The Carbohydrate-binding Specificity and Molecular Modelling of *Canavalia maritima* and *Dioclea grandiflora* Lectins

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The carbohydrate-binding specificity of lectins from the seeds of Canavalia maritima *and* Dioclea grandiflora *was studied by hapten-inhibition of haemagglutination using various sugars and sugar derivatives as inhibitors, including* N-*acetylneuraminic acid and* N-*acetylmuramic acid. Despite some discrepancies, both lectins exhibited a very similar carbohydrate-binding specificity as previously reported for other lectins from* Diocleinae *(tribe* Phaseoleae, *sub-tribe* Diocleinae*). Accordingly, both lectins exhibited almost identical hydropathic profiles and their three-dimensional models built up from the atomic coordinates of ConA looked very similar. However, docking experiments of glucose and mannose in their monosaccharide-binding sites, by comparison with the ConA-mannose complex used as a model, revealed conformational changes in side chains of the amino acid residues involved in the binding of monosaccharides. These results fully agree with crystallographic data showing that binding of specific ligands to ConA requires conformational chances of its monosaccharide-binding site.*

Key words: *Canavalia maritima* - carbohydrate-binding specificity - *Diocleinae* - *Dioclea grandiflora* - lectins - molecular modelling

In recent years, lectins have become very attractive proteins due to their extensive use as probes for both the characterization and isolation of simple and complex sugars (Lis & Sharon 1991) and as useful tools in immunological studies (Moreira et al. 1991). Accordingly, lectins are, by now, the plant proteins of best known three-dimensional structures. Following the pioneering works of Hardman and Ainsworth (1972) and Edelman et al. (1972) on the three-dimensional structure of ConA, the 3D-models of seven lectins have been solved at atomic resolution: PSA from pea (Einspahr et al. 1986); Favin from *Vicia faba* (Reeke & Becker 1986); LoLI from *Lathyrus ochrus* (Bourne et al. 1990a); GSIV from *Griffonia simplicifolia* (Delbaere et al. 1990); EcoL from *Erythrina corallodendron* (Shaanan et al. 1991); PNA from peanut (Banerjee et al. 1993) and LCA from lentil (Loris et al. 1993). In addition, complexes of legume lectins with simple or more complex sugars were solved by X-ray analysis: ConA with mannose (Derewenda et al. 1989); LoLI with glucose and mannose (Bourne et al. 1990b), with a trimannoside (Bourne et al. 1990c), with an octasaccharide (Bourne et al. 1992) of the *N*-acetyllactosaminic type and with Muramic (MurAc) and *N*-acetylmuramic (MurNAc) acids (Bourne et al. 1994a); PSA with a trimannoside (Rini et al. 1993); GSIV with a tetrasaccharide of the Lewis[b] group (Delbaere et al. 1993); LoLII with a glycoprotein (N2) derived from human lactotransferrin (Bourne et al. 1994b), and SBA from soybean with a biantennary analog of the blood group I antigen (Dessen et al. 1995). These complexes have shown that the monosaccharide-binding site of lectins plays a key role in the binding of either monosaccharides or more complex glycans. Along this way, a single monosaccharide unit of the complex glycan binds to the monosaccharide-binding site while other non covalent bonds occurring at the interface of both molecules, e.g. hydrogens bonds often mediated by water molecules and hydrophobic interactions, stabilize the complex between lectins and their ligands (Sharon 1993).

Diocleinae lectins have been recently demonstrated to act as strong inducers of lymphocyte proliferation and γ-interferon production (Barral-Netto

et al. 1992), macrophage stimulation (Rodriguez et al. 1992) and leukocyte imigration (Bento et al. 1993). Although it is clear that the monosaccharide-binding site of *Diocleinae* lectins is involved in these biological activities on the immune system, both possible surface receptors for plant lectins and the resulting biochemical pathways allowing cell stimulation remain largely unknown. Moreover, except for ConA from *Canavalia ensiformis* seeds, no informations are available, until now, on the three-dimensional structures of other *Diocleinae* lectins, that prevents all speculative investigations on the cell-lectin interactions at the molecular level. Studies on molecular modelling and interaction with sugars and sugar derivatives of two lectins from the sub-tribe *Diocleinae, Canavalia maritima* (ConM) and *Dioclea grandiflora* (DGL) were performed in order to provide relevant molecular models of lectin-sugar interaction.

