# Novel structures of plant lectins and their complexes with carbohydrates

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Several novel structures of legume lectins have led to a thorough understanding of monosaccharide and oligosaccharide specificity, to the determination of novel and surprising quaternary structures and, most importantly, to the structural identification of the binding site for adenine and plant hormones. This deepening of our understanding of the structure/function relationships among the legume lectins is paralleled by advances in two other plant lectin families – the monocot lectins and the jacalin family. As the number of available crystal structures increases, more parallels between plant and animal lectins become apparent.

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**Abbreviations**

| | |
|---|---|
| **Con A** | concanavalin A |
| **DBL** | *Dolichos biflorus* lectin |
| **EcorL** | *Erythrina corallodendron* lectin |
| **Fuc** | fucose |
| **Gal** | galactose |
| **GalNAc** | *N*-acetylgalactosamine |
| **Glc** | glucose |
| **GlcNAc** | *N*-acetylglucosamine |
| **Man** | mannose |
| **MPA** | *Maclura pomifera* agglutinin |
| **PNA** | peanut agglutinin |
| **PSP** | porcine seminal plasma spermadhesin |
| **WBA** | winged bean agglutinin |

## Introduction

The past year has witnessed the determination of a wide variety of novel structures of plant lectins, most of them legume lectins, in complex with carbohydrate ligands. This review will, therefore, put emphasis on recent knowledge gained in the domain of the legume lectins, but major results from some other plant lectin families will be discussed briefly as well. Finally, we will discuss some striking similarities that have emerged among the quaternary structures of plant and animal lectins.

## Legume lectin–carbohydrate complexes

A comprehensive comparative study by Sharma and Surolia published in 1997 [1] aimed to rationalize the structural basis of monosaccharide specificity in the legume lectins. Since then, several crystal structures of novel Gal/GalNAc-binding lectins have appeared, against which these views could be tested.

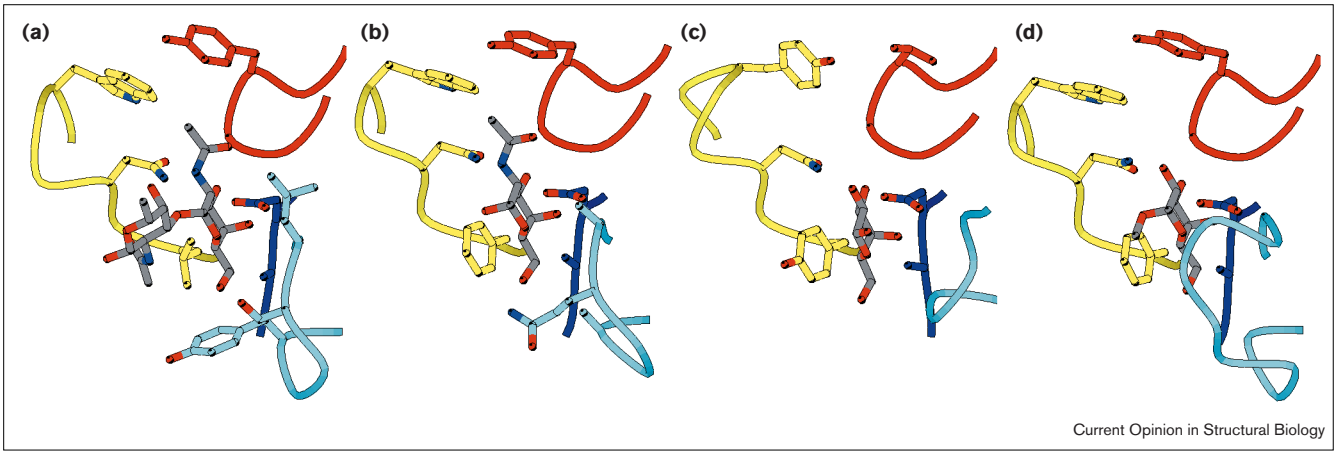
Complexes of the *Dolichos biflorus* seed lectin (DBL) (Figure 1a) with the blood group A trisaccharide {GalNAc(α1-3)[Fuc(α1-2)]Gal} and the Forssman disaccharide [GalNAc(α1-3)GalNAc] [2••] convincingly showed that Leu127 plays an essential role both in the low affinity of DBL for Gal and in its specificity for the Forssman antigen. Leu127 is substituted by an aromatic residue in most other legume lectins. Substitution of this aromatic residue by a shorter, aliphatic leucine or valine residue has been shown to abolish carbohydrate binding in the Gal-specific lectins from *Erythrina corallodendron* (EcorL) [3] and *Robinia pseudoacacia* [4]. Indeed, the mutation L127F increases the affinity of DBL for Gal and GalNAc. The reduction in the contact surface area as a result of the presence of Leu127 is compensated by additional hydrophobic interactions between the lectin and the methyl group of the *N*-acetyl moiety of GalNAc and by an additional hydrogen bond between the *N*-acetyl group and Gly103. Such a mechanism for the specific recognition of *N*-acetylated sugars, whereby the lack of stacking of a large aromatic sidechain against the sugar ring is replaced by compensating interactions with the *N*-acetyl group, seems to be a recurring feature in protein–carbohydrate recognition and was recently observed in the complex of tachylectin-2 with GlcNAc [5••].

