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Review Paper

Lectins, versatile proteins of recognition: a review

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The nomenclature, structure, mode of action and applications of lectins are reviewed. This group of proteinaceous macromolecules has the property of interacting with carbohydrates through binding sites to create complexes. Such complex formation is dependent upon the particular lectin and its specificity for certain carbohydrate structures.

The review deals with the vast array of these known lectins with emphasis on recent literature, and covers such additional aspects as occurrence, purification, specificity, and records the recent advances that have been made in the field since the original discovery of erythrocyte agglutination by plant extracts, and the original extensive work on the original lectin concanavalin A.

INTRODUCTION AND LECTIN DEFINITION

Lectins are ubiquitous natural proteins that hydrophobically bind carbohydrates with characteristic specificities. They have the ability to induce cell agglutination phenomena. Lectins are present in multiple molecular forms and have been mostly obtained in high yield and purity through conventional, affinity or high resolution chromatography. These proteins are of significant use in unravelling biological processes, clinical diagnostic systems and the elucidation of protein and carbohydrate structures.

The promotion of erythrocyte agglutination by plant extracts was first identified by Stillmark in 1888, by searching for toxicity factors in *Ricinus communis* (Barondes, 1988). Ricin, a toxic hemagglutinating protein (RCA), and a protein from *Abrus precatorius*, abrin, with properties similar to ricin, were used by Ehrlich in his immunological studies in the last decade of the 1800s (Sharon & Lis, 1972). Boyd & Shapleigh (1954) called the new group of proteins lectins, from the Latin, *lectus*, to focus on the general property they have of selecting cellular types. Proteins with hemagglutinating activity were referred to as hemagglutinins, phytohemagglutinins or phytoagglutinins (Allen & Brilliantine, 1969). Goldstein *et al.* (1980) defined lectins as proteins or glycoproteins of non-immune origin, interacting with carbohydrates through at least two binding sites, agglutinating vegetable and/or animal cells, and precipitating polysaccharides, glycoproteins or glycolipids. This definition has been debated in relation to the minimal number of carbohydrate binding sites per molecule. Dixon (1981) understood as lectins those proteins with at least one carbohydrate binding site; Barondes (1988) and Sharon and Lis (1990) referred to the existence of additional hydrophobic sites. Hydrophobicity is the main interaction force of lectins with carbohydrates, through the carbohydrate binding sites (Quiocho, 1986), and with proteins, or other substances, through the so-called hydrophobic sites (Roberts & Goldstein, 1983; Kella *et al.*, 1984; Roberts *et al.*, 1986; Barondes, 1988). The cell agglutination effect induced by lectins is represented in Fig. 1.

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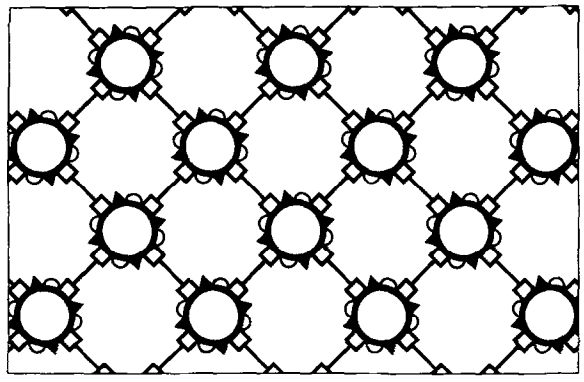


Fig. 1. Schematic representation in 2D of agglutination by lectins. Lectin ⊐—⊏, and its cell surface ligand □, ▲, ▽, carbohydrate or other non-lectin ligands.

NOMENCLATURE OF LECTINS

Lectins have been distinctly designated and some are well known by names originated from the scientific denomination of the species from which they were purified. Thus, the lectin from *Vicia faba* is known as favin (Goldstein & Hayes, 1978; Carrington *et al.*, 1985). Other lectins are known by the common names of the species from which they originated, such as soybean and pea lectins (Lis & Sharon, 1973). Concanavalin A (Con A) a lectin obtained from *Canavalla ensiformis* was named according to a purification protocol (Jones & Johus, 1916; Sumner, 1919). Whenever possible, the designation of lectins should be standardized by using the scientific name of the species from which they were extracted. This nomenclature is used for lectins purified from *Biomphalaria glabrata* (Bretting *et al.*, 1983), *Datura stramonium* (Crowley *et al.*, 1984), *Sarcocystis gigantea* (Macha *et al.*, 1985), *Rana catesbeina* (Nitta *et al.*, 1987), and *Falcata japonica* (Nakajima *et al.*, 1988), among others.

The literature also designates lectins in terms of their monosaccharide specificity e.g. D-galactose-binding hemagglutinin of *Pseudomonas aeruginosa* (Gilboa-Garber *et al.*, 1972); α-D-galactosyl-binding lectin from *Bandeiraea simplicifolia* (Hayes & Goldstein, 1974); D-mannose/D-glucose specific lectin from *Vicia cracca* (Baumann *et al.*, 1982); D-galactose/N-acetyl-D-galactosamine-specific lectin from *Erythrina cristagalli* (Iglesias *et al.*, 1982) and D-mannose-specific lectin from *Galanthus nivalis* (Shibuya *et al.*, 1988). Other authors were also involved in designating the tissue from which the lectin was extracted. For example, Levi & Teichberg (1981) used the designation β-D-galactoside-bending lectin from the electric eel organ of *Electrophorus electricus*; Cammue *et al.* (1985*a*) referred to *N*-acetylgalactosamine-specific lectin from winter aconite (*Eranthis hyemalis*) plant root tubers and Piller *et al.* (1986) described an *N*-acetylgalactosamine-specific lectin from *Salvia sclareae* seeds. A description also used includes the monosaccharide specificity, the tissue from which the lectin is extracted and the species common name, such as: β-D-galactoside-binding lectin from chick embryonic skin (Oda & Kasai, 1983), β-D-galactoside-binding soluble lectin from rat and bovine brain (Caron *et al.*, 1987), and D-galactoside-bending lectin from human brain (Bladier *et al.*, 1989).

