

The crystal structure of *Canavalia brasiliensis* lectin suggests a correlation between its quaternary conformation and its distinct biological properties from Concanavalin A

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Abstract *Canavalia brasiliensis* lectin was isolated from the seeds of a Brazilian autochthonous Leguminosae plant. Despite extensive amino acid sequence similarity with Concanavalin A, *C. brasiliensis* lectin exerts in vitro and in vivo cellular effects that are markedly different from those displayed by Concanavalin A. We have solved the crystal structure of the *C. brasiliensis* lectin at 3.0 Å resolution. The three-dimensional structure of the lectin monomer can be superimposed onto that of Concanavalin A with a root-mean-square deviation for all C α atoms of 0.65 Å. However, this parameter is 0.84 and 1.62 Å when the *C. brasiliensis* lectin dimer and tetramer, respectively, are compared with the same structures of Concanavalin A. We suggest that these differences in quaternary structure may account for the different biological properties of these two highly related Leguminosae lectins.

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Key words: Legume lectin; *Canavalia brasiliensis*; Crystal structure; Quaternary structure

1. Introduction

Carbohydrates act as intermediates of biological communication in biological systems such as differentiation, proliferation, and cell-cell interactions crucially important in both physiological and pathological phenomena [1–3]. The information contained in the enormous variety of oligosaccharide structures normally conjugated to proteins or lipids on cell surfaces (glycocodes) is recognized and deciphered by a specialized group of structurally diverse proteins, the lectins, which exhibit the common property of reversible binding to complex carbohydrates with considerable specificity [4,5]. Other carbohydrate-binding proteins like antibodies, enzymes, and transport proteins are not included in the lectin group.

Lectins are widely distributed in Nature, being found in animals, insects, plants, and microorganisms [4,5]. Hundreds of lectins have been characterized and the number is growing fast. The actual functions of lectins in the organisms that produce them are largely unknown but are undoubtedly as diverse as the proteins themselves. The most thoroughly investigated lectins are those from plants, in particular from the family Leguminosae. Legume lectins are a large group of

structurally similar proteins with distinct carbohydrate specificities. Over 80 of these lectins have been isolated and characterized to varying extents. Proposed functions for plant lectins include a storage or transport role for carbohydrates in seeds, binding of nitrogen-fixing bacteria to root hairs, and inhibition of fungal growth or insect feeding (see [6–8] and references therein). In addition, the existence of hydrophobic sites within the structure of legume lectins, which bind phytohormones, i.e. indoleacetic acid [9] and cytokinins [10], that modulate their hemagglutinating activity [11] and their interaction with carbohydrates [12], suggests a possible role for lectins in aspects of hormonally regulated plant growth and development. Lectins are currently attracting much interest on account of their use as tools in areas of biological, medical, and biotechnological research [13,14]. In addition, legume lectins are excellent models for studying protein-carbohydrate interactions at the atomic level [5,15].

Concanavalin A (ConA), the lectin of *Canavalia ensiformis* (Jack bean) seeds, was the first lectin to have its three-dimensional structure solved [16], and is still the best characterized legume lectin [17,18]. The ConA monomer contains 237 amino acids arranged in two large anti-parallel pleated β -sheets. ConA has a multimeric structure which displays pH-dependent dimer-tetramer interconversion. In crystals of native ConA, the four monomers of a tetramer display perfect crystallographic 222 symmetry. X-ray crystal structures of several other legume lectins, with or without carbohydrate ligand, have been determined, including, apart from ConA, pea lectin, favin, isolectin I from *Lathyrus ochrus* (LOLI), lectin IV from *Griffonia simplicifolia*, *Erythrina corallodendron* lectin, lentil lectin, and soybean agglutinin ([5,19,20] and references cited). The subunits of these lectins differ in glycosylation and in the number of polypeptide chains in each subunit. However, all of them have the same tertiary structure as the ConA monomer, with two metal ions situated in the same locations and conserved residues in their carbohydrate-combining sites occupying invariant positions [5,21]. These lectins are dimers except ConA, peanut lectin, and soybean agglutinin, each being a dimer of a dimer. ConA, pea lectin, favin, LOLI, lentil lectin, and soybean agglutinin dimerize in a similar manner. Dimerization involves antiparallel side-by-side alignment of the two flat β -sheets leading to the formation of a contiguous 12-stranded β -sheet, six strands coming from each subunit. However, different quaternary structures have been found in lectins of *G. simplicifolia* [22], *E. corallodendron* [23], and peanut (*Arachis hypogaea*) [24,25]. Similarly, the crystal structure of the lectin isolated from *Canavalia brasiliensis* seeds (ConBr)

