Glycoconjugate Journal 18, 589–613, 2001

REVIEW

Plant lectins: Occurrence, biochemistry, functions and applications

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Growing insights into the many roles of glycoconjugates in biorecognition as ligands for lectins indicates a need to compare plant and animal lectins. Furthermore, the popularity of plant lectins as laboratory tools for glycan detection and characterization is an incentive to start this review with a brief introduction to landmarks in the history of lectinology. Based on carbohydrate recognition by lectins, initially described for concanavalin A in 1936, the chemical nature of the ABH-blood group system was unraveled, which was a key factor in introducing the term *lectin* in 1954. How these versatile probes are produced in plants and how they are swiftly and efficiently purified are outlined, and insights into the diversity of plant lectin structures are also given. The current status of understanding their functions calls for dividing them into external activities, such as harmful effects on aggressors, and internal roles, for example in the transport and assembly of appropriate ligands, or in the targeting of enzymatic activities. As stated above, attention is given to intriguing parallels in structural/functional aspects of plant and animal lectins as well as to explaining caveats and concerns regarding their application in crop protection or in tumor therapy by immunomodulation. Integrating the research from these two lectin superfamilies, the concepts are discussed on the role of information-bearing glycan epitopes and functional consequences of lectin binding as translation of the sugar code (*functional glycomics*).

Keywords: **affinity chromatography, agglutinin, glycomics, lectin, plant toxin, protein body**

Abbreviations: **Ara: L-arabinose; ConA: lectin from *Canavalia ensiformis*; ER: endoplasmic reticulum; Gal: D-galactose; GalNAc: N-acetyl-*D*-galactosamine; Glc: D-glucose; GlcNAc: N-acetyl-D-glucosamine; GNA: lectin from *Galanthus nivalis* (snowdrop); Fuc: L-fucose; Man: D-mannose; Neu5Ac: N-acetyl-D-neuraminic acid; PHA: lectin(s) from *Phaseolus vulgaris* (common or French bean); SBA: lectin from *Glycine max* (soybean); SDS-PAGE: polyacrylamide gel electrophoresis in sodium-dodecyl-sulfate-containing buffer; WGA: lectin from *Triticum vulgare* (wheat) germ; UEA: lectin from *Ulex europaeus* (gorse).**

1. Introduction

The ubiquity of glycan chains as integral part of various cell constituents is reason to envision fundamental functional roles for these substances. Starting with the monotonous sequence of cell wall and energy storage polymers chitin, cellulose and glycogen, structural analysis of the carbohydrate part of glycoconjugates has taught us that monosaccharides as building blocks for oligomers are better than other compounds (amino acid or nucleotide) in providing diversity. Calculations of the limits for isomer formation with amino acids and sugars easily dispel clichés about the role of carbohydrates being limited to intermediary metabolism. Given 20 letters to form hexamers, 6.4×10^7 different hexapeptides can be devised. For sugars, however, 1.44×10^{15} linear and branched isomers are possible [1]. Adding a sulfate, phosphate or acyl group, the biochemical equivalent of Umlaut generation, to a trisaccharide increases the isomer quantity by one order of magnitude [1]. Since the oligosaccharides often have only limited conformational flexibility in contrast to peptides, a sugar epitope can present itself in one or a few distinct structural topologies, adding the third dimension to structure changes [2,3].

In addition to the unsurpassed structural versatility, two other arguments further support the concept of the sugar code, i.e. the elaborate machinery of glycan assembly and modification

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as well as the spatial accessibility of carbohydrate determinants especially at the ends of branches [4,5]. If sugars can indeed be thought of as the hardware for information storage, then decoding by receptors is expected to be commonplace. Directional hydrogen bonds and stacking/CH-π interactions are pivotal factors governing the selectivity and specificity of binding of carbohydrates to proteins [6]. Several classes of proteins share this ligand capacity, i.e. enzymes acting on carbohydrate substrates such as glycosyltransferases, glycosidases or sulfotransferases, sugar-specific immunoglobulins, proteins binding free mono- or disaccharides (e.g. transport or chemotaxis proteins), and lectins, their definition being explained in detail in Section 3 [7]. Using this concept of the sugar code, the widespread occurrence and hitherto mapped diversity of lectins indicate ways in which this molecular complementarity can be exploited. With growing insights into lectin structures and functions in plants and animals, it is now becoming possible to compare different protein classes. In addition, owing to the popularity of plant lectins as tools in biochemistry, cell biology and medicine, questions arise on their expression, their natural functions and on simple and inexpensive isolation procedures. Herein we present an overview of these sugar receptors starting with a glimpse of the history of plant lectinology followed by a discussion of lectin occurrence, isolation, biosynthesis, structure, functions and applications.

2. Historical aspects

The search for the toxic principle in castor beans (*Ricinus communis*, Euphorbiaceae) prompted one of the most prominent pharmacologists of his time, Rudolf Kobert (1854–1918), to ask his medical student Hermann Stillmark (1860–1923) to study this plant. In his thesis of 1888 at the University of Dorpat/Tartu (Estonia, then one of the Baltic provinces of the Russian Empire), Stillmark described that extracts from castor beans and four other Euphorbiaceae plants are able to agglutinate blood cells from different animals, i.e. rabbits, horses, dogs and cats. He assumed toxicity and agglutinating capability to originate from the same substance, ricin, which he regarded to be an enzyme (in those days "ferment") [8]. As we now know, castor beans contain a tetrameric protein called *Ricinus communis* agglutinin that is able to agglutinate cells but is hardly toxic. A second, dimeric protein, now called ricin or *Ricinus* toxin, is closely related to the agglutinin, and its enzymatic subunit acts as a highly specific RNA N-glycosidase on 28S rRNA but is only a weak agglutinin (see Section 8.1 on *biological functions*). This observation on agglutinating activity resembles that made by S. Weir Mitchell already in 1860, i.e. the activity of rattlesnake (*Crotalus durissus*) venom on pigeon's blood [9].

Soon after Stillmark's discovery, ricin and the related toxin from jequirity beans (*Abrus precatorius*, Leguminosae) played a fundamental role as model antigens in the pioneering studies of Paul Ehrlich [10]. When passing through ricin's history and of note in the era of heightened awareness of biohazard, its extremely potent toxicity even attracted the attention of the Bulgarian secret service. In 1978, they turned it into a deadly weapon in the umbrella homicide of the exile-Bulgarian Georgi Markov who worked for BBC London, prompting infamous headlines for a lectin [11].

Looking back to the beginning of lectin research, progress in the field was hampered in the first decades by the crudeness of the fractionation techniques. The first agglutinin to be isolated was concanavalin A from Jack bean (*Canavalia ensiformis*) seeds. The prominent American biochemist James B. Sumner, well-known for demonstrating that an enzyme (urease) is nothing but a protein (which earned him the Nobel award in 1946), succeeded in purifying also the agglutinating principle from these seeds by crystallization and called it concanavalin A (ConA) [12]. He discovered that ConA is able to interact with red blood cells as well as with starch, glycogen and mucins, and that this interaction can be prevented by low-molecular-weight carbohydrates such as sucrose [13]. This result was the first clear experimental indication that an agglutinin binds carbohydrates and had significant implications for the study of cell membrane constituents. Systematic screening of plant extracts for agglutinating activities led to a breakthrough in haematology and paved the way for coining the term *lectin* (for a review of this development, please see [14]).

In fact, Landsteiner's discoveries of human isoagglutinins in 1900 in Vienna and the species specificity of plant agglutinins as well as his comparison of haemagglutinating/haemolyzing activities with natural antibodies led W. C. Boyd "to test seeds for blood group specificity" [15]. With extracts of the lima bean (*Phaseolus lunatus limensis*, Leguminosae) he found A-type specificity and proposed "the term 'lectin' (from the Latin *legere*, to choose or to pick out) for these and other antibody-like substances" [16]. Drawing on the competitive inhibition of antibody-antigen reactions by compounds structurally related to the haptenic group described by Landsteiner and van der Scheer [17] and the binding of ConA to sugar compounds documented by Sumner and Howell [13], inhibition of haemagglutination mediated by eel (*Anguilla anguilla*) serum and extracts of *Lotus tetragonolobus* seeds by Fuc and of haemagglutination by other plant lectins by GalNAc provided first insights into the chemical nature of the blood group determinants [18]. From this starting point, the chemical nature of the blood group substances as oligosaccharides could be delineated. For the reaction of red blood cells with blood-group-specific lectins, the terminal α-linked monosaccharides GalNAc (group A), Gal (group B) and Fuc (group 0(H)) are decisive. It goes without saying that these studies were further extended thereafter and were crucial for "unravelling the biochemical basis of blood group ABO and Lewis antigenic specificity" [19]. With this focus on haemagglutination it is no surprise that the initial definition of the term *lectin* [16] placed special emphasis on just this aspect. As apparent from the example of the homologous lectin subunits of the *Ricinus* agglutinin/toxin, however, strict application of the criterion of haemagglutination would separate related proteins.

Thus, haemagglutination or precipitation of glycans which depend on at least bivalency are now considered as being only one example of a broad panel of assays able to detect carbohydrate binding [5]. Moreover, lectins ought to be distinguished from other molecules able to clump erythrocytes together.

3. Definition

To qualify as lectin today, a (glyco)protein must meet three distinct requirements [7,20,21].

3.1. A lectin is a (glyco)protein that binds carbohydrate

By this part of the definition, tannins, certain lipids, cationic substances and also cognate carbohydrates in carbohydrate-carbohydrate interactions that agglutinate cells are excluded. Since a carbohydrate recognition domain can be linked to other functional sections in mosaic-like proteins, as encountered especially in animal C-type lectins [21], it is the presence of this distinct binding activity in a multifunctional protein that justifies to call it a lectin, leaving open the possibility to include the protein in other categories, too.

3.2. Lectins are separated from immunoglobulins

Originally, lectins were regarded as "anti-body-like substances" [16,22]. This term refers to the apparent specificity of binding and was not meant to reflect structural similarity. In fact, the term *lectin* was later delimited from immunoglobulins (Ig) that need an antigenic stimulus to be synthesized. Although carbohydrate-binding activity is also seen in the family of I-type animal lectins from the Ig-superfamily [21], all immunoglobulins are thus excluded [7]. Interestingly, lectins are known that can be induced by an external stimulus distinct from an antigenic challenge. Plant lectin expression may increase as a consequence of stress, e.g. virus infection [23], drought [24] or high salt concentration [25,26].

