Eur. J. Biochem. *248*, 43–48 (1997)

Molecular cloning and characterization of ConBr, the lectin of *Canavalia brasiliensis* seeds

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(Received 6 May/19 June 1997) – EJB 97 0650/3

ConBr, a lectin isolated from *Canavalia brasiliensis* seeds, shares with other legume plant lectins from the genus *Canavalia* (*Diocleinae* subtribe) primary carbohydrate recognition specificity for D-mannose and D-glucose. However, ConBr exerts different biological effects than concanavalin A, the lectin of *Canavalia ensiformis* seeds, regarding induction of rat paw edema, peritoneal macrophage spreading in mouse, and *in vitro* human lymphocyte stimulation. The primary structure of ConBr was established by cDNA cloning, amino acid sequencing, and mass spectrometry. The 237-amino-acid sequence of ConBr displays Ser/Thr heterogeneity at position 96, indicating the existence of two isoforms. The mature *Canavalia brasiliensis* lectin monomer consists of a mixture of predominantly full-length polypeptide (α-chain) and a small proportion of fragments 1–118 (β-chain) and 119–237 (γ-chain). Although ConBr isolectins and concanavalin A differ only in residues at positions 58, 70, and 96, ConBr monomers associate into dimers and tetramers in a different pH-dependent manner than those of concanavalin A. The occurrence of glycine at position 58 does not allow formation of the hydrogen bond that in the concanavalin A tetramer exists between Asp58 of subunit A and Ser62 of subunit C. The consequence is that the α carbons of the corresponding residues in ConBr are 1.5 Å closer that in concanavalin A, and ConBr adopts a more open quaternary structure than concanavalin A. Our data support the hypothesis that substitution of amino acids located at the subunit interface of structurally related lectins of the same protein family can lead to different quaternary conformations that may account for their different biological activities.

Keywords: legume lectin; *Canavalia brasiliensis*; nucleotide sequence; amino acid sequence; mass spectrometry.

Carbohydrates encode an enormous information potential due to the many isomeric structures that can be constructed by permutations of (a) ring sites as points of glycosidic attachment, (b) α/β anomerity, (c) pyranose/furanose configuration, and (d) linear/branching structure. A trisaccharide composed of three different hexoses produce 38016 isomer permutations, whereas 27 and three structures can be constructed with three amino acids and three nucleotides, respectively (Laine, 1997). It is therefore not surprising that interactions between carbohydrates and lectins (proteins that decipher glycocodes) are widely recognized to play key roles in a variety of biochemical and cellular processes (Lis and Sharon, 1993; Varki, 1993; Opdenakker et al., 1993; Shur, 1994; Gabius, 1997).

Lectins comprise a structurally diverse group of carbohydrate-binding proteins found in organisms throughout the whole evolutionary scale (Rini, 1995; Weis and Drickamer, 1996). It is well established that bacterial, viral, and animal lectins serve to mediate a wide variety of biological recognition events (Gabius and Gabius, 1997). In contrast, and in spite of being the most thoroughly studied lectins, the function of plant lectins remains enigmatic (Rüdiger, 1997). 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 (reviewed by Rüdiger, 1997). In addition, the existence of hydrophobic sites within the structure of legume lectins, which bind phytohormones, i.e. indolacetic acid and cytokinins, that modulate their hemagglutinating activity and their interaction with carbohydrates, suggests a possible role for lectins in aspects of hormonally regulated

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Abbreviations. ConBr, seed lectin from *Canavalia brasiliensis*; ConA, seed lectin from *Canavalia ensiformis* (Jack bean); MALDI, matrix-assisted laser-desorption ionization; M_{av}, isotope-averaged molecular mass.

Enzymes. Trypsin, (EC 3.4.21.4); chymotrypsin (EC 3.4.21.1); glutanyl endopeptidase (endoproteinase Glu-C) (EC 3.4.21.19); DNA-directed DNA polymerase (EC 2.7.7.7).

Note. The novel ConBr nucleotide sequence data published here have been deposited with the EMBL and SwissProt sequence data banks and are available under accession numbers Y13904 and P55915, respectively.

plant growth and development (reviewed by Cavada et al., 1993).

