REVIEW

Carbohydrate/Glycan-Binding Specificity of Legume Lectins in Respect to Their Proposed Biological Functions

Márcio Viana Ramos[1*], Thalles Barbosa Grangeiro[1], Benildo Sousa Cavada[1], Iain Shepherd[2], Roberval Oliveira de Melo Lopes[1] and Alexandre Holanda Sampaio[1]

[1]Laboratório de Moléculas Biologicamente Ativas (BioMol-Lab). Universidade Federal do Ceará, Campus do Pici, Caixa Postal 6033-CEP 60451-970. Fortaleza-Ce, Brazi; [2]Department of Biochemistry, Irvine Building, University of St. Andrews, St. Andrews, Fife, Scotland

ABSTRACT

The lectins, proteins which specifically recognize carbohydrate moieties, have been extensively studied in many biochemical and structural aspects in order to establish the molecular basis of this non-catalytic event. On the other hand, their clinical and agricultural potentials have been growing fast. Although lectins, mainly those from legume plants, had been investigated for biological properties, studies about the physiological functions of lectins are scarce in literature. Therefore, despite the accumulated data on lectins (as proteins), the role played by these signalizing molecules is poorly discussed. In the light of our accumulated results on legume lectins, specially those obtained from plants belonging to the Diocleinae sub-tribe and available data in literature, we discuss here the main hypothesis of their functions according to their carbohydrate/glycan-binding specificity.

Key Words: Binding-site; biological functions; fine sugar-specificity; legume lectins

INTRODUCTION

Lectins are generically defined as proteins which interact non-covalently with carbohydrate moieties, displaying high affinity and specificity for their ligands. At the present, a large number of lectins have been isolated and their biochemical characteristics established. Recently, an increasing number of three-dimensional structures of plant lectins have been solved from experimental analysis by x-ray diffraction (Bourne *et al*., 1990a; Hamelryck *et al.*, 1999; Chandra *et al.*, 1999) and others are in progress (Calvete *et al.*, 1999). Furthermore, the co-crystallization of some of these proteins with specific ligands has also given information towards the understanding of the interaction between lectins with simple and complex carbohydrates, revealing which amino acids residues are involved in the interaction between molecular partners. Therefore more properly establishing the monosaccharide binding-sites of these proteins (Bourne *et al.*, 1990b; Shaanan *et al.*, 1991; Delbaere *et al.*, 1993; Naismith *et al.*, 1996).

According to their specificity, lectins have been classified as glucose/mannose, N-acetylglucosamine, galactose/N-acetylgalactosamine and fucose-binding specific. Recently, a new group of highly mannose specific lectins was described. The mannose specific lectins constitute a growing group of proteins from *Monocotyledoneae* plants, whose interaction is specially towards mannose, a fact not observed in glucose/mannose lectins (Chandra *et al.*, 1999). In addition, lectins have been demonstrated to interact with syalic acid and some derivatives, although glucose and galactose specific lectins also show ability to interact with this acid (Konami *et al.*, 1994).

More recently, increasing efforts have been made to establish the physiological role of plant lectins and two distinct hypothesis has been placed on the evidence to explain how lectins are involved in the metabolism of their native plants (Van Damme *et al.*, 1998). The first one takes into consideration

* Author for correspondence

the possible involvement of some lectins as molecules of recognition in leguminous plants, displayed in the complex establishment of symbiosis with bacteria *rhizobia* (Diaz *et al.*, 1989). The second describes lectins as defense proteins protecting plants against infections or physical attack caused by microrganisms and predators (Cavada *et al*., 1993). Although these hypothesis have received great adhesion by lectinologists, many questions remain to be solved. How could legume lectins play similar roles when they show broad monosaccharide-binding specificities and thus, how can specificity and functions be correlated? On the other hand, some non legume lectins which are theoretically involved in plant defense usually have similar specificities and related structural domains which provide strong relationships between carbohydrate specificity and function (Cavada *et al.*, 1993). It had also been suggested that lectins are proteins able to recognize foreign molecules or organisms (Ayouba *et al.*, 1992). Although no conclusive data has been obtained in this way, a consensus is accepted independently of the specificity expressed by the lectin that their functions would be in accordance to their ability to interact with specific ligands.

It is now clear that the similarities observed in the tertiary structures of legume lectins are larger than the homology in their amino acid sequences. In fact, legume lectins seem to form an homo- or polyfunctional family of proteins sharing a common three-dimensional folding of their functional subunit, although their quaternary arrangement to form dimmer and tetramer is quit different. Thus, new evidence suggests that conserving the general features of their 3-D structures, legume lectins may be mainly evolved and diverged in the combining-site region to exhibit different specificities, therefore, play specific role in plant metabolism.

In the light of the recent discussion of the lectin definition and new insight achieved by structural and functional studies on the legume lectin monosaccharide/glycan binding-site, we discuss some aspects of lectin functions comparing the amino acid composition of their monosaccharide-binding site and the recent concept of *extended binding site* with the main hypothesis pointing to their functions.

Lectins in the life cycle of leguminous plant

Although relevant information about plant lectins is available, the function of lectins in plants remains without a conclusive statement. Some functions were earlier suggested and investigated. The role of storage or transport proteins was firstly proposed as many legume plants investigated were shown to possess high levels of lectins located in the protein bodies in the seeds, on one hand, and these proteins interacted with sugars, on the other (Etzler, 1986). However no further evidence supported this hypothesis. Einhoff and colleagues proposed that lectins should be involved in the packaging of storage proteins, since various lectins from *Leguminosae* tested, were able to interact with storage proteins belonging to their own plants (Einhoff *et al.*, 1986). Recent results from Wenzel and co-workers (Wenzel & Rudiger, 1995) showed that, pea lectin does interact with vicilin and legumin fraction from the protein bodies of pea seeds, suggesting that the protein body membranes might be a further candidate for lectin interaction (Gers-Barlag *et al.*, 1993). Similar observations were previously obtained from soybean lectins and their storage proteins (Rudiger & Schecher, 1993). Since, up to date, no significant data is available about glycosilation of storage proteins in plants any specific ligand could be attributed to these lectins in seeds. Although it was not emphasized by these authors, a possible role in the maintenance of the protein body structure could be suggested. The search to determine the exactly moment of appearance of the lectin in protein bodies during the development of seeds should be appreciated in the attempt to establish if a parallelism exists between storage protein deposition and lectin appearance in the establishment of protein bodies.

