Research paper

Crystal structure of a pro-inflammatory lectin from the seeds of Dioclea wilsonii Standl.


Aims: Following crystallization and structural determination by standard molecular replacement techniques, DwL was found to be a tetramer based on PISA analysis, and composed by two metal-binding sites per monomer and loops which are involved in molecular oligomerization. DwL presents 96% and 99% identity with two other previously described lectins of Dioclea rostrata (DRL) and Dioclea grandiflora (DGL). DwL differs structurally from DVL and DRL with regard to the conformation of the carbohydrate recognition domain and related biological activities. The structural analysis of DwL in comparison to other Diocleinae lectins can be related to the differences in the dose-dependent pro-inflammatory effect elicited in Wistar rats, probably via specific interactions with mast cells complex carbohydrate, resulting in significant paw edema. DwL appears to be involved in positive modulation of mast cell degranulation via recognition of surface carbohydrates. Since this recognition is dependent on site volume and CRD configuration, edematogenesis mediated by resident cells varies in potency and efficacy among different Diocleinae lectins.

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Abstract

The crystal structure and pro-inflammatory property of a lectin from the seeds of Dioclea wilsonii (DwL) were analyzed to gain a better understanding of structure/function relationships of Diocleinae lectins. Following crystallization and structural determination by standard molecular replacement techniques, DwL was found to be a tetramer based on PISA analysis, and composed by two metal-binding sites per monomer and loops which are involved in molecular oligomerization. DwL presents 96% and 99% identity with two other previously described lectins of Dioclea rostrata (DRL) and Dioclea grandiflora (DGL). DwL differs structurally from DVL and DRL with regard to the conformation of the carbohydrate recognition domain and related biological activities. The structural analysis of DwL in comparison to other Diocleinae lectins can be related to the differences in the dose-dependent pro-inflammatory effect elicited in Wistar rats, probably via specific interactions with mast cells complex carbohydrate, resulting in significant paw edema. DwL appears to be involved in positive modulation of mast cell degranulation via recognition of surface carbohydrates. Since this recognition is dependent on site volume and CRD configuration, edematogenesis mediated by resident cells varies in potency and efficacy among different Diocleinae lectins.
ability to distinguish and crosslink multivalent saccharide ligands or specific cell-surface arrays of glycoconjugates [6,7].

Information on carbohydrate-binding site recognition and specific interactions with different sugars and glycolipids obtained by X-ray crystallography can help establish correlations between cell-surface binding activity and biological activities in different physiological systems [8].

Diocleinae lectins are dimeric or tetrameric structures of dome-shaped monomers consisting of jelly-roll motifs interconnected by turns and loops [9]. According to Rangel and coworkers [10], DwL weighs 25.6 kDa and features 237 residues per monomer. The amino acid sequence identity between DwL (UniProtKB, with accession P86624) [10] and DRL is 96%. Despite the considerable similarity in primary and three-dimensional structure, the proteins in this group differ substantially with regard to biological properties, making them excellent models for the study of structure/function relationships [5,11].

Diocleinae lectins may produce either anti-inflammatory [12,13] or pro-inflammatory effects [14,15], depending on the route of administration. Sugar residues also play an important role, as shown by the reversal of biological activities when lectins are associated with their specific binding sugars. Several reports have described pro-inflammatory activities (mast cell stimulation) for Diocleinae lectins [16–18]. Mast cells are intimately involved in the pathophysiology of allergic diseases and inflammation and their activation promotes the release of chemical mediators responsible for important tissue changes such as vasodilatation, increased vascular permeability, bronchoconstriction and neutrophil or eosinophil chemotaxis [19].

Thus, the objective of this study was to evaluate the pro- and anti-edematogenic activity of a lectin isolated from the seeds of Dioclea wilsonii and the correlation between crystal structure and biological properties, including the participation of resident cells.

2. Materials and methods

2.1. Isolation of D. wilsonii lectin (DwL)

DwL was purified in two steps by dextran affinity chromatography on a Sephadex G-50 column followed by ion exchange chromatography on HiTrap SP XL 01, as described by Rangel and colleagues [10]. The apparent molecular weight of DwL and fragments (β and γ) was estimated by SDS-PAGE in the presence of β-mercaptoethanol [20].