Besides their physiological roles, lectins are attracting increasing interest in biotechnology owing to their ability to bind carbohydrates with considerable specificity. In addition, lectins are excellent tools for dissecting the molecular determinants encoding protein-carbohydrate recognition affinity and specificity (Bittiger and Schnebli, 1976; Lis and Sharon, 1986).

The overwhelming majority of plant lectins that have been isolated and characterized belong to different subfamilies and tribes of the *Leguminosae* family (Sharon and Lis, 1990; Rüdiger, 1997). Members of the legume lectin family show considerable primary structure similarity, but display differences in their carbohydrate-binding specificity (Sharon and Lis, 1990; Rini, 1995; Weis and Drickamer, 1996). The X-ray structures of several lectins from legume seeds have been solved and refined (Hamelryck et al., 1996; Banerjee et al., 1996; see also cited references), including concanavalin A (ConA) from Jack bean (*Canavalia ensiformis*), and those from pea (*Pisum sativum*), lentil (*Lens culinaris*), *Lathyrus ochrus*, *Griffonia simplicifolia*, *Erythrina corallodendron*, peanut (*Arachis hypogaea*), fava (*Vicia faba*), kidney bean (*Phaseolus vulgaris*), and soybean (*Glycine max*). As expected from their taxonomic relationship (see Table 1 in Sharon and Lis, 1990), the monomers of these lectins display rather similar three-dimensional structures called the canonical legume lectin fold, which consists of a dome-shaped sandwich of a flat, six-stranded β-sheet, and a curve, seven-stranded β-sheet (Rini, 1995; Weis and Drickamer, 1996). At physiological pH, these lectins are dimers except for ConA, peanut lectin, and soybean agglutinin, each being a dimer of a dimer. ConA, and the seed lectins of pea, fava, lentil, *Lathyrus ochrus*, and soybean dimerize in a similar manner, which 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 than the canonical subunit association of ConA have been found in lectins of *Erythrina corallodendron* (Shaanan et al., 1991), *Griffonia simplicifolia* (Delbaere et al., 1993), *Arachis hypogaea* (Banerjee et al., 1994), *Glycine max* (Dessen et al., 1995), and *Phaseolus vulgaris* (Hamelryck et al., 1996).

ConA was crystallized by Sumner and Howell in 1936 and was the first lectin to have its primary and three-dimensional structures solved (Edelman et al., 1972; Hardman and Ainsworth, 1972); its crystallographic structure has been recently refined to atomic resolution (Parkin et al., 1996). The many biochemical, biophysical, and structural studies carried out on ConA, both for the native and ConA-saccharide complexes (Reeke and Becker, 1988; Sharon and Lis, 1990; Naismith et al., 1994; Emmerich et al., 1995; Bouckaert et al., 1995; Naismith and Field, 1996; Harrop et al., 1996; Kanellopoulos et al., 1996a, b; Parkin et al., 1996; Hamodrakas et al., 1997; and cited references), make this protein the best characterized plant lectin and an extensively studied representative of its protein family. However, the lectins from *Canavalia ensiformis* and *Dioclea grandiflora* display different fine-saccharide-binding specificity for C-glycosides (Weatherman et al., 1996) and complex carbohydrates (Gupta et al., 1996). In addition, studies from our laboratories indicated that lectins isolated from taxonomically closely related legume plants exert distinct biological activities. Thus, when *Dioclea grandiflora* lectin and *Canavalia brasiliensis* lectin (ConBr) were compared with ConA for their ability to induce paw edema and peritoneal cell immigration in rats, it was found that ConA caused a slight edema with a peak at 1 h after injection and disappeared within 24 h, while *Dioclea grandiflora* lectin and ConBr induced a pronounced and long-lasting (>48 h) edema that reached a maximum at about 6 h (Bento et al., 1993). α-Methylmannoside blocked the edema caused by *Dioclea grandiflora* lectin and ConA, but did not affect that caused by ConBr. At doses that were ineffective in the induction of paw edema, the three lectins caused significant leukocyte accumulation in rat peritoneal cavities. The ability of ConBr to induce polymorphonuclear leukocyte and mononuclear cell immigration was greater than that of *Dioclea grandiflora* lectin and ConA. Although the three lectins display the same specificity for mannose and glucose in hemagglutinating assays, α-methylmannoside impaired ConA- and *Dioclea grandiflora* lectin-induced peritoneal leukocyte immigration, but only partially inhibited cell immigration induced by ConBr (Bento et al., 1993).