3.3. Lectins do not biochemically modify the carbohydrates which they bind

This part of the definition excludes glycosyltransferases, glycosidases and enzymes introducing a substituent like a sulfate group into the carbohydrate. This addition is necessary, because it is known that certain glycosidases agglutinate cells at low temperature, if binding to the cell surface carbohydrates proceeds faster than hydrolysis of glycosidic linkages [27]. Because some plant and also animal lectins can furthermore harbor enzymatic activities that are independent of the lectins' carbohydrate-binding sites, it is now indispensable to demonstrate already at an early stage of investigation whether activities can be attributed to the same or more than one center [21,28–31]. Lectins are furthermore separated from sensors for free mono- or disaccharides acting in chemotaxis or in operon systems and from transport proteins [7].

Having herewith given the criteria for defining a lectin, we can proceed to survey the occurrence of plant lectins.

4. Occurrence of lectins

As is the case with ricin, *Ricinus* agglutinin or ConA, the richest source for most lectins are the seeds or, more generally, the storage organs of plants. These are seeds as in most plants studied so far, but also roots (*Urtica, Phytolacca, Sambucus, Trichosanthes, Calystegia*), tubers or bulbs (*Solanum, Galanthus, Scilla, Allium, Crocus, Tulipa, Iris*), bark (*Sambucus, Sophora, Robinia, Maackia, Laburnum, Cytisus, Cladrastis, Hevea, Abies*) or leaves (*Aloe, Lactuca, Vicia unijuga, Viscum album*) can provide a rich lectin harvest.

Within the cells, lectins are primarily found in protein bodies. They abound in proteins synthesized in the endoplasmic reticulum (ER) and transported via the Golgi apparatus and originate by subdividing the vacuole. Viewed from their origin and their role for protein turnover, protein bodies are related to lysosomes. The main content of protein bodies are storage proteins (vicilin, legumin and convicilin in Leguminosae, related proteins in other plants [32]), lectins, hydrolases (glycosidases, phosphatases) and phytin to store phosphate [33,34]. Protein-body-like and lectin-containing cell organelles are also present in non-seed organs such as e.g. bark [35]. In addition to this major intracellular site lectins have also been found in the cytoplasm [36] and in the intercellular space [37]. The amount of lectin can vary markedly from one plant species to the other [38]. To give the reader an idea of lectin quantities found in seeds, we have compiled data based on purification for various plants and present them in Table 1 along with the carbohydrate specificity of each listed lectin. As alternatives to measure lectin quantities reliably, an immunological assay or tests with neoglycoproteins or neoglycoenzymes [39,40] are routine procedures of high sensitivity. These techniques verify that purification yields have reached a satisfying level.

5. Isolation of lectins

Initially, purification of lectins followed the scheme used for proteins in general without exploiting their special characteristics. The methods used included precipitation by salts, acids and organic solvents. Of course, the preparations thus obtained were far from pure, and results obtained with them have to be interpreted with caution. An exception with celebrity status was Sumner's purification of the abundant ConA by crystallization, as pointed out above [12]. Progress in this area was achieved by the introduction of preparative chromatographic methods with ion exchangers, gel filtration (size exclusion chromatography) media and, above all, affinity adsorbents. Originally developed for isolating enzymes by means of immobilized inhibitors, the principal strategy was transferred to lectinology, and cross-linked dextrans found versatile application in one-step purification schemes for Glc-binding plant lectins [41]. Agarose, a

Table 1. Carbohydrate specificity and content of lectin in selected plants (from [38], modified and extended)

	Carbohydrate specificity		
Plant species	*Monosaccharides*	*Glycans ratio of inhibitory potency compared with the monosaccharide (italics)*	*mg lectin/100 g seeds*
Canavalia ensiformis	Man/Glc	GlcNAcβ2Manα6(GlcNAcβ2Manα3)-Manβ4GlcNAc *4200*	2100
Ricinus communis (Euphorbiaceae)	Gal	Galβ4GlcNAcβ2Manα6(Galβ4-GlcNAcβ2Manα3)Manβ4GlcNAc *50*	1400
Vicia cracca I	Man/Glc	Unknown	1400
Phaseolus vulgaris	No binding monosaccharide known	Galβ4GlcNAcβ2Manα6(GlcNAcβ2-Manα3)(GlcNAcβ4)Manβ4GlcNAc	1200
Griffonia simplicifolia I	Gal/GalNAc	GalNAcα3GalNAcβ3Galα4Galβ4Glc *15 (isolectin A-4)*	700
Griffonia simplicifolia II	GlcNAc	GlcNAcβ4GlcNAc *3.4*	300
Glycine max	GalNAc/Gal	No oligosaccharide known better than GalNAc	300
Robinia pseudoacacia	No binding monosaccharide known	Complex-type N glycan	300
Arachis hypogaea	Gal	Galβ3GalNAc *55*	190
Sophora japonica	GalNAc	GalNAcβ6Gal *16*	170
Phaseolus lunatus	GalNAc	GalNAcα3(Fucα2)Galβ-R *43*	170
Wisteria floribunda/sinensis	GalNAc	GalNAcα6GalNAc *8.8*	160
Vicia cracca II	GalNAc	Unknown	150
Pisum sativum	Man/Glc	Neu5Acα6Galβ4GlcNAcβ2Manα6-(Neu5Acα6Galβ4GlcNAcβ2Manα3)-Manβ4GlcNAcβ4(Fucα6)GlcNAcβAsn *780*	140
Phytolacca americana (Phytolaccaceae) roots	GlcNAc	Galβ4GlcNAcβ6Gal *no ratio available*	125
Dolichos biflorus	GalNAc	GalNAcα3GalNAcα3Galβ4Galβ4Glc *62*	110
Cytisus scoparius	GalNAc	Unknown	82
Euonymus europaeus (Celastraceae)	No interacting monosaccharide known	Galα3(Fucα2)Galβ3/4GlcNAc	75
Lotus tetragonolobus	Fuc	Fucα6GlcNAc *6.5*	65
Lens culinaris	Man/Glc	Neu5Acα6Galβ4GlcNAcβ2Manα6-(Neu5Acα6Galβ4GlcNAcβ2Manα3)-Manβ4GlcNAcβ4(Fucα6)GlcNAcβAsn *6300*	60
Laburnum alpinum	GlcNAc (low affinity)	GlcNAcβ4GlcNAc *no ratio available*	55
Triticum vulgare (Gramineae)	GlcNAc (low affinity)	GlcNAcβ4GlcNAcβ4GlcNAcβ4-GlcNAcβ4GlcNAc *48*	45
Caragana arborescens	GalNAc	GalNAcα3GalNAcβ3Galα4Galβ4Glc *9.5*	35

(*Continued on next page*)

Table 1. (*Continued*).

	Carbohydrate specificity		
Plant species	*Monosaccharides*	*Glycans ratio of inhibitory potency compared with the monosaccharide (italics)*	*mg lectin/100 g seeds*
Vicia faba	Man/Glc	Neu5Acα6Galβ4GlcNAcβ2Manα6-(Neu5Acα6Galβ4GlcNAcβ2Manα3)-Manβ4GlcNAcβ4(Fucα6)GlcNAcβAsn *780*	30
Bauhinia purpurea	GalNAc	Galβ3GalNAc *611*	28
Maclura pomifera (Moraceae)	GalNAc	Galβ3GalNAc *24*	24
Ulex europaeus I	Fuc	Fucα6GlcNAc *4*	16
Ulex europaeus II	GlcNAc (low affinity)	GlcNAcβ4GlcNAcβ4GlcNAcβ4GlcNAc *2.7 (compared with GlcNAcβ4GlcNAc)*	9

Unless indicated otherwise, all plants belong to the family of Leguminosae, and the source of the lectins were the seeds.

commercially available natural polysaccharide containing Gal, was used as an affinity adsorbent for Gal-binding lectins such as those from *Ricinus communis*, *Bauhinia purpurea*, *Glycine max* and *Wisteria floribunda*, and chitin, a polymer of GlcNAc, for the purification of the lectin II from *Griffonia* (formerly *Bandeiraea*) *simplicifolia* [42]. The tacit assumption that the exploited resin has no ligand activity of its own must thus be reconsidered. When used in gel filtration without haptenic sugar in the running buffer, a lectin even if its affinity to the matrix is low will be retarded or even bound, producing erroneous data on its molecular mass.

Since the range of naturally occurring polysaccharides is restricted and does not cover the broad range of carbohydrate specificities of all lectins, further development of methods for immobilizing glycosides, glycopeptides or glycoproteins to any type of resin followed the use of the unmodified matrix. For this purpose, cyanogen bromide or divinyl sulfone activation among various methods have been and are being widely used with high yields of the product [43]. By selecting suitable glycoproteins as ligands, it has even become feasible to devise an affinity matrix presenting a panel of carbohydrate epitopes as potential ligands. Such a matrix can prove its value when screening extracts for lectin activity in one binding step followed by washing and differential elution. For this purpose, gastric mucin or ovomucoid were successfully tested as ligands [38]. Since the reproducibility of purification yields critically depends on the density of the incorporated ligands, it is essential to determine the amount that had been bound to a matrix. If the ligand absorbs UV light as is the case with glycoproteins, the optical density can readily be measured, background turbidity of the matrix to be compensated for by running derivative spectra [44].

For eluting a lectin, the obvious method is to exploit the haptenic sugar as competitive inhibitor following the principle of dissociating antibody-antigen complexes by the soluble hapten group [17]. As far as monosaccharides are concerned this is generally no problem. Certain lectins, however, bind to oligosaccharides which are not readily accessible or are very expensive. In such cases lectin elution can be performed by lowering the pH of the buffer with immediate neutralization of the eluant. When run as a gradient, it has even been possible to fractionate isolectin mixtures [45]. Since not all lectins survive exposure to low pH values, desorption with a borate-containing buffer can represent a reasonable and affordable alternative. Borate competes with the lectins' carbohydrate-binding sites for immobilized carbohydrate by virtue of its affinity to vicinal hydroxyl groups. Similar to changing the pH value of the buffer, this procedure also allows to purify isolectins. When the eluant is applied as a gradient, the five isolectins L_4, L_3E, L_2E_2, LE_3 and E_4 of the common bean (*Phaseolus vulgaris*) were separated [46]. Furthermore, subsequent application of Gal and borate was helpful to resolve from *Griffonia simplicifolia* seed extracts the Gal/GalNAc- and GlcNAc-binding species in one run [38]. After the detection of the lectin by haemagglutination or neoglycoconjugate binding and its isolation further studies can then focus on lectin synthesis and processing, structural aspects and functions.

