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Research paper

Contribution of the carbohydrate-binding ability of *Vatairea guianensis* lectin to induce edematogenic activity

Gabriela F.O. Marques [a, 1], Vinicius J.S. Osterne [b, 1], Livia M. Almeida [a], Messias V. Oliveira [b, 2], Luiz A.C. Brizeno [a], Vanir R. Pinto-Junior [b], Mayara Q. Santiago [b], Antonio H.B. Neco [b], Mario R.L. Mota [c], Luiz A.G. Souza [d], Kyria S. Nascimento [b], Alana F. Pires [a], Benildo S. Cavada [b, *], Ana M.S. Assreuy [a, **]

[a] Institute of Biomedical Sciences, State University of Ceará, Av. Paranjana, 1700, 60740-000, Fortaleza, CE, Brazil
[b] Department of Biochemistry and Molecular Biology, Federal University of Ceará, Mr. Hull s/n Building 907, 60445-970, Fortaleza, CE, Brazil
[c] Department of Dental Clinic, Division of Oral Pathology, Federal University of Ceará, R. Alexandre Baraúna, 949, 60430-160, Fortaleza, CE, Brazil
[d] Instituto Nacional de Pesquisas da Amazônia - INPA, Manaus, Amazonas, Brazil

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ABSTRACT

Vatairea guianensis lectin (VGL), Dalbergiae tribe, is a N-acetyl-galactosamine (GalNAc)/Galactose (Gal) lectin previously purified and characterized. In this work, we report its structural features, obtained from bioinformatics tools, and its inflammatory effect, obtained from a rat paw edema model. The VGL model was obtained by homology with the lectin of *Vatairea macrocarpa* (VML) as template, and we used it to demonstrate the common characteristics of legume lectins, such as the jellyroll motif and presence of a metal-binding site in the vicinity of the carbohydrate-recognition domain (CRD). Protein-ligand docking revealed favorable interactions with *N*-acetyl-D-galactosamine, D-galactose and related sugars as well as several biologically relevant *N*- and *O*-glycans. *In vivo* testing of paw edema revealed that VGL induces edematogenic effect involving prostaglandins, interleukins and VGL CRD. Taken together, these data corroborate with previous reports showing that VGL interacts with *N*- and/or *O*-glycans of molecular targets, particularly in those presenting galactosides in their structure, contributing to the lectin inflammatory effect.

1. Introduction

Widely distributed among living organisms and viruses, lectins are proteins or glycoproteins capable of forming complexes with molecules and biological structures containing saccharides [1]. Since lectins can reversibly bind to carbohydrates, these molecules play major roles in cell communication, such as that occurring in the inflammatory process via glycocode decoding in the structure of soluble and integral cell membrane glycoconjugates [2].

Among lectins, those purified from leguminous plants are widely studied. This group comprises a large family of closely related lectins with similarity in physicochemical and structural properties, but significant differences in their biological activities [3]. Some lectins of the Dalbergieae tribe (Fabaceae, Papilionoideae) have now been purified and characterized. Moreover, the structures of *Pterocarpus angolensis* [4], *Centrolobium tomentosum* [5], *Platypodium elegans* [6], *Arachis hypogaea* [7] and *Vatairea macrocarpa* [8,9] lectins have already been solved. Those lectins possessing binding affinity for *N*-acetyl-glucosamine present anti-inflammatory property, such as the lectin of *Lonchocarpus sericeus* [10–12] and *Lonchocarpus araripensis* [13]. However, those lectins with binding affinity for galactose, such as *Vatairea macrocarpa* lectin, present inflammatory property [14–16]. In addition, the inflammatory effect of *V. macrocarpa* lectin occurs via activation of macrophages with release of cytokines [16].

The *N*-acetyl-D-galactosamine/D-galactose-specific lectin of *Vatairea guianensis* (VGL) is a homotetrameric glycoprotein with

* Corresponding author.
** Corresponding author.
E-mail addresses: messiassigma@gmail.com (M.V. Oliveira), bscavada@ufc.br (B.S. Cavada), anassreuy@gmail.com (A.M.S. Assreuy).
[1] Equal contribution.
[2] Participated in the new docking experiments and in paper writing.

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two *N*-glycosylations at Asn111 and Asn183. It was purified by affinity chromatography and possesses a molecular mass of 120 kDa. VGL presents *in vitro* vasodilator effect, inducing relaxation in endothelialized aorta via nitric oxide [17]. We aimed to gain a better understanding of VGL-saccharide binding (CRD) in the context of VGL biological effects. To accomplish this, we modeled the three-dimensional structure of VGL and focused on its *in vivo* vasodilator effects on a rat paw edema model.

2. Materials and methods

2.1. Lectin isolation

VGL was isolated from *Vatairea guianensis* seeds by ion exchange chromatography (DEAE-Sephacel column), followed by affinity chromatography (guar gum) [17]. The pure protein was diluted in sterile saline (0.9% NaCl) before biological assays.

2.2. Rat paw edema model

VGL (0.01, 0.1 and 1 mg/kg) was administered by subcutaneous (s.c.) route in Wistar rats (150–200 g) as inflammatory stimulus. Controls received sterile saline (0.1 mL/100 g body mass). The experimental protocols were approved by our Institutional Ethical Committee (UECE No. 10130208-8/40).