Differences in the histamine release by rat peritoneal mast cells induced by injection of glucose/mannose-specific lectins isolated from the beans of the plants *C. brasiliensis*, *Dioclea rostrata*, *Dioclea virgata*, *Canavalia maritima*, *Dioclea guianensis*, *Dioclea violacea*, *Dioclea grandiflora*, *Canavalia bonariensis*, *Cratylia floribunda*, and *Canavalia ensiformis*, which belong to the same tribe and sub-tribe (*Phaseoleae* and *Diocleinae*, respectively), have been reported (Gomes et al., 1994). In addition, these lectins display significant variation in their potency of stimulation of human lymphocyte proliferation and interferon γ production by peripheral blood mononuclear cells (Barral-Netto et al., 1992).

The striking distinct pattern of biological effects exerted by homologous lectins prompted us to isolate and characterize the lectin of *C. brasiliensis*.

MATERIALS AND METHODS

PCR amplification and cloning of the ConBr gene. Two oligonucleotides derived from the 5′ and 3′ coding regions of the ConA gene (nucleotides +61 to +933 relative to the transcription initiation site) were used as primers for amplification of the coding region of the ConBr gene by PCR using genomic DNA as template. The sequences of the deoxyoligonucleotide primers were 5′ (forward): 5′-CATATGGCCATCTCAAAGAA-ATC-3′ and 3′ (reverse): 5′-GGATCCTCAAACCACGGTAGC-AAT-3′. Total genomic DNA was purified from embryo axes of germinating *C. brasiliensis* seeds using the method described by Sambrook and colleagues (1989). Amplification was carried out in a 50 µl reaction volume containing 170 ng genomic DNA, 100 pmol of each primer, 200 µM each dNTP, and 2.5 U of *Taq* DNA polymerase (Pharmacia Biotech) in PCR buffer (10 mM Tris/HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$). The reaction mixture was first heated for 5 min at 94 °C in a Perkin Elmer 480 DNA thermal cycler and subsequently submitted to 30 cycles of amplification (1 min at 94 °C, 2 min at 65 °C, and 3 min at 72 °C) followed by 10 min extension at 72 °C. For production of recombinant plasmid-ConBr, PCR products were purified from 1% agarose gel (Qiaex gel extraction kit, Qiagen) and ligated to the linearized plasmid vector pCRII (TA cloning kit from Invitrogen) according to the manufacturer's instructions. Competent *Escherichia coli* DH5α cells were transformed with the pCRII-ConBr construct and plated out on LB agar plates containing carbenicillin and 5-bromo-4-chloro-3-indolyl-β-D-galactoside. Individual transformants were selected by their white colony phenotype. The presence of the insert in the plasmid was determined by small-scale preparation of plasmid DNA following the alkaline lysis method described by Sambrook et al. (1989) followed by restriction analysis and PCR.

DNA sequencing. DNA sequencing was performed by the dideoxynucleotide chain-termination method (Sanger et al.,

1977) using the Auto Read Sequencing kit (Pharmacia Biotech) employing fluorescent primers and a Pharmacia Biotech ALF automatic DNA sequencer. Sequencing was carried out on double-stranded plasmid DNA obtained from pCRIIConBr clones by the large-scale alkaline lysis method (Sambrook et al., 1989). Both strands were sequenced using M13 universal and reverse oligonucleotides or synthetic oligonucleotides as primers.