This contrasts with the situation in EcorL, which has a similar affinity for Gal and GalNAc. In EcorL (Figure 1b), fewer contacts are seen between the lectin and the *N*-acetyl group of GalNAc and, specifically, the hydrogen bond with the conserved Gly107 residue (Gly103 in DBL) is missing [6]. This is a direct result of the slightly different orientation of GalNAc in the binding site of EcorL compared with that in DBL. This is thought to be a consequence of the substitution of Gln219 in EcorL by Ser215 in DBL [2••].

A third variation exists in peanut agglutinin (PNA) (Figure 1c), which, in contrast to DBL, strongly prefers Gal to GalNAc. In PNA, the substitution of Glu129 by aspartic acid converts this strictly Gal-specific lectin to one that binds both Gal and GalNAc [7]. In PNA, Leu127 of DBL is replaced by the more common Tyr125 residue, whereas Glu129 of PNA has no structural counterpart in DBL.

Finally, yet another variation is seen in the complex between Gal and the basic lectin from winged bean, winged bean agglutinin (WBA) (Figure 1d) [8]. WBA has an unusually long, variable 'monosaccharide specificity loop' that is argued to be essential both for the strict preference of WBA for α linkages and for its enhanced affinity for galactoses bearing substitutions (such as an *N*-acetyl group) on C2.

**Figure 1**




Combining (carbohydrate-binding) sites of four Gal/GalNAc-specific legume lectins. The carbohydrate specificity of legume lectins is determined by four loops that may vary in sequence, conformation and length [1]. These are the specificity loop (loop D by Sharma and Surolia [1]) in cyan, the conserved *cis*-peptide in dark blue (loop A by Sharma and Surolia [1]), the metal-binding loop (loop C by Sharma and Surolia [1]) in yellow and the loop containing the conserved glycine (loop B by Sharma and Surolia [1]) in red. The bound carbohydrates are shown in gray. Selected sidechains are shown in ball-and-stick representation, with oxygen atoms in red, nitrogen atoms in blue and carbon atoms, as well as bonds, in the color of the corresponding loop. These sidechains are Asp85, Tyr104, Leu127, Asn129 and Trp132 of DBL and their equivalents in EcorL, WBA and PNA. Also shown in ball-and-stick form are Ser215 of DBL and the equivalent Gln219 residue of EcorL that, together with Leu127 (DBL) and Phe131 (EcorL), are thought to be responsible for their different relative affinities for Gal and GalNAc. **(a)** DBL in complex with the Forssman disaccharide [GalNAc(α1-3)GalNAc]. The interaction between the reducing GalNAc and Tyr218 is believed to be responsible for the high affinity of DBL for the Forssman disaccharide. **(b)** EcorL in complex with GalNAc. **(c)** PNA in complex with Gal(β1-4)GlcNAc. The GlcNAc residue is not shown for clarity. **(d)** WBA in complex with Gal. All figures prepared using Bobscript [34].