The results from our studies with *Diocleinae* lectins during seeds germination showed that the mobilization of these glucose/mannose specific lectins are always retarded when compared to storage proteins, independently, of the seedlings being grown in presence or absence of light (Moreira & Cavada, 1984; Cavada *et al.*, 1990; Cavada *et al.*, 1994). Indeed, traces of these lectins could be detected until the last day of examined germination, when a small amount of other proteins were present in cotyledons (Cavada *et al.*, 1994). When germinated in the light, again, seed lectins were preserved although high molecular weight proteins were mobilized to establish the

seedlings (Cavada *et al.*,1990). During maturation of *Canavalia brasiliensis* seeds, the lectin was detected only after high molecular weight protein appearance (Moreira *et al.*, 1993). A lectin precursor that bound to polydextran was detected previously, displaying extremely weak hemagglutinating activity. If the lectin is synthesized concomitantly to the storage proteins, perhaps it is accumulated as a pre-protein, not completely active and could become active in a specific stage of seed development. It should be taken in account that many of these lectins investigated are from a closely related group of plants and one could reasonable consider these results are of low significance. However, recently a lectin from a distantly related taxonomic group of *Leguminosae* belonging from the *Erythrinae* tribe, in which all lectins investigated are galactose-specific, showed similar behavior when seeds were challenged to germinate in absence of light (Oliveira *et al.*, 1998).

Considering all these results, legume lectins may be involved in the protein body structure, but it does not implicate that they are storage proteins. Indeed, results of these two different approaches are in agreement that lectins are unlikely to be storage proteins although a correlation between an endogenous receptor, specificity and functions could not be evaluated for them. If legume lectins may support protein body membranes, the lectins from these two groups which exhibit differences in their monosaccharide-specificity should share some other molecular ability to act in a similar form. In fact, pioneer studies on the complex binding-specificity of lectins, elegantly showed that lectins possessing differences in monosaccharide-specificity could interact with the same complex glycan which will be discussed later (Debray *et al.*, 1981).

Lectins as a recognition factor in leguminous plant

Leguminoseae is the main source of all lectins isolated and characterized so far (Van Damme *et al.*, 1998). Also, within this family are the greater number of the lectins for which three-dimensional structures have been described (Mourey *et al.*, 1998) (Table I). Although these proteins belong to the same taxon and present many common biochemical characteristics, they exhibit different monosaccharide/glycan binding specificities and quaternary arrangement (Debray *et al.*, 1981). A proposed role for *Leguminoseae* lectins as molecules of recognition in the initial events of symbiosis between the *Leguminoseae-Rhizobium* interaction arose from the observation of the strong specificity expressed between plant and microrganism to establish functional symbiotic nodules and the detection of lectins in root hair of various leguminous plants (Kijne *et al.*,1992). Rhijn *et al.* (1998) using trangenic *Lotus corniculatus* plants expressing soybean lectin gene observed the involvement of legume lectins in the symbiont process. This hypothesis became more attractive after the discovery that *Rhizobium* bacteria produced an extra-cellular factor known to be involved in the initial events of symbiosis, which always contained a carbohydrate moiety in its structure and could eventually interact with root hair lectin (Lerouge *et al.*,1990). Indeed, these lipo-oligosaccharide signalizing molecules, named NOD factors, synthesized as a result of the expression of rhizobial *nod* genes, a key factor hosts in the initial events of symbiosis (Rhijn *et al.*, 1998). Therefore, the ability of a NOD factor to induce root-hair curling and infection, leading to nodule formation, have been demonstrated (Lerouge *et al.*, 1990).

More incisive data to attribute legume lectins with the role of recognition in symbiosis came from work of Diaz *et al* (1989) who showed that trangenic roots of white-clover expressing the pea lectin gene could be noduled by *R. leguminosarum*, a specific symbiont of pea roots, although no true functioning nodules were obtained. Later, Fabre *et al.* (1994) showed through molecular modelling and docking experiments that the legume lectin from *Lathyrus ochrus* could interact with NOD factor expressed by *R. leguminosarum* bv. *vicieae*. All these observations have supported the legume lectins as possible candidates to recognize the NOD factor *in vivo*.

Table 1. Lectins from *Leguminoseae* family distributed in various tribes showing specificities for different monosaccharides.

Tribe or sub-tribe	Species	Specificity
Diocleinae	*Canavalia ensiformis*	Man/Glc
	C. brasiliensis	Man/Glc
	Dioclea grandiflora	Man/Glc
	Cratylia floribunda	Man/Glc
Hedysareae	*Onobrychis vicifolia*	Man/Glc
Dalbergieae	*Vatairea macrocarpa*	Gal
Vicieae	*Pisum sativum*	Man/Glc
	Vicia faba	Man/Glc
	V. villosa-B4	GalNAc
	Lens culinaris	Man/Glc
	Lathyrus ochrus	Man/Glc
Genisteae	*Crotalaria striata*	GalNAc
	Ulex europaeus I	L-Fucose
	Ulex europaeus II	GlcNAc
Trifolieae	*Medicago sativa*	Gal
	M. truncatulata	Man/Glc
	Trifolium repens	Gal
Glycineae	*Glycine max*	GalNAc
Phaseoleae	*Phaseolus vulgaris*	Complex
	Dolichos biflorus	GalNAc
	Arachis hypogaea	Gal
Hedysareae	*Onobrychis vicifolia*	Glc/Man
Shophoreae	*Shophora japonica*	GalNAc
Erytrineae	*Erythrina velutina*	Gal
Lotae	*Lotus tetragonolobus*	L-Fucose
Bauhinieae	*Griffonia simplicifolia*	GalNAc
	Bauhinia purpurea	GalNAc
Parkieae	*Parkia platycephala*	Man/Glc

