Biochemical and Biophysical Research Communications 408 (2011) 566–570

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Structural analysis of ConBr reveals molecular correlation between the carbohydrate recognition domain and endothelial NO synthase activation

Eduardo Henrique Salviano Bezerra [a], Bruno Anderson Matias Rocha [a,f], Celso Shiniti Nagano [b], Gustavo de Arruda Bezerra [a,c], Tales Rocha de Moura [a], Maria Júlia Barbosa Bezerra [a], Raquel Guimarães Benevides [a], Alexandre Holanda Sampaio [b], Ana Maria Sampaio Assreuy [d], Plínio Delatorre [e], Benildo Sousa Cavada [a,*]

[a] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Fortaleza, Brazil
[b] Departamento de Engenharia de Pesca, Universidade Federal do Ceará, Fortaleza, Brazil
[c] Institute of Molecular Biosciences, University of Graz, Humboldtstrasse, Graz, Austria
[d] Universidade Estadual do Ceará, Fortaleza, Brazil
[e] Departamento de Biologia Molecular, Universidade Federal da Paraíba, João Pessoa, Brazil
[f] Programa de Pós-Graduação em Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, Brazil

ARTICLE INFO

*Article history:*
Received 7 April 2011
Available online 19 April 2011

*Keywords:*
*Canavalia brasiliensis*
Carbohydrate recognition domain
Lectin
Endothelial NO synthase
Smooth muscle

ABSTRACT

Diocleinae lectins are highly homologous in their primary structure which features metal binding sites and a carbohydrate recognition domain (CRD). Differences in the biological activity of legume lectins have been widely investigated using hemagglutination inhibition assays, isothermal titration microcalorimetry and co-crystallization with mono- and oligosaccharides. Here we report a new lectin crystal structure (ConBr) extracted from seeds of *Canavalia brasiliensis*, predict dimannoside binding by docking, identify the α-aminobutyric acid (Abu) binding pocket and compare the CRD of ConBr to that of homologous lectins. Based on the hypothesis that the carbohydrate affinity of lectins depends on CRD configuration, the relationship between tridimensional structure and endothelial NO synthase activation was used to clarify differences in biological activity. Our study established a correlation between the position of CRD amino acid side chains and the stimulation of NO release from endothelium.



## 1. Introduction

Lectins are ubiquitous proteins/glycoproteins binding specifically and reversibly to mono- or oligosaccharides [1]. They are responsible for deciphering sugar codes through complex surface interactions and play a central role in a number of biological processes, such as infections, cell communication and cell growth [2]. Specific carbohydrate recognition by proteins is mediated by many interactions and is a complex process [3]. Legume lectins are widely used as structural models for studying carbohydrate/protein interactions due to their high sequence conservation in different organisms and wide range of carbohydrate specificities [4]. Seed lectins of the Diocleinae subtribe are highly homologous, notwithstanding their distinct biological activities, such as the stimulation of histamine secretion and nitric oxide production [5]. A range of activities of Diocleinae lectins are associated with minor changes in the amino acid sequence related mainly to the carbohydrate binding site [6]. Thus, the mere substitution of Pro202 for the residue Ser202 causes structural modifications in the carbohydrate binding site of the *Canavalia maritima* lectin (ConM), changing its affinity for disaccharides [7,8]. The relative position of the carbohydrate binding site and the pH-dependent dimer–tetramer equilibrium have also been shown to contribute to these changes [9,10].

Originally, Sanz-Aparicio et al. determined the crystal structure of the native *Canavalia brasiliensis* lectin (ConBr) at 3.0 Å resolution and concluded that the structure of ConBr differed from that of *Canavalia ensiformis* lectin (ConA) with regard to the relative orientation of the carbohydrate binding site and the overall configuration responsible for specific activities [11]. The authors also hypothesized that ConBr and ConA have different carbohydrate cross-linking characteristics [11]. Delatorre et al. [7] provided new information on carbohydrate cross-linking, describing the interaction of legume lectins with disaccharides. In addition, the interaction patterns at the carbohydrate binding site were described in a structural study of lectins complexed with dimannosides [8], and a new binding site for α-aminobutyric acid (Abu)—probably related to plant defense—was identified in a comprehensive structural analysis of *Canavalia gladiata* lectin (CGL) [12].

* Corresponding author. Address: BioMol-Lab, Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, P.O. Box 6043, 60455-970 Fortaleza, Ceará, Brazil. Fax: +55 85 3366 9818.
*E-mail address:* bscavada@ufc.br (B.S. Cavada).