The high affinity of DBL for the disaccharide GalNAc(α1-3)GalNAc was shown to be as a result of the shielding of a hydrophobic patch on the lectin surface by the *N*-acetyl group of the reducing GalNAc that is positioned outside of the monosaccharide-binding site [2••]. This is broadly in agreement with earlier modeling and NMR studies [9,10]. Shielding of hydrophobic surface has also been invoked as the prime reason for the four-fold increase in the binding affinity of Man(α1-3)Man for concanavalin A (Con A) compared with α-D-mannose [11•]. In this complex, the reducing mannose also does not form specific hydrogen bonds to the protein.

Several more reports have appeared on Con A [11•,12,13,14•] and the related lectin from *Dioclea grandiflora* [15] that focus on the relationship between structure and thermodynamics. This relationship has proven to be a difficult one to rationalize, as, in most complexes, a trade-off is observed between van der Waals and hydrogen-bond interactions on the one hand and conformational strain in the glycosidic linkages and the destabilization of binding in the monosaccharide-binding site on the other hand [11•,13,14•]. Thus, it is not surprising that computational studies aimed at predicting and understanding experimental thermodynamic parameters show only poor success

**Figure 2**

A comparison of the quaternary structures of **(a)** DBL, **(b)** DB58 and **(c)** the canonical legume lectin dimer. The DBL tetramer (with its C-terminal α helices buried in the central hole of the tetramer) is shown in relation to its two 'halves', which form the DB58 dimer and the canonical legume lectin dimer. Two adenine molecules bound to the DBL tetramer are shown as ball-and-stick models. Each DB58-like half of DBL contains two potential adenine-binding sites, of which only one can be occupied.

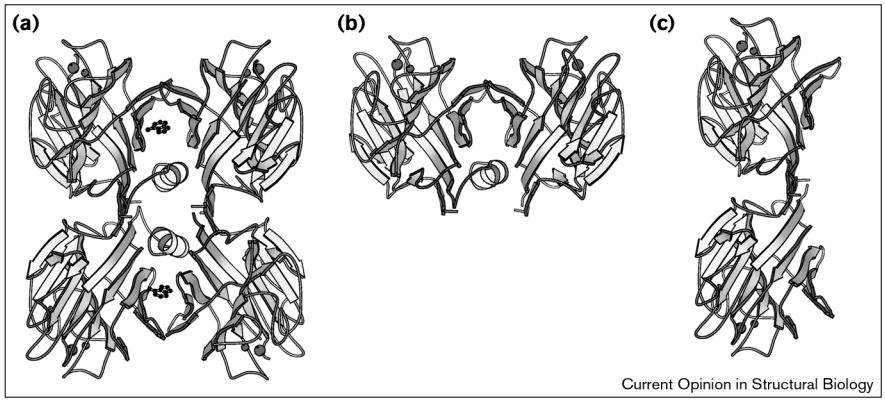
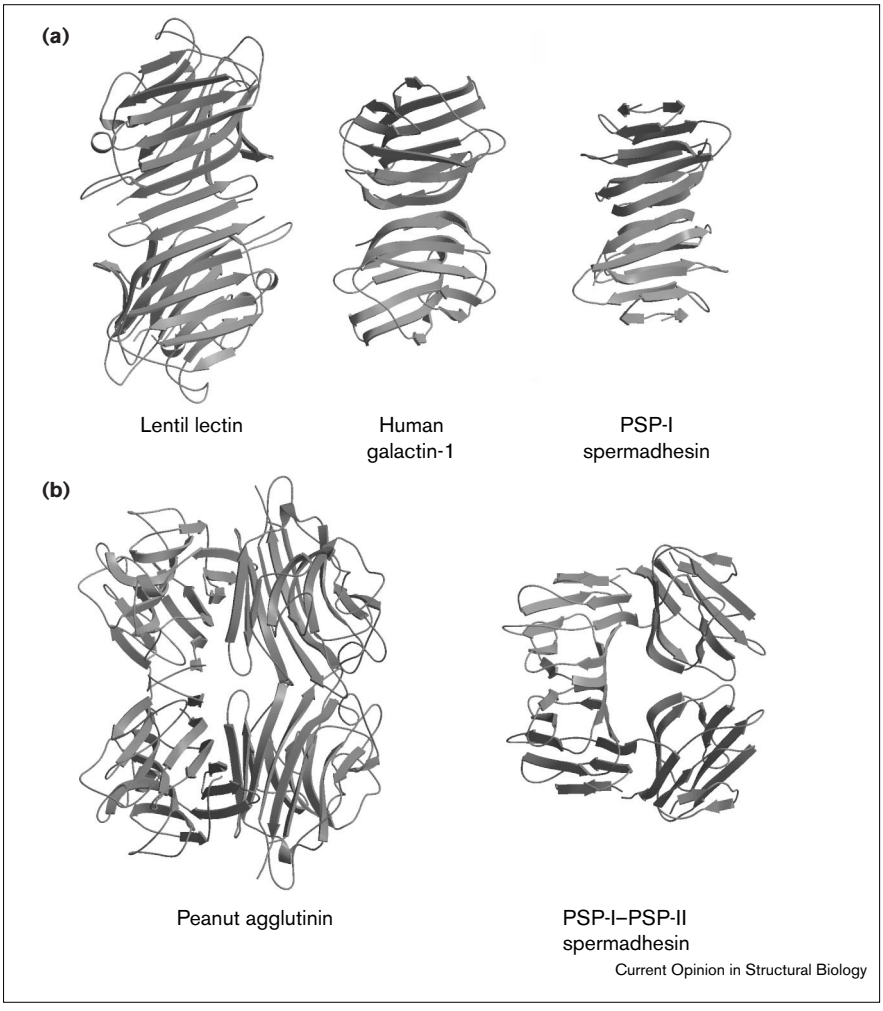