Other designations found for these proteins are endogenous lectins, used for vertebrate lectins present in specific cell types (Raz & Lotan, 1981; Hinek *et al.*, 1988; Allen *et al.*, 1991); gel-lectins mentioned by Raz *et al.* (1988), for endogenous lectins from normal and malignant tissues that bind D-galactosides; soluble lectins, for proteins non-integrated with membranes (Hirabayashi *et al.*, 1987; Barondes *et al.*, 1988; Jia & Wang, 1988), but with free movement in intra- and inter-cellular aqueous compartments (Barondes, 1984; Powell & Harrison, 1991).

The concomitant presence of toxicity and hemagglutinating activity in plant extracts has been well documented. Stead *et al.* (1966) furnished evidence that the toxicity and hemagglutinating activity found in extracts from *Phaseolus vulgaris* are promoted by distinct factors which are chromatographically separable; Carlini *et al.* (1988) analysed seed extracts from 16 *Fabaceae* species and observed that 81% of the materials studied exhibited these two biological properties. The data obtained, together with studies of a toxic protein from *Canavalia ensiformis*, canatoxin (Carlini & Guimarães, 1981) suggested the use of the term hemilectin for monovalent toxic proteins with a unique carbohydrate binding site (Carlini *et al.*, 1988).

OCCURRENCE AND SOURCES OF LECTINS

Lectins are widely distributed in nature, being found in micro-organisms (Gilboa-Garber *et al.*, 1972; Yamaguchi *et al.*, 1981; Glick & Garber, 1983; Harrison, 1991; Lu-Lu *et al.*, 1992; Sasmal *et al.*, 1992), animals (Tatsumi *et al.*, 1982; Lerivray *et al.*, 1985; Nitta *et al.*, 1987; Bladier *et al.*, 1989; Ali & Salahuddin, 1989; Aveliana-Adalid *et al.*, 1990; Suzuki *et al.*, 1990; Weis *et al.*, 1991; Menghi *et al.*, 1992) and plants. In the latter the lectins have been purified from leaves (Suzuki *et al.*, 1979; Cammue *et al.*, 1985*b*; Yanagi *et al.*, 1990), fruits (Allen, 1979; Kitagaki *et al.*, 1985; Anantharam *et al.*, 1986; Kaku *et al.*, 1990; Vozarihampe *et al.*, 1992; Xu *et al.*, 1991), roots (Horejsi & Kocourek, 1978; Gade *et al.*, 1981; Kalsi *et al.*, 1992; Yamashita *et al.*, 1992*a*, 1992*b*), tubers (Allen & Neuberger, 1973; Cammue *et al.*, 1985*a*) and, predominantly, from seeds (Frost *et al.*, 1975; Pueppke, 1981; Iglesias *et al.*, 1982; Young *et al.*, 1982; Moreira *et al.*, 1983; Tollefsen & Kornfeld, 1983; Moreira & Cavada, 1984; Richardson *et al.*, 1984; Rougé & Cavada, 1984; Carrington *et al.*, 1985; Cavada *et al.*, 1985; Yarwood *et al.*, 1985; Piller *et al.*, 1986;

Wantyghem *et al.*, 1986; Tabary *et al.*, 1987; Nakajima *et al.*, 1988; Ahmed & Chatterjee, 1989; Matsuda *et al.*, 1989; Rinderie *et al.*, 1989; Cavalcanti & Coelho, 1990; Suvachittanont & Peutpaiboon, 1992). The broad occurrence of lectins in different species, tissues or cells shows the importance of these molecules in nature.

DETECTION OF LECTINS

The presence of lectins is mainly detected through a hemagglutinating assay (Frost *et al.*, 1975; Beyer *et al.*, 1980; Qu *et al.*, 1986; Bladier *et al.*, 1989; Kellens *et al.*, 1989; Ohsawa *et al.*, 1990; Ozeki *et al.*, 1991). In this assay a serial dilution of the lectin is performed before an incubation with human or other animal erythrocytes. Additionally, to increase the sensitivity of the cells to lectin agglutination, an enzymatic (trypsin, papain or neuraminidase) or a chemical (glutaraldehyde or formaldehyde) treatment can be performed (Stead *et al.*, 1966; Sharon & Lis, 1972; Lotan *et al.*, 1973; Frost *et al.*, 1975; Lis & Sharon, 1981*a*; Caron *et al.*, 1987; Castro *et al.*, 1987; Ahmed & Gabius, 1989; Ali & Salahuddin, 1989; Lee *et al.*, 1990; Yanagi *et al.*, 1990; Ozeki *et al.*, 1991). Other methods can also be used to identify lectin activity, such as a photometric assay (Teichberg *et al.*, 1988), precipitation of polysaccharides or glycoproteins (Delmotte & Goldstein, 1980; Shibuya *et al.*, 1989) and affinity electrophoresis (Goldstein & Hayes, 1978; Lis & Sharon, 1981*a*).