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Abbreviations: ConBr, *Canavalia brasiliensis* lectin; ConA, Concanavalin A; r.m.s., root-mean-square

[26] presented here shows a quaternary conformation distinct from that of ConA. The possible correlation of this finding with the different biological effects exerted by ConBr and ConA is discussed.

2. Materials and methods

Mature seeds of *C. brasiliensis* were collected from plants growing widely in the state of Ceará, north-east Brazil. ConBr was isolated by fractional precipitation with ammonium sulphate followed by affinity chromatography on Sephadex G-50 as described [26]. SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli [27]. Amino acid and amino sugar analyses were performed using a Pharmacia Alpha Plus analyzer after sample hydrolysis with 6 N HCl at 110°C for 18 h in sealed, evacuated ampoules. N-terminal sequence analysis was performed with an Applied Biosystems Procise sequencer following the manufacturer's instructions.

For crystallization, lyophilized ConBr was dissolved in 50 mM 2-(*N*-morpholino)ethanesulphonic acid (MES) pH 6.0 at about 10 mg/ml and MnCl₂ and CaCl₂ were added to a final concentration of 10 mM. Crystals were grown at constant room temperature (22°C) in 4 days to 5 months by the hanging drop vapour diffusion method using 11–15% polyethylene glycol (PEG) 6000, 0.1 M MES, pH 5–6.2 as precipitant. X-ray intensity data collection was performed with a MAR-Research (Hamburg) Imaging Plate System, with a crystal-to-detector distance of 140 mm, using CuK α radiation generated by an Enraf-Nonius rotating anode operating at 3.6 kW. A total of 91 275 distinct reflections were measured from 86 oscillation frames at 1.2° intervals and 15 min exposure per frame. Reflections were integrated using the program MOSFLM version 5.2 [28], and a formatted reflection file was processed using the CCP4 program package [29]. The R_{merge} was 0.15 for 16 811 unique reflections to 3 Å resolution, which represents 90% completeness.

The structure of ConBr was solved by molecular replacement with the program AMORE [30] using the coordinates of native ConA at 1.95 Å resolution (PDB accession code 2CTV) as the search model. Rotation and translation searches, carried out using 15–3.5 Å resolution range, led to unique independent solutions for the four molecules of the asymmetric unit. The position of the four molecules was refined using the rigid-body option of AMORE. The partial model was initially refined using the energy minimization protocols of XPLOR [31]. Non-crystallographic restraints of 500 kJ mol⁻¹ Å⁻² were employed between the four subunits in the asymmetric unit. The starting R factor was 0.34 for data to 3.5 Å resolution. After 400 cycles of refinement, which led to an R factor of 0.238, restrained individual B factors were also refined including data to 3 Å, decreasing thereby the R factor to 0.213. At this stage, $2F_oF_c$ maps were computed and the two amino acid residues which are different between ConBr and ConA (G⁵⁸, G⁷⁰) [32] were changed in the sequence using the program O [33]. All programs were run on a Silicon Graphics Indy Work Station. After examination of the maps and manual model building,

the last cycles of minimization converged to an R factor of 0.201. 383 water molecules were included, and the final model refined to an R factor of 0.163 (R_{free} 0.246).