Isolation and biochemical characterization of ConBr. *Isolation of ConBr.* Mature seeds of *C. brasiliensis* Mart. were collected from plants growing in the state of Ceará, north-east Brazil. ConBr was isolated by fractional precipitation with ammonium sulfate followed by affinity chromatography on Sephadex G-50 as described (Moreira and Cavada, 1984). The concentration of ConBr was determined either spectrophotometrically at 280 nm using an absorption coefficient ($\varepsilon_{1\,cm,\,280\,nm}$) of 13.7 for a 10 mg/ml solution in 20 mM sodium phosphate, 150 mM NaCl, pH 7.2. This absorption coefficient, which has the same value as reported for ConA (Goldstein and Poretz, 1986), was determined by amino acid analysis [carried out with an AlphaPlus amino acid analyzer (Pharmacia) after sample hydrolysis with 6 M HCl, at 110°C for 24 h in evacuated, sealed ampoules] of aliquots of ConBr solutions of defined absorbance. ConBr chains were isolated by reverse-phase HPLC on a Lichrospher RP-100 C_{18} column (250 mm×4 mm, 5 μm particle size) (Merck) eluting at 1 ml/min with a mixture of 0.1% trifluoroacetic acid in water (solution A) and acetonitrile (solution B), first isocratically (25% B) for 5 min, followed by 25−70% B for 45 min.

Electrophoresis. The purity of the isolated lectin was determined by SDS/PAGE (Laemmli, 1970).

Edman degradation. N-terminal sequence analysis (using an Applied Biosystems Procise sequencer).

Carbohydrate analysis. For amino sugar and neutral sugar analyses, the samples were hydrolyzed at 110°C with 4 M HCl for 4 h or 2 M HCl for 2 h, respectively. Sialic acid was determined after sample hydrolysis for 1 h at 80°C with 0.2 M trifluoroacetic acid. Monosaccharides were resolved on a CarboPac PA1 column (0.4 cm×25 cm) eluting at 1 ml/min with either 16 mM NaOH (amino and neutral sugars) or 20 mM NaOH in 60 mM sodium acetate and analyzed using a Dionex DX-300 carbohydrate analyzer equipped with a pulsed amperometric detector and the AI-450 chromatographic software (Anumula and Taylor, 1991).

Enzymatic cleavages and isolation of peptides. ConBr (2−5 mg/ml in 100 mM Tris/HCl pH 8, 150 mM NaCl, 1 M guanidine hydrochloride) was digested with tosylphenylalanylchloromethane-treated-trypsin, α-chymotrypsin (Sigma), or glutamyl endopeptidase (endoproteinase Glu-C; Boehringer Mannheim) overnight at 37°C with an enzyme/substrate ratio of 1:100 (by mass). Proteolytic fragments were isolated by reverse-phase HPLC on a Lichrospher RP-100 C_{18} column (250 mm×4 mm, 5 μm particle size) (Merck) eluting at 1 ml/min with a mixture of 0.1% trifluoroacetic acid in water (solution A) and acetonitrile (solution B) and the following chromatographic conditions: (a) for tryptic peptides, isocratically (5% B) for 5 min, 5−50% B for 135 min, 50−70% B for 20 min; (b) for chymotryptic degradation, isocratically (10% B) for 5 min, 10−50% B for 100 min, 50−70% B for 20 min; and (c) for the endoproteinase Glu-C digest, isocratically (10% B) for 5 min, 10−20% B for 5 min, 20−50% B for 90 min, and 50−70% B for 20 min. Elution was monitored at 220 nm, fractions were collected manually, dried in a SpeedVac (Savant), and analyzed by amino acid analysis, N-terminal sequencing (as above), and mass spectrometry.

Mass spectrometry. The molecular masses of ConBr α-, β-, and γ-chains were determined by electrospray ionization mass spectrometry using a Sciex API-III LC/MS/MS triple quadrupole instrument. Molecular masses of proteolytic peptides were measured by matrix-assisted laser-desorption ionization (MALDI) mass spectrometry using a Kratos (Shimadzu) MALDI-I instrument and α-cyanohydroxycinnamic acid saturated in acetone as the matrix.