0006-291X 
doi:10.1016/j.bbrc.2011.04.061

Currently available information on the structure of ConM and CGL allows us to hypothesize that lectin–carbohydrate affinity is directly related to carbohydrate binding site design and that different distances between specific amino acids result in different biological properties. In order to clarify the relationship between ConBr, ConA and ConM, we analyzed the new crystal structure of ConBr at 2.1 Å resolution and related the carbohydrate recognition domain of each lectin to the respective level of biological activity—in this case, the ability to activate the endothelium NO synthase present in the smooth muscle of rat aorta.

## 2. Material and methods

### 2.1. Crystallization and data collection

ConBr was purified as described by Cavada et al. [13] and solubilized in deionized water at a concentration of 12.5 mg $ml^{-1}$ in all crystallization experiments. Crystallization conditions for ConBr were screened using the hanging-drop vapor diffusion method with a commercially available crystallization solution (Hampton Research Screens I and II, Hampton Research, Riverside, CA, USA) at room temperature (293 K). Drops were prepared by mixing 2.0 μL sample solution and 2.0 μL reservoir solution, followed by equilibration against 300 μL reservoir solution [14]. Small crystals were obtained using crystallization condition No. 32 from the Crystal Screen II kit (0.1 M sodium chloride, 0.1 M Hepes, pH 7.5, and 1.6 M ammonium sulfate). The initial crystallization condition was optimized by increasing the concentration of sodium chloride, ammonium sulfate and pH. The best crystals were obtained with 0.2 M NaCl, 0.1 M Hepes, pH 8.5, and 1.8 M ammonium sulfate. Crystals suitable for X-ray diffraction grew in one week to maximum dimensions of approximately 0.4 × 0.5 × 0.4 mm.

X-ray diffraction data were collected at low temperature (100 K) and a maximum resolution of 2.1 Å, using 30% glycerol as cryoprotectant to avoid ice formation. The data were read at 1.42 Å wavelength at the MX1 beamline of the Laboratório Nacional de Luz Síncrotron (LNLS, Campinas, Brazil) using a MARCCD 165 detector (MAR research) placed 90 mm from the crystal. A set of 360 images (0.5° oscillation) was recorded. Diffraction data were indexed, integrated and scaled using MOSFLM [15] and SCALA [16]. Crystals belongs to the orthorhombic space group I222 and the Matthews coefficient of 2.43 $Å^3$ $Da^{-1}$ indicated the presence of a monomer in the asymmetric unit [17].

### 2.2. Molecular replacement and refinement

The new ConBr crystal structure was determined by molecular replacement using the program MolRep [18]. Coordinates of the native ConBr structure (PDB Code 1AZD) [11] were used as search model, with the best results indicating a correlation coefficient of 69.4% and an $R_{factor}$ of 41.2%. The Abu and glycerol coordinates were obtained using the program PRODRG [19]. The initial structures were refined using REFMAC5 [20]. Some main chain loop regions (68–70, 117–123, 148–151 and 202–205) were adjusted to satisfy the electron density map, and 139 water molecules were added to the model using Coot [21].

Two densities surrounding Arg228, observed at 5$\sigma$ on the $2f_o - f_c$ map (a region extensively available to the solvent surface), were explained by two chloride ions. One of these was coordinated with the nitrogen in side chain of Arg228 and the nitrogen atom of Ala236; the other was coordinated with a water molecule and two nitrogen atoms from the side chains of Arg228 and Asn14. A restrained refinement was subsequently performed resulting in 20.4% $R_{factor}$ and 25.3% $R_{free}$. An omit map contoured at 2$\sigma$ was generated for α-aminobutyric acid (Abu) using the program CCP4 Omit [20]. The crystallography refinement statistics are shown in Table 1.

**Table 1**
Statistics of data collection, refinement and structure quality.

| Parameter | Value |
|---|---|
| *Data collection* | |
| Space group | I222 |
| Unit cell parameters (Å) | |
| A | 68.3 |
| B | 73.0 |
| C | 99.5 |
| Total reflections | 339.768 |
| Number of unique reflections | 14.784 |
| Molecules per asymmetric unit | Monomer |
| Resolution limits (Å) | 58.9–1.8 |
| $R_{merge}$ (%) | 6.9 (19.6)[a] |
| Completeness (%) | 99.2 (99.2)[a] |
| Multiplicity | 6.7 |
| $(I)/\sigma$ | 6.9 (3.5)[a] |
| *Molecular replacement* | |
| Correlation coefficient | 69.4 |
| $R_{factor}$ (%) | 41.2 |
| *Refinement* | |
| Resolution range (Å) | 36.510–2.1 |
| $R_{factor}$ (%) | 20.4 |
| $R_{free}$ (%) | 25.3 |
| Number of residues in asymmetric unit | 237 |
| Number of water molecules | 139 |
| *Temperature factor* | |
| Average $B$ value for whole protein chain ($Å^2$) | 31 |
| *Ramachandran plot* | |
| Residues in most favored regions (%) | 86.4 |
| Residues in additional allowed regions (%) | 13.6 |
| Residues in generously allowed regions (%) | 0 |

[a] Values in parentheses represent the high resolution shell.