6. Lectin biosynthesis

6.1. General

The biosynthesis of most plant lectins studied so far proceeds via the secretory pathway [34]. In brief, the lectin is synthesized at ribosomes attached to the endoplasmic reticulum (ER), enters the lumen of the ER, and is further transported through the Golgi apparatus. In contrast to secretory proteins in a narrow

sense which move to the cell membrane by vesicular transport and then leave the cell by exocytosis, lectins (and other proteins as typical storage proteins) end up in the vacuole. During development of the resting seeds this organelle finally divides into smaller parts called storage vacuoles or protein bodies. This routing is disturbed by the presence of monensin, an ionophore. It intercalates into membranes and allows flow of monovalent cations including protons, thereby interfering with the intracellular traffic. Monensin effectively impairs the separation of transport routes to the outer cell membrane and to the vacuolar membrane, and in turn a lectin will accumulate extracellularly under these conditions [47].

6.2. Processing

On the way from the site of synthesis to its final destination, a lectin is subject to a series of covalent modifications that are common for proteins on this route: the N-terminal signal sequence of about 20 to 30 amino acid responsible for initiating transmembrane passage is split off cotranslationally as soon as a sizeable segment of the newly synthesized protein has entered the lumen of the ER. A further reaction occurring cotranslationally in many lectins is N-glycosylation (O-glycosylation is found in Solanaceae lectins, please see Section 6.4). Consensus sequences (Asn-X-Ser/Thr) for N-glycosylation serve as targets for transfer of membrane-bound dolichol-linked oligosaccharides ($Glc_3Man_9GlcNAc_2$). They are trimmed in the ER and can be reglucosylated with one Glc residue as part of the quality control system to ensure proper folding [48,49]. The $Glc_1Man_{7\text{-}9}GlcNAc_2$-binding ER-proteins calnexin and calreticulin prove in this process that lectins can be efficient molecular chaperones. Besides removing N-terminal signals and trimming N-glycans, the protein part can undergo rearrangements and deletions (please see Sections 6.3.1.3 and 6.3.2). Thus even lectins that are not decorated by carbohydrate in their mature state can originate from glycosylated precursors. Modification reactions continue to take place up to the arrival in the protein bodies where further proteolysis and in a few cases transpeptidations are carried out.

6.3. Legume lectins

In this respect, legume lectins and their biosynthesis have been studied thoroughly. This family of lectins sharing extended sequence homology displays an unusual interspecies variability of sugar target selection (see Table 1). Typically, specificities for Glc, GlcNAc, Man, but also for Gal, GalNAc, Fuc and complex-type oligosaccharides have been detected. Ascribing the capacity to bind sialic acids, a family of monosaccharides not synthesized by plants [50], to certain lectins should be viewed with caution. So-called sialic-acid-binding plant lectins actually bind Gal or lactose but do not interact with free Neu5Ac [51]. Their affinity to Gal or lactose, however, is noticeably enhanced if an acidic group is in close vicinity. Linking a sialic acid or sulfate moiety to the Gal unit which occupies the primary binding site provides instructive examples for the supplementary role of the negatively charged sugar group [52,53]. A notable exception is wheat germ agglutinin and its binding to both GlcNAc and Neu5Ac. Evidently, this sialic acid satisfies the stereochemical requirement determining selectivity for GlcNAc, i.e. presence of an equatorial N-acetyl group and an adjacent equatorial hydroxyl group [54]. Based on their quaternary structure, legume lectins are traditionally subdivided into two categories. One group consists of lectins with identical or nearly identical subunits, while the other category is characterized by different subunit types. A small subgroup of the first category is subject to the previously mentioned posttranslational transpeptidations. Though there are borderline cases which question this subdivision, it is maintained in the present article for practical reasons.

6.3.1. Single-chain lectins

6.3.1.1. Phaseolus lectins. A well-studied example of the first group is given by the lectin fraction from the common or French bean, *Phaseolus vulgaris*, its purification and isolectin resolution referred to above [46]. Infamous as toxic ingredients of insufficiently cooked beans, this lectin fraction can cause maladsorption and irritations in the digestive tract [55]. Another factor contributing to its popularity in research was the abundance and ease of isolation (ranking it on position four in Table 1) giving it the status of a role model. Thus, it has been dubbed "phytohaemagglutinin" (PHA), a name that strictly speaking applies to plant lectins in general [56]. PHA consists of two types of subunits. They are products of tandemly linked genes with 82% sequence identity on the amino acid level. Being frequently designated as E- and L-subunits reflects their preferential binding to erythrocytes and leukocytes, respectively. They are synthesized concomitantly in the ER and assemble to form tetramers at random, thus giving rise to the known series of the five isolectins mentioned above. As likewise noted, the release of the N-terminal signal sequence is common to PHA as for other proteins of the secretory pathway. Even at the C-terminus, proteolysis may occur but in a comparatively less precise manner. This leads to the so-called "ragged ends" which have been found not only in PHA-E but also in the lectins from the Leguminosae *Erythrina corallodendron*, *Glycine max*, *Arachis hypogaea* and *Dolichos biflorus* and in lectins from other plant families, e.g. the Gal-binding agglutinin/toxin from *Viscum album*. Recombinant lectins, too, have ragged ends but these can differ from those occurring under natural conditions. The biological meaning of this type of modification, if not considered to be due to degradative steps after homogenization, is unclear.

Both PHA subunits contain the characteristic N-glycosylation sequons [48], subunit E at Asn_{12}, Asn_{60} and Asn_{80}, subunit L only at Asn_{12} and Asn_{60}. In the mature proteins, only the first two sites are actually glycosylated. The glycan at Asn_{12} belongs to the high-Man type, that at Asn_{60} to the complex type containing xylose and Fuc in the stem

region [57]. High-Man type glycans near the N-terminus are uncommon among animal glycoproteins [51]. To elucidate whether presence of N-glycans affects further transport, lectin biosynthesis has been performed in the presence of tunicamycin, an inhibitor of the first step in N-glycosylation. The lectins were synthesized in a non-glycosylated form in the presence of the inhibitor but were processed and deposited normally [58]. This is in contrast to the conditions in the Lima bean, *Phaseolus lunatus limensis*, where, though belonging to the same genus, the lectin precursor is completely dependent on its glycans for successful maturation. Inhibition of N-glycosylation by tunicamycin prevents the lectin from assembling correctly. Instead, it is retained in the ER and forms mixed aggregates together with phaseolin, a vicilin-type storage protein which normally is also glycosylated, and the chaperone BiP. Notably, processing and transport of another storage protein, legumin, which is not glycosylated, is not affected by tunicamycin [59].

6.3.1.2. Glycine max (soybean) lectin (SBA). To illustrate implications of glycan presence beyond folding and routing, the delineation of a different function for glycans of the soybean lectin is instructive. The Gal/GalNAc-binding lectin from *Glycine max* (soybean agglutinin, SBA) is a glycoprotein with a high-Man type glycan [60]. Apparently, the glycan moiety is necessary for keeping the subunits together: SBA denatured by guanidinium chloride recombines after dilution. It fails, however, to do so after removal of the glycan or in the presence of the competing high-mannose glycopeptide $Man_9GlcNAc_2Asn$ [61,62]. Using various further methods, the authors demonstrated conclusively that the non-reducing ends of the SBA-bound glycans are buried between the subunits whereas the core regions are exposed [63]. Thus, SBA combines two carbohydrate-binding sites in a single molecule: one specific for high-Man-type oligosaccharides and directed towards its own core oligosaccharide, the other one specific for Gal and GalNAc, exposed on the surface and directed towards external carbohydrates. A report describing the recombinant expression in *Escherichia coli* of N-glycan-free but nevertheless active SBA [64] cannot be interpreted unambiguously. As pointed out by Masaoka et al. [63], the small amount of SBA renatured from the inclusion bodies (0.2 to 0.4%) may have been stabilized by the inductor isopropyl-β-D-thiogalactopyranoside similarly to the known effect of GalNAc on chemically deglycosylated SBA.

6.3.1.3. Concanavalin A (ConA). Concanavalin A (ConA), the lectin from the Jack bean which had initially been purified in 1919 by crystallization [12], is one of the most abundant lectins known (Table 1). Analysis of its synthesis and processing was rendered comparatively unproblematic by this strong expression. When its amino acid sequence was compared with that of other legume lectins, a high degree of homology was found. Surprisingly, alignments to achieve optimal homology arranged the polypeptide chains in such a way that the N- and C-termini of ConA face certain residues in the middle of the chains of other lectins and vice versa [65]. The reason for this seemingly strange phenomenon called *circular permutation* remained unclear for several years. In 1985, it was found that the ConA precursor displays the "normal" sequence, and that the circular permutation is due to an up to that time unprecedented posttranslational event in plants, in which the peptide chain is split at one site and annealed at another one [66]. Though known from the biosynthesis of bacterial murein, transpeptidations had never been observed in higher organisms until then. Even up to now the detection of similar events has remained exceptional in eukaryotes [67], although they are quite common among bacteria [68]. *Canavalia ensiformis* belongs to the Diocleinae subtribe of the Leguminosae. Presumably, the lectins from other members of this group (*Canavalia maritima*, *C. gladiata*, *Dioclea grandiflora*, *D. lehmanni*, *D. guianensis*, *D. floribunda*) with amino acids sequences similar to ConA undergo a similar processing step [69].

In addition to this rearrangement of peptide stretches, ConA processing involves an intriguing aspect with respect to glycosylation, as indicated at the end of Section 6.2. Mature ConA is not glycosylated. Its precursor pro-ConA, however, contains a high-Man-type glycan but does not bind carbohydrate. The glycan is localized on a segment of 15 amino acid residues which is lost during maturation [70]. A further peptide segment is concomitantly deleted and the new peptide bond closed, completing a process that takes place in the developing protein bodies [71]. Presumably, this process (like protein splicing) does not require significant refolding because the residues participating are in close proximity already on the level of the precursor. It is very likely that transpeptidation is an enzymatically catalyzed process. If the cDNA coding for pre-pro-ConA is expressed in *Escherichia coli*, the product pre-pro-ConA is not processed at all [72]. This result intimates that not yet known factors in the plant are responsible for ligation. A more direct hint to mechanistic details comes from experiments in which pro-ConA isolated from immature *Canavalia* seeds was subjected to the action of either an extract of immature cotyledons or a commercial asparaginyl endopeptidase [73]. In each case, the product was mature ConA with its full-length chain of 30 kDa together with the so-called fragments of 16.2 and 14.2 kDa which actually arise from incomplete processing. Asparagine-specific vacuolar processing enzymes are not uncommon in the plant kingdom [74].

The fact that the biosynthesis of ConA is severely distorted by tunicamycin underscores that transient presence of the glycan on the level of the precursor is not without functional implications. In the presence of this inhibitor, most of the unglycosylated pro-ConA is retained in the ER, and the small fraction that is transported is processed very slowly. In contrast, the storage protein canavalin which is unglycosylated under normal circumstances is transported in the common way even in the presence of tunicamycin [71]. The differences in the influence

of tunicamycin on processing and routing of PHA, Lima bean lectin and ConA reveal that it is hardly possible to draw general conclusions from a limited number of investigations, and that each case has to be studied separately.