Analytical ultracentrifugation. Analytical ultracentrifugation was performed at 20°C using a Beckman XL-A centrifuge with absorption optics. For equilibrium measurements, six-channel cells were used allowing the simultaneous analysis of nine samples containing initially around 1 mg/ml ConBr in the following buffers supplemented with 1 mM of each $CaCl_2$ and $MgCl_2$: (a) 20 mM Tris/HCl, 0.1 M NaCl, pH 8.5, and pH 7.5; and (b) 20 mM sodium citrate, 0.1 M NaCl, pH 6.5, 5.5, 4.5, 3.5, and 2.5.

RESULTS AND DISCUSSION

Cloning and sequencing of the ConBr gene. We took advantage of the large structural similarity displayed by *Canavalia* lectins to design oligonucleotide primers derived from the N- and C-termini of the prepro-ConA precursor DNA sequence (Carrington et al., 1985) to amplify the coding region of the ConBr gene. The N-terminal 23-nucleotide primer 5′-CATATG-GCCATCTCAAAGAAATC-3′ corresponds to nucleotides coding for the first seven amino acids of the ConA precursor signal peptide, and an additional three nucleotide anchor that, together with the ATG initiation codon, produce a restriction site for *Nde*I. The C-terminal 24-nucleotide primer 5′-GGATCCT-CAAACCACGGTAGCAAT-3′ codes for the last six amino acids of prepro-ConA plus an additional six nucleotide anchor sequence incorporating a restriction site for enzyme *Bam*HI. Since most of the characterized legume lectin-encoding genes so far characterized are devoid of introns, including the genes from *Canavalia ensiformis* lectin (Carrington et al., 1985; Min et al., 1992) and *Canavalia gladiata* lectin (Yamauchi and Minamikawa, 1990), the ConBr coding sequence was amplified using *C. brasiliensis* genomic DNA as the template. This yielded a single 870-bp PCR product. This is the size of the ConA precursor, confirming the absence of intervening sequences in the ConBr gene. The PCR product was cloned into the pCRII vector and its complete DNA sequence was determined by sequencing both strands of the pCRIIConBr insert. The ConBr DNA sequence differs from that of ConA (EMBL nucleotide data bank accession number X01632, G17979) in ten nucleotides. Seven nucleotide changes are silent substitutions (which in ConBr and ConA are A310→C, T360→A, C393→A, A417→G, C421→T, T423→G C522→T, A624→G), while two, A662→G and C698→G, generate amino acid differences between ConA and ConBr, which in the mature proteins correspond to replacements of Asp58 (ConA) to glycine (ConBr) and Ala70 (ConA) to glycine (ConBr), respectively.

Protein chemical characterization of isolated ConBr. SDS/PAGE of purified ConBr showed that the protein consists of a major 30-kDa band and small proportions of 16-kDa and 12-kDa fragments. N-terminal sequence analysis of isolated ConBr showed the following two amino acid sequences: ADTIVA-VELDTYPNTDIGDPSYPHIGIDIKS (major) and STHET-NALHFMFNQFSKDQKDLILQ (minor). N-terminal amino acid sequencing of electroblotted samples demonstrated that the 30-kDa and the 16-kDa bands displayed the same N-terminal amino acid sequence (ADTIVA) and that the 12-kDa fragment had the N-terminal sequence STHET. This indicated that, like ConA, pro-ConBr is post-translationally cleaved into two dis-

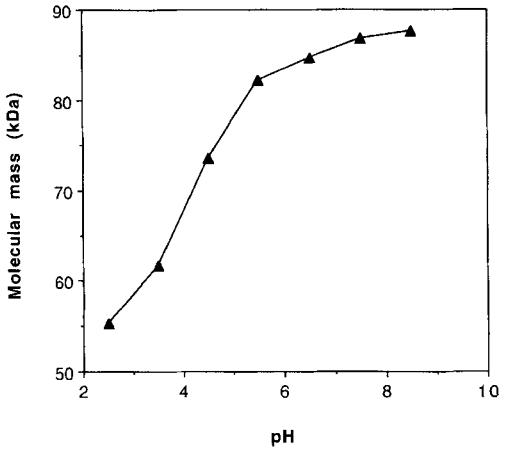

Fig. 1. pH-dependent oligomerization of ConBr determined by analytical centrifugation equilibrium sedimentation.

