6586 *J. Agric. Food Chem.* **2002,** *50,* 6586–6591

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

How Proteins Bind Carbohydrates: Lessons from Legume Lectins

NATHAN SHARON* AND HALINA LIS

Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

The pioneering studies of Irvin Liener on soybean agglutinin (SBA) in the early 1950s served as the starting point of our involvement in lectin research during the past four decades. Initially we characterized SBA extensively as a glycoprotein and showed that its covalently linked glycan is an oligomannoside commonly present in animal glycoproteins. We have also introduced the use of the lectin to the study of normal and malignant cells and to the purging of bone marrow for transplantation. Our recent work focuses on the combining site of *Erythrina corallodendron* lectin, closely related to SBA. In this legume lectin, as in essentially all other members of the same protein family, irrespective of their sugar specificity, interactions with a constellation of three invariant residues (aspartic acid, asparagine, and an aromatic residue) are essential for ligand binding. Lectins from other families, whether of plants or animals, also combine with carbohydrates by H-bonds and hydrophobic interactions, but the amino acids involved may differ even if the specificity of the lectins is the same. Therefore, nature finds diverse solutions for the design of binding sites for structurally similar ligands, such as mono- or oligosaccharides. This diversity strongly suggests that lectins are products of convergent evolution.

KEYWORDS: ***Erythrina corallodendron*** **lectin; hydrogen bonds; hydrophobic interactions; monosaccharides; oligosaccharides; soybean agglutinin; specificity; three-dimensional structure**

INTRODUCTION

The pioneering studies of Irvin Liener on soybean agglutinin (SBA) in the early 1950s served as the starting point of our involvement in lectin research during the past four decades. It was also the beginning of a long-lasting friendship, one outcome of which was the treatise on lectins that he initiated and planned and then edited together with Irwin J. Goldstein and one of us (N.S.) (*1*).

Around 1960, we became interested in the nutritional properties of soy proteins, and at the same time also in glycoproteins, about which little was then known and the existence of which in plants was in doubt. We were soon intrigued by Liener's early publications on soy proteins (*2*−*5*) and chose to focus on the hemagglutinin he isolated from soybean oil meal because it seemed to meet both of the above interests. In these publications he provided evidence that soybean hemagglutinin (later renamed by us as soybean agglutinin, SBA) may have a deleterious effect on the nutritional properties of raw soybeans and soybean meal. He also found that SBA contained glucosamine and suggested that it might be a glycoprotein. Last, but not least, he indicated that SBA is the only biologically active protein present in soybeans in considerable amount (∼1%).

Quite early we lost interest in the nutritional effects of SBA and turned our attention to its physicochemical properties, carbohydrate specificity, and applications. Among others, we proved that SBA contained not only glucosamine but also mannose (*6*), another typical constituent of glycoproteins, and that the carbohydrate is linked to the protein via *N*-acetylglucosaminylasparagine (*7*), known at the time to occur only in animal glycoproteins (all sugars mentioned are of the D configuration except for fucose, which is L). The complete structure of the carbohydrate of SBA was elucidated in collaboration with the group of J. F. G. (Hans) Vliegenthart as the branched oligomannoside $Man_9(GlcNAc)_2$ already known to be present in animal glycoproteins, thus providing evidence for the evolutionary conservation of such protein-linked glycans (*8*). We also determined that SBA is made up of four nearly identical subunits (*9*), found that it is specific for *N*-acetylgalactosamine, and proposed that its combining site is small, of the size of a monosaccharide (*10*). In another series of investigations we demonstrated that the lectin reacts with certain malignantly transformed cells, but not with the normal parental ones (*11*), and that it can be employed for the fractionation of mouse lymphocytes (*12*) as well as for the isolation of bone marrow stem cells (*13*). This is the basis for the routine application of the lectin for purging of human bone marrow for transplantation into "bubble children" (i.e., children born with immune deficiency), resulting in the saving of the lives of many of them, and for its experimental use for the treatment of end-stage leukemia patients (*14*). Recently we cloned SBA and expressed

* Author to whom correspondence should be addressed (telephone 972-8-934-3605; fax 972-8-946-8256; e-mail nathan.sharon@weizmann.ac.il).

10.1021/jf020190s CCC: $22.00 © 2002 American Chemical Society
Published on Web 09/24/2002

Table 1. Properties of Legume Lectins

>100 isolated, ∼50 sequenced, >20 three-dimensional structures solved, many more with ligands
consist of two or four subunits of 25–30 kDa, each with one Ca^{2+} and one Mn^{2+} required for activity
tertiary structures are superimposable; quaternary structures are different
possess diverse carbohydrate specificities
have a single combining site per subunit with low K_a for monosaccharides (∼10^3 M^{-1})

it in both bacterial and mammalian cells (*15*). There is no doubt that Irvin Liener deserves credit for much of this.

Our involvement with SBA led us to the investigation of other lectins, mainly leguminous ones, such as peanut agglutinin (PNA) and *Erythrina corallodendron* (coral tree) lectin (ECorL), and eventually to attempts to understand how, at the molecular level, proteins bind carbohydrates (*16*). Here we summarize the current status of this problem, as illustrated by our studies of ECorL. First, a brief survey of legume lectins in general is presented.