The NOD factors are lipo-oligosaccharide signal molecules structurally organized as tri-, tetra- or pentasaccharides of *N*-acetylglucosamine, generally sulfated at C-6 position in the reducible termini and modified by the presence of an acylated *N*-acetylglucosamine termini (Lerouge *et al.*, 1990). Recently, different NOD factors of other symbiotic bacteria have been isolated and characterized. These compounds show similar structural features and only few changes in general structure are observed (Spaink *et al.*, 1995). Some symbiotic bacteria, which have had NOD factors isolated and their structure determined, are listed in Table 2, associated to the specific host-plant.

Although different pieces of evidence pointed some legume lectins as biological agents to interact with NOD factors, how could the role of its specific recognition be attributed to these proteins; whether *Leguminoseae* family possesses a large number of lectins exhibiting a broad sugar specificity on one hand, and NOD factor studied until now, exhibit only a *N*-acetylglucosamine backbone in its structure? As non interaction apparently occurs between research groups investigating lectins and NOD factor, this question remains obscure.

Few lectins used to test this hypothesis were from the group glucose/mannose specific, which also interact with *N*-acetylglucosamine. Indeed, although some legume lectins such as *Ulex europaeus* lectin-I, *Arachis hypogaea* and *Erythrina corallodendron* are well studied, their physiological functions have not been frequently questioned and there are no comments present to relate these lectins to NOD factor. These lectins have fucose, galactose and galactose specificity, respectively. On the other hand, the symbiosis process was extensively studied in *Medicago sativa* and *Glycine max*, which expressed galactose specific lectins. They are noduled by *R. melioti* and *R. fredii*, respectively, which produce NOD factors without galactose or N-acetyl galactosamine units (Kijne *et al.*, 1992). Besides this, no effort has been made to provide the lectin interaction with NOD factor, expressed by their respective symbiotic bacteria.

In *Bradyrhizobium japonicum* - *Glycine max* system, the lectin recognition hypothesis failed in the analysis of interaction between soybean root lectin and its symbiont. Strong evidence showed that a lectin isolated from *B. japonicum* surface, named BJ38, might be responsible for the attachment of bacteria to soybean roots (Kijne *et al.*, 1992). Recently, the hypothesis of lectin mediating symbiosis was again tested using transgenic plants expressing the soybean galactose-specific lectin (Rhjin *et al.*, 1998). However, they showed that the purified or synthetic *Bradyrhizobium japonicum* NOD factor induced nodule formation on both transgenic *Lotus corniculatus* expressing soybean lectin gene (*Le1*) or nontrasgenic *L. corniculatus*, suggesting that lectin could change the host specificity of *L.corniculatus* in a NOD-factor independent way. This result point out the hypothesis that the lectin could be a specific NOD-

factor receptor in the host plant. Although the involvement of legume lectin in symbioses remains in evidence, this possible biological activity still requires more detailed investigations as at the moment the sugar binding specificity of lectins seems to be not related to this specific function.

Table 2. Specificity of nodulation expressed by host-plants and symbiotic bacteria of known NOD factor structure.

Specie or biovar (bv)	Host-plants	+/-
Rhizobium leguminosarum	*Lathyrus*	+
Foreiers et al. (1981)	*Lens*	+
	Pisum	+
	Viciae	+
Rhizobium meliloti	*Melilotus**	Nd
Spaink et al. (1995)	*Medicago*	–
	*Trigonella**	Nd
Rhizobium fredi	*Glicine*	–
Spaink et al. (1991)	*Vigna*	–
Rhizobium sp. NGR234	*Macroptilum**	Nd
Bec-Ferté et al. (1992)		
Rhizobium loti	*Lotus corniculatus*	Nd
Bradyrhizobium japonicum	*Glicine*	–
Price et al. (1992)	*Vigna*	–
Rhizobium etli	*Phaseolus*	Nd
Azorhizobium caulinodans	*Sesbania**	Nd
"Cowpea" rhizobia	Wide range of tropical legumes	Nd

*- Plants which lectins have not been isolated, nd - not determined (+/-) the monosaccharide inhibitor of the lectin is present or absent in the structure of NOD factor

Assuming that the ability to specifically recognize a receptor moiety is related to function, only limited replacement of amino acids in the binding-site of legume lectins was permitted during evolution to conserve the same specificity. A good example is ConA and DGL lectins, compared to *Vicieae* lectins. Phenylalanine and glycine residues, completely conserved within *Vicieae* lectins, correspond to arginine and tyrosine, respectively in *Diocleinae* lectins although the same specificity was conserved. On the other hand, regarding LOL and EcorL, the changing of three amino acids in the binding-site led to a new specificity, although the two lectins showed very similar three-dimensional folding and the spatial arrangement of the amino acids in the binding-site were almost identical. In the case of LOL and PSL, which exhibited the same amino acids in the binding-site and have the same specificity, the monosaccharide, mannose, was directly bound by a network of hydrogen bonds in different forms (Eijsden *et al.*, 1994). Thus, one may presume that apparently major changes of features in amino acid composition of the carbohydrate binding-site may lead to different specificity and consequently to differential recognition too. Considering all these facts, what could be the role played by fucose or Gal/GalNAc specific legume lectins in their native plants and what is the actual significance of a particular lectin within its plant? Whether legume lectins play a role of recognition in the initial phase of symbiosis process, perhaps different specificity represent a strategy to interact with specific symbionts in a specific way, mediated or not by NOD factor, thus assuring a highly specific recognition. Presently, this hypothesis constitutes a challenge to be demonstrated. The evidence presented by Ho and Kijne (Kijne *et al.*, 1992) in soybean-*Bradyrhizobium* system and pea-*Rhizobium leguminosarum* system pointed out this and an increasing number of results showed the involvement of lectins in the symbiosis process, although their role had been almost undetermined.