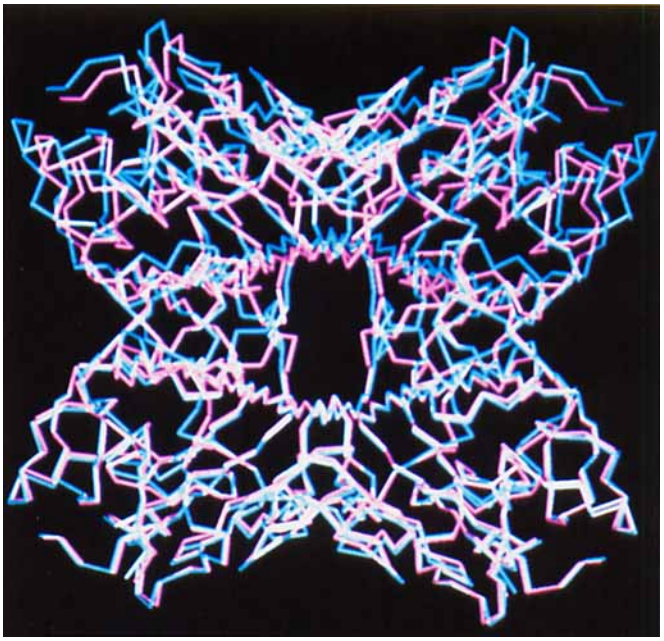

Fig. 2. Comparison of the three-dimensional structures of ConA (magenta) and ConBr (blue and white) tetramers. For superposition, the coordinates of ConA (Brookhaven Protein Data Bank accession code 2CTV) and the coordinates of ConBr (Sanz-Aparicio et al., 1997) were used.

tinct chains that are religated by transpeptidation to form the mature lectin (30-kDa α-chain) in which the alignment of residues 1−118 and the sequence from positon 119 to the C-terminus is reversed from that of the precursor (Min and Jones, 1994). Since this process is not quantitative, the 16-kDa and 12-kDa fragments (β- and γ-chains, respectively) are most probably unligated peptides rather than proteolytic products of the 30-kDa polypeptide.

The complete amino acid sequence of ConBr derived by DNA sequencing was confirmed by peptide mapping of isolated ConBr. In addition, a dimorphism (Ser/Thr) at position 96 was found, indicating the existence of two isolectins in the ConBr preparation. Amino acid analysis together with MALDI mass spectrometric analysis of tryptic peptides T7 ($M+H^+$ 692 Da) and T8 ($M+H^+$ 706 Da) showed that these proteolytic products corresponded to the same polypeptide stretch (NMQNGK, positions 41−46) but differed in by 14 Da, indicating that Met42 was oxidized in T8. This point was confirmed by mass spectrometric analysis. Reverse-phase HPLC separation in combination with electrospray ionization mass spectrometric analysis of isolated ConBr showed the following species (in daltons) whose N-terminal sequence is shown in parentheses: 12864±2 (ADTIVA); 12676±6 (STHETN); 12878±4 (ADTIVA); 25527±8 (ADTIVA); 25554±4 (ADTIVA). The 12676-Da species corresponds to the polypeptide encoded by the PCR-amplified ConBr DNA product from nucleotides 88−444 [calculated isotope-averaged molecular mass (M_{av}) 12680 Da]. The fragment with molecular mass 12864 Da corresponds to the product encoded by the ConBr gene between nucleotides 490−843 (calculated M_{av} 12865 Da). These data indicate that the protein with molecular mass 25527 Da (α1-chain) is made up by ligation of the 12864-Da (β1-chain) and the 12676-Da (γ-chain) species. The 12878-Da fragment may correspond to the same sequence as the β1-chain with Thr96 and oxidized Met42 (calculated M_{av} 12879 Da). This species was designated as the β2-chain. Finally, the protein of 25554 Da (α2-chain) may consist of covalently linked β2-chain and the 12676-Da γ-chain.