MATERIALS AND METHODS

Carbohydrates, *N*-acetylmuramic (MurAc) and *N*-acetylneuraminic (NANA) acids were purchased from Sigma. Haemagglutination and inhibition of haemagglutination were performed in standard microplates (Flow Laboratories) as previously reported (Ayouba et al. 1993).

The lectins were isolated by affinity cromatography on Sephadex G-50 as reported by Moreira et al. (1983) and purity was judged by SDS-PAGE.

Exposed/buried regions along the amino acid sequences of both ConM and DGL lectins were delineated on hydropathic profiles built according to Kyte and Doolittle (1982).

InsightII, Homology and Discover programs (Biosym Technologies, San Diego, CA, USA) were used to build and refine the three-dimensional models of ConM and DGL monomers from X-rays coordinates of ConA (Protein Data Bank code 2CNA, Brookhaven, NY, USA). The HCA (Hydrophobic Cluster Analysis) (Gaboriaud et al. 1987) method was used to delineate, along the amino acid sequences of both ConM and DGL, the structurally conserved regions homologous to those of ConA.

To built the three-dimentional models of ConM and DGL, the sequences of these lectins were aligned with ConA sequence (Fig. 1). Conserved segments of the sequences, predicted by HCA
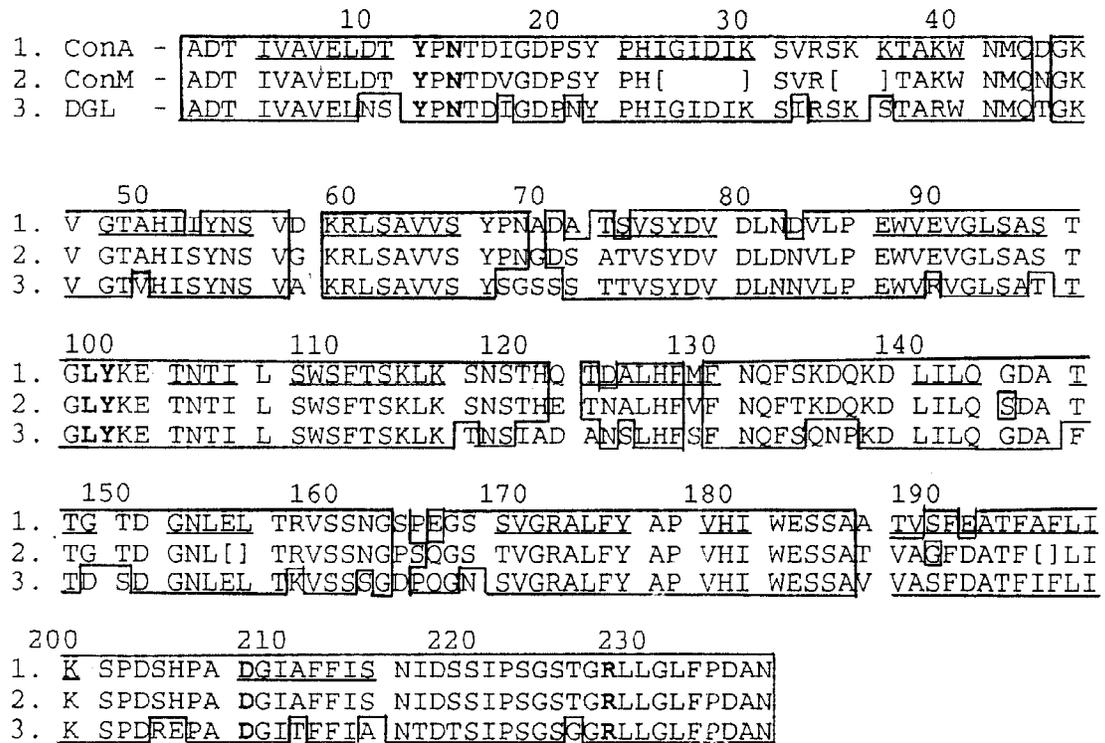
Fig. 1: the amino acid sequences of *Canavalia ensiformis*, *Canavalia maritima* and *Dioclea grandiflora* lectins. Amino acids in headlines form the binding site of ConA, as determined by crystallographic studies. Identical amino acids are shown in blocks. Segments underlined in ConA sequence represent the β-structure of this protein which is strongly conserved in other *Diocleinae* lectins. Brackets represent segments of ConM sequence not determined.