LEGUME LECTINS

Legume lectins represent the largest and most thoroughly studied family of these sugar-binding proteins. Over 100 members of this family are known, almost all isolated from seeds of the plants (*17*−*19*). Because they are readily obtainable in purified form and exhibit an amazing variety of sugar specificities, these lectins are eminently suitable for tackling the question of how proteins bind carbohydrates. Fundamental insights obtained from the study of these lectins have been widely applied to lectins of other families, for instance, the pharmacologically important C-type animal lectins. Furthermore, well-established concepts such as subsite multivalency were first formulated and the formation of cross-linked lattices was first observed during studies of legume lectins and proved to be valid outside the legume lectin family. An important additional reason for the interest in legume lectins is their structural similarity to lectins from other sources, such as the galectins of animals.

Typically, the members of this protein family consist of two or four subunits (or protomers), either identical or slightly different, each with a single, small carbohydrate combining site with the same specificity (**Table 1**). They also contain one ion each of tightly attached calcium and manganese per subunit, which are required for carbohydrate binding. The subunits are of 25−30 kDa and are commonly composed of a single polypeptide chain of ∼250 amino acids. Most, but not all, are glycosylated and carry up to three asparagine-linked oligosaccharides per subunit (*20*). The primary structures of the lectins of this family are remarkably homologous, with ∼20% of invariant amino acids and close to 20% of similar ones. The conserved amino acids include several of those that participate in hydrogen bonding or hydrophobic interactions with the monosaccharide held in the combining site and almost all residues that coordinate the metal ions.

Currently, well over 100 three-dimensional structures of more than 20 legume lectins, without as well as with various carbohydrate ligands, have been elucidated by high-resolution X-ray crystallography (*21*). The legume lectin subunits, all of which are nearly superimposable, are in the shape of a dome. They consist largely of antiparallel sheets and possess a topology related to the jelly roll fold that is known as "the lectin fold" (*22*). The same fold has been observed in a number of other proteins from diverse sources, including the mammalian galectins.

The carbohydrate-binding sites of the legume lectins, similarly to those of other lectins (*23*), are in the form of shallow depressions on the surface of the protein. They are located at the top of each subunit and are accessible not just to monosaccharides but to oligosaccharides as well. The Ca^{2+} and Mn^{2+} are 4.25 Å apart and are in close proximity (9−13 Å) to the carbohydrate-binding site. The sites appear to be preformed because few conformational changes occur upon carbohydrate binding.

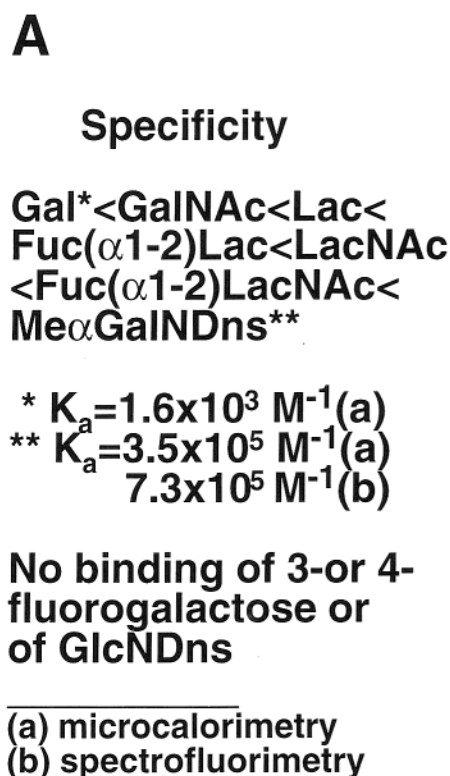
COMBINING SITE OF ECORL

Over the recent years, our laboratory has focused on the combining site of ECorL. The lectin is Gal/GalNAc specific and binds these sugars, as well as other ones, with association constants in the range of 10^2 M^{-1} to ∼5×10^4 M^{-1} (**Figure 1A**).

The high-resolution three-dimensional structure of the ECorL−lactose complex, in which only the galactose part of the ligand was clearly seen, was solved by Boaz Shaanan in collaboration with us (*28*) (**Figure 1B**) and further refined and extended in his laborarory to complexes with other sugars, including *N*-acetyllactosamine (LacNAc) (*29*). Information on the amino acids having side chains involved in ligand binding was also obtained by site-directed mutagenesis (*26*). On the basis of these results, in conjunction with data from the literature on related lectins, we concluded that in general legume lectins, irrespective of their specificity, bind monosaccharides through the side chains of a constellation of three invariant combining site residues: an aspartic acid, an asparagine, and an aromatic amino acid (phenylalanine, tryptophan, or tyrosine) (*23*). The only exceptions to this generalization are *Dolichos biflorus* lectin (DBL) (*30*) and PHA-L (*31*), in the combining site of which the invariant aromatic acid is replaced by leucine, and the *Maackia amurensis* lectins MAL and MAH, in which the conserved asparagine is replaced by aspartic acid (*20*). Glycine, the fourth amino acid that is invariant in the combining site of almost all legume lectins, participates in hydrogen bonding of the monosaccharide via its main-chain amide; it is replaced by arginine in concanavalin A (*32*) and by lysine in MAL and MAH (*20*). Whether this bond is essential for ligand complexation is not known.