### 2.3. Molecular docking and structure analysis

The solved ConBr structure was aligned with original native ConBr (PDB Code 1AZD), ConM (PDB Code 2CWM) and ConA (PDB Code 1JBC). Significant differences in primary structure and structure characteristics, such as loops, amino acid side chain availability, CRD configuration and distances between amino acids were investigated and the volume of each CRD was calculated by the program Q-SiteFinder [22]. The obtained data allowed grouping the lectins according to structural features and relate differences in structure to the level of biological activity.

Molecular docking was performed with MolDock—an interactive molecular graphics program [23]. α-D-Mannosyl-1,2-methyl-α-D-mannopyranoside was used to verify the carbohydrate binding properties of ConBr. MolDock is based on a search algorithm combining differential evolution with a cavity prediction algorithm. The program takes hydrogen bond directionality into account as an additional term in the docking scoring function. A re-ranking procedure was added to increase docking accuracy [24].

The biological activity used to compare lectins was their ability to induce *in vitro* relaxation of rat aortic rings, as previously described by our group, using the same experimental setting [6,27].

## 3. Results and discussion

### 3.1. Overall structure of ConBr

The new crystal structure of ConBr (PDB Code 3JU9) reveals some differences from the previously deposited native ConBr

structure (PDB Code 1AZD) [11]. ConBr biological assemble is a tetramer. The refined structure consists of a ConBr monomer, 139 water molecules, two chloride ions, a glycerol in the carbohydrate recognition domain and a single Abu molecule in the hydrophobic pocket. When the new structure is solved at 2.1 Å, its main chain configuration is largely similar to that of the low-resolution native ConBr (1AZD). However, highly unstable segments such as the ConA-like conserved loop in region 117–123—commonly described as a region of low electron density [24]—shows considerable improvement on electron density maps. The most significant differences between the native and the refined structure, including changes in the position of amino acid residues, were observed in loop regions 68–70, 117–123, 148–151 and 202–205.

The $f_o - f_c$ map shows a density surrounding Leu115, Leu126 and Val179 corresponding to a non-protein amino acid (Abu) commonly co-purified with Diocleinae lectins, the coordinates of which were previously described for *C. gladiata* lectin (CGL) by Delatorre et al. [12]. The crystal structure of CGL (PDB Code 2D7F) features a hydrophobic pocket which explains the interaction.

The Abu molecule is tightly bound through hydrophobic and hydrophilic interactions in the ConBr crystal structure. The hydrophobic pocket (Fig. 1A) shows a region accessible to the solvent surface, allowing hydrophilic interactions to occur. These hydrophilic interactions (Fig. 1B) occur through hydrogen bonds between the nitrogen atom in Abu and the nitrogen atom in His180 (main chain), and between the oxygen atom in Abu and the oxygen atom in Ala125 (main chain) or the nitrogen atom in Ala125 (peptide bonds). Abu also interacts with two water molecules, involving oxygen and nitrogen at 2.76 and 2.86 Å, respectively. In addition, Abu mediates the contacts between the components of the canonical dimer, establishing hydrogen bonds with Asp139 and Met129 (Table 2). The interaction between Abu and ConBr is shown in Fig. 1. An omit map contoured at $2\sigma$ agreed with the α-aminobutyric acid (Abu) coordinates.

**Table 2**
Distances between Abu and the pocket amino acid residues at canonical dimmers interface.

| Abu atom | Amino acid atom[a] | Chain[a] | Distance (Å)[a] |
|---|---|---|---|
| N1 | HIS180 N1 | A | 2.7 |
| O | ALA125 N | A | 3.4 |
| O | ALA125 O | A | 3.4 |
| OTX | ASP139 OD2 | B | 2.6 |
| O | MET129 O | B | 3.4 |

The residues Leu115, Leu125, Ala126 and Val179, which make up the hydrophobic pocket, are conserved in legume lectins [12]. The interactions between ConBr and Abu increase structure stability, producing a decrease in vibrational scattering and improving electron density in loop extremities. Atom positions were less clear in previous ConA structures due to the poor electron density of this surface loop [25].