method, were placed in boxes following the assignment of the α-carbons coordinates from ConA to each segment. The segments of ConM primary sequence not determined were modelled by homology with other lectins from *Canavalia* genus which show 100% of identity in these regions. Docking experiments were carried out using the coordinates of the ConA-α-methyl-mannopyranoside complex (Derewenda et al. 1989). Molecular modelling and docking were performed on a Silicon Graphics Personal Iris 4D25G workstation. As a result of the high identity among the primary sequences of the studied lectins, Ramachandran plots from ConM and DGL models were very similar to that of ConA structure. Only few bad contacts were identified between amino acids in the models. The bad distances were corrected by altering the dihedral angles involving side chain atoms.

RESULTS

The comparison of amino acid sequences of ConM and DGL to that of ConA showed a high degree of both identity and homology (Fig. 1). Accordingly, the hydropathic profiles (Fig. 2) and the HCA plots (results not shown) of all these *Diocleinae* lectins looked very similar, except for a few discrepancies. As a result, the molecular models of ConM and DGL built up from the coordinates of ConA strongly resembled that of ConA. Each monomer exhibited a flattened dome-shaped structure, the walls of which correspond to seven (front face) and six (back face) strands of antiparallel β sheet interconnected by reverse turns and loops (Fig. 3). At the top of each monomer, a few convergent loops forming a small pocket constitute a monosaccharide-binding site responsible for the binding of simple sugars (mannose, glucose) to the lectins.

Both ConM and DGL similarly reacted with simple sugars and their derivatives, including *N*-acetylmuramic acid and *N*-acetylneuraminic acid (Table). However, few discrepancies occurred with some of the checked carbohydrates, i.e., raffinose, glucose and fructose.

Docking experiments performed with both mannose and glucose showed that the monosaccharide-binding sites of ConM and DGL are quite similar to that of ConA (Fig. 4). In this respect, a network of hydrogen bonds identical to that described for ConA (Derewenda et al. 1989) connects the sugar residues to the amino acids forming the monosaccharide-binding site. However, conformational changes in the side chain of some residues were observed (Fig. 5), which could explain some of the discrepancies reported in inhibition of haemagglutination (see Table).
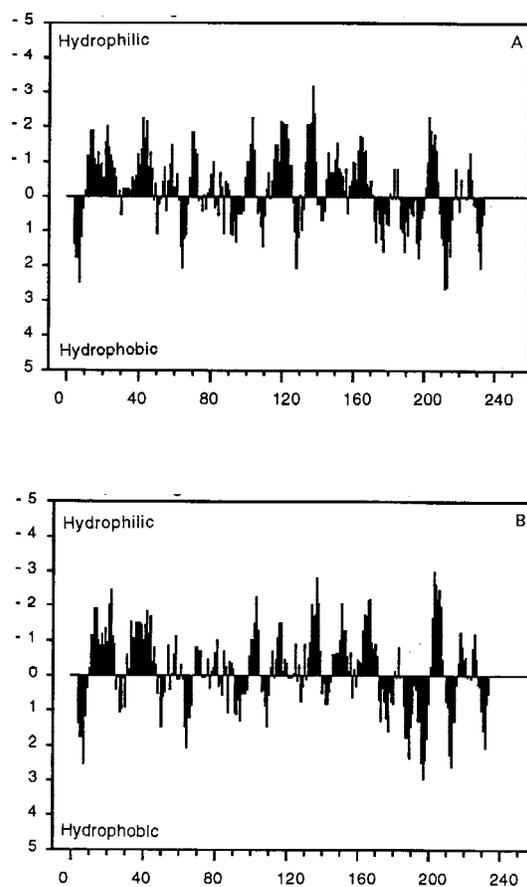
Fig. 2: comparison of the hydropathic profiles of *Canavalia maritima* (A) and *Dioclea grandiflora* (B) lectin plots according to Kyte and Doolittle (1982).
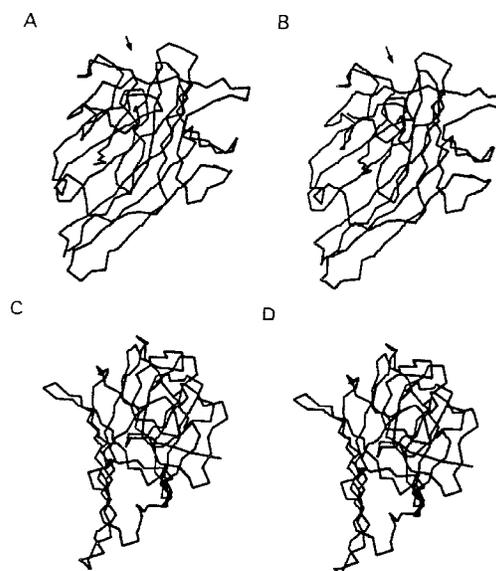
Fig. 3: three-dimensional models showing the front faces of *Dioclea grandiflora* (A) and *Canavalia maritima* (B) lectins and the side views of DGL (C) and ConM (D). The location of the monosaccharide-binding sites is indicated by arrows.