2.2. Crystallization and X-ray data collection

DwL was diluted homogenously to a concentration of 10.0 mg mL⁻¹ in ultrapure water and incubated during 1 h with 3 mM X-Man (5-bromo-6-chloro-3-indolyl-alpha-D-mannopyranoside) for all crystallization experiments. Crystallization conditions for DwL were screened using the hanging-drop vapor diffusion method with Hampton Research Crystal Screens I and II (Hampton Research, Riverside, CA, USA) [21] at room temperature (293 K). Crystals were obtained using condition #41 of Crystal Screen II (0.01 M Nickel (II), 0.1 M Tris pH 8.5, 1.0 M lithium sulfate monohydrate). Crystal improvement was achieved by lowering the pH value to 8.0 of the condition #41 [10].

X-ray data were collected from a single orthorhombic crystal (point group I222) cooled to 100 K. Crystals were soaked in a cryoprotectant solution containing 30% glycerol to avoid ice formation and data were collected using synchrotron radiation (MX1 station, National Laboratory of Synchrotron Light—LNLS, Campinas, Brazil) of 1.47 Å wavelength. A complete data set was obtained using a CCD detector (MAR Research) with 120 frames with 40 s of exposure per frame and a 1° oscillation range. Data had to be cut at 2.3 Å due to ice ring issues. I/σI was 0.0 in the next shell (2.23 Å). The data set extending to a maximum resolution of 2.3 Å was indexed and integrated using XDS [22] and scaled with SCALA [23].

2.3. Molecular replacement and refinement

The primary sequence of DwL was aligned with that of other ConA-like lectins. Thus, the lectins of Dioclea violacea (DVL), Dioclea guianensis (Dgui) and Dioclea rostrata (DRL) presented 96%, 95% and 96% identity with DwL, respectively [10]. DRL (PDB code: 2ZBJ) was the best model in the molecular replacement study using Phaser [24]. After this, it was observed R_factor = 0.3565 and R_free = 0.356.

Several rounds of iterative refinement were performed with the PHENIX suite [25], adding water molecules and defining the optimal X-ray weight. The residues GLY58, GLY70, VAL129, ASP192 and SER202 were replaced and the side chain conformations were roughly adjusted to satisfy the electron density map. A total of 92 water molecules were added. Manual adjustments in Coot [26] were made after each refinement step and X-Man molecules were manually added to the model. The structure was validated using the MolProbity server [27]. The temperature factors for ligands were 24.71 (X-Man) and 23.46 (Abu).

2.4. Biological assays

2.4.1. Animals

Wistar rats (180–250 g) were kept in cages (6 in each) in a controlled environment (circadian cycle, 25 °C, food and water ad libitum). The experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of Ceará (UECE, Fortaleza, Ceará, Brazil) under No. 10130208-8/40, following the recommendations of the Guide for the Care and Use of Laboratory Animals of the US Department of Health and Human Services (NIH publication No. 85–23, revised 1985).

2.4.2. Drugs and reagents

α-methyl-α-mannoside (α-C3H), α-glucose, indomethacin, N4-nitro-L-arginine methyl ester (LNAME), pentoxifylline, pyrilamine, thioglycolate, compound 48/80 and Evans blue were purchased from Sigma (St. Louis, MO, USA). All drugs were solubilized directly in sterile saline, except for indomethacin, which was initially dissolved in dimethyl sulfoxide up to 10% of the total volume, then in saline.

2.4.3. Rat paw edema model

Paw volume was measured with a hydroplethysmometer at baseline (immediately before s.c. injection of inflammatory stimuli into the hind paw) and at 0.5, 1, 2–8 and 32 h. The results were expressed as paw volume variation (mL) in relation to baseline values. The area under the time-course curve (AUC) was expressed in arbitrary units [28].

2.4.4. Evaluation of edematogenic activity

In order to evaluate the edematogenic effect of DwL, paw edema was induced by s.c. injection of DwL (0.01, 0.1, 1 mg/kg) in a final volume of 0.1 mL/100 g body mass. The increase in vascular permeability was indirectly measured by quantifying protein leakage from inflamed paws [29] induced with 1 mg/kg DwL s.c. The animals were injected i.v. with Evans blue (25 mg/kg) 1 h before euthanasia, coinciding with the peak of edematogenic activity. The paws were then sectioned at the ankle, weighed and incubated with 2 mL formamide for 72 h at 37 °C. The optical
density (A600 nm) of the extracted dye was expressed as mean µg Evans blue/g tissue ± SEM.