SPECIFICITY

Landstein & Raubischek (1908) were the first to discuss the specificity of lectins, and observed that several legume seed extracts promoted different hemagglutination properties when assayed with erythrocytes from distinct animals. In relation to human erythrocytes, some lectins do not interact whereas others show weak agglutination properties or cell specificity. Lectins of the latter type were isolated from *Ulex europaeus*-UEA 1, *Dolichos biflorus*, *Bandeiraea simplicifolia*-BSI-B and BSI-A4 as well as *Plecoglossus altivelis* (Table 1). Specificity inside the Rh factor was a characteristic of *Clerodendro trichotomum* lectin (Bird & Wingham, 1968).

Lectins with specificity to different human blood cell types are more strongly inhibited by saccharides present in the immunodeterminant glycoproteins. For example, *N*-acetyl-D-galactosamine and L-fucose strongly inhibit lectins specific for type A and O erythrocytes, respectively (Sharon & Lis, 1972; Sikdar & Chatterjee, 1990). Lectins non-specific to type A, B or O from the ABO system may exhibit mono and/or oligosaccharide specificity (Horejsi & Kocourek, 1978; Renwrantz & Berliner, 1978; Iglesias *et al.*, 1982; Richardson *et al.*, 1984; Rougé & Cavada, 1984; Cammue *et al.*, 1985*b*, Piller *et al.*, 1986; Vasta & Marchalonis, 1986; Wantyghem *et al.*, 1986; Castro *et al.*, 1987; Tabary *et al.*, 1987; Al-Mahmood *et al.*, 1988; Atta *et al.*, 1989, 1990; Kellens *et al.*, 1989). In the case of a new lectin of undefined carbohydrate specificity, panels of cells and lectins of defined carbohydrate recognition can be used to unravel the nature of the lectin binding site (Cavalcanti *et al.*, 1990).

STRUCTURES OF LECTINS AND THE MECHANISM OF THEIR INTERACTION WITH CARBOHYDRATES

There is great diversity in lectin structural organization. The number of subunits per molecule is variable and the nature of polypeptides can be distinct (RCA I) or similar (wheat germ agglutinin, WGA), as shown in Table 2. Hydrophobic interactions, disulfide bridges and hydrogen bonds can be involved in the subunit association.

The dissimilar polypeptides can have distinct functions. For example, RCA I contains two types of subunits, chains A and B; the enzymatic activity is in subunit A and the carbohydrate binding site is in subunit B (Houston & Doyle, 1982).

Another characteristic of the broadness of lectin structure is the distribution of the carbohydrate binding sites per subunit (Table 2). The amino acid residues of neighbouring promoters can participate in binding site formation (Quiocho, 1986).

The carbohydrate binding sites of lectins recognise and adjust to the ligand carbohydrate through a lock and key type mechanism, involving complex networks of hydrogen bonds (Fig. 2). As discussed by Quiocho (1986), the formation of a carbohydrate–protein complex involves the displacement of water molecules associated with the protein polar groups and around the highly polar sugar, with the establishment of new hydrogen bonds; these latter bonds and Van der Waals contacts are the dominant forces in binding stability.

MULTIPLE MOLECULAR FORMS

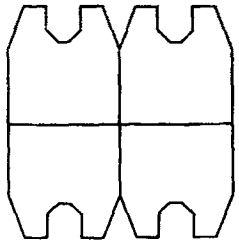
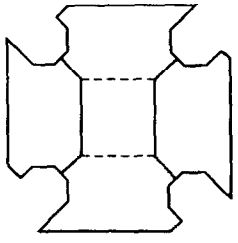
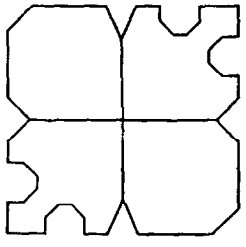
In proteins, multiple molecular forms are a frequent phenomenon. In enzyme proteins they could originate at the level of the genes defining the polypeptide structures or be covalent and non-covalent post-translational modifications. In addition *in vitro* modifications could result in artefactually heterogeneous preparations (Moss, 1982). According to the Biological Nomenclature Commission of IUPAC-IUB (1977), isoenzymes are multiple molecular forms of an enzyme occurring within a single species, resulting from the presence of more than one structural gene. Non-isoenzymic multiple forms of enzymes may also be referred to as 'secondary isoenzymes' (Moss, 1982). Apart from some incorrect designations, evidence has accumulated that the so-

Table 1. Lectin specificities evaluated with carbohydrates (monosaccharides and oligosaccharides) and human erythrocytes