Spaink *et al.* (1995) have recently suggested that the recognition of NOD factors by the host legume could be related to the different hydrophobicities exhibited by fatty acids present in NOD factor structure, thus opening the question if the chitin unit from NOD factors plays same role in the recognition process. Presently there are only a few available structures of NOD factors produced by symbiotic bacteria, although a new NOD factor showing an additional *O*-metyl-fucoside moiety in its structure was reported (Sanjuan *et al.*, 1992; Cohn *et al.*, 1998). Many others could be isolated and their structures established, especially in bacteria which interact with legume plants which do not express glucose/mannose lectins and the actual interaction between lectin and NOD factor (if possible) should be evaluated.

The set of amino acid shown in Table 3 also suggested that a well conserved triad Asp-Asn-Gly present in almost sequences was responsible for carbohydrate recognition despite the monosaccharide specificity. Although Gly is replaced by Arg in ConA and other *Diocleinae* lectins the later plays similar role in the complex with mannose or glucose. A critical study on the molecular basis of legume lectin carbohydrate/ glycan interaction has showed that the monosaccharide specificity of this lectins is

determined by a loop length and conformation corresponding to Thr-97 to Glu-102 in ConA (Thr216 to Glu 224 in EcorL and Thr-28α to Ala-33α in the lectins from pea, lentil and *Lathyrus*) (Sharma & Surolia, 1997; Loris *et al.*, 1998). The results suggested that both length and conformation of this loop defined the monosaccharide-specificity of legume lectins. In this view, differences in monosaccharide specificity became more interesting and attractive. Studies have been shown that affinity of these lectins for oligosacchaides was still stronger Brewer & Gupta, 1994). Debray (1981) interpreted these data structurally. The complexity of lectin-glycan interaction have been attributed to the existence of an extended-binding site surrounding the monosaccharide site, including few additional residues on the lectin that formed addition hydrogen bounds directly or *via* water with the sugar units in the glycan structure (Imberty & Perez, 1994; Bourne *et al.*, 1994; Dessan *et al.*, 1995). Although the molecular basis of this phenomena has been investigated, its biological relevance has not been considered.

Legume lectins as defense proteins

The genetic program of plant defence against a broad wild spread predator has been investigated by many different groups. Not surprisingly, almost of these genetic programs seem to be built up of a highly integrated system involving closely related proteins possessing very precise function whose sometimes work on a cooperative or complementary way. It is now understood that some plants synthesizes defense proteins only under adverse conditions as salt or water stress and fungi or insect attack.

Within legume lectins already studied, ConA and other ConA-like lectins were shown to protect artificial seeds against the beetle *Callosobruchus maculatus* (Gatehouse *et al.*, 1995). However, it is not certain that the intrinsic toxic activity which leads insect to die or to a delay in its development is due the carbohydrate-binding activity of the lectins. The lectins from seeds of *Canavalia brasiliensis*, *Dioclea grandiflora*, *D. rostrata* and *Cratylia floribunda* which have identical monosaccharide specificity (Glc/Man) exhibit differences when tested to *C. maculatus* (Gatehouse *et al.*, 1995). The former being totally ineffective and the two latter being toxic. Although at present any structural basis could explain the ineffectiveness of two of these lectins, the toxic effects of the other have been credited to the ability of the lectins to binding to membrane glycoproteins in mouth and/or epithelial cells in the mid-gut inducing changes in performance of feed. Recently, Zhu-Salzman and colleagues (1998) reported the carbohydrate binding and resistance to proteolysis control insecticidal activity of *Griffonia simplicifolia* lectin II in order to investigate its toxic activity. In the case of ConA which does not appear to be toxic to mammals, at least at low levels of dietary inclusion, its gene could be used in an transgenic plant project against *C. maculatus*. However, the first lectin reported to possess insecticidal activity was PHA. It was attributed the inability of the cowpea bruchid beetle, *C.maculatus*, to attack the seeds of *Phaseolus vulgaris* due the presence of PHA, the haemagglutinating lectin present in the seeds. Nevertheless, many years latter, the toxic effects were more attributed to the isecticidal protein arcelin and to α-amylase inhibitor, two lectin-like proteins occurring in *Phaseolus vulgaris* seeds exhibiting sequence homology to PHA (Mirkov *et al.*, 1994; Schroeder *et al.*, 1995; Fabre *et al.*, 1998). The PHA protein family seems to be a classical example of a integrated defense system which each protein has a precise role to protect seeds, although undefined yet. Structural studies of arcelin, PHA and α-amylase inhibitor have proved that these proteins share very similar three-dimensional folding but differing in quaternary structure and in their active binding site whose are ready distinct (Bompard-Gilles *et al.*, 1996; Hamelryck *et al.*, 1999; Mourey *et al.*, 1998). Once, the activity and function do seem to be defined by the combine site-region. Apart of present status of the investigation about the lectins as tools in crop biotechnology, a condition sine qua non for their use is the test to engineered plant for resistance in field. In this way, some non legume plants have already been used (Rao *et al.*, 1998).