To measure the anti-edematogenic effect of DwL, the most pro-inflammatory concentration of DwL was injected i.v. 30 min prior to s.c. administration of carrageenan (300 µg/paw), a well-known phlogistic agent [30]. Following the same protocol, positive controls received carrageenan while negative controls received sterile saline only. An additional group of animals received the standard anti-inflammatory, dexamethasone (1 mg/kg, i.p.).

2.4.5. Modulation of edematogenic activity

The mechanism of edematogenesis was investigated by administering indomethacin (5 mg/kg; s.c.), L-NAME (30 mg/kg; i.v.) pyrilamine (10 mg/kg; i.p.) or pentoxifylline (90 mg/kg; s.c) 30 min prior to injection with 1 mg/kg DwL. The controls received the same volume of sterile saline.

Compound 48/80 was injected i.p. over 4 days (on the first 3 days at 0.6 mg/kg, then at 1.2 mg/kg) to achieve systemic mast cell depletion. The animals also received thioglycolate i.p. for 4 days (on the first day at 3%/10 mL, then at 1.5%/10 mL) to induce systemic increase in macrophage population [31,32]. The paws were injected s.c. with DwL (1 mg/kg) 24 h after subchronic treatment with compound 48/80 or thioglycolate. Controls were injected with saline.

2.4.6. Investigation of the lectin domain involvement

DwL at the most pro-inflammatory dose was incubated with its binding sugars glucose (0.5 M) or α-CH2 (1 M) for 1 h at 37 °C to allow for lectin–sugar interaction before the experiment. Lectin and sugar controls were incubated individually under similar conditions.

2.4.7. Statistical analysis

Results were expressed as mean values ± SEM for each group of 5–7 animals. Differences were analyzed with ANOVA or Student’s t test, as appropriate. The level of statistical significance was set at p < 0.05.

3. Results

3.1. Purification and crystallization of DwL

DwL was purified and crystallized as described by Rangel and coworkers [10]. SDS-PAGE revealed the presence of DwL (25 kDa) and fragments (b and γ: 12 kDa).

The crystals belonged to point group I222. The Matthews coefficient [33] indicated the presence of a monomer in the asymmetric unit. X-ray data collection statistics are shown in Table 1. The structure factors and coordinates were deposited in the Protein Data Bank under accession code number 3SH3.

The graph confirmed the absence of residues in disallowed regions and the deviations were within the normal range. Molecular replacement and refinement data are shown in Table 1. The structure factors and coordinates were deposited in the Protein Data Bank under accession code number 3SH3.

2.3 Å).

![Fig. 1. Interaction between X-Man and the residues of the carbohydrate-binding domain of Dioclea wilsonii lectin. The figure shows 2Fo–Fc map contoured at 1σ for X-Man and dash lines represent polar contacts between X-Man with CRD of DwL.](image-url)
The structure of DwL includes a metal-binding site containing the conserved residues ASN14 and TYR12 (which interact with calcium), GLU8 and HIS24 (which interact with manganese) and ASP10 and ASP19 (which interact with both). In addition, the peptide bond of ALA207 and ASN208 in the cis configuration is isomerized due to the presence of divalent metals changing the side chain orientation of ASN14 and ASP208 [7].

The difference electron density map shows a feature in the vicinity of LEU115, LEU126 and VAL179, corresponding to a non-protein amino acid (Abu) commonly co-purified with Dioclea lectins [35]. The hydrophobic pocket shows a region accessible to the solvent surface, allowing hydrophilic interactions to occur. These hydrophilic interactions occur through hydrogen bonds between the nitrogen atom in Abu and the nitrogen atom in HIS180 (main chain) at 2.7 Å, and between the oxygen atom in Abu and the oxygen atom in SER125 (main chain) or the nitrogen atom in SER125 (peptide bonds) at 3.1 Å. Abu also interacts with two water molecules, involving oxygen and nitrogen at 2.7 Å and 2.8 Å, respectively. In addition, Abu mediates between the components of the canonical dimer, establishing hydrogen bonds with ASP139 and SER129 (Fig. 2).