Lectins		Specificities			References
		Monosaccharides	Oligosaccharides	Blood groups	
Seeds					
Amaranthus caudatus		GalNAc	Galβ1,3GalNAcα-O$(CH_2)_3$-CO_2CH_3	Non-specific	Rinderle *et al.* (1989)
Arachis hypogaea		β-D-Gal; Methyl-α-D-Man; D-Fuc; L-Fuc; *a*-D-Gal; α-D-GalNAc	D-Gal(1-3)D-GalNAc	Non-specific	Lotan & Sharon (1978)
Bandeiraea simplicifolla	BS-I	α-D-Gal; α-D-GalNAc	—	B, A	Hayes & Goldstein (1974); Murphy & Goldstein (1977); Wood *et al.* (1979)
	BSI-B4	Methyl-α-D-Gal; α-D-Gal	—	B	Hayes & Goldstein (1974); Murphy & Goldstein (1977); Wood *et al.* (1979)
	BSI-A4	Methyl-α-D-GalNAc; α-D-GalNAc	—	A	Hayes & Goldstein (1974); Murphy & Goldstein (1977); Wood *et al.*, (1979)
	BS-II	α-D-GlcNAc; β-D-GlcNAc	*N*,*N*′-Diacetylchitobiose; *N*,*N*′,*N*″ Triacetylchitotriose	B	Wood *et al.*, (1979); Ebisu & Goldstein (1978)
Canavalia ensiformis		α-D-Man; α-D-Glc; α-D-GlcNAc	Isomaltose, maltose, sucrose	Non-specific	Goldstein & Hayes (1978)
Dolichos biflorus		Methyl-α-D-GalNAc	GalNAc, GalNAc	A_1	Etzler & Kabat (1970); Etzler (1972); Backer *et al.* (1983)
Glycine max		α-D-GalNAc; β-D-GalNAc		Non-specific	Lolan *et al.* (1974); Gordon *et al.* (1972)
Lens culinaris		α-D-Man; α-D-GlcNAc; α-D-Glc	Maltose, isomaltose	Non-specific	Howard *et al.* (1971); Goldstein & Hayes (1978)
Phaseolus vulgaris — E		—	Galβ4GlcNAcβ2Manα6 GlcNAcβ4—Manβ-GlcNAcβ4GlcNac GlcNAcβ2Manα3	Non-specific	Koernfeld *et al.* (1972); Yamashita *et al.* (1983)
Phaseolus vulgaris — L		—	Galβ4GlcNAcβ2 Man Galβ4GlcNAcβ6	Non-specific	Leavitt *et al.* (1977); Hammärstrom *et al.* (1982)
Psophocarpus tetragonolobus		D-Gal	—	Non-specific	Pueppke (1979)
Salvia sclarea		*N*-Acetylgalactosamine	GalNacβ1-4Galβ1-3GalNAc; GalNAcβ1-3Galα1-4Galβ1-4Glc	Non-specific	Piller *et al.* (1986)
Triticum vulgaris		β-D-GlcNac; NeuNAc	β-D-GlcNAc(1-4)β-GlcNAc(1-4)β-D-GlcNAc	Non-specific	Goldstein & Hayes (1978); Allen *et al.* (1973)
Ulex europaeus — UEA-I		α-L-Fuc	L-Fucα2Galβ4GlcNAc; L-Fucα2Galβ3GlcNH$_2$	H	Matsumoto & Osawa (1969); Hindsgaul *et al.* (1982)
Ulex europaeus — UEA-II		GlcNAc	$(D\text{-GlcNAc})_2$	Non-specific	Matsumoto & Osawa (1970)
Root tubers					
Eranthis hyemalis		α-D-GalNAc	Lactose	Non-specific	Cammue *et al.* (1985*a*)
Solanum tuberosum		—	*N*,*N*′,*N*″-Triacetylchitotriose	Non-specific	Goldstein & Hayes (1978)
Marine sponge					
Desmapsama anchorata		D-Gal; D-GalNAc	Raffinose, mellibiose	Non-specific	Atta *et al.* (1990)
Fish					
Plecoglossus altivelis		L-Rha; L-Man; L-Lyx	—	B	Sakakibara *et al.* (1985)
Frog					
Rana catesbeiana		D-Gal	Lactose; thiodigalactoside	Non-specific	Ozeki *et al.* (1991*a*)

Fuc, fucose; Gal, galactose; Glc, glucose; GlcNAc, 2-Acetamido-2-deoxyglucose; GalNAc, 2-Acetamido-2-deoxygalactose; Lyx, lyxose; Man, mannose; NeuNAc, *N*-Acetylneuraminic acid; Rha, rhamnose.

Table 2. Example of subunit structural organization in lectins

Lectin	Diagram of the tertiary/ quaternary structure[a]	References
Glycine max[b] (Soybean)		Deboeck *et al.* (1984) (not cited)
Triticum vulgaris[b] (WGA)		Goldstein & Hayes (1978); Quiocho (1986)
Ricinus communis[c] (RGA I)		Houston & Doyle (1982)

[a]Constructed on the basis of the quoted references. [b]Identical or [c]distinct subunits are represented by polygons without or with one or two carbohydrate binding sites. Dashed lines represent the back view of one subunit.

called isolectins result from the expression of different genes. To evaluate isolectins the amino acid sequence has been established for the genus Lathyrus (Cavada *et al.*, 1985; Yarwood *et al.*, 1985), to *Psophocarpus tetragonolobus* (Kortt, 1985) and to *Phaseolus vulgaris* (Miller *et al.*, 1975). Lectin multiple forms, resulting in characteristically distinct electrophoretic mobilities, have been attributed to minor enzymic modifications of the major hemagglutinin or differences in the carbohydrate side chains of lectins that are glycoproteins. They could also be formed prior to or during isolation, as a result of side chain modifications such as hydrolysis of the amide group of glutamine or asparagine in the proteins (Lis *et al.*, 1966; Entlicher *et al.*, 1971; Howard *et al.*, 1971; Sharon & Lis, 1972; Lis & Sharon, 1981*b*). If the heterogeneity is of genetic origin or has not been defined, the term isoform could be the correct term when different lectin molecular forms occur in the same species (Palva & Coelho, 1992).

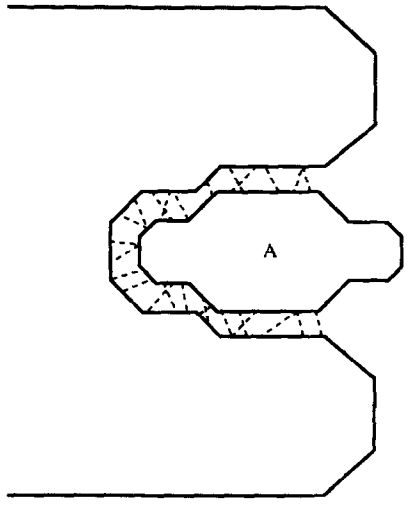


Fig. 2. Schematic representation of the carbohydrate-binding domain in lectins. The cleft usually results from association between protein structural domains. Dashed lines indicate hydrogen bonds among the carbohydrate ligand (A) and the lectin binding site.