A set of amino acid to define the monosaccharide specificity

In a view of the understanding of the role of legume lectins under the symbiosis establishment or any other activity, the differences in their active combining site and specificity for carbohydrate should be considered. As shown in Table III the set of amino acid which compose the monosaccharide binding-site of legume lectins

present some interesting features. Although the well conserved triad Asp-Asn-Gly support the saccharide in the site, the other residues have been not strongly conserved during evolution. The replacement of amino acid residues is unlikely to have generated different specificities among the lectins as the contribution of these semi-conserved and non conserved residues is made by hydrogen bonds established with the sugars *via* the CO and NH groups or architecture well conserved (Loris *et al.*, 1998). Nevertheless, minor differences may change the affinity of lectins by structurally related ligands. Lectins from the same group (Gal/GalNAc) can interact
more strongly with a sugar derivative than with the simple monosaccharide as demonstrated by, EcorL and DBL both specific for galactose but DBL binds more tightly to *N*-acetyl-galactosamine. This phenomena is also true to the lectins from *Diocleinae* which are similar over 90% by pairwise alignment (Ramos *et al.*, 1996). Although the lectins are gifted of at least a monosacharide-binding site, it is reasonable to suppose that their biological ligand are larger than a single monosaccharide. Indeed, lectins not only bind to oligosacchaides, but this interaction is tighter than that to their monosacchaide inhibitor (Konami *et al.*, 1994; Do & Lee, 1998; Dam *et al.*, 1998). Although the ability to interact with oligosacchaide had been proved early, the first complex of a lectin with a complex glycan was established later by crystallographic studies (Bourne *et al.*, 1990b, 1994; Naismith *et al.*, 1996).

These crystallography works clearly identified the involvement of additional amino acid residues in the vicinity of the monosaccharide-binding site, with the glycans complexes. The hydrogen bounds occurring between the glycans and other amino acid residues (also mediated by water) are responsible for the correct anchorage of the larger receptor and by the magnitude of the affinity constant determined to be so far strong than to that for monosaccharides.

The new set of amino acid composing these sites in legume lectins has been named *extended binding-site* and has been proved to occur in all lectins which structure has been investigated in complex with oligosaccharides (Imberty & Pérez, 1994; Naismith & Field, 1996).

Although the completeness of the complexes between legume lectins and complex glycans is of an extraordinary elegance, of most intrinsic and biological relevance seems to be the ability of lectins possessing different monosaccharide specificity to binding the same receptor, through attachment in different epitope present in the same glycan structure (for review see Debray *et al.*, 1981). This very interesting biological event puts under suspect the actual role of the monosacchaide-binding site. According to these results, we could speculate that despite the monosaccharide specificity, legume lectins (and probably non legume lectins) could display similar roles as they can interact with complex glycans broadly distributed in naure. In this context, the monosacchaide-binding site would serve essentially to attach the ligand, being the specificity of the interaction defined by different epitope in the structure. Although the available results allow speculations on basis of the molecular structure of legume lectins and their specificity, identification of endogenous receptors remains obscure and up to date, our attempt to identify endogenous receptor on free-lectin fractions from seed lectins of some members of *Diocleinae* lectins has failed.

Table 3. Comparison of the carbohydrate specificity and amino acid composition of the monosaccharide-binding site of legume lectins of known structure.

Lectin	Binding-site composition

	Specificity	Strongly conserved Amino acids		Semi-conserved Amino acids		Not conserved Amino acids	
ConA[a]	Man/Glc	N-14	D-208	R-288	Y-12	L-99	Y-100
DGL[b]	Man/Glc	N-14	D-208	R-228	Y-12	L-99	Y-100
CFL[b]	Man/Glc	N-14	D-208	R-228	Y-12	L-99	Y-100
OVL[c]	Man/Glc	N-131	D-89	G-107	F-129	D-214	L-215
AHL[b]	Gal	N-127	D-83	G-104	Y-125	L-212	G-213
VFL[a]	Man/Glc	N-126	D-82	G-100	F-124	A-211	E-212
PSL[a]	Man/Glc	N-125	D-81	G-99	F-123	A-210	E-211
UEL-I[c]	L-Fucose	N-135	D-87	G-105	F-127	T-219	Y-220
UEL-II[c]	GlucNAc	N-139	D-88	S-106	F-130	V-222	G-223
LCL[c]	Man/Glc	N-127	D-82	G-99	F-123	A-189	E-190
LOL[a]	Man/Glc	N-125	D-81	G-99	F-123	A-210	E-211
GSL-Iv[a]	GalNAc	N-135	D-89	G-107	W-133	V-221	G-222
ECL[a]	Gal	N-133	D-89	G-107	F-131	A-218	Q-219
VML[d]	Gal	N-129	D-87	G-105	F-127	L-213	S-214
DBL[a]	GalNAc	N-129	D-85	G-103	L-127	L-214	S-215
GML[a]	GalNAc	N-130	D-88	G-106	F-128	G-218	E-219

a - determined by three-dimensional structure, b and c predicted by alignment to ConA and GML, respectively. d-predicted by alignment to ECL. ConA (Naismith et al., 1996), DGL (Cavada et al. 1993), CFL (Cavada et al. 1999), OVL (Sharon & Lis, 1990), AHL (Sharma & Surolia, 1997), VFL (Reeke et al., 1986), PSL (Bourne et al., 1990b), UEL-I, II (Sharma & Surolia, 1997), LCL (Sharon & Lis, 1990), LOL (Bourne et al., 1990b), GSL-IV (Delbaere et al., 1993), ECL (Shaanan et al., 1991), DBL (Imberty et al., 1994), GML (Dessan et al., 1995). VML (Calvete et al. 1998; Ramos et al. 1999) Lectins are designed by initial letter of the plant names.

CONCLUSIONS

Legume plants, are the most rich source of lectins and the continuous investigation of new lectins in members of this group may give new insight on the lectin function in nature. Although investigations under the physiological role of lectins in plant have received few attention, results from biochemical and structural features and mainly the understanding of the molecular basis of the specificity of new lectins may help the discussion. The hypothesis discussed here focused only the interaction of lectins with carbohydrate/glycan. It should be take in account that some legume lectins have been shown to possess a hydrofobic cavity forming a combining site other than that to carbohydrate/glycan, which has a high affinity to adenine and some adenine-related compounds, including plant hormones substances (Loris *et al.*, 1998). In fact the legume *Dolichos biflorus* lectin has recently been successfully crystallized and its complex with adenine solved (Hamelryck *et al.*, 1999). The capability to bind adenine was earlier showed (Gegg & Etzler, 1994; Puri & Surolia, 1994). It should be expected that a new field to discuss the polyfunctional features of these proteins will be initiated fast, adding new data to the present discussion.