### 3.2. Three-dimensional CRD configuration and its influence on endothelium NO synthase activation

Diocleinae lectins display a high degree of identity in their primary structures and share many biochemical and structural features, the most characteristic of which is conserved regions. Thus, the amino acid residues of the carbohydrate binding site (Tyr12, Asn14, Leu99, Tyr100, Asp208 and Arg228), the metal binding site (Glu8, Asp10, Tyr12, Asn14, Asp19, His24, Val32, Ser34, Asp208 and Arg228) and the hydrophobic cavity (Tyr54, Leu81, Leu85, Val89, Val91, Phe111, Ser113, Val179, Ile181, Phe191, Phe212 and Ile214) conserved in the primary structure of ConBr are similarly conserved in many other Diocleinae lectins [5].

The high level of CRD conservation in Diocleinae lectins does not seem to determine the specificity of carbohydrate interaction.

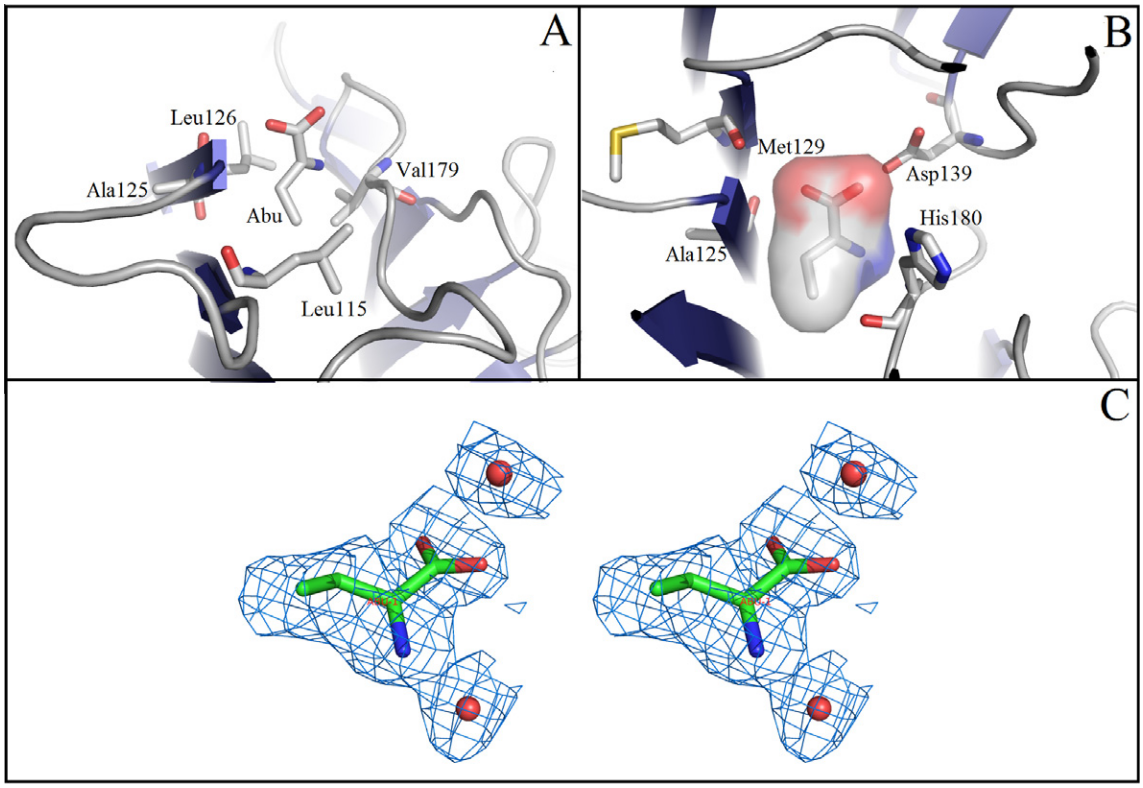


**Fig. 1.** Abu binding pocket interactions. (A) Region of hydrophobic interaction and the "tie" formed to stabilize loop 117–123 promoted by Abu. (B) Main hydrophilic interactions between Abu and residues of the canonical dimer. (C) Omit map stereo view representation of α-aminobutyric acid (Abu) binding pocket.