LECTIN PURIFICATION

An initial step in purifying lectins from different sources involves preparation of extracts in saline or in a buffer solution. This has been performed with: bacteria (Yamaguchi *et al.*, 1982; Glick & Garber, 1983); yeast (Al-Mahmood *et al.*, 1988); fungae (Eifler & Ziska, 1980; Lin & Chou, 1984; Kochibe & Matta, 1989; Kawagishi & Mizuno, 1988; Kawagishi *et al.*, 1988; Kellens *et al.*, 1989; Kellens & Peumans, 1990); protozoa (Kobiler & Mirelman, 1980); Macha *et al.*, 1985; Tannich *et al.*, 1991); marine sponge (Atta *et al.*, 1989, 1990); snail (Bretting *et al.*, 1983); insect hemolymph (Komano *et al.*, 1980; Castro *et al.*, 1987; Gomes *et al.*, 1991; Kamiya *et al.*, 1992); tunicates (Vasta & Marchalonis, 1986; Suzuki *et al.*, 1990); fish eggs (Sakakibara *et al.*, 1985); lung, liver and intestine of rat, mouse, chicken, sheep, goat, buffalo and human (Beyer *et al.*, 1980; Leffler *et al.*, 1989; Ali & Salahuddin, 1989); porcine heart (Merkle *et al.*, 1989) and human plasma, serum, placenta and brain (Hamazaki, 1986; Taylor & Summerfield, 1987; Ahmed & Gabius, 1989; Bladier *et al.*, 1989). In plants, the extracts have been prepared from tubers (Cammue *et al.*, 1985*a*; Shet & Madaiah, 1988; Hegde *et al.*, 1989); cereals (Shen *et al.*, 1984); fruit (Kitagaki *et al.*, 1985; Anantharam *et al.*, 1986; Mach *et al.*, 1991); bulbs (Shibuya *et al.*, 1988; Kaku *et al.*, 1992); bark (Shibuya *et al.*, 1989; Harada *et al.*, 1990; Kaku *et al.*, 1990; Tazaki & Yoshida, 1992). leaves (Cammue *et al.*, 1985*b*; Yanagi *et al.*, 1990) and seeds (Murphy & Goldstein, 1977; Tollefsen & Kornfeld, 1983; Kortt, 1985; Crowley *et al.*, 1984; Piller *et al.*, 1986; Wantyghem *et al.*, 1986; Maliarik *et al.*, 1987; Nakajima *et al.*, 1988; Rinderle *et al.*, 1989; Ahmed & Chatterjee, 1989). Whenever necessary, a pre-extraction with organic solvents is carried out (Delmotte & Goldstein, 1980;

Tabary *et al.*, 1987; Rinderle *et al.*, 1989). For example Ozeki *et al.* (1991) treated a preparation of sea urchin eggs containing a D-galactoside-specific lectin with cold acetone. The latter would interfere with the subsequent separation of protein on an affinity column since non-specific hydrophobic adsorption of impurities constitutes an impediment to specific binding of the desired protein.

Many extracts containing lectin activity are still partially purified by exhaustive dialysis (Sage & Green, 1972), pH dependent fractionation (Suzuki *et al.*, 1979) or salt fractionation (Gould & Scheineberg, 1970; Delmotte & Goldstein, 1980; Moreira & Oliveira, 1983; Borrebaek & Rougé, 1986; Wantyghem *et al.*, 1986; Tabary *et al.*, 1987; Atta *et al.*, 1989, 1990; Sikdar & Chatterjee, 1990; Yanagi *et al.*, 1990; Ruggiero-Lopes *et al.*, 1992). A protein's solubility in aqueous salt solution is dependent upon the ion type and the salt concentration. Some purification can be achieved by the recovery of fractions precipitated in different salts. Ammonium sulphate is frequently used for salting out proteins since very high ionic strengths can be obtained. This salt can precipitate proteins while stabilizing the native structure of the protein since the hydrophobic forces are strengthened and the tendency of water to expel proteins is increased (Voet & Voet, 1990). Even after a long period of storage, ammonium sulphate precipitation can stabilize the hemagglutinating activity (Montelione *et al.*, 1981).

Some ions, such as I^-, Li^+, Mg^{2+}, Ca^{2+} and Ba^{2+} increase the protein solubility; these ions, also said to be chaotropic, tend to denature proteins because of their abilities to disrupt hydrophobic interactions (Voet & Voet, 1990).

Several conventional techniques contribute to the purification of lectins, based on protein charge or size. In ion exchange chromatography proteins are adsorbed to the matrix, mainly due to ionic interaction with the adsorbent (Peterson & Sober, 1956). Disruption of interactions by pH dependent modifications, or by agents competing with the adsorbents' charged groups (Lis *et al.*, 1966; Entlicher *et al.*, 1970; Moreira & Ainouz, 1981; Genaud *et al.*, 1982; Cammue *et al.*, 1985*b*; Piller *et al.*, 1986; Wantyghem *et al.*, 1986; Nitta *et al.*, 1987; Ohsawa *et al.*, 1990; Yanagi *et al.*, 1990; Benkirane *et al.*, 1992) lead to the release of proteins in distinct fractions, dependent on the binding of each sample component to the matrix. For lectin purification, exchangers such as DEAE–cellulose (Moreira & Alnouz, 1981; Genaud *et al.*, 1982; Tatsumi *et al.*, 1982; Lin & Chou, 1984; Sakakibara *et al.*, 1985; Piller *et al.*, 1986; Wantyghem *et al.*, 1986; Nitta *et al.*, 1987; Rinderle *et al.*, 1989), CM–cellulose (Genaud *et al.*, 1982; Lin & Chou, 1984; Sakakibara *et al.*, 1985; Nitta *et al.*, 1987) CM–Sephadex (Hegde *et al.*, 1989), DEAE–Sephadex (Ohtani *et al.*, 1980), SP–Sephadex (Kortt, 1984; Cammue *et al.*, 1985*b*; Hegde *et al.*, 1989) and DEAE–Sepharose (Kolberg & Sletten, 1982) have been successfully used.