6.3.2. *Two-chain lectins*

As already alluded to above, legume lectins, in particular those from the subtribe Vicieae, can be composed of two non-homologous types of subunit and therefore are called two-chain lectins, in contrast to the single-chain lectins as PHA, ConA or SBA. Most lectins from the various *Vicia* species, from *Pisum*, *Lens* and *Lathyrus* belong to this group. Following the molecular size order in SDS-PAGE, the smaller one is designated as α-, the larger one as β-subunit. As seen for other lectins, biosynthesis starts with a signal sequence which is not part of the mature protein, then the β- and finally the α-subunits follow. This order of sequence has been substantiated in several cases and is backed up by the occurrence of small amounts of a β/α-precursor in pea lectin preparations and by a comparison between the amino acid sequences of two-chain- and single-chain lectins (for a review see [75]). The final proteolytic cleavage of the chains occurs in the protein bodies, together with C-terminal processing which can produce isoforms [76]. The strict distinction between single-chain and two-chain lectins is more or less fortuitous. The genus *Lathyrus* belongs to the Vicieae tribe, and monitored plant species predominantly synthesize two-chain lectins with the exception of *Lathyrus nissolia* which produces a single-chain lectin. If the *L. nissolia* lectin is incubated with a lectin-free extract of immature *L. ochrus* seeds at an acidic pH as it prevails in developing protein bodies, the *L. nissolia* lectin is split into two dissimilar subunits as is common among other Vicieae lectins [77]. Apparently, it is not the lectin *per se* which determines to which group it belongs but rather the presence or absence of an appropriate endopeptidase. Since the β-subunits of two-chain lectins bear asparagine residues at their C-termini, such an enzyme is likely an asparaginyl endopeptidase [74]. The second position of the α-chain's N-terminus is threonine. It thus appears that the endopeptidase cleaves the protein right in the middle of a potential N-glycosylation site. It is neither known whether this site has been transiently glycosylated nor whether a glycan if transferred at this position assists in cleavage. It is interesting to note that the site in ConA where transpeptidation occurs is Asn_{118}-Ser_{119}-Thr_{120}, a potential glycosylation sequon [73]. Taking a look at those positions which correspond to the cleavage sites of two-chain lectins, substitutions to residues other than asparagine modify this site in most one-chain lectins such as SBA or PHA, another argument in favor of the assumed endopeptidase specificity.

6.4. Solanaceae lectins

While biochemical properties and carbohydrate binding of Solanaceae lectins are well investigated, their biosynthetic route has not been studied in detail. These lectins form a group by themselves and are not related to legume lectins. All of them bind GlcNAc and its oligomers. The best studied member of this group is the lectin from potato tubers. Its biosynthesis starts very early during tuber development, and the lectin is abundantly present in the epidermis [78]. It is a glycoprotein, but in contrast to the well-studied legume lectins which if they are glycoproteins bear N-linked glycans, the highly glycosylated (52.3%) potato lectin contains O-glycans linked to hydroxyproline (Ara) and serine (Gal) residues [79]. This is a very uncommon structure among plant lectins, whereas it is quite frequently encountered in typical plant cell wall proteins [80]. The observation that potato lectin was detected, at least in part, also in the cell wall intimated to count this lectin to the family of cell wall glycoproteins [81]. More recently, however, the lectin was reported to reside in the cytoplasm although in close proximity to the inner wall surface. Upon wounding the tuber, the synthesis of a new chitin-binding protein is induced which despite being related to the lectin is the product of a different gene [82]. As a *bona fide* carbohydrate-binding protein, it is justified to call this new protein a lectin, too. One might note that its expression enhanced by a lesion is slightly evocative of immune reactions. The upregulation of lectin expression after virus infection has already been cited earlier [23].

6.5. Euphorbiaceae lectins

Of the Euphorbiaceae lectins, the toxic ricin is studied most intensively [83]. Similar to other plant lectins, pre-pro-ricin's sequence starts with a 24-residues' N-terminal targeting signal followed by the toxic A-chain, then a peptide of 12 residues which does not appear in the mature lectin follows linking the Gal-binding B-chain with the A-chain [84]. The precursor of the *Ricinus* agglutinin has an almost identical sequence [85] with only a few amino acid exchanges. Nevertheless, these will account for the already given differences of both proteins in subunit aggregation as di- or tetramers and in biological activities [83]. Biosynthesis starts at the ER, the precursors are glycosylated and transported to the protein bodies where they are cleaved to yield the subunits [86,87]. As documented recently, the presence of the linker peptide is essential for correct targeting to the vacuole and the protein bodies [88]. Apparently, N-glycosylation is not necessary, because presence of tunicamycin does not impair the transport of the unglycosylated proteins [89]. This is similar to the events in the processing of the *Phaseolus vulgaris* lectins [58]. A pertinent question concerns the way how the plant protects itself against the toxic principle. Ricin is a potent toxin not only to animals but, albeit to a lesser extent, also to plants including castor beans if tested in cell-free systems or if applied exogenously [90]. Since mature and active ricin is generated exclusively in the protein bodies and thus does not show up in the cytoplasm, this strict compartmentalization apparently prevents the *Ricinus* toxin from being a *horror autotoxicus*.

6.6. Lectins from monocot plants

From the monocot plants, special emphasis has been placed on cereals, in particular on wheat. The lectin from wheat, usually designated as wheat germ agglutinin (WGA), is a rather small and heat-resistant protein established by hevein domains (please see Section 7.2). It interacts with GlcNAc (and also Neu5Ac, see in Section 6.3), preferentially with its $\beta 1 \rightarrow 4$-linked oligomers, the constituents of the polysaccharide chitin. By the way, binding to the natural product chitin offers a clue for defining natural target molecules (please see Section 8.1.2 on *biological functions*). WGA is synthesized in the ER as a glycosylated precursor of 23 kDa, in the presence of tunicamycin of only 20 kDa. Upon maturation, the glycan is lost together with a C-terminal segment [91] and subunits of 18 kDa result which assemble to a 36 kDa dimer. Structural aspects of this lectin will be continued to be discussed in the Section 7.2 on *lectin structure*. The lectin is present in the embryo where it is deposited in storage vacuoles preferentially at the periphery [92], whereas typical storage organs as the endosperm, scutellum and aleuron layer are devoid of it. On germination, *de novo* synthesis starts in the roots where it is localized at the tip. The synthesis of WGA, as already mentioned in Section 3, is enhanced under stress conditions as drought [24] or osmotic strain [25,26]. Rice (*Oryza sativa*), a further important cereal, contains a similar lectin. While still in the ER, it displays a M_r of 23 kDa in SDS-PAGE but is then processed to first yield an 18 kDa chain which finally is split into 8 kDa and 10 kDa subunits forming the mature lectin [93]. In rice, a chitin-binding endosperm-specific lectin which is similar to but not identical with the embryo-specific lectin has also been found [94,95].

In the search for alternatives to chemical pesticides in pest control, the insecticidal action of the snowdrop (*Galanthus nivalis*) bulb lectin GNA [96] has prompted numerous studies on its properties, biosynthesis and application. At the monosaccharide level, this lectin binds Man but in contrast to Man-binding legume lectins such as ConA it does not accommodate Glc into its carbohydrate-binding site. Binding to microvilli in the tomato moth larvae is instrumental for the transport into cells of the gut and malphigian tubules [97]. The mature lectin consists of 13 kDa subunits which combine to tetramers [98]. Its biosynthesis was followed in detail in developing ovaries. Comparable to many other lectins, the pre-pro-lectin's sequence starts with a signal stretch of 23 residues and contains a C-terminal extension of 29 residues. Expectedly, biosynthetic processing takes place in the ER and the final deposition site are the storage vacuoles [99].

6.7. Uncommon locations

Up to this point, the take-home message apparently emerges that most plant lectins travel intracellularly along the secretory pathway after synthesis in the ER with final destination in protein bodies or storage vacuoles. Growing evidence, however, has been gathered that there are exceptions to this rule, as already indicated above. Nearly two decades ago, two different yet related lectins from the Leguminosa *Dolichos biflorus* were detectable at different sites. Whereas the "classical" seed lectin is deposited in the protein bodies, the second lectin which is found in stem and leaves resides at the inner periphery of the cells in loose association with the cell wall [37], a characteristic similar to that of the potato agglutinin [81]. More recently, a lectin was isolated from rhizomes of the Convolvulacea hedge bindweed (*Calystegia sepium*) that binds to Man [100]. When its cDNA sequence was determined, it turned out that the mature lectin's protein sequence corresponds to the open reading frame of the cDNA and that it is neither preceded by a signal sequence nor undergoes proteolytic processing [101]. The lack of a signal sequence suggested a localization different from most other lectins. Therefore, the intracellular localization was probed in *Calystega* rhizomes by immunofluorescence. Indeed, the vacuoles which constitute most of the cell volume are entirely free of lectin whereas the thin cytoplasmic layer between the vacuole and cell membrane/wall is stained by the antibody [36]. Deduced from its amino acid sequence, the *Calystegia* lectin is related to jacalin, a lectin from the Moracea *Artocarpus integrifolia*. This plant is taxonomically distinct from *Calystegia*. Jacalin, however, binds Gal rather than Man and is processed and deposited in the "normal" way, i.e. in protein bodies [36]. Thus, jacalin and the *Calystegia* lectin, though possessing homologous amino acid sequences, display different carbohydrate specificities and are localized in different intracellular compartments, a phenomenon that is also known from C-type lectins of animals binding to Man/GlcNAc or Gal [21]. Further monitoring of lectin properties disclosed that the *Calystegia* lectin belongs to an extended family. Lectins with similar carbohydrate specificities and similar "jacalin-related" amino acid sequences have been found in several plants that are taxonomically far apart such as those in the Asteracea Jerusalem artichoke (*Helianthus tuberosus*) [102] and in the Musacea banana (*Musa acuminata*) [103]. The occurrence of a similar lectin in salt-stressed rice plants is of particular interest due to possible economic implications [104,105]. Regarding the situation in animals, it is notable that intrafamily diversity can lead to expression of various family members in the same cell and to a complex network of functional additivity/synergism or divergence that is beginning to be analyzed, for example for galectins [106,107]. Interestingly, this animal lectin family has a folding profile also seen in leguminous agglutinins (please see Section 7.1 and Figure 1).

7. Lectin structures

So far, sequences have primarily been considered to infer evolutionary relationships and to reflect processing routes. Access to sequences is also an essential step on the way to solve folding patterns of plant lectins and their modes how to accommodate carbohydrate ligands in their binding pockets.