3. Results and discussion

ConBr, the lectin from *C. brasiliensis*, a legume from the Diocleinae sub-tribe, has 99% amino acid sequence identity with ConA and like this latter lectin displays the same primary specificity for glucose/mannose [32]. However, ConBr shows different biological effects from ConA regarding induction of rat paw edema [34], peritoneal macrophage spreading in mouse [35], and in vitro human lymphocyte stimulation [36]. To establish the structural basis of the biological activity of ConBr, the lectin was crystallized and its structure solved by molecular replacement using ConA coordinates. Although a variety of three-dimensional structures of lectins and lectin-saccharide complexes have been reported [4,5,19–21], providing a detailed picture of lectin-carbohydrate interactions, ConA is the only legume lectin of the Diocleinae subtribe (Phaseolae tribe) whose structure has been solved.

3.1. The crystal structure of *C. brasiliensis* (ConBr) lectin: structural differences from *C. ensiformis* (ConA) lectin

A full data set to 3.0 Å resolution was collected from polyhedral crystals (maximum dimension of 0.2 mm) of *C. brasiliensis* lectin (ConBr). Refined cell dimensions were $a = 71.3$ Å, $b = 71.7$ Å, $c = 191.0$ Å. The space group was P2₁2₁2₁, and the Matthews number [37] of the crystal was 2.1, which corresponds to 40% solvent assuming 4 ConBr subunits in the asymmetric unit. The three-dimensional structure of the ConBr subunit can be superimposed onto a ConA monomer with r.m.s. deviation (all C α atoms) of 0.65 Å. However, when the ConBr dimer and tetramer were compared with the respective oligomers of ConA (Fig. 1), the r.m.s. deviations were 0.84 and 1.62 Å, respectively. This clearly indicates that ConBr possesses a slightly though significantly different quaternary structure from that of ConA. This point is also illustrated comparing the distances between the metal ions and the carbohydrate binding domains (CRDs) of the subunits of ConBr and ConA tetramers (Table 1). Furthermore, the number of hydrogen bonds and van der Waals contacts between subunits forming the dimers (AB and CD) and be-

Table 1
Relation between structural characteristics of ConBr and ConA

Pair of subunits	Ca-Ca	Mn-Mn	CDR-CDR	H-bonds	Van der Waals
(ConBr-ConA)					
AB	−0.09	0.16	0.07	−5 (−39%)	−50%
AC	−0.89	−1.33	−1.10	+3 (+30%)	+20%
AD	2.98	2.66	3.21		
[Saccharide-free ConA-(α MP-ConA)]					
AB	0.18	0.21	0.42	0	−20%
AC	0.24	−0.45	−0.24	0	−36%
AD	1.83	1.67	1.74		

For relative disposition of subunits in the tetramer, see Fig. 2. Ca-Ca and Mn-Mn denote the difference of distances between calcium and manganese atoms, respectively, between ConBr and ConA tetramers; CRD-CRD, differences of the distance between carbohydrate recognition domains of ConBr and ConA subunits: for each CRD, the centroid of the distance between the atoms directly involved in α -D-mannopyranoside binding was calculated (the following atoms were considered: Asn-14 ND2, Leu-99 N, Tyr-100 N, Asp-208 OD1, Asp-208 OD2, and Arg-228 N) [17]. All distances are in Å. H-bonds and van der Waals, difference of the number of hydrogen bonds and van der Waals contacts, respectively, between ConBr and ConA subunits (in parentheses, percentages of total bonds). Values represent the subtraction of ConA parameters from those of ConBr. For comparison, the same values calculated by superposition of ConA/ α -D-mannopyranoside complex (α MP) with native ConA are shown.



Fig. 1. Stereo pair comparing the structures of the ConBr (thick line) and the ConA (thin trace) tetramers. The comparison was generated superimposing subunits A (left upper corner, see Fig. 2A) of both lectins.