In the ECorL−lactose complex, the hydrophobic surface formed by the C3−H, C4−H, and C5−H of galactose is stacked over the aromatic ring of Phe131 (**Figure 1B**). Stacking of the hydrophobic face of sugar rings on aromatic side chains is a common feature of the complexes with carbohydrates of other lectins, for instance, of the galectins, and certain bacterial toxins, such as *Escherichia coli* lytic toxin, and also of non-lectin proteins (*33*). Replacement of the aromatic amino acid that stacks against the carbohydrate with a smaller, nonaromatic residue (*26*, *34*) afforded an inactive protein, illustrating the essential role of this kind of interaction for ligand binding.

Coming back to the key H-bonding amino acid side chains, it was found that substitution, by site-directed mutagenesis, of Asp89 or Asn133 in ECorL by alanine resulted in loss of sugar-binding ability (*26*). It is in accord with the X-ray crystallography data that Asp89 forms H-bonds with the 3- and 4-OH of galactose in the combining site and that Asn133 is H-bonded with the 4-OH of this sugar, and also with the inability of the lectin to interact with 3- or 4-fluorogalactose. Mutation of asparagine and/or aspartic acid located at equivalent positions in other legume lectins, such as PHA-L (*35*), pea (*Pisum sativum*) lectin (PSL) (*36*), and *Griffonia simplicifolia* lectin II (34) abolished their activity. The key role of these two amino



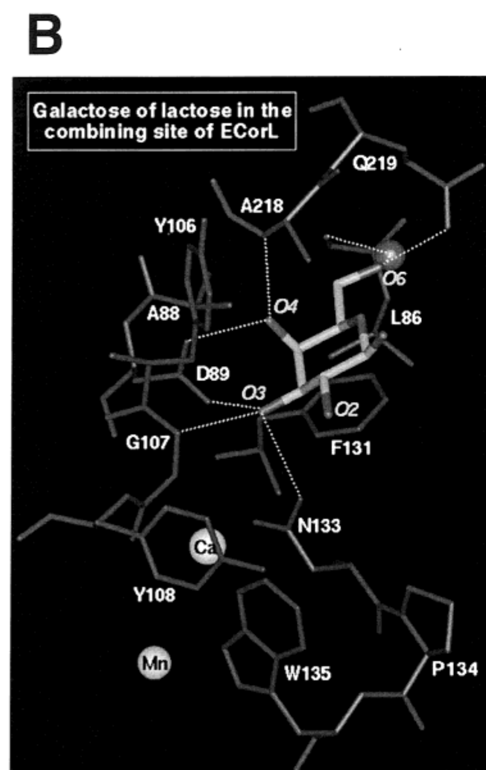


Figure 1. (A) Specificity of ECorL (*24–27* and unpublished results). (B) Model of galactose of lactose in the combining site of the lectin based on that of Shaanan et al. (*28*).

acids in ligand binding has been similarly demonstrated for the homologous, mannose-specific animal lectin MR60/ERGIC-53 (*37*).

The asparagine just mentioned also coordinates the Ca^{2+} present in all legume lectins and thus helps to position this residue in the correct place for ligand binding (*23*). The Ca^{2+} and the adjacent Mn^{2+} are both linked by coordination bonds to two conserved aspartic acid residues. Binding of the two metal ions is further stabilized by four conserved water molecules, two for each of the ions (*38*, *39*). One of the water ligands of the calcium ion forms a bridge with the carbonyl group of the carbohydrate-binding aspartic acid carboxylate. An additional characteristic of the combining site of legume lectins is the presence of a rare *cis*-peptide bond between this aspartic acid and the preceding amino acid, which is almost always alanine. This bond is stabilized by the metal ions and is required for the proper orientation of the aspartic acid in the combining site.

Water molecules serve not only for ligating the metal ions to the legume lectins but also for mediating the interactions of the lectins with sugars. Thus, in the ECorL−lactose complex (*25*), as well as in the complexes of the lectin with other ligands (*26*), a water molecule bridges between the 6-OH of galactose and the main-chain oxygen of Leu86.