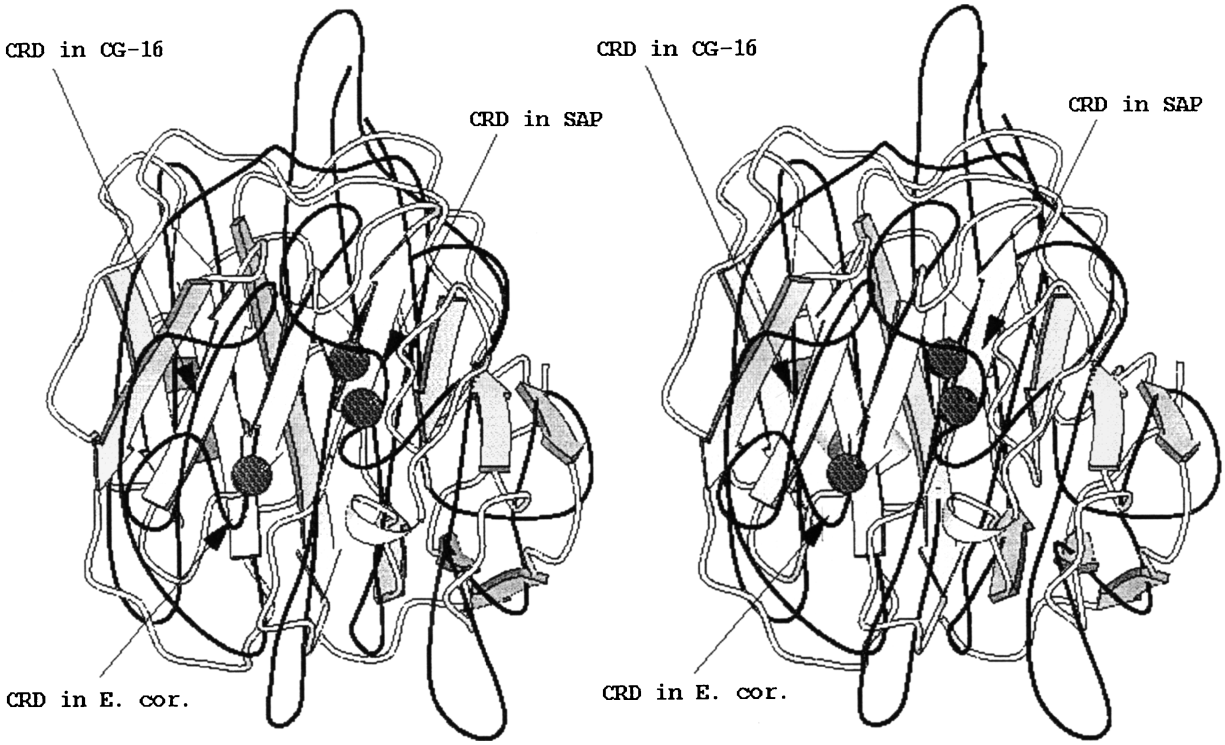

Figure 1. Appearance of a common folding pattern in plant and animal lectins. Illustration of the versatile jelly-roll motif with its antiparallel β-strand arrangement in a pentraxin (1SAC) with its bound Ca^{2+}-ions and in a legume lectin (1AXY). While sharing the general folding pattern the binding sites for the ligand in the pentraxin (SAP), plant lectin (*Erythrina corallodendron*) and galectin (CG-16, from [116]) are at different places, shown by arrows (kindly provided by A. Romero, Madrid).

7.1. Legume lectins

As other fields of lectin research, structural investigations began with the lectin from the Jack bean *Canavalia ensiformis*, and the method used was x-ray crystallography [108]. ConA turned out to be mainly built up of β-sheets that are connected by loops whereas α-helical elements are almost absent. In the following years, this folding pattern was likewise seen for various other legume lectins by x-ray crystallography, circular dichroism and calculation of hydropathic profiles from amino acid sequences. Over the years, however, x-ray crystallography proved to be the method of choice to map plant lectin structure. Despite their diversity in carbohydrate-binding specificity, the folding patterns of subunits of legume lectins share secondary and tertiary structures to the extent that they are superimposable [109–113]. They consist of a flat six-stranded back sheet, a curved seven-stranded front sheet and a smaller five-stranded sheet (S-sheet) that holds the two larger sheets together. The structures resemble flattened bell-shaped domes containing a shallow pocket at their apex which forms the carbohydrate-binding site. The bottom of the pocket contains binding sites for bivalent metal ions. If demetallized ConA requires to be reconstituted, binding of manganese has to precede that of calcium ions. The presence of these ions is essential for correct folding and internal arrangements of the carbohydrate-binding site to reduce entropic expenses by restricting mobility for the orchestrated ligand contact. The metal ions do not participate directly in the interaction with the ligand as is the case in C-type animal lectins. In addition to the metal-ion- and carbohydrate-binding sites, many legume lectins harbor binding sites for hydrophobic molecules [75]. When the overall folding pattern of legume lectins is run through computer searches to pick up similarities, the jelly-roll motif is seen (or predictable) for galectins and serum pentraxins as shown in Figure 1, also for some bacterial glycohydrolases (e.g. *Bacillus* 1,3-1,4-β-glucanase) and for the intracellular lectin ERGIC-53 (endoplasmic reticulum-Golgi-intermediate-compartment protein at 53 kDa) and calculated for VIP-36 (vesicular integral membrane protein at 36 kDa), with ligand- and also metal-ion-binding sites (if present) at variable positions [21,114,115].

As a special feature of ConA, binding of Ca^{2+} induces an isomerization of the non-proline Ala_{207}-Asp_{208} peptide bond from the *trans* to the *cis* conformation allowing Asp_{208} to flip into its carbohydrate-binding position [116]. Though not studied as detailed as ConA, the subunits of lectins from the Leguminosae *Vicia faba, Pisum sativum, Lathyrus ochrus, Erythrina corallodendron* and *Griffonia simplicifolia* (lectin IV) adopt an identical structural fold with only minor changes. The question arose as to whether this structure will invariably lead to carbohydrate binding or whether changes in the actual binding domain can be tolerated. In the course of extensive structural

work it was discovered that non-lectin proteins occurring in *Phaseolus* species, namely arcelin and the α-amylase inhibitor, resemble the *Phaseolus* lectin in their primary, secondary and tertiary structures [117,118]. Thus, legume lectins apparently belong to a larger protein family that includes also proteins that lack contact sites to bind carbohydrate, a situation similarly seen for C-type animal lectins and related proteins with C-type lectin-like domains without carbohydrate-binding capacity [5]. On the other hand, lectins from other plant families though sharing with legume lectins their ability to bind carbohydrate are definitely not structurally related to them [110,111].

In contrast to similarities of folding patterns of the subunits, remarkable diversity is observed in the mode of association of subunits. Table 2 gives a brief overview of known dimeric structures. Within the class of legume lectins, fully active species can be either dimers or tetramers resulting from dimer association, Table 3 giving an insight into different ways of dimer assembly governed by protein-protein interactions. Among plant lectins, the diversity of the legume lectins in carbohydrate specificities is unique. As structural studies show, conserved amino acid residues are responsible for the affinity to a given monosaccharide whereas the specificity is determined by variable lengths of loops [109]. When oligosaccharides or glycosides with hydrophobic aglyconic moieties are bound, additional residues in the vicinity of the primary carbohydrate-binding site participate to accommodate the ligand's noncarbohydrate tail. Concerning lectins from non-legume plants, general features for comparison have also been elucidated and will be outlined in the next sections.

7.2. Lectins from other plants

WGA, the wheat germ agglutinin, has already been introduced. It belongs to the superfamily of chitin-binding proteins including not only lectins but also certain enzymes (chitinases). Hevein from the rubber tree (*Hevea brasiliensis*) with its sequence of 43 amino acids is the prototype of this protein class [119]. WGA is composed of two 18 kDa subunits each

Table 2. Association of legume lectin subunits

Type	*Description*	*Occurrence*
Canonical	Side by side association of the back sheets which leads to a continuous arrangement of twelve β-strands.	ConA, lectins from *Pisum sativum, Vicia faba, Lathyrus ochrus* (α-amylase inhibitor from *Phaseolus vulgaris*)
GS IV type	Back sheets are packed face to face with the β-strands running perpendicularly to each other.	Lectin IV from *Griffonia simplicifolia*
EcorL type	The interface between both subunits consists mainly of the side chain of the "upper" β-strands of the back sheets.	Lectins from *Erythrina corallodendron* and *Psophocarpus tetragonolobus*
DB58 type	The subunits associate with their flat back sheets facing each other while the side chains intercalate. The C-terminal peptide of one subunit is positioned in the central cavity. This peptide is not present in the other subunit.	Leaf and stem lectin from *Dolichos biflorus*

Table 3. Assembly of legume lectin dimers to tetramers

Type	*Description*	*Occurrence*
ConA type	Two canonical dimers are packed against each other with the central part of their continuous twelve strand β-sheets turned around by 90°.	ConA
PNA type	An association of two GS-IV dimers where a subunit of the first dimer combines with a subunit of the second dimer in a canonical way.	Lectin from *Arachis hypogaea*
PHA-L type	The tetramer can be regarded either as a dimer of two canonical dimers or alternatively as a dimer of two DB58 dimers, depending on which subunits are combined to form dimers.	Lectins L_4 from *Phaseolus vulgaris*, B_4 from *Vicia villosa*, II from *Ulex europaeus*, from *Dolichos biflorus* and *Glycine max*
GS I-B_4 type	Interaction of the monomers by comparatively small buried interfaces provided mainly by nonpolar residues.	Lectin I-B_4 (demetallized) from *Griffonia simplicifolia* [115]

consisting of four independently folded and helically assembled hevein-like domains held together by four disulfide bridges lending stability to the molecule. Its four carbohydrate-binding sites for GlcNAc (or Neu5Ac) are located at the interface between the subunits [54]. Besides crystallographic analyses, the hevein domains and their binding of sugar have been studied in solution by NMR spectroscopy using proton-proton distance constraints for hevein, pseudohevein and the B domain of WGA and explaining the multivalent chitin binding of these defense proteins in structural terms [120, 121 and references therein]. Positioning of the disulfide bridges and key aromatic residues, which are crucial for ligand contact in plant hevein domains [122] and shown in Figure 2, as well as superimposable folds give reason to attribute the origin of chitin-binding lectins in invertebrates to convergent evolution [123]. As noted above in the section on *biosynthesis*, another target, i.e. Man, can be implicated in defense by a lectin leading to the discussion of the structure of GNA.