tween subunits of the dimers which interact to form the tetramer (AC and BD) (Fig. 2A) also differ between ConBr and ConA (Table 1). Thus, the subunits forming the dimers (AB and CD) adopt a more open conformation in ConBr than in ConA (schematically depicted in Fig. 2B). This relative shift does not affect significantly the relative distance between the metals and the centroids of the respective carbohydrate binding domains, but diminishes the number of both the hydrogen bonds and van der Waals contacts between the subunits (Table 1). On the other hand, in ConBr the distances between metal ions and CRDs between the more proximal subunits in the tetramer (AC and BD, Fig. 2A) are shorter than in the ConA, and the ConBr AC and BD interfaces display more hydrogen bonds and van der Waals contacts than the corresponding subunit interaction in ConA (Table 1). This relative movement of dimers AB and CD is shown in Fig. 2B. Finally, the distances between metal ions and CRDs of the more distant, diagonally positioned subunits are greater in the ConBr than in the ConA tetramer (Table 1). Hence, in comparison with ConA, the change in the relative disposition of subunits forming the ConBr dimer is further amplified by tetramerization. Similarly, Banerjee and colleagues [25] have concluded that the differences between the tetrameric arrangements in ConA and peanut lectin necessarily follow from their different modes of dimerization.

Interestingly, crystals of saccharide-free ConA belonged to space group I222 and the asymmetric unit contained one subunit [38,39], whereas Concanavalin A complexed with methyl α -D-mannopyranoside crystallized, like ConBr, in the $P2_12_12_1$ space group [17]. Saccharide-free ConA is a tetrameric structure composed of crystallographically identical subunits with exact 222 symmetry. In the saccharide complex, the ConA subunits are (like in ConBr) related by non-crystallographic 2-fold axes, and the tetrameric association is different from that of native ConA in that the monomers tend to separate (see Fig. 7 in [17]). Like ConBr, there are fewer changes for the AB and CD than for the AC and BD dimers. However, despite the striking similarities between the crystal structures of native ConBr and ConA-saccharide complex, both structures are significantly different (Table 1). Altogether, the

above data and our results indicate that ConA and ConA homologues can adopt different quaternary structures.

Although the structural basis of the different quaternary structures of ConBr and ConA needs further, high-resolution data, it is worth noting that the amino acid at position 58 (glycine in ConBr and aspartic acid in ConA) is the only different residue between ConA and ConBr involved in tetramer formation. At this position, replacement of Asp by Gly disrupts the hydrogen bond between Asp-58 of subunit A and Ser-62 of subunit C in ConA, and the α carbons of the corresponding residues in ConBr are 1.5 Å closer than in ConA. The other amino acid which is different in ConBr (Gly-70) is located in the poorly resolved loop 67–71, which interacts with region 117–123 in the AC and BD subunit interactions. The electron density of our ConBr crystals is poor at this region and hampers a closer structural analysis. Nonetheless, it would be interesting to carry out mutagenesis studies to investigate if replacement of ConBr Gly-58 by aspartic acid and/or Gly-70 by alanine would produce a molecule with the structure and functional characteristics of ConA.

3.2. Possible biological relevance

Structural studies with other lectins have revealed the importance of subunit multivalency as determinant of the biological activity of lectins, since the characteristics of binding can be tuned by alteration of the individual saccharide residues or their relative orientation [40]. Our data, suggesting that changes of the relative orientation of carbohydrate-binding sites within highly homologous lectins, i.e. ConA and ConBr may be the structural basis for explaining their distinct biological activities, are in accordance with the above proposition. We hypothesize that ConBr and ConA may bind to carbohydrate structures, which may be similar or identical though differently exposed on cell surfaces, triggering thereby the response of different cell populations or different quantitative effects on the same cells. The different carbohydrate crosslinking characteristics of ConBr and ConA might be further explored by higher-order lectin multimerization.