Acknowledgements. This work was supported by CNPq, BNB, FINEP, PADCT, FUNCAP, CAPES/COFECUB and BioTools Ecological Foundation.

RESUMO

As lectinas, proteinas que especificamente reconhecem estruturas que contém carboidratos, têm sido extensivamente estudadas em muitos aspectos bioquímicos e estruturais, objetivando estabelecer as bases moleculares deste evento não-catalítico. Por outro lado, os potenciais clínicos e agriculturais destas proteínas têm crescido rapidamente. Embora as lectinas, principalmente aquelas de legumes tenham sido bastante investigadas em suas propriedades biológicas, estudos sobre as funcões fisiológicas de lectinas são escassos na literatura. Além disto, a despeito da quantidade de dados acumulados sobre lectinas (como proteínas), o papel desempenhado por estas

moléculas de sinalização é pobremente discutido. Valendo-se de nossos estudos sobre lectinas de leguminosas, principalmente da sub-tribo *Diocleinae*, e outros dados presentes na literatura, discutimos aqui, as principais hipóteses de suas funções com base na especificidade por carboidratos e glicanos complexos.

REFERENCES

Ayouba, A.; Martin, D. & Rougé, P. (1992), Recognition of muramic acid and *N*-acetylmuramic acid by *Leguminosae* Lectins: possible role in plant-bacteria interactions. *FEMS*, **92:**41-46

Bompard-Gilles, C. Rouseau, P.; Rougé, P. & Payan, F. (1996), Substrate mimicry in the active center of a mammalian amylase: structural analysis of an enzyme-inhibitor complex. *Structure*, **4:**1441-1452

Bourne, Y.; Abergel, C.; Cambillau, C.; Frey, M.; Rougé, P. & Fontecilla-Camps, J-C. (1990a), X-ray crystal structure determination and refinement at 1.9 A^o resolution of isolectin I from the seeds of *Lathyrus ochrus*. *J. Mol. Biol.*, **214**: 571-584

Bourne, Y.; Rougé, P. & Cambillau, C. (1990b), X-ray structure of a (α-Man (1-3)β-Man(1-4)GlcNac)-lectin complex at 2.1A^o resolution. *J. Biol. Chem.* **265 (30)**: 18161-18165

Bourne, Y.; Mazurier, J.; Legrand, D.; Rougé, P.; Montreuil, J.; Spik, G. & Cambillau, C. (1994), Structures of a legume lectin complexed with the human lactotranferrin N2 fragment, and with an isolated biantennary glycopeptide: role of the fucose moiety. *Structure*, **2**:209-219

Brewer, C.F. & Gupta, D. (1994), Mutivalent carbohydrate-protein interactions. A new dimension of binding specificity. *Current Topics in Peptide & Prot. Res.*, **1**:177-191

Calvete, J.J., Santos, C.F., Mann, K., Grangeiro, T.B., Himtz, M., Urbanke, C. & Cavada, B.S. (1998), Amino acid sequence, glycan structure, and proteolytic processing of the lectin of *Vatairea macrocarpa* seeds. *FEBS Letters*, **425(2)**: 286-292.

Calvete, J.J., Thole, H.H., Raida, M., Urbanke, C., Romero, A., Grangeiro, T.B., Ramos, M.V., Rocha, I.M.A., Guimarães, F.N. & Cavada, B.S. (1999), .Molecular characterization and crystallization of *Diocleinae* lectins. *Biochem. Biophys. Acta*, **1430**:367-375

Cavada, B.S.; Vieira, C.C.; Silva, L.M.A.; Oliveira, J.T.A. & Moreira, R.A. (1990), Comportamento da lectina de sementes de *Canavalia brasiliensis* Mart. durante a germinação em presença de luz. *Acta Bot. Bras*. **4 (2)**:13-20

Cavada, S.B.; Moreira, R.A.; Oliveira, J.T.A. & Granjeiro, T.B. (1993). Primary structures and functions of plan lectins. *R. Bras. Fisiol. Veg*. **5 (2)**: 193-201

Cavada, B.S.; Grangeiro, T.B.; Ramos, M.V.; Crisostomo, C.V.; Silva, L.M.A.; Moreira, R.A. and Oliveira, J.T.A. (1994), Lectin from *Dioclea guianensis* var. *lasiophylla* Duke seeds mobilization during germination and seedling growth in the dark. *R. Bras. Fisiol. Veg.* **6 (1)**: 21-25

Cavada, B.S., Nogueira, N.A.P., Farias, C.M.S.A., Grangeiro, T.B., Ramos, M.V., Thole, H.H., Raida, M., Rougé, P. & Calvete, J.J. (1999), Primary structure and kinetic interaction with glycoproteins of the lectin from seeds of *Cratylia floribunda*. *Prot. Peptide Letters* **6(1)**: 27-34

Chandra, N.R., Ramachandraiah, G., Bachhawat, K., Dam, T.K., Surolia, A. & Vijayan, M. (1999), Crystal structure of a dimeric mannose-specific agglutinin from Garlic: quaternay association and Carbohydrate specificity. *J. Mol. Biol.* **285(3)**:1157-1168

Cohn, J., Bradley, D.R. & Stacey, G. (1998), Legume nodule organogenesis. *Trends in Plant Science*, **3**:105-110

Dam, T.K.; Cavada, B.S.; Grangeiro, T.B.; Santos, C.F.; Sousa, F.A.M.; Osacarson, S. & Brewer, C.F. (1998), Diocleinae lectins are a group of proteins with conserved binding sites for the core trimannoside of asparagine-linked oligosaccharide and differential specificities for complex carbohydrates. *J. Biol. Chem.*, **273(20)**:12082-12088