Several Man-binding lectins from monocot species (Amaryllidacae, Liliaceae, Alliaceae, Orchidaceae, Araceae) have been studied. Their main representative is the snowdrop lectin GNA

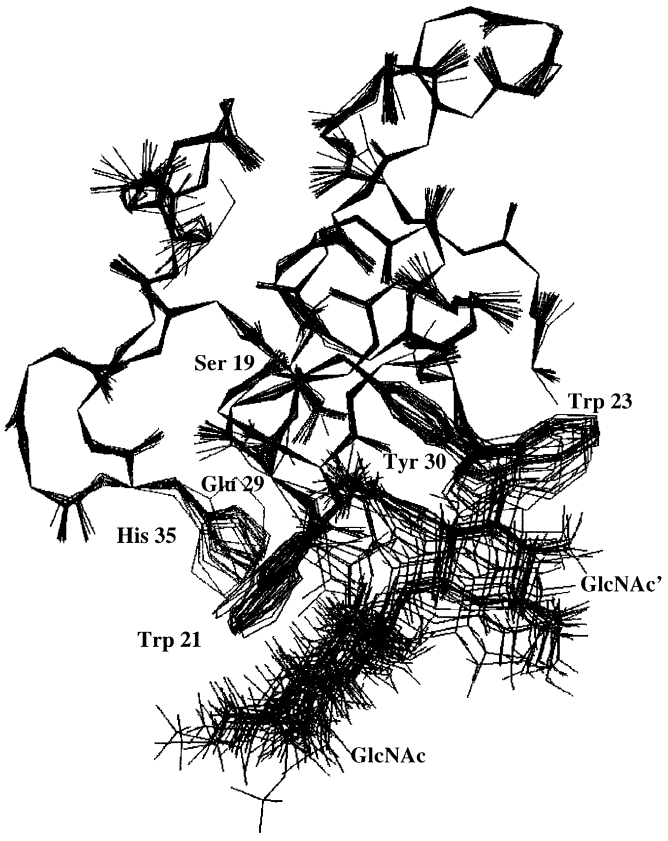

Figure 2. Depiction of the positioning of amino acid residues in a binding site of a plant lectin. Illustration of the structure of the hevein-$(GlcNAc)_2$ complex as obtained by molecular dynamics simulations, kindly provided by M. Frank, H.-C. Siebert and C.-W. von der Lieth (Heidelberg/Munich). The contact between the ligand and key aromatic residues is readily visible.

(*Galanthus nivalis*), whose insecticidal action has been referred to above. GNA (50 kDa) is a tetramer constituted by four 13 kDa subunits. Each subunit consists of three subdomains forming a twelve-stranded β-barrel structure. Each subdomain is formed by a bundle of four antiparallel strands of β-sheets connected by loops. The subunits associate to form a crownshaped tetramer. The twelve Man-binding sites are provided by clefts on the surface of each subdomain [124].

An instructive example for the adaptability of a folding pattern is given by the β-prism fold. Jacalin from Jackfruit (*Artocarpus integrifolia*, Moraceae) is a lectin that binds preferentially to the oncofetal TF-antigen Gal$\beta \rightarrow$ 3GalNAcα. The subunits consist of an unusually small (24 residues) β-chain and a larger (133 residues) α-chain which are derived from a precursor by posttranslational processing. Together both chains form the subunits which consist of three bundles of four β-sheets strands arranged into the β-prism structure [125]. Subunits associate to dimers and tetramers. The carbohydrate-binding site involves the N-terminus (Gly) of the α-chain which is generated by processing [110,125]. In contrast to most other lectins, jacalin has a rather broad specificity interacting also with Man and Glc. A study making use of of surface plasmon resonance, x-ray crystallography and molecular modeling revealed that the unusually broad specificity can be attributed to a carbohydrate-binding cleft that is much more extended than in comparable lectins [126]. A similar architecture has been found for the lectin from the osage orange (*Maclura pomifera*), another Moracea [127]. Interestingly, this folding pattern is shared by lectins binding other sugars (e.g. Man) from other plants, for example the Asteracea *Helianthus tuberosus* [128]. As already noted above, a basic fold can be adapted to various targets by site-specific sequence alterations.

Amaranthus caudatus and other Amaranthaceae species contain lectins that similarly to jacalin blind the TF antigen Gal$\beta \rightarrow$ 3GalNAcα. The lectin's subunits of 34 kDa combine to homodimers of 64.5 kDa. Their subunits are oval in shape and composed of an N-terminal and a C-terminal domain connected by a short helical segment. Each domain forms a β-trefoil fold established by six strands of antiparallel β-sheets which form a β-barrel. Both subunits assemble to dimers in a head-to-tail fashion, and the carbohydrate-binding sites are formed by two shallow invaginations at the interface between the subunits [110]. For ricin, subunit assembly by a disulfide bridge brings the Gal-binding B-chain and the toxic A-chain together [83]. The lectin part's structure—like that of hevein-domain-containing lectins—is another example of the origin of domains by gene duplication with two homologous domains forming the lectin, each containing three homologous 40-residue folding units which pack around a pseudo-threefold axis [129]. The outlined way how the plant protects itself from the toxic action of ricin (please see Section 6.5 on *lectin biosynthesis*) and the cooperation of the two subunits to exert the potent toxic activity render it likely to assume that ricin can be considered as protection against animal predators. This comment

opens the discussion on the functional significance of plant lectins.

8. Biological functions

A central question which has often been asked but up to now not yet been answered definitively is that on the biological function(s) of plant lectins. Not surprisingly, reviews have dealt with this issue with increasing level of experimental input over the last two decades [110,130–134]. Similar to the situation in animals [21,135], there are evidently diverse families of lectins and intrafamily divergence adapted to distinct roles, to be elucidated case by case. Even for lectins with homologous sequences as those from the Leguminosae (or the galectins in animals), a common function cannot be readily ascribed to them because individual parameters such as carbohydrate specificities, location and time of appearance differ. Therefore, every description of a function requires clear-cut evidence which cannot be substituted by analogy considerations. The main lines of current ideas in this area are divided into two groups: one in which a lectin is assumed to bind ligands from foreign sources such as animal, fungal or bacterial aggressors or symbionts, and another one in which a lectin interacts with ligands from the plant.

8.1. External activities

It is reasonable to regard toxic lectins such as those from *Ricinus communis* and from *Phaseolus vulgaris* as protectants against animal predators. Ricin toxicity is brought about by an elaborate transport mechanism akin to that of bacterial AB_5 toxins: the lectin subunit binds to the cell surface and makes the molecule penetrate the cell membrane. The imported protein then moves on a retrograde migration route along the exocytic pathway of eukaryotic cells and finally arrives at the ribosomes. Here it turns on its deadly enzymatic activity by splitting off an exposed and unpaired adenine at position 4324 from the 28S rRNA (rat) which is essential for binding of elongation factors and thus for protein synthesis in general [136]. This mechanism of action is shared by several other plant proteins, generally combined by the designation "ribosome-inactivating proteins class II" (RIP II). Whether other presumed enzymatic activities, specifically nucleolytic capacity, might contribute to the final consequence, will have to be clarified with preparations "that are guaranteed free of contaminations" [137]. The mechanism by which ricin acts on cells fully justifies Stillmark's original statement of *ricin* as being a highly toxic enzyme (=ferment), referred to in Section 2 on *historical aspects*. Agglutination of red blood cells by *Ricinus* extracts, however, is brought about by its accompanying agglutinin.

The following description is intended to accentuate lectin interactions with insects. Due to their interaction with the digestive tract, the lectins from *Phaseolus vulgaris* (PHA) are toxic for mammals in general [55]. The same does not hold true for insects. Initially, it was assumed that also the cowpea weevil (*Callosobruchus maculatus*) is harmed by feeding a PHA-containing diet but later it was found that sufficiently purified PHA is actually nontoxic to this insect [138], and that the active compound of *Phaseolus vulgaris* beans that is toxic to the cowpea weevil is the α-amylase inhibitor [139]. On the other hand, PHA, in particular the isolectin E_4, is apparently toxic to the potato leafhopper (*Empoaca fabae*) [140]. As shown by light and electron microscopy, PHA-E_4 binds to the midgut epithelial cells and leads to severe disorganization and finally to occlusion of the lumen [141]. Whereas PHA is not active against the cowpea weevil, the wheat germ lectin (WGA) is [138]. Also, the GlcNAc-specific lectin II from *Griffonia simplicifolia* (GSA-II) inhibits growth of the cowpea weevil [142] by virtue of its carbohydrate-binding site [143]. WGA, though also binding GlcNAc, does not harm the potato leafhopper except at high doses [144]. Anti-nutritive effects of WGA in humans are not known. With 450 mg lectin per kg present in wheat germ (Table 1) the natural concentration is more than an order of magnitude lower than the effective dosage for insects. Designed to mimic a diet under these conditions it is disconcerting to read that rats fed on a fully-balanced semi-synthetic diet with 93 g lactalbumin/kg and 7 g WGA/kg for 10 days showed reduced utilization of dietary proteins and growth [145]. These selected and representative examples demonstrate that interactions between plant lectins and insects are quite specific and can neither be predicted nor generalized. They also intimate that genetic manipulation to obtain transgenic crop plants rich in insecticidal lectin may not be without consequence for humans.

8.1.1. Protection from insects; transgenic plants

During the last decade, feeding trials to assess the insecticidal action of lectins as potential biological pesticide have focused on the Man-binding lectin GNA, as already mentioned. This lectin has a rather broad insecticidal activity. After initial experiments in which various insects were fed with artificial diets supplemented with GNA, transgenic plants containing the GNA gene were increasingly used. Transgenic plants include potato [96,146], rice [147–149] and wheat [150]. Insects affected are aphids [96,150,151], moths [146,152] and plant hoppers [147,148]. In this context it is essential to underscore that the implications of lectin presence in an ecosystem is not confined to the desired effect in crop protection. In a tritrophic study in which ladybirds fed on aphids that had been sucking on transgenic GNA-containing potato plants, adverse effects on the development of the coleopterous predators were observed [151]. In related studies where the moth *Lacanobia oleracea* reared on GNA-potatoes was exposed to the wasp *Eulophus pennicornis*, no significant [152] or even a beneficial effect [153] on the wasp progeny occurred, demonstrating that long-term effects in complex systems will have to be evaluated very carefully and patiently prior to rushing to claims for safe and efficient application. A similar reasoning will be presented in Section 9.2 concerning the

immunomodulatory effects of lectins and claims for clinical benefit.