**Figure 3**




Similarities among the quaternary structures of legume lectins and those of animal lectins exhibiting a related fold. Several animal lectins not only possess a monomeric fold that is related to the legume lectin fold, but also adopt surprisingly similar quaternary structures. **(a)** The canonical legume lectin dimer compared with similar dimers of human galectin-1 and of the spermadhesin PSP-I. **(b)** The 'open' tetramer of peanut agglutinin compared with the crystallographic tetramer formed by the spermadhesin PSP-I–PSP-II heterodimer.

rates, even when applied to the very simple system of Man versus Glc binding to Con A [12].

## Novel quaternary structures of legume lectins

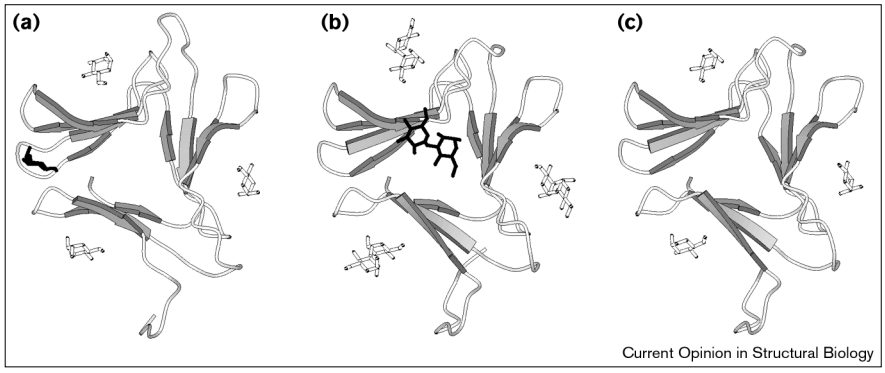
The legume lectin family is known to exhibit a number of different quaternary structures. Several recent reports have now further extended this variety. WBA [8] is the second lectin to have been found to adopt the EcorL dimer structure. In contrast to EcorL, glycosylation cannot be invoked to explain the noncanonical association of the WBA monomers. In their paper, Prabu *et al.* [8] present a detailed discussion of noncanonical association in legume lectin dimers.

Most interesting are the structures of two related lectins from *Dolichos biflorus* (DBL, from the seeds, and DB58, from the stem and leaves) (Figure 2) [2••]. These lectins each consist of an equimolar mixture of full-length and C-terminally truncated subunits. In the crystal structures of DBL and DB58, these C termini were shown to adopt an α-helical conformation and their α helices are packed between the back β sheets of two opposing monomers. In the case of DB58, this leads to the formation of a novel kind of dimer. The C-terminal helices not only mediate quaternary structure formation, but also play an important role in establishing the adenine/hormone-binding site found in many legume lectins.

