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Lectin Structure−Activity: The Story Is Never Over

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Advances in plant lectin biochemistry have made great strides during the past decade. Technical advances in biophysical techniques and molecular biology, the availability of synthetic oligosaccharides, and characterization of lectins with unique carbohydrate-binding properties are responsible for these advances. Studies in this laboratory support the view that interesting new discoveries are yet to be made. A new lectin was recently isolated from a fungus (*Polyporous squamosus*) that recognizes the Neu5Ac α2,6 Gal β1,4 GlcNAc/Glc trisaccharide epitope with high affinity. The lectin does not interact with α2,3-linked Neu5Ac or Neu5Ac α2,6 GalNAc groups as occur in ovine submaxillary mucin. An unusual lectin with two distinctly different carbohydrate-binding sites is present in tubers of *Xanthosoma sagittifolium* (L). One species of sites recognizes clusters of oligomannosyl residues. The other type of binding site best accommodates a nonsialylated, triantennary oligosaccharide having LacNAc or Lacto-*N*-biose (Gal β1,3GlcNAc) groups at its three nonreducing termini. The banana lectin has also been studied. It recognizes both α and β1,3-linked glucosyl oligosaccharides, generates a precipitin curve with the branched trisaccharide Man α1,6[Man α1,3]Man, and binds to β-glucans containing β1,6-glucosyl end groups.

KEYWORDS: Lectins; sialic acid-binding lectins; banana lectin; mushroom lectin

Progress in plant lectin biochemistry has exploded during the past decade (*1*). New lectins with interesting properties have been isolated and characterized; a great many lectins have been cloned and homologies in their amino acid sequences and similarities in their molecular structures established. The X-ray crystallographic structure of a host of these lectins has been solved at high atomic resolution. The biosynthesis of many lectins has been elucidated. Many lectins have been expressed in bacteria and eukaryotic cells and their mutant forms studied to determine changes, if any, in their carbohydrate-binding specificity. A number of lectins have also been transfected into food crops with the intention of conferring resistance to various insect vectors. Still, there are many unanswered questions: What is the function, if any, of the rather precise carbohydrate-binding sites, which occur in plant lectins (*2*)? Do they represent part of a primitive immune system? Are they, as has been suggested by many, a device for resisting the ravages of microorganisms, insects, and rodent pests? Why do some plants appear to lack lectins while others may have two or more lectins with totally different types of carbohydrate-binding sites?

It is interesting to note that there are only a rather restrictive number of sugars that plant lectins recognize. These include the monosaccharides D-glucose, D-galactose, D-mannose, L-fucose (6-deoxy-L-galactose); two amino sugars, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine; and the keto sugars D-fructose and L-sorbose, the latter two because of their structural relationships to reactive monosaccharides. Interestingly, no plant lectin that binds pentose sugars has been reported, although concanavalin A binds D-arabinose with low affinity. Some lectins recognize di- and trisaccharides with greater affinity than the monomeric sugars. For example, wheat germ agglutinin binds *N*,*N*′-diacetylchitobiose and *N*,*N*′,*N*″-triacetylchitotriose with binding constants that are approximately 130 and 6000 greater than that of *N*-acetyl-D-glucosamine (*3*−*5*); lectins from *Datura stramonium* and *Erythrina cristagalli* bind *N*-acetyllactosamine 50−150 times stronger than *N*-acetyl-D-glucosamine or galactosamine, respectively (*6*, *7*). Concanavalin A recognizes the branched trisaccharide Man α1,3 [Man α1,6] Man ∼40 times greater than methyl α-mannoside (*8*, *9*), and the polypore mushroom *Polyporus squamosus* binds Neu5Ac α2,6Gal β1,4GlcNAc with $K_a = 1.38 \times 10^6$ M^{-1} (*10*).

Anomeric preference is another factor involved in lectin−carbohydrate-binding activity. For example, all mannose/glucose-binding lectins show a high preference for the α-anomeric form (*1*) as do the *Griffonia simplicifolia* GS-I isolectins (*1*), whereas the lectin from *Ricinus communis* binds more avidly to β-galactosides. On the other hand, the *Wisteria floribunda* and GS-II lectins display an indifference in the binding to anomers of *N*-acetyl-D-galactosamine- and *N*-acetyl-D-glucosamine-containing oligosaccharides, respectively (*1*).

Most plant lectins recognize and interact with terminal nonreducing units of oligo- and polysaccharides and complex polymers such as glycoproteins and glycolipids. Notable exceptions include wheat germ agglutinin, which recognizes internal GlcNAc residues linked β1,4, β1,6, or both β1,4 and 1,6 (*11*), and concanavalin A, which bind to internal Glc or Man residues linked α1,2 (*12*, *13*).

Despite the great activity in the field of plant lectinology, there are still many surprises that I believe await us. These include the discoveries of new lectins with unique carbohydrate-

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Table 1. Specificity of Some Sialic Acid-Recognizing Plant Lectins

lectin	specificity
Sambucus nigra (SNA)	Neu5Ac, α2,6 Gal/GalNAc
Trichosanthes japonica (TJA1)	Neu5Ac α2,6GTal β1,4GlcNAc
Polyporus squamosus (PSA)	Neu5Ac α2,6Gal β1,4Glc/GlcNAc
Agrocybe cylindracea	Neu5Acα2,3Galβ1,3/1,4BlcNAc/GalNAc
Maakia amurensis (MAL)	Neu5Ac α2,3Gal β1,4GlcNAc/Glc

binding activities, newly discovered biological functions of plant lectins such as those explored by Etzler and colleagues (*14*), and further discoveries of higher orders of organization by which lectins bind to and cross-link oligosacchaides, glycoproteins, and biological receptors of the kind pioneered by Brewer et al. (*15*).