8.1.2. Protection from fungi

With the insecticidal action of plant lectins the spectrum of external functions is certainly not yet completely covered [154]. Binding to cell wall constituents in fungi can interfere with their growth. Fungal cell walls contain chitin, the $\beta 1 \rightarrow 4$ linked polymer of GlcNAc. As already suggested in Section 6.6 when discussing sugar binding by WGA, it is, therefore, likely that a fungicidal action is exerted by GlcNAc-binding lectins. In fact, this is the case for several lectins *in vitro*. In order to unequivocally demonstrate the fungicidal action of a lectin, however, it is important to avoid the use of lectins that are contaminated by other fungicidal proteins such as for example chitinases [132].

8.1.3. Symbiosis with bacteria

Plant lectins are not only assumed to be part of the defense system. Regarding bacteria and animal lectins, this aspect prevails with serum and surfactant collectins and also ficolins as molecular guards against infection [21,155]. In the case of plant lectins, however, their interaction with cell surface compounds is also considered to initiate new and desired contacts. Several plants, in particular Leguminosae, are known for their ability to establish a symbiosis with soil bacteria of the genus *Rhizobium* and related genera which are able to fix atmospheric nitrogen, rendering plants independent of supply of external nitrogen fertilizer. This symbiosis is species specific: a given *Rhizobium* strain will nodulate only a single legume species or at best a limited number. It is thus suggestive, together with the widespread and abundant occurrence of lectins in the seeds of Leguminosae, that lectins might participate in establishing the symbiosis between plant roots and rhizobial bacteria. Initial studies appeared to support this hypothesis but were questioned by further scrutiny [130]. These experiments were performed with seed lectins. Naturally, they will not contact soil bacteria *in vivo*.

Recent studies focused on root lectins, some of them being similar to seed lectins. Indeed, it could be shown that by transferring the pea lectin gene to white clover roots, lectin expression enables them to host also pea-specific bacteria [156], and that this effect requires an intact sugar-binding site of the lectin [157]. This topic has been dealt with extensively in several reviews [133,158–160]. A new aspect emerged with the detection of the nodulation (Nod) factors, i.e. lipochitooligosaccharides that are produced by the bacteria as a response to plant-derived stimuli (flavonoids). That a lectin is part of the communication network involved in nodulation is substantiated by the trnsformation of clover roots with the pea lectin gene [156,157]. This manipulation broadens the specificity of the roots to various Nod factors enabling them to respond with enhanced cortical cell growth [161]. Presumably, this is an indirect effect since the pea lectin, being specific for α-linked Man or Glc residues will not bind to β-linked GlcNAc residues which form the backbone of Nod factors [162]. Work is currently in progress to identify the receptors of Nod factors which by definition are lectins. From roots of alfalfa (*Medicago sativa*) plants, proteins were isolated by affinity chromatography on immobilized Nod factor analogues (GlcNAc or its trimer) which interact with authentic Nod factors *in vitro* [163]. From horse gram (*Dolichos biflorus*) roots, a lectin known for many years and originally described to bind GalNAc similar to the seed lectin of the same plant [164] was recently found to weakly interact with GlcNAc, better with chitooligomers and best with authentic Nod factors [29]. Since the phosphatase activity, a further novel property of this lectin, was enhanced in the presence of Nod factors, this protein might function as a signal transducer [29]. With this functional connection, evocative of the signal-triggering properties of animal lectins [165], the separation between external and internal functions becomes blurred. Interestingly, genome analysis of *Arabidopsis thaliana* has uncovered a further trait of animal lectins in a plant gene, i.e. association of a legume-lectin-like extracellular domain with a receptor-like serine/threonine kinase motif (*Ath.lecRK-al-a4*) [166].

8.2. Internal activities

8.2.1. Interaction with storage proteins

This leads our considerations to a further set of hypotheses. According to them lectins act within the plant. A rather passive function as storage proteins is self-evident, because seed lectins are generally degraded during germination. This proposal, however, does not yet satisfactorily answer the question why they possess binding sites for carbohydrates and other substances. Clues to address this issue could be gained by characterizing the nature of lectin ligands, in the case of animal lectins for example matrix glycoproteins to aid cell attachment [21,167]. When pea or lentil seed extracts were passed over a column with an affinity adsorbent containing (pea, lentil) lectin, two fractions of lectin-binding material were obtained, one which is bound by ionic interaction and the other one which binds via the lectin's carbohydrate-binding site. Electrophoretic analysis revealed that the lectin-binding material belongs to the storage proteins, the first fraction being a mixture of legumin and non-glycosylated vicilin, the second one consisting of glycosylated vicilin [168–171], a result corroborated independently [172]. Differences in affinity of the lectin-binding fractions towards the lectin as compared to the bulk storage proteins are also reflected by physicochemical (calorimetry [173], nephelometry [170]) and immunochemical [174,175] properties. How these disparities translate into differences in routing or packaging is unclear at the moment.

8.2.2. Interaction with enzymes

The interaction of lectins with storage proteins is clearly only one aspect of the interactions of lectins within the protein body. In addition to storage proteins, also hydrolytic enzymes

(glycosidases [169,170] and phosphatases [176]) are bound by the lectin in a carbohydrate-, ion strength- or pH-dependent manner. A striking example is the α-mannosidase from *Canavalia ensiformis* seeds which despite being a glycoprotein reacts with the lectin from the same plant, ConA, not via its carbohydrate moiety but by ionic interaction most effectively at pH 5 [177,178]. When isolated protein body membranes are brought into contact with lectin, pH- or carbohydrate-dependent interactions between them are observed [179,180]. Calcium ions which stabilize the conformation of the protein (please see Section 7.1) are released when lowering the pH. This is probably the reason why the interaction of Ca^{2+}-dependent lectins with binding partners is abolished at low pH values [116]. Moreover, pea protein body membranes contain a protein that is cross-reactive with the lectin [181]. Its presence may result from residual firm lectin binding to the membrane, a phenomenon also demonstrated by *in vitro* studies [182], or it may represent a modified form of the lectin.

The dual binding ability of lectins to storage proteins and to protein body membranes suggests that lectins might form a reversible glue between protein and membrane, an idea that is supported by the time course of lectin and storage protein biosynthesis during seed maturation [183]. Such interactions could be modulated by small alterations of the ionic or pH environment within the protein bodies. In consequence, participation of lectins in organizing the protein body content has been suggested, a concept also discussed for *Erythrina indica* [184] and for rice (*Oryza sativa*) seeds [95,185] as well as for soybean (*Glycine max*) leaves [186]. This mechanism to bridge an effector to a target might also be at work for a lectin with myrosinase-binding capacity in seeds of *Brassica napus*, possibly ensuring that products of glucosinolate hydrolysis are made available in the vicinity of an aggressor traced by the lectin [187]. *Mutatis mutandis*, the principle to take advantage of bi- or oligofunctional lectins is also illustrated by the concept that comitin, a 24 kDa actin-binding protein, might link actin cytoskeleton to Man-presenting Golgi vesicles [188].

Lectins may not only bind enzymes but also modify their activities. More than a decade ago it was observed that wheat germ and potato lectins are able to activate endogenous phosphatases from the respective plant [189,190]. More recently, the lectin from a mushroom (*Pleurotus ostreatus*) turned out to activate a phosphatase from the same source in a carbohydrate-dependent manner [30,191]. In addition, this lectin is closely associated with an α-galactosidase activity [30]. At present, it is not possible to provide a straightforward molecular explanation for these phenomena. It may be instructive to note reports on unexpected activities of proteins originally described as lectins. The B-chain of ricin is reported to display a lipolytic activity which is asserted to contribute to ricin's toxicity [192] under the precondition that the preparation employed was completely pure [137]. As mentioned above, a root lectin from the legume *Dolichos biflorus* recently shown to bind Nod factors exhibits a nucleotide phosphohydrolase (apyrase) activity which is enhanced in the presence of haptenic carbohydrates [29]. Actually, the modulation of properties of a second independent site by the lectin domain is similarly operative in animal lectinology, for example in the case of the 67 kDa elastin-/laminin-binding protein or CBP70 interacting with galectin-3 [21,193]. Further association of a lectin-like domain with a hydrolytic activity (β-galactosidase, endo-β1,4-glucanase) is seen in two enzymes of ripening strawberries [31]. Certain bacterial lectins, too, were shown to modify enzymatic activities [194] or to harbor enzymatic activities of their own [195–197]. It is attractive to envision a cooperation of the two active sites, the lectin's domain guiding the enzyme's activity to a site of action (see above), as likewise suggested for sperm's proteolytic acrosin by its Fuc-binding site homing in on zona pellucida glycans [21]. Cellulases and xylanases of aerobic microorganisms with non-catalytic cellulose-binding domains optimizing enzyme-substrate proximity add to the list of modular proteins harboring enzyme and lectin activity [198].

8.2.3. Knock-out experiments

Most studies aiming at defining the biological function of lectins have been performed with isolated proteins *in vitro*. Recently, genetic engineering was introduced to addressing this question by knocking out their expression. Transgenic alfalfa (*Medicago sativa*) plants were constructed with engineered antisense genes of the (putative) lectin genes *MsLEC1* and *MsLEC2* [199]. As KO animals for lectins have provided clues for *in vivo* functions [21], this approach is valuable for delineating answers to the same question in plants. In antisense plants, embryogenesis was severely impaired and both vegetative and reproductive development was disturbed whereas development and growth were normal in controls (vector control, sense-transgenic plants) [199]. This result points to an involvement of the targeted lectins already at early stages, a challenge for further investigations in this field. They will enhance our knowledge in terms of a broader panel of species studied and with regard to the individual steps that bring about the observed phenomena both outside and inside the plant. In what respect the mitogenic activity of plant lectins, initially detected by Nowell for PHA and resting lymphocytes [200], might play a role in development *in situ* will have to be clarified. Regarding plant lectins as tools, this example together with the blood-group-specific binding discussed above attests the potential of lectins in medical applications.

