
Neisseria meningitidis	$(Neu5Ac\alpha2,3)_{0,1}$Galβ4GlcNAc – β3Galβ4GlcNAc	GSL
Salmonella typhimurium	Man	GP
Streptococcus pneumoniae	$(Neu5Ac\alpha2,3)_{0,1}$Galβ4 - GlcNAcβ3Galβ4GlcNAc	GSL
Streptococcus sanguis	Neu5Acα2,3Galβ3GalNAc	GP
Streptococcus suis	Galα4Galβ	GSL

[a]Predominant form of ligand on cells: GP, glycoproteins; GSL, glycosphingolipids. Unless otherwise noted, for references, see Roussel & Lamblin, 1996; Sharon, N. & Ofek, 2000. [b]Ruiz-Palacios et al., 2003

from *Rhizobium lupinii*, and from *Agrobacterium tumefaciens*, also a member of the Rhizobia family. In rare cases the lectins are predominantly intracellular. Two such lectins, PA-IL and PA-IIL, have been isolated from *Pseudomonas aeruginosa* (Gilboa-Garber et al., 1997).

3.2.5.c. Viruses

Viruses contain sugar-specific surface proteins or glycoproteins that act as hemagglutinins and are therefore classified as lectins (Table 3.5) (Sharon & Lis, 1997). Much information is available on the influenza and polyoma

viruses, belonging to the orthomyxoviruses and papoviruses, respectively. Similar lectins that are less well defined are found in myxoviruses, such as those of Newcastle disease, Sendai and rotavirus. Other viral lectins include those of foot-and-mouth disease (Fry et al., 1999), HIV (Haidar et al., 1992)

Table 3.6 Viral lectins

Virus	*Specificity*	*Refs*
Corona viruses		
Bovine	Neu5,9Ac_2	(1)
Herpes viruses		
Herpes simplex	Heparan sulfate	(2)
Myxoviruses		
Orthomyxo		
Influenza A & B (human strains)	Neu5Acα2,6Gal[β4Glc(NAc)]$_{0,1}$	(3)
Influenza A & B (porcine strains)	Neu5Acα2,3/6Gal[β4Glc(NAc)]$_{0,1}$	(3)
Influenza C	Neu5,9Ac_2	(4)
Paramyxo		
Newcastle disease	Neu5Acα2,3Gal[β4Glc(NAc)]$_{0,1}$	(5)
Sendai	Neu5Acα2,8Neu5Ac	(6)
Rotavirus	Neu5Ac[a]	(7)
Papoviruses		
Polyoma	Neu5Acα2,3Gal[β4Glc(NAc)]$_{0,1}$	(8)
	Neu5Acα2,3Galα3(Neu5Acα2,6)-GalNAc	(8)
Picornaviruses		
Foot-and-mouse disease	Heparan sulfate	(9)
Retroviruses		
HIV	Man-OS; heparin; dextran sulfate	(10)

[a]Valid only for some animal strains; (1) Schultze et al., 1996; (2) Spillmann, 2001; (3) Wiley & Skehel, 1987; (4) Rogers, G. N. et al., 198) (5) Lamb & Kolakofsky, 1996; (6) Markwell et al., 1981; (7) Ciarlet & Estes, 1999;; Dormitzer et al., 2002a; (8) Freund et al., 1991; (9) Fry et al., 1999; (10) Haidar et al., 1992; Mbemba et al., 1994; Rider, 1997.

and herpes simplex (Spillmann, 2001).

3.3 ISOLATION AND PURIFICATION

Purified lectins are essential in order to establish their molecular properties and is highly desirable for their many applications. In the past, lectins have been obtained solely from native sources, but they can now be produced also by recombinant techniques (section 3.3.2).

3.3.1 From natural sources

Isolation of a lectin begins commonly with extraction of the tissue or organ in which it is present. This is quite simple in the case of plants, especially their seeds (Fig. 3.9) (Goldstein & Poretz, 1986; Rüdiger, 1993).