DISCRIMINATION BETWEEN GALACTOSE AND ITS 4-EPIMER

The fact that the key amino acids involved in the binding of the carbohydrate are highly conserved in all legume lectins and have an identical spatial disposition raises the puzzling question of how they distinguish between galactose and its 4-epimer mannose (or glucose). Comparison of the three-dimensional structures of monosaccharide complexes of galactose and mannose/glucose-specific lectins revealed that this is achieved by positioning the ligand in the former lectins in a different

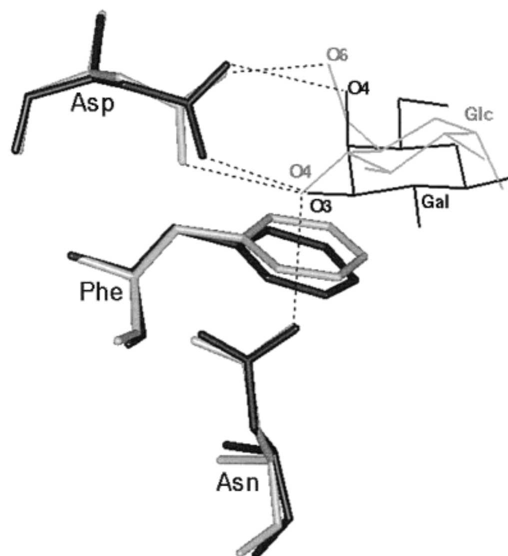


Figure 2. Superposition of β-galactose in the combining site of ECorL on α-glucose in the combining site of LOL-I. Note that the galactose is hydrogen-bonded to the conserved combining site residues via its 3- and 4-OH, whereas the glucose is bonded to the same residues via its 4- and 6-OH. The stacking of the ligands on the combining site phenylalanine of the respective lectins is also evident.

orientation than in the latter ones (**Figure 2**). For instance, in concanavalin A (*32*, *40*) and the lectins from *Lathyrus ochrus* (LOL-I) (*41*) and from lentil (*42*), mannose and glucose are oriented so that oxygens of the aspartic acid side chain are hydrogen-bonded to 6-OH and 4-OH of the monosaccharide, whereas the amide of the asparagine side chain forms such a bond to the 4-OH. In addition, the main-chain amide of glycine (or of arginine in the case of concanavalin A and of the other Diocleae lectins) hydrogen bonds with the 3-OH of the

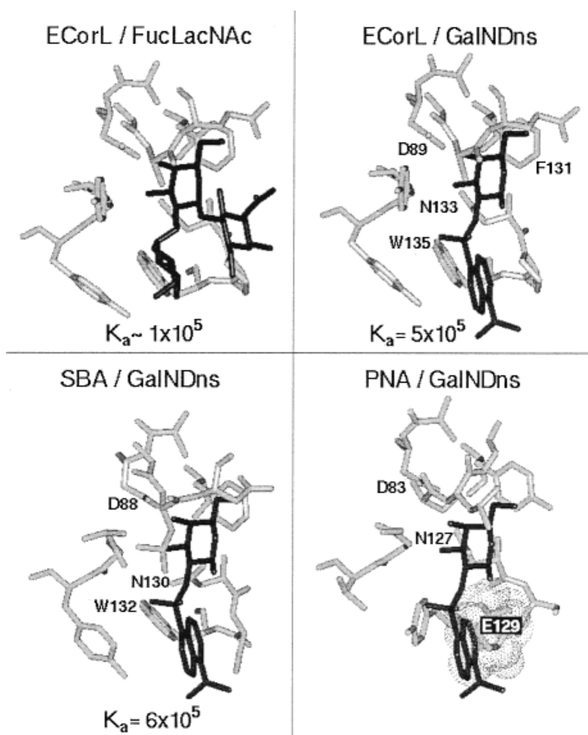


Figure 3. Models of of Fuc(α1-2)Gal(β1-4)GlcNAc in the combining site of ECorL and of *N*-dansylgalactosamine in the combining sites of ECorL, SBA and PNA.

monosaccharide. On the other hand, in lectins specific for galactose, such as ECorL (*25*, *26*), SBA (*43*), and PNA (*44*), the above oxygens of the combining site aspartic acid are H-bonded with the 4-OH and 3-OH, whereas the amide of the asparagine and the NH of glycine are both bonded to the 3-OH. In this way, the same constellation of three highly conserved residues in legume lectins, correctly positioned by the two metal ions either directly or via water molecules, provides the framework required for binding of diverse monosaccharides.

HIGH AFFINITY FOR *N*-DANSYLGALACTOSAMINE

Among the Gal/GalNAc-specific lectins, the ratio of affinities for the two sugars varies widely. Extreme cases are PNA, which does not bind *N*-acetylgalactosamine at all, and DBL, which has little if any ability to interact with galactose. Between are lectins such as ECorL, which binds *N*-acetylgalactosamine only ∼2 times better than galactose, and SBA, which binds the former monosaccharide some 30 times better than the latter. Both ECorL and the very similar *Erythrina cristagalli* lectin (ECL or ECA), as well as SBA, exhibit exceptionally high affinities for *N*-dansylgalactosamine, ∼250 times higher than for *N*-acetylgalactosamine in the case of the *Erythrina* lectins (*24*, *25*, *45*) and 20 times higher in the case of SBA (*46*); on the other hand, PNA does not bind *N*-dansylgalactosamine. The high affinity of the dansyl derivatives to ECorL and SBA is strictly sugar specific, because these lectins do not bind *N*-dansylglucosamine (H. Streicher, private communication).