The above data show that ConBr is a mixture of two isolectins ($\alpha 1 = \beta 1\gamma$, and $\alpha 2 = \beta 2\gamma$). Carbohydrate analysis showed that ConBr is not glycosylated. ConBr isoforms α1 and α2 differ in a single amino acid (Ser/Thr) at position 96 and in two (α1) and three (α2) residues, respectively, from the amino acid sequence of ConA.

pH-dependent oligomerization of ConBr. The oligomerization state of ConBr as a function of the pH of the solution was assessed by equilibrium sedimentation. The apparent molecular mass of the lectin continuously increased from 55300 Da at pH 2.5 to a maximum value of 87600 Da at, or above, pH 6.5 (Fig. 1). The nominal molecular mass expected for the ConBr tetramer (102 kDa) was not obtained, suggesting that even at pH 8.5 ConBr displays a mixture of dimers and tetramers. This oligomerization behavior is different from that reported for ConA, which is a homogeneous dimer at pH 5 (Agrawal and Goldstein, 1968) and a dimer of dimers above pH 7 (Kalb and Lustig, 1968).

Noteworthy, the amino acid at position 58 (glycine in ConBr and aspartic acid in ConA) is involved in tetramer formation. X-ray crystallographic analysis of ConBr indicated that replacement of aspartic acid by glycine at this position disrupts the hydrogen bond between Asp58 of subunit A and Ser62 of subunit C in ConA, and the α-carbons of the corresponding residues in ConBr are 1.5 Å closer than in ConA (Sanz-Aparicio et al., 1997). The consequence is that the subunits forming the dimers (AB and CD) adopt a more open conformation in ConBr than in ConA (Fig. 2). Thus, in ConBr the distances between metal ions and carbohydrate recognition domains between the more proximal subunits in the tetramer are shorter than in ConA, and the ConBr AC and BD interfaces display more hydrogen bonds and van der Waals' contacts than the corresponding subunit interaction in ConA. In addition, the distances between metal ions and the carbohydrate recognition domains of the more distant, diagonally positioned subunits are 3.0 Å greater in ConBr than in the ConA tetramer.

We hypothesize that the different quaternary structures of ConBr and ConA may account for their different pH-dependent dimer-tetramer equilibrium. Relevant to this point, it has been reported that *Dioclea grandiflora* lectin is a tetramer at all pH values (Moreira et al., 1983; Richardson et al., 1984). *D. grandiflora* lectin has an alanine residue at position 58, which could stabilize the dimer-dimer interface through hydrophobic interaction. Crystallographic studies are underway in our laboratories to clarify this point.

Although lectins bind monosaccharides rather weakly, they employ common strategies for enhancing both the affinity and specificity of their interactions for more complex carbohydrate ligands, including extended secondary binding sites and subunit multivalency (Rini, 1995; Drickamer, 1995). Our data suggest that substitution of properly positioned amino acids may change the relative orientation of the carbohydrate-binding sites of highly similar lectins, i.e. conA and ConBr, and may be the structural basis for explaining their distinct biological activities (see Introduction). We hypothesize that ConBr and ConA bind to carbohydrate structures, which may be similar or identical though differently exposed on cell surfaces, thereby triggering the response of different cell populations or promoting different quantitative effects on the same cells.

The primary structure of lectin of *Canavalia gladiata* differs in two amino acids from that of ConBr, and displays a glycine residue at position 58 (Yamauchi and Minamikawa, 1990). Comparison of the physicochemical and biological characteristics of this lectin with those of conA and ConBr will provide further clues to dissect the effects of amino acid substitutions on the structure and function of homologous lectins.

This work was supported by grants from *Programa de Apoio ao Desenvolvimento Científico e Tecnológico* (PADCT), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Financiadora de Estudos e Projetos* (FINEP), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), *International Foundation for Science* (IFS), and *Fundação Cearense de Amparo à Pesquisa* (FUNCAP), Brasil, and grant PB95-0077 from *Dirección General de Investigaciones Científicas y Técnicas*, Madrid, Spain.

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