The carbohydrate-binding site is occupied by X-Man. DwL interacts with mannose in much the same way as other ConA-like lectins (Table 2). A structural comparison between DwL and DVL (PDB code: 3AX4) can help clarify the interaction between DwL and X-Man. By superimposing these structures, differences may be observed in the interaction between X-Man and the hydrophobic sub-site (Fig. 3). Thus, in DwL, the side chain of LEU99 is positioned at an angle preventing hydrophobic interaction. This could lead to interaction with more complex carbohydrates — a process requiring the involvement of this sub-site.

### 3.3. Biological activities

DwL evoked dose-dependent paw edema at all doses tested, beginning at 30 min, peaking at 4 h and subsiding at 24 h (Fig. 4A). Injection of 0.01, 0.1 and 1 mg/kg DwL increased paw volume 4.8 times (AUC: 7.61 ± 0.93), 5.7 times (AUC: 9.01 ± 0.87) and 7 times (AUC: 11.21 ± 0.8), respectively, compared to saline (AUC: 1.58 ± 0.63 arbitrary units) (Fig. 4B). The highest peak of paw edema (1 mg/kg at 5 h) was paralleled by a 3-fold increase in vascular permeability (67.2 ± 3.5 μg) compared to saline (21.29 ± 10.9 μg Evans blue/g paw wet weight) (Fig. 4C). On the

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>X-Man</th>
<th>D. wilsonii PDB: 3SH3</th>
<th>D. violacea PDB: 3AX4</th>
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<tr>
<td>TYR 12 CD2</td>
<td>O4</td>
<td>3.44</td>
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<tr>
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<td>O6</td>
<td>3.21</td>
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<td>O6</td>
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<td>C9</td>
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<td>N1</td>
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<td>–</td>
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<td>ALA 207 CB</td>
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<td>C4</td>
<td>3.23</td>
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</tr>
<tr>
<td>ASP 208 OD1</td>
<td>C4</td>
<td>–</td>
<td>3.49</td>
</tr>
<tr>
<td>ASP 208 OD1</td>
<td>C6</td>
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<td>3.07</td>
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<tr>
<td>ASP 208 OD2</td>
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<td>3.31</td>
<td>3.25</td>
</tr>
<tr>
<td>GLY 227 CA</td>
<td>O3</td>
<td>–</td>
<td>3.25</td>
</tr>
<tr>
<td>ARG 228 CG</td>
<td>O3</td>
<td>3.18</td>
<td>–</td>
</tr>
<tr>
<td>ARG 228 CB</td>
<td>O4</td>
<td>3.27</td>
<td>3.27</td>
</tr>
</tbody>
</table>

![Fig. 2. α-Aminobutyric acid (Abu) binding site. The electron density shows 2fο–f esp map contoured at 1σ for Abu and the amino acid residues involved in the site are also demonstrated.](image)

![Fig. 3. Structural comparison between DwL (green) and DVL (PDB code: 3AX4, in blue). Differences in the interaction between X-Man and the hydrophobic sub-site may be observed after structures superimposition. In DwL, the side chain of Leu99 is positioned at an angle preventing hydrophobic interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)
other hand, i.v. injection of DwL (AUC: 14.01 ± 0.87) did not inhibit carrageenan-induced edema (AUC: 15.2 ± 0.8) (Fig. 4D).

The edematogenic effect of saturated solution of DwL with glucose (AUC: 3.43 ± 0.1) or α-CH₃ (AUC: 3.72 ± 0.2) was similar to that of DwL (AUC: 3.31 ± 0.14) (Fig. 5A) per se. Neither glucose (AUC: 0.20 ± 0.05) nor α-CH₃ (AUC: 0.22 ± 0.03) induced edema when administered alone compared to saline (AUC: 0.19 ± 0.08 arbitrary units).

The administration of pyrilamine (histamine antagonist) reduced the edematogenic effect of DwL (AUC: 2.71 ± 0.19) by 47% (AUC: 1.44 ± 0.29); indomethacin (non-specific enzyme cyclooxygenase inhibitor) by 32% (AUC: 1.85 ± 0.18); L-NAME (non-selective NOS inhibitor) by 31% (AUC: 1.99 ± 0.17) and pentoxifylline (non-specific cytokine inhibitor) by 53% (AUC: 1.28 ± 0.21 arbitrary units) (Fig. 5B), suggesting an edematogenesis mediated by mechanisms involving histamine, prostaglandins, nitric oxide, IL-1 and TNF-α.