Examination of the three-dimensional structure of ECorL made it clear that the ability of the lectin to accommodate galactose derivatives with bulky substituents at C-2 depends on the presence of a cavity of appropriate size facing this part of the galactopyranose ring (**Figure 3**) (*28*). A similar cavity is present in SBA (*41*), but in PNA it is partially blocked by the side chain of a glutamic acid (*47*). Evidence for this role of the glutamic acid was obtained in our laboratory before the three-dimensional structure of the PNA−carbohydrate complex was solved. Comparison of the primary structure of PNA with that of ECorL, together with the crystallographic data on the structure of the ECorL−lactose complex, suggested to us that in the former lectin two extra amino acids, glutamic acid and asparagine, are present that are not found in the region of the cavity of the latter lectin. Introduction of these two residues into recombinant ECorL, although decreasing only to a small extent (∼4 times) its affinity for *N*-acetylgalactosamine, diminished it markedly (up to some 40 times) for the much bulkier *N*-dansylgalactosamine (*23*). Molecular modeling showed that in the complex of ECorL with methyl α-*N*-dansylgalactosaminide the dansyl moiety of the ligand fits snugly into the cavity and lies close to, and in parallel with, the indole group of Trp135, resulting in the formation of a charge-transfer complex between the two (*37*). This could account in part for the high affinity of the *N*-dansyl derivatives for the lectin. However, other factors may also play a role, because replacement of Trp135 by alanine decreased this affinity no more than 10-fold and the resultant mutant still bound methyl α-*N*-dansylgalactosaminide 70 times better than galactose. Replacement by alanine or threonine of Tyr108, the other aromatic residue lining the cavity, affected only marginally this affinity, ruling out the possibility that the tyrosine interacts to any significant extent with the dansyl moiety.

COMPLEXES WITH OLIGOSACCHARIDES

Most lectins, whether from legumes or from other sources, have a higher affinity for oligosaccharides than for monosaccharides, suggesting the occurrence of additional interactions with the parts of the ligand located outside the monosaccharide-combining site. The amino acids involved in these interactions are not necessarily the same as the key residues discussed above. The higher affinity of ECorL for lactose (by a factor of ∼2) appears to be due to an H-bond between the amide of Gln219 and the 3-OH of the glucose moiety of the disaccharide. This was suggested on the basis of site-directed mutagenesis of ECorL (*26*) and confirmed by the refined X-ray cystallographic analysis of the ECorL−lactose complex (*29*). The above studies also led to the conclusion that an H-bond between the same glutamine and the carbonyl of the acetyl of *N*-acetyllactosamine may account for the 4−8 times higher affinity of the lectin for the latter disaccharide as compared to lactose.

Among all oligosaccharides tested, Fuc(α1-2)Gal(β1-4)-GlcNAc is the best natural ligand for ECorL [2−5 times better than *N*-acetyllactosamine (*25*, *48*)]. No crystal structure of the complex of the trisaccharide with ECorL is available, but molecular modeling revealed a perfect fit of the fucose in the cavity extending from the monosaccharide-binding site of the lectin (**Figure 3**). The amide of Asn133 that H-bonds with the 3-OH of galactose interacts similarly with the 2-OH and with the glycosidic oxygen of the fucose. The same fucose hydroxyl may also H-bond with the backbone NH of Gly107. The modeling indicated in addition such a bond between the 3-OH of fucose and the hydroxyl of Tyr108. Also, Pro134 and Trp135 lining the cavity make hydrophobic contacts with the CH_3 of this monosaccharide, as well as with its 2- and 3-OHs, and together with Tyr108 they close the binding site. The extensive network of bonds notwithstanding, the fucose makes only a small contribution to the affinity of the trisaccharide to the lectin. This contrasts with the much larger contribution of the dansyl group of *N*-dansylgalactosamine, which in the complex of the latter compound with the lectin occupies the same site as the fucose. Here we have an illustration of the fact that affinities

Table 2. Different Lectins Combine with the Same Ligand by Different H-Bonding Side Chains

ligand	lectin[a]	H-bonding residues	ref
mannose	Con A	Asn, Asp	see text
	GNL	Asn, Asp, Gln, Tyr	*50*
	MBP-A	Asn, Glu	*51*
galactose	ACA	Asn, His, Thr	*52*
	ECorL	Asn, Asp	see text
	galectin 1	Arg, Asn, His, Glu	*53*

[a] Con A, concanavalin A; GNL, *Galanthus nivalis* lectin; MBP-A, mannose-binding protein; ACA, *Amaranthus caudatus* agglutinin; ECorL, *Erythtrina corallodendron* lectin.

(or energetics) of protein−ligand interactions cannot be deduced from structural considerations (*49*).