9. Applications of plant lectins

In addition to the increasingly sophisticated description of the occurrence and structural characteristics of plant lectins, this aspect has been and continues to be a driving force for the growth of literature in glycosciences [201]. Numerous questions in basic and medical sciences have been and are being addressed by exploiting plant lectins (Table 4). Laboratory manuals illustrate that lectin-involving methods have well matured and

Table 4. Common applications of plant lectins as tools in basic and medical sciences

Biochemistry
Detection of defined carbohydrate epitopes of glycoconjugates in blots or on thin-layer chromatography plates
Purification of lectin-reactive glycoconjugates by affinity chromatography
Glycan characterization by serial lectin affinity chromatography
Glycome analysis (glycomics)
Quantification of lectin-reactive glycoconjugates in enzyme-linked lectin-binding assays (ELLA)
Quantification of activities of glycosyltransferases/glycosidases by lectin-based detection of products of enzymatic reaction
Cell biology
Characterization of cell surface presentation of glycoconjugates and their preceding intracellular assembly and routing in normal and genetically engineered cells
Analysis of mechanisms involved in correct glycosylation by lectin-resistant cell variants
Fractionation of cell populations
Modulation of proliferation and activation status of cells
Model substratum for study of cell aggregation and adhesion
Medicine
Detection of disease-related alterations of glycan synthesis
Blood group typing and definition of secretor status
Quantification of aberrations of cell surface glycan presentation, e.g. in malignancy
Cell marker for diagnostic purposes incl. infectious agents (viruses, bacteria, fungi, parasites)

reached the status of routine handling [202,203]. Together with carbohydrate-specific monoclonal antibodies lectins afford the possibility to profile glycan populations. Mindful of the conclusion that oligosaccharides are "ideal for generating compact units with explicit informational properties" [204], complexity in the pattern of code units (the *glycome*) with cell type selectivity and modulation of their expression in response to changes of the status of differentiation or onset of disease (e.g. malignant transformation) can be predicted. In this sense, plant lectins have made themselves known to assess a defined aspect of activity of the complex machinery for glycan assembly and modification, starting with synthesis of nucleotide sugars and their transport into the Golgi lumen [4,48].

9.1. Profiling of glycosylation

Indeed, the plethora of studies in this research area confirms the expectation that glycan synthesis fulfils the requirements of a flexible process. Figuring prominently, it is definitely capable to generate a wide variety of sugar determinants. Sorting through disease states as an example, multiple alterations of N- and O-glycan structures were disclosed worthy of further tests whether they can eventually be referred to as markers with clinical relevance [205–207]. Analogous lessons were drawn from the study of development in various systems [208]. As compiled in Table 4, the structural changes cannot only be detected but also quantified and characterized on the molecular level by plant lectins. The development of serial lectin affinity chromatography had a major impact on glycan isolation and gained access to salient sequence information [209,210]. The topological route how these lectin-reactive epitopes are transported following their synthesis and then presented on the cell surface as well as their lateral movements in the membrane are readily followed by lectin cytochemistry using fluorescent or biotinylated lectins or lectin-coated colloidal gold granules [211,212]. Examples for the ample return after investing efforts into basic research on plant lectins are given in the next paragraphs.

In diagnostic pathology, especially the possibilities to stain vascular endothelia and tumors by the Fuc-specific lectin UEA-I and to distinguish pathogens, for example fungi such as *Aspergillus fumigatus, Candida albicans* or *Rhizosporus oryzae*, underscore the usefulness of these sugar-specific probes (Table 4) [213–217]. However, care is to be exercised and methodological aspects closely scrutinized before clinically predictive power of lectin binding is claimed, as thoughtfully discussed by Walker in a special case [218]. Regarding limits of plant lectin application in histochemistry, distinct epitopes with functional relevance in mammalian cell adhesion such as sulfated Lewis^x [193,219] cannot be monitored, because no plant source for a lectin of this specificity is known. In other words, plant lectins should not be relied upon to completely cover the wide variety of sugar code words in mammalian glycoconjugates. Consequently, to close this gap tissue lectins have been introduced to classical lectin histochemistry [220,221]. The endogenous lectins together with carrier-immobilized carbohydrate ligands and plant lectins establish the complete panel of tools in current glycohistochemistry [135,222,223]. Owing to the immunogenicity of plant lectins it is similarly reasonable to include endogenous lectins (bioaddressins) in work on targeted drug delivery [224].

9.2. Ligand cross-linking and mediator release

In cell biology, toxic lectins (ricin) are potent tools to select resistant cell variants on the way to pinpoint genetic defects of the glycosylation machinery (Table 4) [225]. As model substances to induce cell aggregation and adhesion, their cell-binding capacity enables to assay synthetic inhibitors such as glycodendrimers and to dissect signaling cascades relevant for these processes [226,227], the same aim as can be attained with mitogenic lectins in the study of molecular mechanisms of proliferative control and mediator release [228]. In such instances, the cross-linking capacity of lectins which form ordered networks with ligands is vital to accomplish initiation of the signaling cascade [165,229]. By activating immune cells, inducing apoptosis or modulating their cytokine secretion, the respective plant lectins effectively function as biological response modifiers. An example is given by the Gal-binding lectin from mistletoe, an

abundant agglutinin related to ricin/*Ricinus communis* agglutinin with similar capacity to induce secretion of proinflammatory cytokines from mononuclear cells at non-toxic quantities [230]. Unquestionably, immunomodulatory activity should not be assumed to translate automatically into clinical benefit [230]. We have already alerted the reader to an analogous concern not to rush to lectin application prematurely in the context of noting the lack of knowledge concerning ecological long-term effects in lectin-dependent crop protection. In fact, the spectrum of lectin-reactive cells responding to the mitogenic stimulus can include tumor cells, and activation of immune cells may render growth-promoting factors available to let tumor cells thrive more rapidly and/or aggressively than without immunomodulation [231]. In fact, intratumoral macrophages reactive with the lectin could release proinflammatory cytokine in a paracrine manner (Figure 3). When the mistletoe lectin, a component of proprietary extracts from alternative/complementary medicine, was tested at its immunomodulatory dosis in tumor models, this treatment modality indeed led to tumor growth stimulation *in vitro* with certain lines and a part of the tested histotypic cultures, *in vivo* in mice after transplantation and in rats after chemical carcinogenesis [232–235]. In a recent report on a trial with melanoma patients receiving a proprietary mistletoe extract (Iscador®) it was concluded that "for patients with lymph node metastases, treatment with Iscador may accelerate and alter the course of the disease; a significant increase in brain metastases and a significant decrease in overall survival rates were observed for this very high-risk group" [236]. Although it is not clear whether the clinically examined preparation contained the immunomodulatory dose of the lectin, these data seriously question the practice to exclude treatment modalities of alternative/complementary medicine from rigorous safety tests [237]. They also underline the principally double-edged character of lectin-induced immunomodulation in tumor biology.

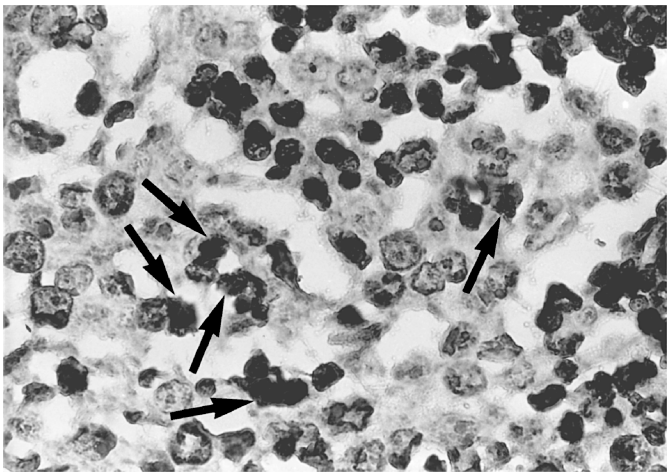

Figure 3. Illustration of localization of lectin-reactive cell surface glycoconjugates using a biotinylated probe. Localization of intratumoral macrophages reactive with the immunomodulatory mistletoe lectin (arrows) in a section of an axillary lymph node metastasis from a mammary carcinoma. Lectin binding can elicit *in situ* cytokine release with its inherent ambivalence, as described in the text.

9.3. Nuclear transport and perspectives

An intriguing example for plant lectin application in cell biology and glycan analysis comes from the detection of O-GlcNAcylation of nucleocytoplasmic proteins by WGA [238]. From the panel of plant lectins, only Gramineae proteins detect this special type of O-glycosylation separate from mucin-type O-glycans [48,54,239,240]. Their availability enables not only monitoring of dynamic changes on the extent of this modification but also blocking studies to infer a role of this sugar e.g. in nuclear transport. This application is a further example epitomizing the very handy target specificity of lectins when dealing with complex-glycan-containing samples (Table 4). It is therefore a good advice to keep an updated list of lectin specificities on the desk [241] to select the proper tool when needed—and further additions to the list of plant lectins with new properties are certainly always welcome to match an upcoming problem with a proper tool. The abundance of lectins in plants (Table 1), the availability of efficient purification methods (see Section 5 on *isolation of lectins*) and their stability (probably connected to the outlined external functions in defense and conferring eminent resistance to detergents and aprotic solvents to them [242,243]) render it likely that the literature on lectin applications will continue to grow. Conceptually, the equivalent of genome and protein analysis, termed *glycomics* or *glycome* profiling [5,244] (Table 4), will gear up the output of pertinent publications on the way to correlate cellular glycan (code word) display with functions (*functional glycomics*).

10. Conclusions

Plant lectins are established laboratory tools with applications for example in glycan profiling in cyto- and histochemistry and as elicitors of cellular activities such as mitosis. These properties in signaling have contributed to delineation of mechanisms of signal transduction from initial cell binding and ligand cross-linking to the final response. In this respect, they can mimic properties of endogenous lectins. These insights pave the way for growing appreciation of the range of activities of protein-carbohydrate recognition *in situ* and help to familiarize with this concept. Equally important, the analysis of plant lectin structures teaches intriguing lessons also relevant for the strategic design of animal lectins. As discussed, the spatial integration of enzymatically active and lectin sites in modular proteins provides a means to target the catalytic center to distinct places via the lectin activity. Considering structure, the occurrence of the jelly-roll-like folding pattern in plant and animal lectins affords a notable role model to define distinct ways how a binding site for carbohydrate ligands can be positioned into the same basic structure. Moving from the analysis of the carbohydrate-binding site's architecture to the level of the complete protein,

it next appears possible that occupancy of this site can influence the functionality at other parts of a mosaic-like molecule. Finally, the effectiveness of plant lectins to interact with animal cells can now rightfully be viewed in an ecological context, e.g. as protection of the plant against predators.

Acknowledgments

We gratefully acknowledge the contributions and enthusiasm of our coworkers and collaborators in the course of the studies in our laboratories. We also want to express our sincere gratitude to Dr. J.H. Wu (Taipei, Taiwan) for promptly and precisely sending published data to be included in Table 1, to Drs. M. Frank and C.-W. von der Lieth (Heidelberg, Germany), Dr. A. Romero (Madrid, Spain) and Dr. H.-C. Siebert (Munich, Germany) for kindly providing ilustrations, and to Dr. B.B. Namirha (Munich, Germany) for competently polishing the text. A sincere apology is directed to colleagues whose original work could not be fully given reference to due to the very strict space limitations further enforced during the review process. The financial support from the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, the Dr.-M.-Scheel-Stiftung für Krebsforschung, the VW-Stiftung and the Wilhelm-Sander-Stiftung as well as DAAD programs and NATO fellowships for travel grants are also gratefully acknowledged.

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Received 24 October 2001; revised 29 July 2002;
accepted 30 July 2002