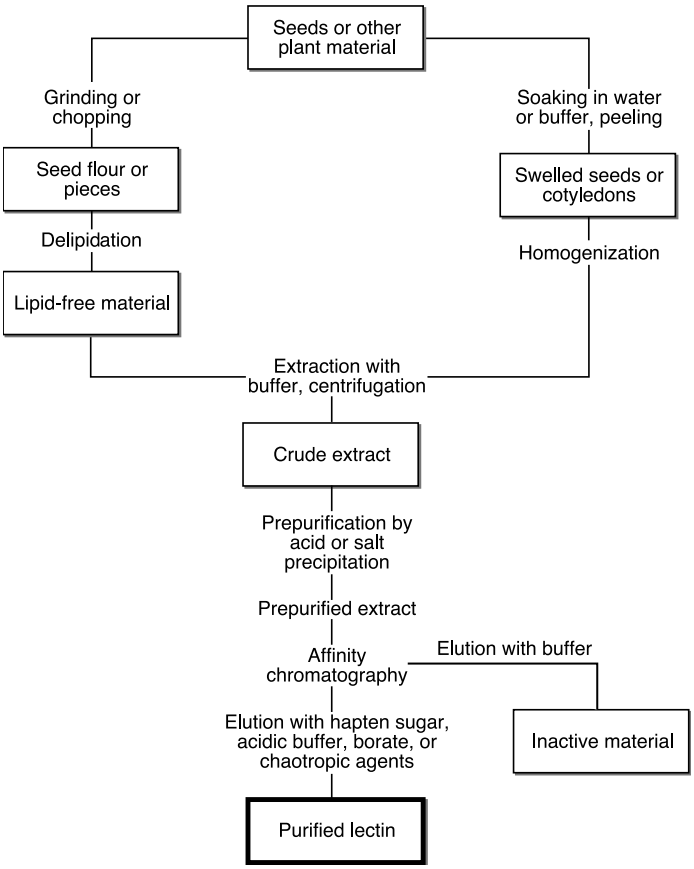

Fig. 3.9 Scheme for lectin purification. reproduced with permission from Rüdiger, 1993; copyright 1993 Springer Verlag..

The seeds are ground and the meal obtained is extracted with a neutral buffer. Often it is advisable to pre-extract the dry meal with an organic

solvent, such as petroleum ether, to remove colored materials derived from the seed coat and lipids that may be present in large amounts. Animal tissues are either homogenized directly in the extraction buffer or the tissue is extracted first with acetone to remove water and lipids. The extraction buffer should preferably contain protease inhibitors to prevent degradation of the lectin during purification, and, in the case of membrane bound lectins, a detergent as well.

Preliminary fractionation of the crude extract (e.g., by ammonium sulfate precipitation) is often done to obtain a protein fraction devoid of other constituents (e.g., polysaccharides in the case of plants). Final purification is achieved by affinity chromatography on a suitable adsorbent (Fig. 3.10).

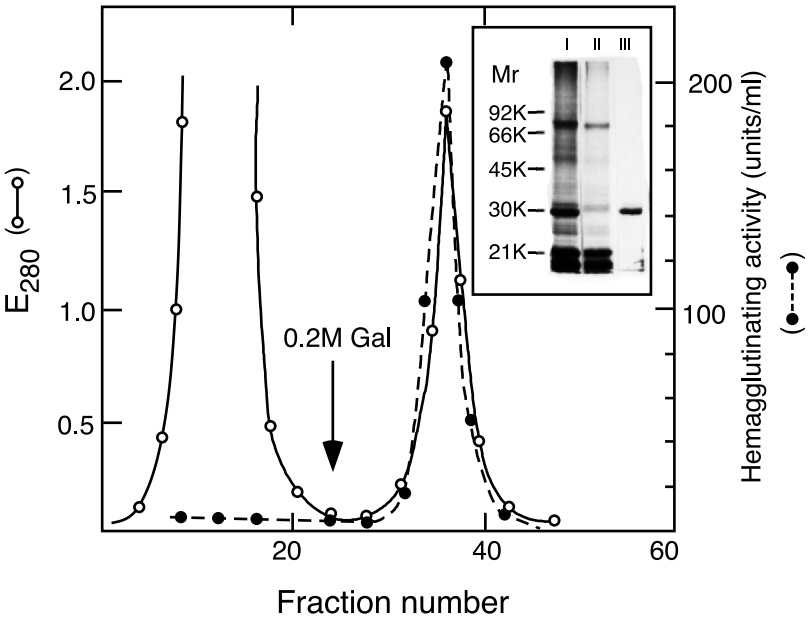

Fig. 3.10 Isolation of *Erythrina corallodendron* lectin from an ammonium sulfate fraction of a seed protein preparation by affinity chromatography on a column of galactose-derivatized Sepharose 4B. The first peak is of inactive protein eluted with phosphate buffered saline, and the second peak is the pure lectin, eluted with galactose. o--o, protein; ●--●, hemagglutinating activity. Inset, analysis by polyacrylamide gel electrophoresis of: I, crude protein preparation applied to column; II, first peak; III, second peak. M, molecular weight markers.