COMPARISON WITH NONLEGUMINOUS LECTINS

Lectins other than those of the legumes also combine with carbohydrates, primarily by a network of hydrogen bonds and hydrophobic interactions. However, different amino acids are often employed for the same purpose, even for combination with the same ligand (**Table 2**). In fact, essentially all side chains capable of forming hydrogen bonds with sugars do so in one lectin−carbohydrate complex or another. Although carboxylic and carboxyamino acids are most common, basic and hydroxyamino acids, as well as aromatic ones, also participate in ligand binding. For instance, in the crystal structures of galectin 2 from human spleen in complex with lactose and of galectin 1 from bovine spleen in complex with *N*-acetyllactosamine, the side chains of three amino acids (histidine, asparagine, and arginine), invariant among all galectins sequenced, are hydrogen bonded to the 4-OH of the terminal, nonreducing galactose, and a likewise conserved tryptophan is stacked against the sugar ring; the 6-OH is also hydrogen bonded with the protein, but neither the 2-OH nor the 3-OH is.

In addition to the diversity of amino acids taking part in the complexation of carbohydrates, metal ions may in rare cases play a direct role in ligand binding, as found in the selectins (*54*). Because most saccharides are uncharged, ionic (charge−charge) interactions are not often found in lectin−saccharide complexes.

The above brief survey clearly demonstrates the great diversity of the carbohydrate-binding sites of lectins. This diversity strongly suggests that lectins are products of convergent evolution (*55*).

LITERATURE CITED

(1) Liener, I. E., Sharon, N., Goldstein, I. J.. Eds. *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Academic Press: Orlando, FL, 1986; 600 pp.

(2) Liener, I. E.; Pallansch, M. J. Purification of a toxic substance from defatted soy bean flour. *J. Biol. Chem.* **1952**, *197*, 29−36.

(3) Wada, S.; Pallansch, M. J.; Liener, I. E. Chemical composition and end groups of the soybean hemagglutinin. *J. Biol. Chem.* **1958**, *233*, 395−400.

(4) Liener, I. E. Inactivation studies on the soybean hemagglutinin. *J. Biol. Chem.* **1958**, *233*, 401−405.

(5) Liener, I. E. From soybeans to lectins: a trail of research revisited. *Carbohydr. Res.* **1958**, *213*, 1−5.

(6) Lis, H.; Sharon, N.; Katchalski, E. Isolation of a mannose-containing glycopeptide from soybean hemagglutinin. *Biochim. Biophys. Acta* **1964**, *83*, 376−378. Soybean hemagglutinin, a plant glycoprotein. I. Isolation of a glycopeptide. *J. Biol. Chem.* **1966**, *241*, 684−689.

(7) Lis, H.; Sharon, N.; Katchalski, E. Identification of the carbohydrate−protein linking group in soybean hemagglutinin. *Biochim. Biophys. Acta* **1969**, *192*, 364−366.

(8) Dorland, L.; van Halbeek, H.; Vliegenthart, J. F. G.; Lis, H.; Sharon, N. Primary structure of the carbohydrate chain of soybean agglutinin; a reinvestigation by high resolution ^{1}H-NMR spectroscopy. *J. Biol. Chem.* **1981**, *256*, 7708−7711.

(9) Lotan, R.; Siegelman, H. W.; Lis, H.; Sharon, N. Subunit structure of soybean agglutinin *J. Biol. Chem.* **1974**, *249*, 1219−1224.

(10) Lis, H.; Sela, B. A.; Sachs, L.; Sharon, N.; Sachs, L. Specific inhibition of *N*-acetyl-D-galactosamine of the interaction between soybean agglutinin and animal cell surfaces. *Biochim. Biophys. Acta* **1970**, *211*, 582−585.

(11) Sela, B. A.; Lis, H.; Sharon, N.; Sachs, L. Different locations of carbohydrate-containing sites in the surface membrane of normal and transformed mammalian cells. *J. Membrane Biol.* **1970**, *3*, 267−279.

(12) Reisner, Y.; Ravid, A.; Sharon, N. Use of soybean aagglutinin for the separation of mouse B and T lympocytes. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 585−1591.

(13) Reisner, Y.; Itzicovitch, L.; Meshorer, A.; Sharon, N. Hemopoietic stem cell transplantation using mouse bone-marrow cells fractionated by lectins *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 2933−2936.

(14) Nagler, A.; Morecki, S.; Slavin, S. The use of soybean agglutinin (SBA) for bone marrow (BM) purging and haematopoietic progenitor cell enrichment in clinical bone-marrow transplantation. *Mol. Biotechnol.* **1999**, *11*, 181−194.

(15) Adar, R.; Streicher, H.; Rozenblatt, S.; Sharon, N. Synthesis of soybean agglutinin in bacterial and mammalian cells. *Eur. J. Biochem.* **1997**, *249*, 684−689.

(16) Sharon, N.; Lis, H. The structural basis for carbohydrate recognition by lectins. *Adv. Exp. Med. Biol.* **2001**, *491*, 1−16.

(17) Sharon, N.; Lis, H. Legume lectins—a large family of homologous proteins. *FASEB J.* **1990**, *4*, 3198−3208.

(18) Van Damme, E. J. M.; Peumans, W. J.; Pusztai, A.; Bardocz, S. *Handbook of Plant Lectins: Properties and Biomedical Applications*; Wiley: Chichester, U.K., 1998; 452 pp.