GlcNAc-specific lectin II from *Ulex europeaus* has recently been crystallized and a preliminary structure determination reveals a tetramer resembling PHA-L (phytohaemagglutinin-L from *Phaseolus vulgaris*), SBA (soybean agglutinin) and DBL [16]. In contrast to DBL, no central helix is present between the two back β sheets. Rather, the tetrameric arrangement is stabilized by interprotomer disulfide bridges (R Loris, unpublished data).

Electron microscopy and X-ray diffraction studies at low resolution suggested a novel, tetrameric arrangement for fucose-specific isolectin A from *Lotus tetragonolobus* [17•]. This work constitutes the first detailed characterization of a type-II two-dimensional cross-linked lattice between a lectin and a bivalent carbohydrate, the oncofetal dimeric Lewis$^x$ antigen. Fitting the canonical legume lectin dimer to the 19 Å electron diffraction data resulted in a tetramer

**Figure 4**




Carbohydrate-recognition domains (CRDs) of different monocot lectins. The monocot lectins are composed of subunits that possess pseudo-threefold internal symmetry. Each of these three subdomains contains a carbohydrate-binding site. The structures of the garlic and daffodil lectins show that, in addition to these three binding sites per subunit, additional binding sites may be present that probably function as high-affinity subsites involved in oligosaccharide recognition. **(a)** Subunit A of garlic lectin in complex with Man. **(b)** Daffodil agglutinin complexed with mannobiose Man(α1-3)Man. **(c)** Snowdrop lectin complexed with Man. Each time, the sugars bound in the three symmetrically disposed and structurally equivalent CRDs are shown as ball-and-stick models with open bonds, whereas the additional carbohydrate in garlic and daffodil lectins is shown as black ball-and-sticks.

in which the two canonical dimers are rotated 20° relative to their mutual orientations in the Con A tetramer.

Recently, an attempt was made to rationalize quaternary structure formation in legume lectins in terms of buried hydrophobic surface area, shape complementarity and calculated interaction energy [18]. Neither of these parameters allows a completely unambiguous discrimination between arrangements that are experimentally observed and those that are not; however, globally, each parameter tends to favor the observed arrangements.

## Structural link between plant and animal lectins

Legume lectins, galectins, pentraxins and spermadhesins are all carbohydrate-binding proteins that share a related monomeric fold. In all four families, a variety of quaternary structures is observed, giving rise to different spacings among the binding sites of different monomers (for the most recent additions to the universe of lectin quaternary structures, see [2••,19–22]). The spacing and orientation of the sugar-binding sites of a lectin become important when binding multivalent epitopes [23,24].

That evolutionarily related proteins adopt different quaternary structures is not unusual as such. What is unusual is that, among these lectins, variation in quaternary structure is possible despite a large degree of conservation in amino acid sequence and in monomeric structure. Furthermore, some of the individual quaternary structures observed for evolutionarily unrelated legume lectins, galectins and spermadhesins are strikingly similar (Figure 3). The first crystal structures of the galectin dimer showed a striking resemblance to the canonical legume lectin dimer [25,26]. Similarly, in the crystal structure of heterodimeric PSP-I–PSP-II (porcine seminal plasma spermadhesin), two PSP-I monomers associate to form a structure that resembles a canonical legume lectin dimer [20]. Given that sheet extension is an often-used mechanism for dimer formation in many unrelated proteins, this observations could still be attributed to chance. On the other hand, the similarities in subunit association among the spermadhesins and GS-IV (*Griffonia simplicifolia* lectin IV) or PNA are amazing to say the least. The PSP-I–PSP-II spermadhesin heterodimer strongly resembles the dimer formed by the legume lectin GS-IV [20]. Together with the above-mentioned crystallographic association of the PSP-I subunits, a PNA-like tetramer is formed. PNA was the first protein to be shown to adopt an 'open' quaternary structure [27]. Such structures were, up to a few years ago, considered 'forbidden' by nature.