The edematogenic effect of DwL was reduced about 65% (AUC: 3.29 ± 0.18) by mast cell depletion evoked with compound 48/80 (AUC: 1.02 ± 0.08), but not by thioglycolate-induced macrophage accumulation (AUC: 2.85 ± 0.17) (Fig. 5C).

4. Discussion

Like most legume lectins, DwL has a protein chain in the asymmetric unit featuring 237 residues. Diocleinae lectins consist of one intact subunit (α) and two fragments (β and γ) originated from the post-translational processing of the lectin precursor during seed development [10]. The mannose moiety of X-Man binds to the CRD, stabilizing the protein. This leads to a more highly ordered crystal arrangement allowing the formation of better-quality crystals, as observed for other legume lectins such as Par-ky a plathycephala lectin – PPL [36].

Being a tetramer, DwL is formed by the intersection of two perpendicular crystallographic two-fold axes and consists of two dimers in canonical β-sheet conformation. Referred to as “jelly-roll”, this quaternary arrangement is found in all ConA-like lectins. Abu has previously been identified in lectins, including CGL [35] and ConBr [37]. It has been suggested this interaction is part of a plant defense mechanism.

In respect to lectin effect in the inflammatory process, DwL displayed edematogenic activity paralleled by increased vascular permeability, but failed to produce anti-inflammatory effects. Diocleinae lectins may produce either anti-inflammatory [12,13] or pro-inflammatory effects [14,15,38] depending on the route of administration. However, for some lectins, such as DwL, no anti-inflammatory activity has yet been observed. The same is observed for the lectins of Canavalia brasiliensis (ConBr) and Dioclea grandi flora [13], the later shows 99% of identity with DwL. The edematogenic effect of DwL was of short duration, representing a typical manifestation of acute inflammation like other Diocleinae lectins.

Several studies have shown that resident cells may participate in leukocyte recruitment via synthesis of inflammatory mediators [39–41]. In our study, DwL-induced edema was reversed by treatment with compound 48/80, but not with thioglycolate, which
...is evidence of the influence of mast cells in the lectin effect. In fact, mast cell activation could be implied in the neutrophil recruitment to rat peritoneal cavity induced by DwL. Our findings are supported by several studies on mast cell recruitment promoted by plant lectins: *Araucaria angustifolia* lectin, a GlcNAc-binding protein, produced edematogenic effect via activation of inflammatory mediators from resident mast cells [42]; the plant lectin KM+ was observed to activate and degranulate mast cells, providing an amplification loop for the neutrophil recruitment induced by this lectin [43]; and Diocleinae lectins (ConM, ConA, ConBr, DvirL, DRL, DVL, Dgui and CRL I) have been observed to induce histamine release in rat peritoneal mast cells [16–18,44].

Carbohydrate recognition is the main activity and potential biotechnological application of Diocleinae lectins. The importance of carbohydrate sites has also been demonstrated for a range of effects produced by Diocleinae lectins in inflammation models [12,13,15,38]. However, saturated solution of ConA and ConBr with glucose also released histamine from Wistar rat peritoneal mast cells [16]. Likewise, our results show that the edematogenic activity of DwL was not reversed by its binding sugars, suggesting that mast cell activation involves interactions with complex glycans that commonly depend on nonpolar interactions (Table 2). The observed interaction between DwL and X-Man sheds light on some important aspects of carbohydrate recognition by ConA-like lectins in general. The number of hydrogen bonds in DwL is similar to that of the LEU199 side chain conformation of DwL and DwL. Thus, the two interactions with CD2 and the binding-enhancing hydrophobic contacts observed in DwL are absent in DwL. (Fig. 3). This can explain why despite high identity of DwL with DVL (99%–SwissProt accession code: P58909) and Dgui (95%–SwissProt accession code: P81637) [10], DwL do not present anti-inflammatory effect [13] (Table 3). Comparing DwL edematogenic effect with other Diocleinae lectins, it can be observed that DwL show the best potency, increasing the edema in 12 times in relation to controls (Table 3). The conformation of the hydrophobic sub-site in DwL together with lack of affinity for monosaccharides may explain why this lectin interaction with complex glycans in mast cells lead to better efficacy for edematogenic activities and why the monosaccharides were unable to prevent the edematogenic effect of this lectin.