Paw edema was measured by hydroplethysmometry immediately before VGL injection (zero time), and from 0.5 to 72 h thereafter and was expressed as the variation in paw volume (mL) or area under curve (arbitrary units) [18] compared to zero time.

The participation of inflammatory mediators in the lectin effect was evaluated by treating the animals with the following pharmacological inhibitors: nitric oxide synthase (N-Nitro-L-arginine methyl ester/L-NAME; 25 mg/kg; intravenous), cyclooxygenase (indomethacin; 5 mg/kg; subcutaneous) andinterleukin-1β (thalidomide; 45 mg/kg; intraperitoneal) [19] 30 min before VGL administration (1 mg/Kg; s.c.).

The participation of the lectin carbohydrate-recognition domain (CRD) was evaluated by the injection of the most active dose of VGL (1 mg/kg) after incubation (30 min/37 °C) with its binding sugar galactose (0.1 M). Galactose was individually incubated at the same conditions as control.

2.3. Histological analysis

Paw tissues were removed 6 h after VGL (1 mg/kg) administration, fixed with 10% buffered formalin for 24 h, embedded in paraffin, cut into 5-μm thick slices, stained with hematoxylin & eosin (HE) and analyzed by light microscopy coupled to image acquisition systems (ScopePhoto; Image Manager 50). The intensity of tissue inflammation was graded according to the following scores: 0. normal tissue (no distinguishable change, 0%), absence of inflammatory infiltrate; 1. discrete tissue changes (initiation of changes, up to 30%), slight inflammatory infiltrate; 2. moderate tissue changes (patent changes, 31–60%), moderate inflammatory infiltrate; and 3. severe tissue changes (widespread changes, 61–100%), severe inflammatory infiltrate.

2.4. Immunohistochemistry

Fragments of paw tissue were sectioned to a thickness of 3 μm, placed on silanized slides and processed as described in the following protocol. Samples were deparaffinized, subjected to rehydration and antigen-recovery using citrate buffer (pH 6.0), incubated (10 min; r.t.) with 6% H_2O_2 in methanol (1:1) and washed with TRIS pH 7.6 (TRIS) in order to inactivate endogenous peroxidases. Samples were re-incubated for 1 h (r.t.) with the primary antibody (Ab) against IL-1β (monoclonal; Abcam "AB9787"; 1:100), washed and further incubated (30 min; r. t.) with biotinylated immunoglobulin (Ig; DAKO E0468) and streptavidin (DAKO P0397). Diaminobenzidine chromogen (DAKO K3469) was applied for 10 min, and Mayer's hematoxylin was used for counterstaining. Samples were dehydrated (ethanol and xylene) and cover-slipped with permanent Mounting medium. Parallel sections were treated with control IgG instead of the primary Ab.

For semi quantitative immunohistochemical evaluation, sections were randomly selected in 5 fields (400x magnification) in areas of greater concentration of immunostained cells located in connective or epithelial tissue. The percentage of cells with cytoplasmic or nuclear expression was scored as follows: (0) no positive cells; (1 - mild) 1–33% positive cells; (2 - moderate) 34–66% positive cells; (3 - intense) 67–100% positive cells (adapted from Minal Chaudhary et al. 2012) [20].

2.5. Statistical analysis

The *in vivo* data were presented as mean ± SEM, and the statistical analysis was performed by ANOVA, followed by Bonferroni's test. P values < 0.05 were considered significant. Histopathological and immunohistochemical data were expressed as median (maximum and minimum) and analyzed by Mann-Whitney test.

2.6. Template determination and secondary structure prediction

VGL sequence was downloaded from the Universal Protein Resource (Uniprot) (ID: P86893). The template for homology modeling was obtained from BLASTp search on the Protein Data Bank (PDB) with default parameters. Proteins most similar to VGL were chosen and ranked based on their resolution and geometric parameters.

Secondary structure prediction was carried out using PsiPro server, an automated system for secondary structure prediction [21]. The secondary structure was applied as one of the validation factors for homology model selection.

2.7. Homology modeling and validation

The 3D structure of VML in complex with Tn antigen (PDB ID: 4U36) was downloaded from PDB as the template structure. The homology model of VGL was built with MODELLER v.9.16 [22]. VGL and VML structures were aligned using salign module, followed by manual optimization. Initially, a hundred models were generated and ranked based on the Modeller objective score function (molpdf) and Discrete Optimized Protein Energy (DOPE) scores. Several models with lower molpdf and DOPE were selected and submitted to validation of stereochemical properties like Ramachandran plot, steric overlaps, Cβ deviation parameters, rotamers, and bond angle deviations using PROCHECK [23]. Side-chain acceptability was obtained by the Verify3D server [24]. QMEAN and Z-scores were also assessed by Protein Structure and Assessment tools [25–27]. The model having the best values in all validations was selected and applied in the subsequent analyses. Molecular drawings were prepared with PyMol (Shrodinger, LLC).

2.8. Molecular docking

Molecular docking was applied to verify the VGL ability to interact with several sugars. In order to perform the dockings, energy minimized saccharides structures were downloaded from PubChem [28]. Simulations were carried out with CLC Drug Discovery Workbench (CLC Bio; Boston, MA, USA), a software that uses