A

B
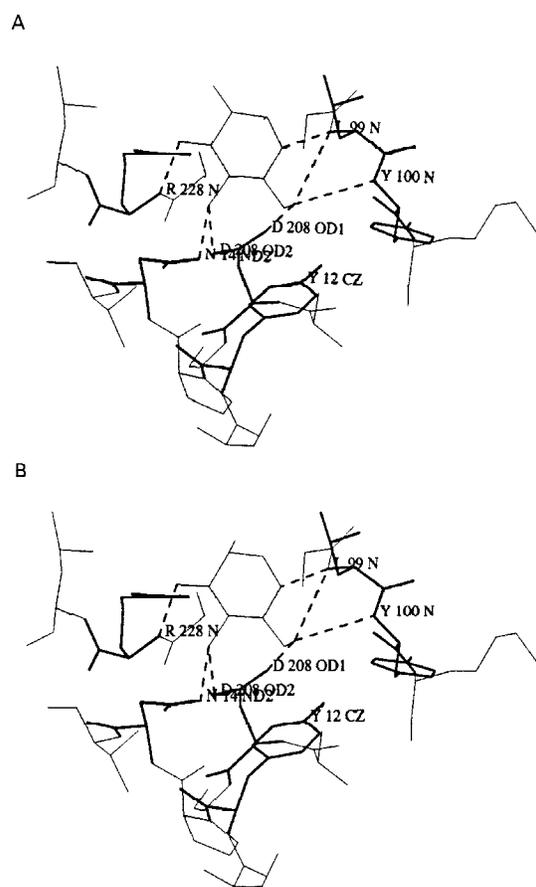
Fig. 4: molecular docking in the monosaccharide-binding site of the studied lectins (thick lines) with glucose (A) and mannose (B).
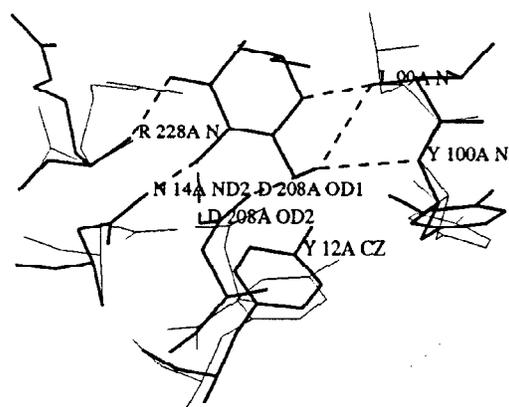
Fig. 5: superimposition of the monosaccharide-binding sites of *Dioclea grandiflora* (thin lines) and *Canavalia ensiformis* lectins complexed with α-methyl-mannopyranoside (thick lines) showing the changes in side chain disposition in the complexed structure.