Molecular exclusion chromatography, introduced by Polson (1961), is based on percolation of a protein sample in the pores of inert matrices of controlled porosity such as crosslinked dextran and polyacrylamide, amongst others (Relland, 1971), with subsequent separation of the components by differential elution, according to the molecular size. A small loss of activity can occur due to the adsorption of material on the support, a phenomenon observed by Li & Li (1972). Sephadex (Marchalonis & Edelman, 1968; Nicholson *et al.*, 1974; Pusztal *et al.*, 1981) and Bio-Gel P (Nicolson *et al.*, 1974; Powell, 1980; Pusztal *et al.*, 1981; Moreira *et al.*, 1983) have been used as molecular exclusion matrices in lectin purification.

Affinity chromatography is one of the most powerful techniques for protein purification. It is advantageous since a biomolecule is selected on the basis of its biological function or affinity, with high specificity and recovery. The technique has been used for the purification of receptors (Bischoff & Lodish, 1987; Halberg *et al.*, 1987; Wrenn *et al.*, 1988), enzymes (Cuatrecasas *et al.*, 1968; Hirota & Shimamura, 1985) and other proteins (Castro Novo *et al.*, 1992; Wales *et al.*, 1992), as well as different cell types (Overveld *et al.*, 1988); it represented a landmark in the isolation of lectins (Ohtani *et al.*, 1980; Pusztal *et al.*, 1981; Genaud *et al.*, 1982; Peumans *et al.*, 1982; Lin & Chou, 1984; Sakakibara *et al.*, 1985; Wantyghen *et al.*, 1986; Kellens *et al.*, 1989; Koshte *et al.*, 1990).

The application of affinity chromatography to lectin purification is mainly based on the protein's ability to bind carbohydrates in a specific and reversible way. The lectins have the advantage of not modifying the compounds with which they interact and binding is not so strong that it is irreversible (Lis & Sharon, 1981*b*).

Different affinity matrices for lectins are chosen according to the lectin specificity to carbohydrates, which can be defined through inhibition assays of the hemagglutinating activity, by using simple monosaccharides or complex carbohydrates. In relation to the recognition specificity, lectins have been classified in the following groups: I, mannose/glucose; II galactose/*N*-acetylgalactosamine; III, *N*-acetylglucosamine; IV, L-fucose; V, sialic acid and X, interacting only with oligosaccharides. Members of each group may differ in their affinity to a variety of monosaccharide analogues and derivatives. Many lectin binding sites do not recognize a change in substitution at positions C2 and C3 of the ligand carbohydrate, however, the position of the OH group attached to C4 seems critical (Sharon & Lis, 1990). Lectins specific to glucose/mannose or their derivatives, may use Sephadex with distinct exclusion limits as their matrices. The most commonly used are G-50–G-200 (Agrawal & Goldstein, 1967, 1972; Entlicher *et al.*, 1970; Kolberg & Sletten, 1982; Young *et al.*, 1982; Moreira *et al.*, 1983; Moreira & Cavada, 1984; Rougé & Cavada, 1984; Borrebaek & Rougé, 1986). Con A, a group I

lectin, binds to Sephadex G-50, -75, -100 and 200, but is eluted in the column void volume of Sephadex G-10 and -25. These are the most crosslinked Sephadex, and it appears that the required lectin binding site is unavailable. Sephadex G-50 was the most appropriate for Con A purification: this matrix also gives the highest flow rate. Lectins with a specificity to *N*-acetyl-D-glucosamine and its oligosaccharides can use chitin as an affinity matrix (Bloch & Burger, 1974; Iyer *et al.*, 1976; Kobiler & Mirelman, 1980; Shen *et al.*, 1984; Shibuya *et al.*, 1986; Kochibe & Matta, 1989). Those lectins specific to galactose and its derivatives can be affinity purified using agarose (Nicolson & Blaustein, 1972), guar gum (Shet & Madaiah, 1988), Sepharose (Rosen *et al.*, 1973; Komano *et al.*, 1980; Kawagishi & Mizuno, 1988; Atta *et al.*, 1989; Datta & Basu, 1981; Vasta & Marchalonis, 1986; Qu *et al.*, 1986; Shet & Madaiah, 1989) and acid-treated Sepharose (Bhattacharyya *et al.*, 1981). Several affinity matrices have been prepared by coupling carbohydrates or glycoproteins to different supports (Table 3).