Thus, ConM and ConA have different patterns of interaction with dimannosides, despite containing the same amino acid residues in the CRD. One possible determinant of this specificity is the substitution of Pro202 for Ser202, an amino acid residue proximal to the CRD. This increases significantly the ConM carbohydrate-binding capacity by reducing the interference of His205 (which is closer to Tyr100 than to Tyr12) [8]. When a dimannoside is introduced in the ConBr carbohydrate binding domain by docking, the interactions between the mannoses and the amino acids are formed mainly by polar and van der Waals contacts, and the binding is fully stabilized by hydrophobic interactions with Tyr12, Leu99 and Tyr100. Twelve H-bonds are formed between the dimannoside and six amino acids of the carbohydrate binding domain (Leu99, Tyr100, Ser168, Asp208, Thr226 and Arg228) (Fig. 2) and van der Waals interactions are established between the dimannoside and Tyr12, Leu99, Ala207 and Asp208 (Table 3).

The ability of ConA-like lectins (including ConBr and ConM) to activate NO synthase is variable. According to some authors, ConM is the most potent of the three [6,26,27]. The fact that these lectins have a conserved CRD suggests that differences in biological activity are due to differences in spatial arrangement. Thus, whereas the CRD structure of ConM and ConBr is very similar, the residues that make up this cavity (Arg228-Asn14, Arg228-Tyr12, Tyr12-Tyr100, Tyr12-Tyr14, Leu99-Tyr100 and Arg228-Leu99) are closer together than Arg228-Tyr100 (Table 3), making the CRD narrower and deeper in both structures.

Although the level of NO synthase activation induced by ConBr and ConA is similar, ConBr is geometrically more akin to ConM. Delatorre and co-workers reported that a substitution of Pro202 modifies the configuration at loop 202–205, possibly explaining the movement of Leu99 and Tyr100 [6,7]. However, though no such substitution is visible in ConBr, the loop geometry is similar to that of ConM. This loop configuration promotes a close interaction between the Asp203 side chain and the Tyr100 main chain, easing the steric hindrance in close contact with Tyr12. This is made possible by a reduction in the distance between Gly227 and Tyr12, which influences the short ranges between other carbohydrate residues and the carbohydrate binding pocket. ConBr and ConM are very similar with regard to site design, whereas the site in ConA is more open, associated with a reduced ability to induce nitric oxide production.

**Table 3**
Distances (Å) between amino acid residues involved in interaction with carbohydrates.

| | | ConBr 3JU9 | ConM 2CWM | ConA 1JBC |
|---|---|---|---|---|
| Tyr12 CZ | Gly227 CA | 8.67 | 8.97 | 9.61 |
| Leu99 CG | Tyr12 CZ | 6.85 | 7.64 | 9.6 |
| Leu99 CG | Asn14 ND2 | 9.11 | 9.08 | 10.29 |
| Leu99 CG | Arg228 CZ | 11.86 | 11.16 | 10.53 |
| Tyr100 CZ | Tyr12 CZ | 5.45 | 5.26 | 4.37 |
| Tyr100 CZ | Asn14 ND2 | 9.9 | 9.48 | 8.62 |
| Tyr100 CZ | Arg228 CZ | 14.25 | 13.7 | 10.87 |

[a] This distances correspond to the residue atoms which are involved in interaction with carbohydrate.

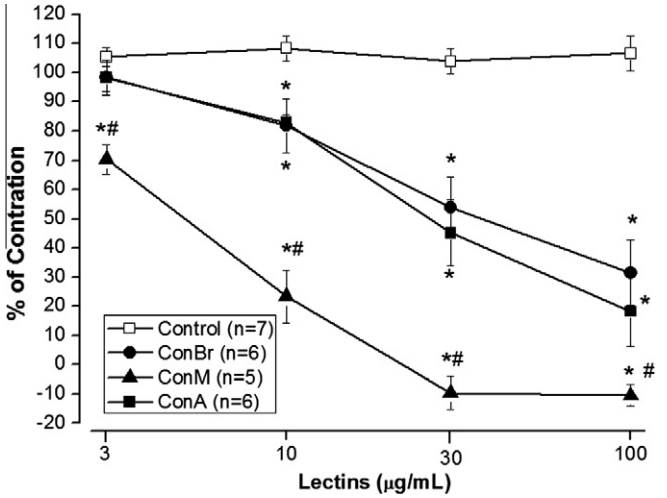


**Fig. 3.** *Canavalia* lectins induce relaxation in endothelized aortic rings precontracted with phenylephrine (Phe). Data comparing the responses of CGL, ConBr, ConA and ConM to control (100% Phe contraction). Values expressed as mean ± SEM; $^{*}p < 0.05$ versus control, $^{\#}p < 0.05$ versus ConBr and ConA. (Adapted from Assreuy et al. [27] and Gadelha et al. [6].)