(19) Loris, R.; Hamelryck, T.; Bouckaert, J.; Wyns, L. Legume lectin structure. *Biochim. Biophys. Acta* **1998**, *1383*, 9−36.

(20) Imberty, A.; Gautier, C.; Lescar, J.; Perez, S.; Wyns, L.; Loris, R. An unusual carbohydrate binding site revealed by the structures of two *Maackia amurensis* lectins complexed with sialic acid-containing oligosaccharides. *J. Biol. Chem.* **2000**, *275*, 17541−17548.

(21) Bettler, E.; Loris, R.; Imberty, A. 2001 3D lectin database, www.cermav.cnrs.fr/databank.lectine.

(22) Srinivasan, N.; Rufino, S. D.; Pepys, M. B.; Wood, S.; Blundell, T. L. A superfamily of proteins with the lectin fold. *Chemtracts Biochem. Mol. Biol.* **1996**, *6*, 149−164.

(23) Lis, H.; Sharon, N. Lectins: carbohydrate-specific proteins that mediate cellular recognition. *Chem. Rev.* **1998**, *98*, 637−674.

(24) Kinzy, W.; Belenky, D. M.; Loontiens, F. G.; Sharon, N. Synthesis of methyl α- and *β*-*N*-dansylgalactosaminides, probes for the combining sites of GalNAc-specific lectins. *Glycoconjugate J.* **1992**, *9*, 225−227.

(25) Arango, R.; Rodriguez-Arango E.; Adar, R.; Belenky, D.; Loontiens, F. L.; Rozenblatt, S.; Sharon, N. Modification by site-directed mutagenesis of the specificity of *Erythrina corallodendron* lectin for galactose derivatives with bulky constituents. *FEBS Lett.* **1993**, *330*, 133−136.

(26) Adar, R.; Sharon, N. Mutational studies of the combining site residues of *Erythrina corallodendron* lectin. *Eur. J. Biochem.* **1996**, *239*, 668−674.

(27) Moreno, E.; Teneberg, S.; Adar, R.; Sharon, N.; Karlsson, K.-A.; Ångström, J. Re-definition of the carbohydrate specificity of *Erythrina collarodendron* lectin based on solid-phase binding assays and molecular modeling of native and recombinant forms obtained by site-directed mutagenesis. *Biochemistry* **1997**, *36*, 4429−4437.

(28) Shaanan, B.; Lis, H.; Sharon, N. Structure of a lectin with ordered carbohydrate, in complex with lactose. *Science* **1991**, *254*, 862−866.

(29) Elgavish, S.; Shaanan, B. Structures of the *Erythrina corallodendron* lectin and its complexes with mono- and disaccharides. *J. Mol. Biol*. **1998**, *277*, 917−932.

(30) Hamelryck, T. W.; Loris, R.; Bouckaert, J.; Dao-Thi, M.-H.; Strecker, G.; Imberty, A.; Fernandez, E.; Wyns, L.; Etzler, M. E. Carbohydrate binding, quaternary structure and a novel hydrophobic binding site in two legume lectin oligomers from *Dolichos biflorus*. *J. Mol. Biol*. **1999**, *286*, 1161−1177.

(31) Hamelryck, T. W.; Dao-Thi, M.-H.; Poortmans, F.; Chrispeels, M. J.; Wyns, L.; Loris, R. The crystallographic structure of phytohemagglutinin-L. *J. Biol. Chem.* **1996**, *271*, 20479−20485.

(32) Derewenda, Z.; Yariv, J.; Helliwell, J. R.; Kalb (Gilboa), A. J.; Dodson, E. J.; Papiz, M. Z.; Wan, T.; Campbell, J. The structure of the saccharide-binding site of concanavalin A. *EMBO J.* **1989**, *8*, 2189−2193.

(33) Rini, J. Lectin structure *Annu. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 551−577.

(34) Zhu, K.; Bressan, R. A.; Hasegawa, P. M.; Murdock, L. L. Identification of *N*-acetylglucosamine binding residues in *Griffonia simplicifolia* lectin II. *FEBS Lett.* **1996**, *390*, 271−274.

(35) Mirkov, T. E.; Chrispeels, M. J. Mutation in of Asn128 to Asp of *Phaseolus vulgaris* leucoagglutinin (PHA-L) eliminates carbohydrate-binding and biological activity. *Glycobiology* **1993**, *3*, 381−387.

(36) Van Eijsden, R. R.; De Pater, B. S.; Kijne, J. W. Mutational analysis of the sugar-binding site of pea lectin. *Glycoconjugate J.* **1994**, *11*, 375−380.

(37) Roche, A.-C.; Monsigny, M. Trafficking of endogenous glycoproteins mediated by intracellular lectins: facts and hypotheses. *Chemtracts Biochem. Mol. Biol.* **1996**, *6*, 188−201.

(38) Loris, R.; Stas, P. P. G.; Wyns, L. Conserved waters in legume lectin crystal structures. The importance of bound water for the sequence-structure relationship within the legume family. *J. Biol. Chem*. **1994**, *269*, 26722−26733.