Desorption of affinity matrices has been performed biospecifically, using the competing carbohydrate or non-biospecifically by changing the pH or ionic strength (Table 3). Adsorbed Con A has been eluted from Sephadex with glucose, D-fructose, D-mannose, sucrose and methyl-β-D-glucopyranoside (Agrawal & Goldstein, 1967). The low molecular weight of the inhibitors permits diffusion through the matrix and hence competition with the lectin binding site. The efficiency of the process is due to the fact that the carbohydrates possess the required configuration to compete with the interaction of Con A with the polysaccharide. Water has been used to desorb protein antigens in immunoaffinity chromatography (Bureau & Daussant, 1983) and lectins. A process called affinity-repulsion chromatography was used to purify lectins and Con A isoforms (Teichberg *et al.*, 1988). In this technique the proteins are loaded on the affinity matrix at relatively high ionic strengths and are efficiently eluted by the addition of deionized water. Some affinity-binding proteins were isolated by adsorption to matrices in the presence of calcium and the lectin detachment was performed with EDTA (Hamazaki, 1986; Haltiwanger & Hill, 1986; Taylor & Summerfield, 1987; Umetsu *et al.*, 1991). This synthetic amino acid has a high affinity for metal cations with two or more positive charges. It is necessary to irrigate the matrix with excess EDTA to remove the calcium involved in the lectin–matrix binding, through the formation of a Ca $EDTA^{2-}$ chelate.

Among the high resolution techniques used to obtain pure lectins are also FPLC and HPLC. The surface lectin of pathogenic *Entamoeba histolytica* was isolated by affinity chromatography followed by anion exchange chromatography on a Mono Q HR 5/5 column using the FPLC system (Tannich *et al.*, 1991). HPLC can be utilized for molecular weight estimations of purified lectins (Yanagi *et al.*, 1990), for protein fractionation (Eloumami *et al.*, 1990; Pratt *et al.*, 1990; Suzuki *et al.*, 1990) and for separation of peptide fragments obtained by digestion of purified lectins by endoproteinases (Konami *et al.*, 1991; Kusui *et al.*, 1991).

LECTIN APPLICATIONS

The availability of a great number of lectins with distinct and different carbohydrate specificities has

Table 3. Affinity matrices containing carbohydrates or glycoproteins coupled to different supports

Lectins	Affinity matrices		Eluents	References
	Support	Ligand		
			Biospecific	
Wheatgerm agglutinin (*Triticum vulgaris*)	Sepharose	α-D-GlcNAC	α-D-GlcNAC	Bloch & Burger (1974)
Cultured soybean cells (*Glycine max*)	Sepharose	D-Gal	D-Gal	Malek-Hedayat *et al.* (1987)
Marine sponge (*Desmapsama anchorata*)	Sepharose	Raffinose	Raffinose	Atta *et al.* (1990)
Ayu fish eggs (*Plecoglossus altivelis*)	Sepharose	L-Rha	L-Rha	Sakakibara *et al.* (1985)
Frog eggs (*Rana castebeland*)	Sepharose	Asialofetuin	Lactose	Ozeki *et al.* (1991*a*)
Seeds (*Bandeiraea simplicifolia*)	Bio-Gel	Mellobionate	α-D-GalNAc	Murphy & Goldstein (1977)
Winged-bean seeds (*Psophocarpus tetragonolobus*)	Bio-Gel	Lactosyl-AE	D-Gal	Patanjali *et al.* (1988)
			Non-biospecific	
Potato (*Solanum tuberosum*)	Sepharose	*N*,*N*′*N*″-Triacetylchitotriose	Ammonium hydroxide	Matsumoto *et al.* (1983)
Winter aconite root tubers (*Eranthis hyemails*)	Agarose	Fetuin	Distilled water	Cammue *et al.* (1985*a*)
Seeds (*Amaranthus caudatus*)	Synsorb	Galβ1,3GalNAcα-0-$(CH_2)_8$-CO_2CH_3	Acetic acid, pH 3·0	Rinderle *et al.* (1989)

Gal, galactose; GlcNAc, 2-Acetamido-2-deoxyglucose; Rha, rhamnose; GalNAc, 2-Acetamido-2-deoxygalactose.

resulted in the use of these proteins as tools in medical and biological research. Lectins can be utilized to explore cellular surfaces by binding to the carbohydrate portion of glycoproteins or glycolipids projected from the cell (Cuatrecasas & Tell, 1973; Nicolson, 1974; Rapin & Burger, 1974; Ito *et al.*, 1985; Alroy *et al.*, 1987; Sahter *et al.*, 1990; Rossell *et al.*, 1990; Stern *et al.*, 1990; Clark, 1991; Fontes *et al.*, 1991; Sarkar *et al.*, 1991). These versatile molecules have been used for red blood cell typing (Murphy & Goldstein, 1977; Krajhanze *et al.*, 1978; Sakakibara *et al.*, 1985; Nakajima *et al.*, 1988); as mitogenic agents (Nowell, 1960; Barker & Farnes, 1967; Novogrodsky & Katchalski, 1971; Suzuki *et al.*, 1979; Iglesias *et al.*, 1982; Kolberg & Sletten, 1982; Wantyghem *et al.*, 1986; Atta *et al.*, 1989; Freir & Rudger, 1990; Licastro *et al.*, 1991; Ryder *et al.*, 1992); and to detect alterations during transformation (Ishibashi *et al.*, 1982; Walker, 1985; Ravindranaths *et al.*, 1988). Lectins have been used to characterize distinct stages of development from the trypanosomatides:adult forms from *Schistosomoa mansoni* (MacGregor *et al.*, 1985) and stage-specific differences during *in vitro* conversion from amastigote to promastigote, from *Leishmania donovani* (Wilson & Pearson, 1984). Changes in cell surface saccharides during development of cysts from zoospores of the *Phytophtora cinnamomi* fungus were also detected by lectins (Bacic *et al.*, 1985).