A wide variety of affinity adsorbents, to suit any taste or purse, have been described in the literature and many of them can be purchased ready-made (Table 3.7). These include polysaccharides such as Sephadex, a polymer of glucose employed for the purification of concanavalin A and pea lectin;

agarose (or Sepharose), a polymer of galactose, for the purification of the lectins from castor bean; acid-treated Sepharose for the purification of SBA; and chitin, a polymer of *N*-acetylglucosamine, for the purification of WGA. In the absence of readily available polysaccharides, use can be made of adsorbents consisting of carbohydrates or glycoproteins as such, or in the form of a synthetic derivative, that are covalently attached to an insoluble carrier. For instance, lactose coupled to Sepharose is the reagent of choice for

Table 3.7 Adsorbents for affinity chromatography of lectins[a]

Matrix	*Ligand*	*Specificity of lectin*
Type 1: Polysaccharides[b]		
Chitin	-	GlcNAc
Insolubilized guaran	-	Gal
Sephadexes	-	Glc/Man
Sepharoses	-	Gal
Type 2: Matrix-bound glycoproteins[b]		
Sepharose	Bovine submaxillary mucin	
	Fetuin	
	Hog gastric mucin	
	Ovomucoid	
	Thyroglobulin	
Type 3: Matrix-bound mono- or oligosaccharides[c]		
Sepharose	Carbohydrate derivatives with a free amine	
CH-Sepharose (derivatized with 6-aminohexanoic acid)	Carbohydrate derivatives with a free amine	
Divinylsulfone-activated Sepharose[d]	Any sugar	
Epoxy-activated Sepharose[d]	Any sugar	

Table 3.7 Adsorbents for affinity chromatography of lectins[a]

Matrix	*Ligand*	*Specificity of lectin*

[a]Modified from Lis & Sharon, 1981a. Compilations of methods for the affinity chromatography of lectins are found in *Methods in Enzymology*, volumes 28, 34, 50, 83, 138, 179 and 230; [b]immobilized polysaccharides serve both as supports and as ligands for lectins with suitable specificities; [c]columns of immobilized glycoproteins, preferably desialylated, can be used for the purification of lectins with different sugar specificities, or those that do not interact with simple sugars. In the latter case elution is done with solutions of low or high pH. Desorption of Ca^{2+}-requiring lectins can be achieved by the addition of EDTA; [d]the specificity of the lectins isolated depends on the carbohydrate used as ligand.

the purification of the lectins from peanut, eel electric organ or calf heart muscle. *N*-Acetylglucosamine bound to the same support serves for the purification of potato lectin and WGA, whereas immobilized porcine AH blood type substance is employed for the purification of the blood type A specific DBL and HPA. When working with lectins of an uncommon specificity, adsorbents have to be tailor made, as for example Sepharose-bound asialoglycophorin for the purification of the blood type N-specific

Table 3.8 Immobilized supports used for the affinity purification of PNA

Matrix	*Ligand*
Acrylamide gel	*ε*-Aminocaproyl *N*-glycosylamine of galactose
Aminoethylpolyacrylamide gel	Lactose
Cross-linked arabinogalactan	-
Cross-linked desialylated erythrocyte stroma	-
Divinylsulfone-activated Sephadex	Galactose
Insolubilized guaran	-
Sepharose	Asialofetuin

lectin from *Vicia graminea*. Often, a number of techniques are available for the purification of the same lectin, as illustrated in Table 3.8 for PNA.

3.3.2 By recombinant techniques

An alternative approach for the preparation of lectins has been made possible by the advent of recombinant DNA technology. It is based on the isolation of the cDNA or genomic DNA of the lectin, its insertion into a suitable vector and expression in an appropriate host cell. Isolation of the cDNA requires knowledge of at least part of the primary sequence of the

lectin itself or of a structurally similar one. By this technique, several plant lectins, among them of pea (Stubbs et al., 1986; van Eijsden et al., 1992), *Erythrina corallodendron* (Arango et al., 1993), peanut (Sharma & Surolia, 1994) and *Griffonia simplicifolia* (Zhu et al., 1996) have been expressed in *E. coli*. Expression of plant lectins was also achieved in other systems, e.g. WGA in *Saccharomyces cerevisiae* (Nagahora et al., 1992), PHA and GNA in *Pichia pastoris* (Raemaekers et al., 1999), PNA in insect cells (Kumar et al., 1999) and SBA in monkey cells (Adar et al., 1997); (for a more complete listing of recombinant plant lectins, see Streicher & Sharon, 2003). Examples of lectins from non-plant sources, that have been expressed in *E. coli* include those of the slug *Limax flavus* (Kurachi et al., 1998) and from the mushroom *Marasmius oreades* (Kruger et al., 2002). Recombinant techniques are essential for the preparation of mammalian lectins that occur in tissues or cells in tiny amounts and are obtainable in sufficient quantities only by expression in heterologous cells, primarily fibroblasts. Using such techniques it is now also possible to engineer novel specificities into lectins by directed and random mutations (see 6.3.2) (Yim et al., 2001).