Perhaps the lectin folds were chosen during evolution exactly because they allow the easy evolution of quaternary structures, in order that they can adapt easily to the different spacing of carbohydrate ligands presented on, for example, cell surfaces. As a result of the intrinsic flexibility of oligosaccharides, high-affinity binding poses a specific problem for carbohydrate-binding proteins. Thus, it is possible that multivalency is nature's solution to this problem and that the rapid evolution of quaternary structure is a necessary consequence.

## Monocot lectins

Three recent reports provide insights into the structural basis of the oligosaccharide specificities of the monocot lectins, as well as into quaternary structure formation [28•,29•,30]. The dimeric lectin from garlic (*Allium sativum*) is the second monocot lectin for which a detailed structural analysis has become available [28•]. Seven mannoses are found bound to this dimer: one bound to each subdomain of the threefold pseudo-symmetric monomer

and one further mannose bound to a high-affinity subsite that is thought to be relevant to the recognition of a high mannose structure (Figure 4a). The differences in carbohydrate specificity between dimeric garlic lectin and tetrameric snowdrop lectin appear to be a direct consequence of their different quaternary structures. Thus, the monocot lectins are the first example of a protein family that uses variation in subunit association as a mode of achieving oligosaccharide specificity.

The crystal structure of daffodil lectin in complex with Man(α1-3)Man also reveals an additional binding site that is distinct from the three carbohydrate-recognition domains symmetrically arranged on each monomer [29•]. This fourth binding site is located in a different position to that of garlic lectin (Figure 4b) and was also proposed to play a role in the recognition of larger oligosaccharide ligands, as is further supported by a comparison with the snowdrop lectin–pentasaccharide complex [31] (Figure 4c). For the 'primary' binding sites, the disaccharide can bind with either its reducing or nonreducing end in the monosaccharide-binding site, a situation similar to the Man(α1-2)Man–Con A complex.

## The jacalin family

The crystal structure determination of a second member of the jacalin family, *Maclura pomifera* agglutinin (MPA) [32], resulted in the first view of how the T-antigen disaccharide [Gal(β1-3)GalNAc] is specifically recognized by this lectin family. Interestingly, the bulk of the interactions between the carbohydrate and the lectin occur via the reducing end of GalNAc, whereas the nonreducing Gal only interacts with the protein via a small number of van der Waals contacts and a single water bridge. Thus, the preference of MPA for Gal(β1-3)GalNAc over GalNAc is explained essentially by the occurrence of the shielding of exposed hydrophobic surface, as has also been observed recently with GalNAc(α1-3)GalNAc binding to DBL [2••] and Man(α1-3)Man binding to Con A [11•].

A plausible model for discrimination between Man and Gal by KM+, a mannose-specific member of the jacalin family, has been presented based on homology modeling [33•]. A key role in Man and Gal distinction by KM+ may be played by Asp141. In contrast to jacalin and MPA, all known mannose-specific lectins from the same family are single-chain lectins that are not post-translationally cleaved into an α and β chain. In KM+, Gly14 of the linker peptide between the virtual α and β chains forces Asp141 into a different rotamer conformation than observed for the equivalent Asp125α in the jacalin–Gal complex. In this conformation, Asp141 could make a hydrogen bond with an equatorial oxygen on C4, as found in Man and Glu, but not with an axial O4, as in Gal.

## Conclusions

The legume lectins continue to profile themselves as the model system of choice to study the fundamentals of protein–carbohydrate interaction. The analogies one continues to find among these plant lectins and several families of animal lectins justify this choice. A full understanding of the structural basis of carbohydrate specificity and quaternary structure formation in the legume lectin family requires detailed studies on members belonging to other specificity groups (e.g. Fuc or GlcNAc), as well as detailed mutagenesis and calorimetric analysis. Several groups are indeed working on this aim.

Other plant lectin families have now also been structurally characterized in more detail. While even a year ago, only a single member of the jacalin family and the monocot lectins was known, relatives with different specificities can now be compared. Several crystallization reports suggest that our understanding will also grow rapidly in the coming years.

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- •• of outstanding interest

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This detailed structural analysis of a dimeric monocot lectin indicates its quaternary structure as a major determinant of the different oligosaccharide specificities between heterodimeric garlic lectin and homotetrameric snowdrop lectin. The A subunit of this lectin contains four, rather than the expected three, saccharide-binding sites.

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