Con A (a lectin specific to α-D-mannosyl and α-D-glucosyl terminal residues), lentil lectin (specific to α-methylmannoside), *Bandeiraea simplicifolia* I-BSI (that interact with α-linked D-galactosyl and *N*-acetyl-D-galactosaminyl terminals), *Helix pomatia* and *Ricinus communis* II-RCA (specific to *N*-acetylgalactosamine), wheatgerm agglutinin (specific to *N*-acetylglucosamine and sialic acid), jacalin (specific to α-galactopyranosides, such as mellibiose and methyl-α-D-galactopyranoside) *Ricinus communis* I-RCA I (that interact with β-D-galactosyl terminal residues), were immobilized on inert matrices and used as affinity matrices to purify glycoproteins (Hock *et al.*, 1980; Blake & Goldstein, 1982; Gioamnini *et al.*, 1982; Tsuji *et al.*, 1983; Torres & Smith, 1988; Vynow *et al.*, 1988; Delanghe *et al.*, 1989; Lejeune *et al.*, 1989; Hortin & Trimpe, 1990; Sarkar *et al.*, 1991). A galactomannan from *Cassia alata* seeds was purified in a single step by using an α-D-galactopyranosyl-binding lectin from *Bandeiraea simplicifolia* coupled to cyanogen bromide-activated Sepharose 4 B (Ross *et al.*, 1976). Affinity high-performance liquid chromatography was utilized for structured analysis of *N*-glycanase-released oligosaccharides using columns of silica-bound Con A, leucoagglutinating phytohemagglutinin, (L-PHA), *Datura stramonium* agglutinin (DSA), *Vicia viliosa* agglutinin (VVA), and *Ricinus communis* I and II (RCA I and RCA II) (Green *et al.*, 1987; Green & Baenziger, 1989).

Lectins with distinct specificities have been conjugated with fluorescein isothiocyanate (FITC) to evaluate the distribution of lectin receptors in postimplantation mouse embryos (Kimber, 1989); for detection of fungi in tissue sections (Karayannopoulou *et al.*, 1988) and for analysis of the surface of the nitrogen-fixing bacterium *Azospirillum brasiliense* (Yagoda-Shagam *et al.*, 1988). Lectins labelled with radioactive isotopes have been used for a comparison of *Leishmania* glycoconjugates (Rossell *et al.*, 1990) and to study the interactions with glycoconjugates of different zymodemes of *Trypsanosoma cruzi* (Stevens *et al.*, 1988). Lectins were also labelled with digoxigenin and used for carbohydrate structure studies of blotted glycoproteins (Haselbeck *et al.*, 1990). A gold-complexed *Aplysia depilans* gonad lectin, specific to galacturonic acid, was used to study the carbohydrate distribution in some pathogenic fungi (Benhamou, 1989). Colloidal gold-labelled lectins specific to *N*-acetyl-D-galactosamine (soybean, *Helix pomatia* and *Dolichos biflorus* agglutinins) and to D-galactose (*Griffonia simplicifolia* lectin), were used for electron microscopic observations of blood cells (Eguchi *et al.*, 1989). Lectins conjugated to peroxidase and specific to L-fucose (*Ulex europaeus* I and *Tetragonolobus lotus* lectin), to galactose (peanut lectin) and to mannose (Con A) were useful in blotting and in histochemistry studies (Ching & Rhodes, 1988; Kijimoto-Ochiai *et al.*, 1989; Ishikawa *et al.*, 1989). Two-dimensional O'Farrell gel electrophoresis, followed by Western blotting, was used for the analysis and identification of plated glycoproteins by treating nitrocellulose blots with avidin–biotin-conjugated lectins of different specificities (Kehrel *et al.*, 1987).

According to Slavin (1987), lectins have been used for the identification of some infections caused by anaerobic bacteria, through the interaction between lectin and cryptantigens, which is essentially hydrophobic. The exposure of red cell cryptantigens may result from the action of microbial enzymes in the bloodstream, such as neuraminidase (T) or an endo-β-galactosidase (Tk), or by incomplete biosynthesis (Tn). Also, lectins have been exploited to characterize and quantify serum glycoproteins, in the control of rheumatoid arthritis, to evaluate the response of treatment to anti-inflammatory drugs and as an important immunosuppressive in bone marrow transplantation. Lectins have been used to explore specific binding sites in the midgut of the mosquitoes. *Anopheles stephensi liston* and *Aedes aegypti* L. (Diptera, Gulicidae) and to differentiate regions in mosquito salivary glands through characteristic bindings (Perrone *et al.*, 1986; Rudin and Hecker, 1989); also, to investigate the effect on *Ricinus communis* invertase (Vattuone *et al.*, 1991).

A lectin of *Phaseolus vulgaris*, leucoagglutinin (PHA-L), was used as an anterograde marker to reveal morphological details in neurons, their axons and terminals (Gerfen & Sawchenko, 1984), to identify neural connections (Thompson & Thompson, 1988) in chemically specified circuits in the central nervous

system (Gerfen and Sawchenko, 1985) and as retrograde and anterograde markers in the central nervous system of the frog (Antal & Pektó, 1990).

Antiserum to lectins has been developed in rabbit and utilized to detect the binding to tissues. It is always preferable to use purified immunoglobulin G (IgG) to whole antiserum. The interaction can be performed with IgG conjugated to peroxidase, or indirectly, by using anti-lectin IgG followed by a step using anti IgG conjugated to peroxidase, to amplify the immunological reaction (Leatham, 1986). Anti-lectin IgG may also be used to evaluate, in a preliminary approach, the homologies among lectins of the same or distinct species (Genaud *et al.*, 1982; Peumans *et al.*, 1982; Wantyghem *et al.*, 1986; Hirabayashi *et al.*, 1987; Tabary *et al.*, 1987).

The amplitude of carbohydrates in cell recognition (Sharon & Lis, 1993) is a demonstration of lectins' actions *in vivo*. Their uses *in vitro* show that these molecules are a central tool in deciphering the saccharide code in biomolecules.

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