During the past several years, my laboratory has made several interesting discoveries. These include the characterization of a lectin with two distinctly different types of carbohydrate-binding sites (*16*), a polypore mushroom lectin that binds with high affinity to a trisaccharide terminated by an α2,6-linked sialic acid group (*10*), and, most recently, a lectin that recognizes internal α1,3-linked glucosyl residues, as well as the β1,3-linked glucobiose (laminaribiose) and β-glucans containing β1,6-linked glucosyl end-groups.

The lectin present in the tubers of *Xanthosoma sagittifolium* (L), purified on two different affinity columns—asialofetuin- and invertase-Sepharose—is a heterotetrameric protein ($\alpha_2\beta_2$) composed of four 12 kDa subunits linked by non-covalent bonds (*16*). Using the techniques of quantitative precipitation and hapten inhibition of precipitation, we established that the lectin contains two different types of carbohydrate-combining sites: one type for oligomannoses, which preferentially bind to a cluster of nonreducing terminal α1,3-linked mannosyl residues; the other type for complex *N*-linked carbohydrates (*16*). These sites recognize nonsialylated, triantennary oligosaccharides having LacNAc (Galβ1,4GlcNAc-) or Lacto-*N*-biose (Galβ1,-3GlcNAc-) groups at their nonreducing termini.

Sialic acids are present on the surface of all vertebrate cells and occupy a key terminal position on many glycoproteins and glycolipids. Lectins that recognize the sialic acids serve as valuable reagents for the glycobiologist. They can be employed for the detection and preliminary characterization of sialic acids on cell surfaces and on complex carbohydrates in solution. As affinity matrices, they can be used for the isolation and resolution of sialo-containing glycoconjugates. Strangely, although there is no report of sialic acid occurring in plants (sialic acids have been found in fungi) (*17*), several plant lectins have been reported to recognize sialic acid-containing oligosaccharides, some with great specificity. **Table 1** presents a list of such lectins. It is to be noted that some of these sialic acid-binding plant lectins can distinguish between α2,3- and α2,6-linked sialic acids (*10*, *18*−*20*).

Recently we discovered a polyporous mushroom (*Polyporus squamosus*) growing on a decayed elm stump. From this fungus we isolated a cell-agglutinating protein with lectin-like activity (*10*). It was isolated on a β-D-galactosyl-Synsorb affinity column, followed by ion exchange chromatography on DEAE-Sephacel. At first we believed we had isolated a lectin with a rather mundane specificity because there are many lectins with similar carbohydrate-binding specificities (for example, it bound lactose and other β-galactosides similarly to ricin). However, when we examined its reactivity with several sialylated oligosaccharides, we were surprised to discover that it bound Neu5Ac α2,6Galβ1,4GlcNAc/Glc with $K_a = 1.88 \times 10^6\ M^{-1}$, which is 2000-fold stronger than toward galactose (*10*). It did not bind to α2,3-linked sialylated oligosaccharides as occur in many glycoproteins. Nor did it bind to a variety of glycolipids, all of which have sialic acid linked α2,3. The lectin does not bind sialic acid itself or Neu5Ac-linked α2,8 and the corresponding colominic acid (*10*). The *P. squamosus* lectin is also unique in that it recognizes only Neu5Acα2,6Galβ1,4Glc/GlcNAc trisaccharide sequences of asparagine-linked oligosaccharides (*10*, *20*) unlike the *Sambucus nigra* lectin, which also binds to Neu5Acα2,6Gal/GalNAc residues that occur in *O*-Ser/Thr-linked glycoproteins (*18*).

Finally, I would like to briefly mention our latest results on the carbohydrate-binding properties of the banana lectin. Although its isolation was reported a decade ago by Koshite and his colleagues (*21*), we have discovered many unique features that have gone unnoted. They characterized the lectin as a mannose-binding protein. Our studies also indicated that it appeared to be a typical glucose/mannose-binding protein in that it gave precipitation curves with branched α-mannans (yeast mannan) and α-glucans (dextrans, glycogens, and starches) and that these precipitation reactions were inhibited by D-glucose and D-mannose and their derivatives (*22*). These properties were very reminiscent of concanavalin A. However, the comparison ends there, for we discovered that, unlike concanavalin A, the banana lectin binds 3-*O*-methylglucose and recognizes *internal* α-glucosyl residues linked at the O-3 position; thus, banana lectin precipitates elsinan, a linear polysaccharide containing α1,3- and α1,4-glucosidic bonds in the ratio of 3:1 (*22*). Also consistent with its binding of 3-*O*-methylglucose, we discovered that the banana lectin binds laminaribiose [3-*O*-β-D-glucosyl-D-glucose(Glcβ1,3Glc)], the first plant lectin reported to bind to this disaccharide (*22*, *23*). Finally, we found that the banana lectin precipitates, in a specific manner, β-glucans with multiple β1,6-linked glucosyl end groups, for example, the *Pleurotus ostreatus* mushroom β-glucan (*23*). Concanavalin A displays none of these properties. Finally, the banana lectin generates a precipitin curve with the branched trisaccharide Mana1,3[α1,-6Man]Man, unlike concanavalin A, and a branched pentamannose oligosaccharide, both of which are components of the core region of *N*-Asn-linked glycoproteins. Not surprisingly, these two glucose/mannose-binding lectins from banana and jack beans belong to two very diverse plant families.

Without doubt, there are still many fascinating lectins to be discovered that will keep many of us occupied for the foreseeable future.

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