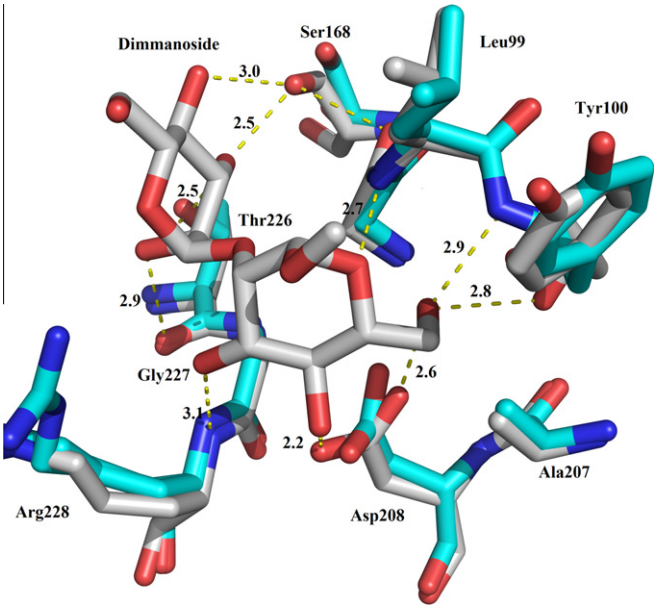


**Fig. 2.** Superposition of the carbohydrate binding domain of ConBr (gray; PDB Code 3JU9) and ConA (cyan; PDB Code 1I3H) with α-D-mannosyl-1,2-methyl-α-D-mannopyranoside introduced into the ConBr structure by docking. H-bonds are represented in yellow with their respective distances in angstroms (Å). Labels represent all amino acids involved in polar contacts and van der Waals interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

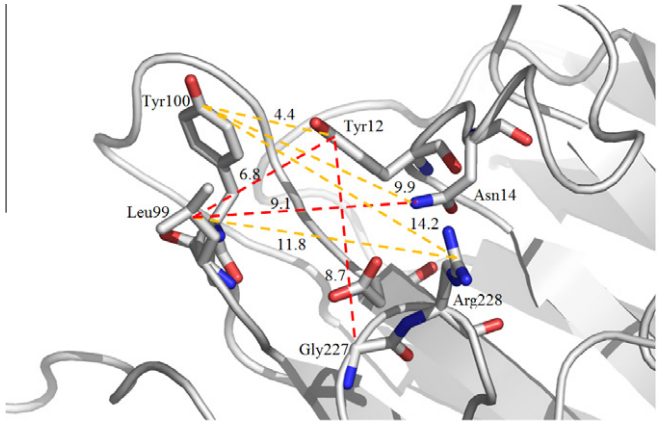


**Fig. 4.** Carbohydrate recognition domain (CDR). Dashes show the distances (Å) between CRD residues involved in geometrical changes of the pocket. In ConBr, distances are shorter between Gly227-Tyr12, Leu99-Tyr12 and Leu99-Asn14 (red dashes) and greater between the residues which characterize efficient inducers of NO production. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.3. CRD volume and biological activity of ConBr

The configuration and reciprocal distances between the residues in the CRD of ConBr suggests this lectin would be a more potent inducer of nitric oxide production than ConM, but in fact ConBr was less efficient than ConA (Fig. 3). In this study, the volume of each CRD was calculated and associated with the geometry of the amino acid side chains. The volumes obtained were ConM = 135 Å$^3$ (PDB Code 2CWM), ConA = 151 Å$^3$ (PDB Code 1JBC) and ConBr = 105 Å$^3$ (PDB Code 3JU9). Although ConBr and ConM have a similar CRD configuration, CRD volume is much smaller in the former, making it less efficient in stimulating NO synthase activation of endothelial cells. The smaller size may be explained by the extrapolation of ideal distances between Tyr100 and Arg228 and the very small distance between Leu99 and Tyr12 in the carbohydrate binding site (Fig. 4, Table 3).

On the surface of endothelial cells are found complex signaling carbohydrates which influence the recognition of lectins with different CRD volume. The number of interactions between the CRD and the endothelial cell carbohydrate makes a significant difference in biological activity.