Debray, H., Decout, D., Strecker, G., Spik, G. & Montreuil, J. (1981), Specificity of twelve lectins towards oligosaccharides and glycoproteins related to *N*-glycoproteins. *Eur. J. Biochem.*, **117**:41-55

Delbaere, L.T.J.; Vandonselaar, M.; Prasad, L.; Quail, J.W.; Wilson, K.S.; & Dauter, Z. (1993), Structures of the lectin IV of *Griffonia simplicifolia* and its complex with the Lewis b human blood group determinant at 2.0 A^o resolution. *J. Mol. Biol.* **230**: 950-965

Dessan, A.; Gupta, D.; Sabesan, S.; Brewer, C.F. & Sacchettini, J.C. (1995), X-ray crystal structure of the soybean agglutinin cross-linked with a biantennary analog of blood group I carbohydrate antigen. *Biochem.*, **34**: 4933-4942

Diaz, C.L.; Melchers, L.S.; Hooykaass, P.J.J.; Lugtenberg, B.J.J. & Kijne, J.W. (1989), Root lectin as a determinant of host-plant specificity in the *Rhizibium*-legume symbiosis. *Nature* **338**: 579-581

Do, Su-Il & Lee, K.Y. (1998), Jacalin interacts with Asn-linked glycopeptides containing multi-antennary oligosaccharide structure with terminal α-linked galactose. *FEBS Letters*, **421**:169-173

Einhoff, W.; Fleischmann, G.; Freier, T.; Kummer, H. & Rudiguer, H. (1986), Interaction of leguminous

seed lectins with seed proteins - lectins as packing aids of storage proteins. In: *Lectins: Biol., Biochem., Clinical Biochem*., ed. Driessche, E.V.; Bog-Hansen, T.C., Textop Denmark, Textop, Denmark, **5**:45-52

Eijsden, R.R.V., Pater, B.S. & Kijne, J.W. (1994) Mutational analysis of the sugar-binding site of pea lectin. *Glycoconjugate J.*, **11**:375-380.

Etzler, M.E. (1986), Distribution and functions of plant lectins. In: *The lectins: Properties, Functions, and applications in Biology and Medicine*, eds Leiner, I.E., Sharon, N. & Goldstein, I.G. New York, Academic Press, 600pp

Fabre, C, Barre, A., Demont, N., Promé J.C. & Rougé, P. Do Leguminosae lectins interact with nod factors? (1994). In: Lectins, Biol. Biochem. Clinical Biochem. ed. Driessche E.van, Fischer, J., Beeckmans, S., Bøg-Hansen, T.C. Textop Denmark, Textop, Denmark, **10**:142-148.

Fabre, C., Causse, H., Mourey, L., Koninkx, J., Riviere, M., Hendriks, H., Puzo, G., Samama, J.P. & Rougé, P. (1998), Characterization and sugar-binding properties of arcelin-1, na insecticidal lectin-like protein isolated from kidney bean (*Phaseolus vulgaris* L. cv. RAZ-2) seeds. *Biochem. J*., **329**:551-560

Gatehouse, A.M.R., Powell, K.S., Peumans, W.J., Van Damme, E.J.M. & Gatehouse, J.A. (1995), Insecticidal properties of plant lectins: their potential in plant protection. Pp 35-58. In: *Lectins: Biomedical Perspectives*, ed. Pusztai, A. & Bardocz, S. Taylor e Francis., London, 331 pp

Gegg, C.V. & Etzler, M.E. (1994), Photoaffinity labeling of the adenine binding sites of two *Dolichos biflorus* lectins. *J. Biol. Chem.* **269**:5687-5692

Gers-barlag, H.; Schecher, S.; Kumar, N.S. & Rudiger, H. (1993), Protein body membranes as a binding partners of lectins In*: Lectins: Biol. Biochem., Clinical Biochem.* ed. Driessche, E.V., Franz, H.; Beeckmans, S.; Pfuller, U., Kallikorm & Bog-Hansen, T.C. Textop, Denmark, **8**:97-100.

Hamelryck, T.W., Loris, R., Bouckaert, J., Dao-Thi, M.H., Wyns, L. & Etzler, M. (1999) Carbohydrate Binding, Quaternary Structure and a Novel Hydrophobic Binding Site in Two Legume Lectin Oligomers from *Dolichos biflorus*. *J Mol Biol.* **286(4)**:1161-1177.

Imberty, A. & Perez, S. (1994), Molecular modelling of protein-carbohydrate interactions. Understanding the specificities of two legume lectins towards oligosaccharides. *Glycobiology*, **4(3)**: 351-366.

Kijne,, J; Diaz, C.; Sylvia de Pater & Lugtenberg, B. (1992), Lectins in the symbiosis between rhizobia andd leguminous plants. In: *Advances in Lectin Research* ed. Franz, H. **5**:15-50.

Konami, Y., Yamamoto, K., Osawa, T. & Irimura, T. (1994), Strong affinity of *Maackia amurensis* hemagglutinin (MAH) for silaic acid acid-containing Ser/Thr-linked carbohydrate chains of N-terminal octapeptides from human glycophorin A. *FEBS Letters*, **342**:334-338.

Lerouge, P., Roche, P., Faucher, C., Truchet, G., Promé, J.C. & Dénarié, J. (1990). Symbiotic host-specificity of *Rhyzobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature*, **344**:781-784.

Loris, R. Hamelryck, T., Bouckaert J. & Wyns, L. (1998). Legume lectin structure. *Biochem. Bioph. Acta*, **1383**:9-36.

Mirkov, T.E.; Waslstrom, J.M.; Hagiwara, K.; Finardi-Filho, F.; Kjemtrup, S. & Chrisppels, M.J. (1994), Evolutionary relationships among proteins in the phytohemagglutinin-arcelin-α-amylase inhibitor family of the common bean and its relatives. *Plant Mol. Biol.*, **26**:1103-1113.

Moreira, R.A. & Cavada, B.S. (1984), Lectin from *Canavalia brasilienses*. Isolation, characterization and Behavior during germination. *Biol. Plant.*, **26 (2)**:113-120.