DVL and DwL also differ in the position 161. DVL contains a serine, and DwL contains an asparagine, although it is located about 24 Å of the monosaccharide binding site. This difference might be important when it comes to interaction with complex carbohydrates, since the activities seem to be caused by interactions with complex carbohydrates, as indicated by the reverse assays. It is

<table>
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<tr>
<th>Table 3</th>
<th>Comparison between anti and pro-inflammatory effects of Diocleinae lectins.a</th>
</tr>
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<tr>
<td>Lectin</td>
<td>Time of edema peak</td>
</tr>
<tr>
<td>ConBr</td>
<td>½ h</td>
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<tr>
<td>CGL</td>
<td>½ h</td>
</tr>
<tr>
<td>ConM</td>
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<td>ConA</td>
<td>½ h</td>
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<td><em>D. virgata</em></td>
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<td>DGF</td>
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<tr>
<td>DwL</td>
<td>5th h</td>
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</table>

N.I.: not investigated.

a Adapted from [6,11,12,15,39].
known that single mutations in amino acids at key positions close to the monosaccharide binding site drastically affects biological activities in Diocleinae lectins [15]. In this study, we report the same feature but occurring relative distant of the monosaccharide binding site, which goes with the fact that the biological activities presented here are caused by complex carbohydrates.

Dwl seems to interact with complex carbohydrates in mast cells surface, which in turn release mediators producing symptoms of inflammation, including vasodilatation, plasma extravasation and neutrophil migration. In this study, pre-treatment of rats with a prostaglandin and thromboxane blocker — indomethacin [45], an NOS inhibitor — L-NAME [46], an H1 histamine receptor antagonist — pyrilamine [47] and a pro-inflammatory cytokine expression down-regulator — pentoxifylline [48] reduced the edematogenic effect of Dwl, evidencing the involvement of prostaglandin, NO, histamine and cytokines (TNF-α and IL-1β). Upon being activated, mast cells possess the ability to release mediators — both preformed and newly synthesized — which serve to alert the immune response or amplify an existing response. Granule-associated mediators, including proteases, histamine, proteoglycans and cytokines (eg TNF-α) are released immediately upon activation, being critical for initiating mast cell-mediated innate immune responses. Newly synthesized mediators include lipids, cytokines and chemokines [19,49,50]. Lipid mediators such as leukotriene C4, leukotriene B4 and prostaglandin D2 are involved in various allergic and pro-inflammatory responses [49,50].

Masl cells produce various cytokines ranging from pro-inflammatory cytokines (eg TNF-α, IL-6 and IL-1β) to several chemokines (eg CCL5 and CXCL8), which recruit immune cells to the site of infection. Finally, mast cells are phagocytic and produce nitric oxide, superoxide radicals and antimalarial peptides, such as cathelicidins [19,51].

Conformational changes in the hydrophobic sub-site have consistently been associated with the strengthening or weakening of interactions between glycans and lectins [3,6]. Another interesting structural aspect reported for legume lectins is the CRD conformation in lectins of the genus Canavalia. It has been suggested that certain geometric aspects of the CRD are related to cell recognition and the effect of these lectins on endothelial cells [52]. A comparison of three lectins of the genus Dioclea revealed discrepancies (Table 4) similar to those previously described for DVL and DRL using the same experimental model. However, the present study is to our knowledge the first to establish three-dimensional structural/functional relationships using a model of edematogenesis. These discrepancies in geometric aspects of the CRD can be involved in differences in inflammation induced by lectins of the Dioclea genus. For example, it should explain why DRL induces neutrophil migration via macrophage activation [14] while Dwl via activation of mast cells.

5. Conclusion

Dwl differs structurally from DVL and DRL with regard to the conformation of the carbohydrate recognition domain and related biological activities. The mechanism of edematogenic activity in Dwl appears to involve positive modulation of mast cell degranulation via recognition of surface carbohydrates. Since this recognition is dependent on site volume and CRD configuration, edematogenesis mediated by resident cells varies in potency and efficacy among different Diocleinae lectins.

Acknowledgments

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References


Table 4

Distances between residues of the carbohydrate-binding domain of lectins from genus Dioclea.

<table>
<thead>
<tr>
<th>Residues</th>
<th>Distance (Å)</th>
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<td>11.07</td>
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