Some differences in biological activity may entail not only differences in primary structure directly related to the specificity site, but also differences in the configuration of the site and adjacent loops. The new ConBr structure with improved resolution shows how geometric parameters in the CRD can determine interactions with carbohydrates and, consequently, biological activity. ConBr and ConM use different strategies (a loop translocation and the substitution of Pro202 for Ser202, respectively) to achieve the same structural change in the CDR, characterized mainly by an approximation between Tyr12 and Tyr100 and distancing of Tyr100 from Arg228. This structural CRD design may be considered the most important factor determining NO induction by Diocleinae lectins, characterized by at least two configurations which promote (ConM) or reduce (ConA) the NO-dependent inflammatory response stimulated by these lectins. The lesser ability of ConBr to induce nitric oxide production seems to be a result of its smaller CRD volume—an essential feature in the assessment of biological activity. It is important to note that while our study assessed biological activity using a model that explores the endothelial NO synthase, experiments assessing other NO synthase isoforms might shed light on this important issue. Potentially, there is much to be learned about lectin activity by studying groups of lectins with similar CRD configuration and volume.

## Acknowledgments

This work was partly financed by Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Laboratório Nacional de Luz Síncrotron (LNLS), Campinas—Brazil. C.S.N., A.H.S., A.M.S.A., P.D. and B.S.C. are senior investigators of CNPq. We thank Dr. Albert Leyva, David Erickson and Jesper Sampaio for helping with the English language editing of the manuscript. Additional funding was provided by the Doktoratskolleg "Molecular Enzymology" Grant W901-B05 from the Austrian Science Fund (to K.G. and G.A.B.)