Moreira, R.A.; Silva, L.M.A.; Horta, A.C.G.; Oliveira, J.T.A. & Cavada, B.S. (1993), Lectin from *Canavalia brasiliensis* Mart. Behaviour during maturation and detection of a lectin precursor. *R. Bras. Fisiol. Veg.* **5 (2)**: 133-138.

Mourey, L., Pédelacq, J.D., Birck, C., Fabre, C., Rougé, P. & Samama, J.P. (1998), Crystal structure of the arcelin-1 dimer from *Phaseolus vulgaris* at 1.9 A° resolution. *J. Biol. Chem.*, **273(21)**: 12914-12922.

Naismith, J.H. & Field, R.A (1996),. Structural basis of trimannoside recognition by Concanavalin A. *J. Biol. Chem.*, **271(2)**: 972-976.

Oliveira, J.T.A., Moraes, S.M.D., Cavada, B.S., Moreira, B.S. & Vasconcelos, I.M. (1998), Protein and lectin mobilization during *Erythina velutina* forma *aurantica* seed germination and seedling growth in the Dark. *R. Bras. Fisiol. Veg.* **10(1)**:25-30.

Puri, K.D. & Surolia, A. (1994), Amino acid sequence of the winged bean (*Psophocarpus tetragonolobus*) basic lectin. Adenine binding and identification of the active-site tryptophan residue. *J. Biol. Chem.*, **269(49)**:30917-26.

Ramos, M.V., Moreira, R.A., Oliveira, J.T.A., Cavada, B.S. & Rougé, P. (1996), The carbohydrate-binding specificity and molecular modelling of *Canavalia maritima* and *Dioclea grandiflora* lectins. *Mem. Inst. Oswaldo Cruz*, **91(6)**:761-766.

Ramos, M.V. Cavada, B.S., Calvete, J.C., Sampaio, A.H., Mazard, A.M., Barre, A., Grangeiro, T.B., Freitas, B.T., Leite, K.B. & Rougé, P. (1999). Specificity of the *Vatairea macrocarpa* lectin towards glycans exhibiting exposed Gal/GalNAc residues. *Protein and Peptide Letters* **6(3)**:163-172

Rao, K.V., Rathore, K.S., Hodges, T.K., Fu, X., Stoger, E., Sudhakar, D., Williams, S., Christou, P., Bharathi,

M., Bown, D.P., Powell, K.S., Spence, J., Gatehouse, A.M.R. & Gatehouse,.A. (1998), Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistence to rice brown plant hopper. *The Plant Journal*, **15(4)**:469-477.

Reeke, G.N.,Jr., & Becker, J.W. (1986), Three-dimentional structure of favin: saccharide-binding cyclic permutation in leguminous lectins. *Science* **234**:1108-1111.

Rhijn, P.V., Goldberg, R.B. & Hirsch, A.M. (1998), *Lotus corniculatus* nodulataion specificity is changed by the presence of a soybean lectin gene. *Plant Cell* **10**:1233-1250.

Rudiger, H. & Schecher, G. (1993), The protein body membrane of soybean seeds as a possible lectin-biding component In: *Lectins: Biol. Biochem., Clinical Biochem*, ed. Driessche, E.V., Franz, H.; Beeckmans, S.; Pfuller, U., Kallikorm and Bog-Hansen, T.C. Textop, Denmark, **8**:101-104.

Sanjuan, J. Carlson, R.W., Spaink, H.P., Bhat, U.R., Barbour, W.M., Glushka, J. & Stacey, G. (1992). A 2 *O*-methylfucose moiety is present in the lipo-oligosaccharide nodulation signal of *Bradrhyzobium japonicum*, *Proc. Natl. Acad. Sci. USA*, **89**:8789-8793.

Schroeder, H.E.; Gollasch, S.; Moore, A.; Tabe, L.M.; Craig, S.; Hardie, D.C.; Chrispeels, M.J.; Spencer, D. & Higgins, T.J.V. (1995), Bean α-amylase inhibitor confers resistance to the pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum* L.) *Plant Physiol.* **107**:1233-1239.

Shaanan, B.; Lis, H. & Sharon, N. (1991), Structure of a legume lectin with an ordered *N*-linked carbohydrate in complex with lactose. *Science* **254**: 862-866.

Sharma, V. & Surolia, A. (1997), Analysis of the carbohydrate recognition by legume lectin: size of the combining site loops and their primary specificity. *J. Mol. Biol.*, **267**:433-445.

Sharon, N. & Lis, H. (1990). Legume lectins - a large family of homologous proteins. *The FASEB Journal* **4**: 3198-3207

Spaink, H.P., Bloemberg, G.V., Brussel, A.A.N.V., Lugtemberg, B.J.J., Drift, K.M.G.V.D., Havwerkamp, J. & Oates, J.E.T. (1995). Host specificity of *Rhyzobium leguminosarum* is determined by the hydrophobicity of highly unsaturated fatty acyl moieties of the nodulation factors. *Molecular Plant-Microbe interaction*, **8**:155-154.

Van Damme, E.J.M., Peumans, W.J., Barre, A. & Rougé, P. (1998), Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Critical Reviews in Plant Science* **17(6)**:575-692.

Wenzel, M. & Rudger, H. (1995), Interaction of pea (*Pisum sativum*) lectin with pea storage proteins. *J. Plant Physiol*. **145**: 191-194

Zhu-Salzman, K., Shade, R.E., Koiwa, H. Salzman, R.A., Narasimhan, M., Bressan, R.A., Haseggawa, P.M. & Murdock, L.L. (1998), Carbohydrate binding and resistance to proteolysis control insecticidal activity of *Griffonia simplicifolia* lectin II. *Proc. Natl. Acad. Sci. USA*, **95(25)**:15123-15128.

Received: October 05, 1999;
Revised: January 06, 2000;
Accepted: April 25, 2000.