Multivalent carbohydrate derivatives have been used to discriminate between related proteins (reviewed in [40]). A recent

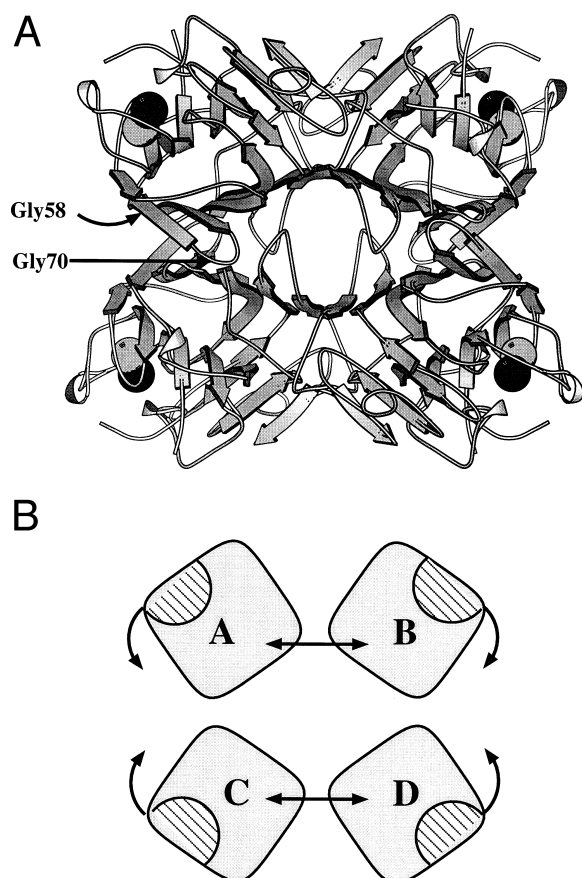


Fig. 2. (A) Ribbon drawing, generated using the program MOLSCRIPT [46], showing the overall structure of ConBr tetramer and the relative location of the four subunits. Gray and black balls represent Mn²⁺ and Ca²⁺ ions at the transition-metal-binding site (S1) and the neighbouring calcium site (S2), respectively [47]. CRD, carbohydrate recognition domain. The positions of residues Gly-58 and Gly-70, which in ConA are Asp and Ala, respectively, are shown. (B) Scheme of the relative movements between subunits of ConBr which differentiate the structure of this lectin from that of ConA. In comparison with native ConA, the ConBr tetramer expands vertically by 0.52 Å and horizontally by 0.43 Å, resulting in the carbohydrate recognition domains of the distant subunits (AD and BC) being 3.21 Å more separated in the ConBr tetramer than in ConA (see Table 1). The overlap of the dimers increases by 3°. The shaded surfaces represent the carbohydrate recognition domain of each subunit.

example [41] involves the homologous carbohydrate-binding proteins, Concanavalin A and the lectin from *Dioclea grandiflora* (75% amino acid sequence identity [42]), which exhibit similar binding preferences for glucose and mannose residues [43]. Comparing the binding properties of the two lectins with seventeen carbohydrate ligands, differences in binding free energies were in all cases less than 1 kcal/mol [44]. However, unlike ConA, *D. grandiflora* lectin (DGL) exists as a homotetramer at all pH values [42,45,48]. Weatherman and co-workers [41] studied the binding of a series of C-glycosides to ConA and DGL by titration microcalorimetry and fluorescence anisotropy. Bisglycosylated glucose and mannose oxanorbornene derivatives afforded complete recognition selectivity for ConA over DGL. The authors found that the stoichiometry of binding of ConA to these dimeric ligands was two ConA binding sites per ligand, and postulated that either ConA but not DGL was crosslinked by the bivalent

carbohydrate derivatives, or that ConA contains a second class of binding sites not found in DGL [41]. The observations of Weatherman and colleagues that monovalent ligands were not bound by ConA, and that none of the norbornyl ligands were bound by DGL, together with our present results and with the fact that *D. grandiflora* and other lectins of Brazilian legumes of the Diocleinae subtribe, including *Dioclea* (*D.*) *virgata*, *D. guianensis*, *Canavalia bonariensis*, *D. rostrata*, and *D. violacea*, display characteristic biological activities [34–36,46], suggest another possibility: that the bivalent glycoside derivatives interact with the two carbohydrate-binding sites of a ConA dimer and that the affinity and selectivity of this interaction requires a spatial arrangement of binding sites found in ConA, which may be different from that displayed by *D. grandiflora* lectin. Clearly, detailed biophysical and structural studies on Diocleinae lectins are needed to establish the structural basis of the diverse biological properties of these ConA-related lectins.

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