TABLE

Inhibition of the haemagglutinating activity of *Canavalia maritima* and *Dioclea grandiflora* lectins by sugars[a]

Sugars	Lectins	
	ConM	DGL
β-D(-)fructose[b]	4.2	16.7
maltose[b]	1.0	4.2
L(-)sorbose[b]	8.3	16.7
D(+)threalose[b]	1.0	2.1
α-D(+)mellibiose[b]	2.1	2.1
sucrose[b]	8.3	16.7
D(+)raffinose[b]	16.7	NI
N-acetylmuramic acid[c]	4.2	4.2
N-acetylneuraminic acid[c]	8.3	8.3
α-D(+)galactose[b]	NI	NI
polygalacturonic acid[d]	NI	NI
P-nitrophenyl-6-O-β-D (+)galactopyranoside[e]	NI	NI
α-D(+)mannose[b]	2.1	2.1
α-D(+)glucose[b]	4.2	16.7

a: minimal concentration required to inhibit 1 haemagglutination unit; NI: not inhibitory even at the following concentrations: 33.3 mM *b*, 8.3 mM *c*, 0.33% *d* and 3.3 mM *e*.

DISCUSSION

Despite some changes occurring in the amino acid sequences of *Diocleinae* lectins, their three-dimensional structures have been nicely conserved during evolution and strongly resemble those of other legume lectins (for review see Rougé et al. 1991). The structural similarities observed among *Diocleinae* lectins confirm these proteins as excellent phylogenetic markers as proposed by Moreira et al. (1995). The amino acid residues involved in the binding of monosaccharides (Tyr^{12}, Asn^{14}, Leu^{99}, Tyr^{100}, Asp^{208} and Arg^{228}) by ConA are completely conserved in other *Diocleinae* lectins. As a result, all these lectins exhibit a quite similar carbohydrate-binding specificity as shown by hapten-inhibition of haemagglutination by simple sugars and derivatives (Table). Their ability to bind *N*-acetylmuramic acid and *N*-acetylneuraminic acid, which has been reported for many legume lectins (Ayouba et al. 1991, 1992), is of special interest since these molecules are surface components of various saprophytic or pathogenic bacteria or fungi. Moreover, LoLI from *Lathyrus ochrus* seeds has been recently co-crystallized with both muramic acid and muramyl-dipeptides (Bourne et al. 1994a).

These results suggest that lectins could play similar biological roles possibly related with recognition processes occurring between plants and microorganisms. However, the analysis of carbo-

hydrate-specificity of ConM and DGL towards mono-, di- and trisaccharides reveals some discrepancies, as previously shown for other lectins from the *Diocleinae* sub-tribe (Ayouba et al. 1993). Our molecular modelling studies confirm that the monosaccharide-binding sites of ConM and DGL are very similar to that of ConA. Similarly, our docking experiments performed with glucose and mannose show that a hydrogen bonding scheme similar to that observed in ConA (Derewenda et al. 1989) connects both sugars to the sites (Fig. 4). As shown in ConA-sugar complexes (Naismith et al. 1994), although the main chain positions of the amino acid residues forming the monosaccharide-binding site of ConA remain very similar in both complexed and native structures of ConA, their side chains are altered in the complexed form. Especially, Leu[99], Tyr[100] and Arg[228] undergo drastic conformational changes upon binding to sugars. These findings suggest that suitable conformational changes occur in *Diocleinae* lectin binding-sites to make possible their interaction with monosaccharides. This monosaccharide-binding site flexibility observed for ConA, ConM and DGL could explain, at least in part, the overall ability of legume lectins to recognize distinct glycan structures. As far as our results of docking experiments are concerned, it is reasonable to speculate that the monosaccharide-binding site of *Diocleinae* lectins may be somehow particular for each one, thus generating slight discrepancies in the affinity of closely related lectins towards identical ligands as showed for raffinose, glucose and fructose (Table) and hence towards complex carbohydrate structures. In addition, preliminary results based on the fine interaction of *Diocleinae* lectins, measured by surface plasmon ressonance, showed that remarkable differences exist in the association of each lectin and a specific ligand.

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