## References

[1] W. Peumans, A. Barre, Q. Hao, P. Rougé, E.J.M. Van Damme, Higher plants developed structurally different motifs to recognize foreign glycans, Trends Glycosci. Glycotechnol. 12 (64) (2000) 83–101.
[2] A. Pusztai, S. Bardocz, S.W. Ewen, Uses of plant lectins in bioscience and biomedicine, Front. Biosci. 13 (2008) 1130–1140.
[3] R. Loris, I. Van Walle, H. De Greve, S. Beeckmans, F. Deboeck, L. Wyns, J. Bouckaert, Structural basis of oligomannose recognition by the *Pterocarpus angolensis* seed lectin, J. Mol. Biol. 335 (5) (2004) 1227–1240.
[4] R. Loris, T. Hamelryck, J. Bouckaert, L. Wyns, Legume lectin structure, Biochem. Biophys. Acta 1383 (1998) 9–36.
[5] B.S. Cavada, T. Barbosa, S. Arruda, T.B. Grangeiro, M. Barral-Netto, Revisiting proteus: do minor changes in lectin structure matter in biological activity? Lessons from and potential biotechnological uses of the Diocleinae subtribe lectins, Curr. Protein Pept. Sci. 2 (2001) 123–135.
[6] C.A.D.A. Gadelha, F.B.M.B. Moreno, T. Santi-Gadelha, J.B. Cajazeiras, B.A.M. Rocha, A.M.S. Assreuy, M.R. Lima Mota, N.V. Pinto, A.V. Passos Meireles, J.C. Borges, B.T. Freitas, F. Canduri, E.P. Souza, P. Delatorre, D.N. Criddle, W.F. De Azevedo Jr., B.S. Cavada, Native crystal structure of a nitric oxide-releasing lectin from the seeds of *Canavalia maritima*, J. Struct. Biol. 152 (3) (2005) 185–194.
[7] P. Delatorre, B.A.M. Rocha, C.A.A. Gadelha, T. Santi-Gadelha, J.B. Cajazeiras, E.P. Souza, K.S. Nascimento, V.N. Freire, A.H. Sampaio, W.F. Azevedo Jr., B.S. Cavada, Crystal structure of a lectin from *Canavalia maritima* (ConM) in complex with trehalose and maltose reveals relevant mutation in ConA-like lectins, J. Struct. Biol. 154 (2006) 280–286.
[8] G.A. Bezerra, T.M. Oliveira, F.B.M.B. Moreno, E.P. de Souza, B.A.M. Rocha, R.G. Benevides, P. Delatorre, W.F. De Azevedo Jr., B.S. Cavada, Structural analysis of *Canavalia maritima* and *Canavalia gladiata* lectins complexed with different dimannosides: new insights into the understanding of the structure biological activity relationship in legume lectins, J. Struct. Biol. 160 (2007) 168–176.
[9] D.A. Wah, A. Romero, F.G. Sol, B.S. Cavada, M.V. Ramos, T.B. Grangeiro, A.H. Sampaio, J.J. Calvete, Crystal structure of native and Cd/Cd-substituted *Dioclea guianensis* seed lectin. A novel manganese-binding site and structural basis of dimer–tetramer association, J. Mol. Biol. 310 (2001) 885–894.
[10] T.M. Oliveira, P. Delatorre, B.A.M. Rocha, E.P. Souza, K.S. Nascimento, G.A. Bezerra, T.R. Moura, R.G. Benevides, E.H.S. Bezerra, F.B.M.B. Moreno, V.N. Freire, W.F. Azevedo Jr., B.S. Cavada, Crystal structure of *Dioclea rostrata* lectins: insights into understanding the pH-dependent dimer–tetramer equilibrium and the structural basis for carbohydrate recognition in Diocleinae lectins, J. Struct. Biol. 164 (2) (2008) 177–182.
[11] J. Sanz-Aparicio, J. Hermoso, T.B. Grangeiro, J.J. Calvete, B.S. Cavada, The crystal structure of *Canavalia brasiliensis* lectin suggests a correlation between its quaternary conformation and its distinct biological properties from Concanavalin A, FEBS Lett. 405 (1997) 114–118.
[12] P. Delatorre, B.A.M. Rocha, E.P. Souza, T.M. Oliveira, G.A. Bezerra, F.B.M.B. Moreno, B.T. Freitas, T. Santi-Gadelha, A.H. Sampaio, W.F. Azevedo Jr., B.S. Cavada, Structure of a lectin from *Canavalia gladiata* seeds: new structural insights for old molecules, BMC Struct. Biol. 7 (2007). article no. 52.
[13] R.A. Moreira, B.S. Cavada, Lectin from *Canavalia brasiliensis* (Mart.). Isolation, characterization and behavior during germination, Biol. Plant. 26 (1984) 113–120.
[14] J. Jancarik, S.H. Kim, Sparse matrix sampling: a screening method for crystallization of proteins, J. Appl. Crystallogr. 24 (1991) 409–411.
[15] A.G.W. Leslie, The integration of macromolecular diffraction data, Acta Crystallogr. D: Biol. Crystallogr. 62 (1) (2006) 48–57.
[16] P.R. Evans, Scaling and assessment of data quality, Acta Crystallogr. D: Biol. Crystallogr. 62 (2006) 72–82.
[17] B.W. Matthews, The solvent content of protein crystals, J. Mol. Biol. 33 (1968) 491–497.
[18] A. Vargin, A. Teplyakov, MOLREP: an automated program for molecular replacement, J. Appl. Crystallogr. 30 (1997) 1022–1025.
[19] A.W. Schuettelkopf, D.M.F. Van Aalten, PRODRG—a tool for high-throughput crystallography of protein–ligand complexes, Acta Crystallogr. D: Biol. Crystallogr. 60 (2004) 1355–1363.
[20] Collaborative Computational Project, Number 4, The CCP4 suite: programs for crystallography, Acta Crystallogr. D: Biol. Crystallogr. 50 (1994) 760–763.
[21] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics, Acta Crystallogr. D: Biol. Crystallogr. 60 (2004) 2126–2132.
[22] A.T. Laurie, R.M. Jackson, Q-SiteFinder: an energy-based method for the prediction of protein–ligand binding sites, Bioinformatics 21 (2005) 1908–1916.
[23] R. Thomsen, M.H. Christensen, MolDock: a new technique for high-accuracy molecular docking, J. Med. Chem. 49 (2006) 3315–3321.
[24] W.F. De Azevedo Jr., MolDock applied to structure-based virtual screening, Curr. Drug Targets 11 (2010) 327–334.
[25] J. Bouckaert, T.W. Hamelryck, L. Wyns, R. Loris, The crystal structures of Man(alpha 1-3)Man(alpha 1-0)Me and Man(alpha 1-6)Man(alpha 1-0)Me in complex with Concanavalin A, J. Biol. Chem. 274 (1999) 29188–29195.
[26] J.L. Andrade, S. Arruda, T. Barbosa, L. Paim, M.V. Ramos, B.S. Cavada, M. Barral-Netto, Lectin-induced nitric oxide production, Cell. Immunol. 194 (1999) 98–102.
[27] A.M.S. Assreuy, S.R. Fontenele, A. De Freitas Pires, D.C. Fernandes, N.V.F.C. Rodrigues, E.H.S. Bezerra, T.R. Moura, K.S. Nascimento, B.S. Cavada, Vasodilator effects of Diocleinae lectins from the *Canavalia* genus, Naunyn-Schmiedeberg's Arch. Pharmacol. 380 (2009) 509–521.