(39) Adar, R.; Ångström, J.; Moreno, E.; Karlsson, K. A.; Streicher, H.; Sharon, N. Structural studies of the combining site of *Erythrina collarodendron* lectin. Role of tryptophan 135. *Protein Sci.* **1998**, *7*, 52−63.

(40) Bradbrook, G. M.; Gleichmann, T.; Harrop, S. J.; Habash, J.; Raftery, J.; Kalb (Gilboa), J.; Yariv, J.; Hillier, I. H.; Helliwell, J. R. X.-Ray and molecular dynamic studies of concanavalin-A glucoside and mannoside complexes. *J. Chem. Soc., Faraday Trans*. **1998**, *94*, 1603−1611.

(41) Bourne, Y.; Roussel, A.; Frey, M.; Rougé, P.; Fonntecilla-Camps, J.-C.; Cambillau, C. *Proteins: Struct., Funct. Genet*. **1990**, *8*, 365−376.

(42) Loris. R.; Casset, F.; Bouckaert, J.; Pletinckx, J.; Dao-Thi, M.-H.; Poortmans, F.; Imberty, A.; Perez, S.; Wyns, L. The monosaccharide binding site of lentil lectin: an X-ray and molecular modeling *Glycoconjugate J.* **1994**, *11*, 507−517.

(43) Dessen, A.; Gupta, D.; Sabesan, S.; Brewer, C. F.; Sacchettini, J. C. X-ray crystal structure of soybean agglutinin cross-linked with a biantennary analogue of the blood group I carbohydrate antigen. *Biochemistry* **1995**, *34*, 4933−4942.

(44) Banerjee, K.; Das, K.; Ravishankar, R.; Suguna, K.; Surolia, A.; Vijayan, M. Conformation of protein-carbohydrate interactions and a novel subunit association in the refined structure of the peanut lectin−lactose complex. *J. Mol. Biol.* **1996**, *259*, 281−296.

(45) De Boeck, H.; Loontiens, F. G.; Lis, H.; Sharon, N. Binding of simple carbohydrates and some *N*-acetyllactosamine-containing oligosaccharides to *Erythrina cristagalli* agglutinin as followed with a fluorescent indicator ligand. *Arch. Biochem. Biophys.* **1984**, *234*, 297−304.

(46) DeBoeck, H.; Lis, H.; van Tilbeurgh, H.; Sharon, N.; Loontiens, F. G. Binding of simple carbohydrates and some chromophoric derivatives of soybean agglutinin as followed by titrimetric procedures and stopped-flow kinetics. *J. Biol. Chem.* **1984**, *259*, 7067−7074.

(47) Sharma, V.; Srinivas, V. R.; Adhikari, P.; Vijayan, M.; Surolia, A. Molecular basis of recognition by Gal/GalNAc specific lectins: influence of Glu129 on the specificity of peanut agglutinin (PNA) towards C2-substituents of galactose. *Glycobiology* **1998**, *8*, 1007−1012.

(48) Lemieux, R. U.; Ling, C. C.; Sharon, N.; Streicher, H. The epitope of the H-Type trisaccharide recognized by *Erythrina corallodendron* lectin. Evidence for both attractive polar and strong hydrophobic interactions for complex formation. *Isr. J. Chem.* **2000**, *40*, 167−176.

(49) Janin, J. Ångstroms and calories. *Structure* **1997**, *3*, 473−479.

(50) Hester, G.; Kaku, H.; Goldstein, I. J.; Wright, C. S. Structure of mannose-specific snowdrop (*Galanthus nivalis*) lectin is representative of a new plant lectin family. *Nat. Struct. Biol.* **1995**, *2*, 472−479.

(51) Weis, W. I.; Drickamer, K.; Hendrickson, W. A. Structure of a C-type mannose-binding protein complexed with an oligosaccharide. *Nature* **1992**, *360*, 127−134.

(52) Transue, T. R.; Smith, A. K.; Mo, H.; Goldstein, I. J.; Saper, M. A. Structure of benzyl T-antigen disaccharide bound to *Amaranthus caudatus* agglutinin. *Nat. Struct. Biol.* **1997**, *4*, 779−782.

(53) Lobsanov, Y. D.; Gitt, M. A.; Leffler, H.; Barondes, S. H.; Rini, J. M. X-Ray crystal structure of the human dimeric S-lac lectin, L-14-II, in complex with lactose at 2.0 Å resolution. *J. Biol. Chem.* **1993**, *268*, 27034−27038.

(54) Weis W.; Drickamer K. Structural basis of lectin-carbohydrate recognition. *Annu. Rev. Biochem*. **1996**, *65*, 441−473.

(55) Drickamer, K. Two distinct classes of carbohydrate-recognition domains in animal lectins. *J. Biol. Chem.* **1998**, *263*, 9557−9560.

Received for review February 12, 2002. Revised manuscript received April 25, 2002. Accepted April 29, 2002.

JF020190S