PURIFICATION AND CHARACTERIZATION OF A LECTIN FROM SEEDS OF VATAIREA MACROCARPA DUKE

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Key Word Index—Vatairea macrocarpa; Leguminosae; lectin; affinity chromatography; d-galactose-binding; amino acid sequence.

Abstract—A lectin from Vatairea macrocarpa Duke seeds (VML) was isolated using affinity chromatography on a guar gum column. The lectin, a glycoprotein without erythrocyte specificity, displays specificity to galactose and some derivatives. On SDS–polyacrylamide gels, V. macrocarpa seed lectin is composed of two major high-Mr bands of 34 and 32 kDa and two minor low-Mr bands of 22 and 13 kDa. N-Terminal sequencing showed that the 34, 32, and 13 kDa products possess identical N-terminal sequence, which display best similarity with the N-terminal portion of Robinia pseudoacacia lectins (RPL). On the other hand, the N-terminal sequence of the 22 kDa band can be aligned with an internal sequence of RPL starting at residue 149 of the cDNA–derived sequence. These data indicate that, like other leguminous lectins, VML is made up of a mixture of one-chain 30–35 kDa glycoforms and of 22 and 13 kDa endogenous C- and N-terminal fragments. Size-exclusion chromatography indicated that, at neutral pH, VML is predominantly a dimeric (70 kDa) protein, although tetramers (115 kDa) and larger aggregates (300 kDa) were also present. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Lectins are (glyco)proteins of non-immune origin that interact reversibly and specifically with carbohydrates [1]. Lectins are widely distributed in Nature and have been found in viruses, micro-organisms, plants and animals. In the Plant Kingdom, seeds of legumes such as beans and peas (Leguminosae) have long been known to be a rich source of lectins [2, 3]. Legume lectins are the best studied group of plant lectins and hundreds of these proteins have been isolated and characterized in relation to their chemical, physicochemical, structural and biological properties. Increasing experimental evidence suggest that seed lectins in legumes are defense proteins that may protect mature seeds against the attack of predators such as insects and mammals [4]. Indeed many legume lectins exhibiting different carbohydrate specificities are insecticidal to important pests of crops. This bio-

logical activity of plant lectins is of great economic potential because lectin genes are good candidates to confer insect resistance to transgenic crops. Therefore, the purification and characterization of lectins from new sources may reveal novel genes with the potentiality to be used in the genetic improvement of crops. The genus Vatairea (Leguminosae: Papilionoideae) comprises only 7 species of leguminous trees, which are widespread in Brazil, Guiana, and the Atlantic coastal regions of tropical Central America and Mexico. Here, we report the purification and some properties of a lectin from seeds of V. macrocarpa Duke, a species found in northeastern Brazil. This is the first report of purification of a lectin from a species of the genus Vatairea.

RESULTS AND DISCUSSION

Crude extracts of Vatairea macrocarpa seeds contain measurable amounts of a galactose-specific lectin. The purification procedure of the lectin from V. macrocarpa seeds is summarized in Table 1. Vatairea mac-
Table 1. Overall recovery of protein and haemagglutinating activity from *Vatairea macrocarpa* seeds. The seed flour was extracted with 0.15 M NaCl, centrifuged and the clear supernatant submitted to affinity chromatography on guar gum column.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total agglutination activity (titre g⁻¹ flour)*</th>
<th>Specific agglutination activity (titre mg⁻¹ protein)</th>
<th>Extent of purification (times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>28 100</td>
<td>81 900</td>
<td>2 920</td>
<td>1.0</td>
</tr>
<tr>
<td>PII (guar gum column)</td>
<td>2.6</td>
<td>2 480</td>
<td>7 880</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*Titre defined as reciprocal of highest dilution exhibiting haemagglutinating activity with native rabbit blood cells in 0.15 M NaCl.

*V. macrocarpa* lectin (VML), quantitatively extracted with 0.15 M NaCl from defatted ground seed, was purified in a single-step by affinity chromatography on guar gum, a galactomannan polysaccharide. The affinity chromatography step on a column of guar gum is depicted in Fig. 1. Isolation of β-Gal–specific lectins using guar gum has been described for other plant lectins like those from *Ricinus communis* [5], *Glycine max* [6], and *Erythrina velutina* forma *aurantiaca* [7]. After washing the column with 0.15 M NaCl, the lectin was recovered from the guar gum column as a sharp peak eluting with 0.15 M NaCl containing 0.1 M galactose or, alternatively, by washing the column with glycine–HCl, pH 2.6, containing 0.15 M NaCl (Fig. 1). The flow-through fraction was devoid of haemagglutinating activity indicating the complete adsorption of the lectin to the matrix. The overall yield was usually ca. 260 mg of lectin per 100 g of dry seed ground.

The lectin from *V. macrocarpa* seeds showed no haemagglutinating specificity when tested with blood cells from sheep, pig, cow, rabbit, and human (groups A, B, and O). However, it did not induce haemagglutination of goat erythrocytes, and the strongest haemagglutinating activity was exhibited against pig erythrocytes, followed by those from rabbit (Table 2). For practical reasons, rabbit red blood cells were subsequently used in haemagglutination and hapten-inhibition assays. The agglutinating activity of the purified lectin was specifically inhibited by carbohydrates containing β-galactose residues (Table 3). Among the carbohydrates tested, β-galactose and lac-

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**Fig. 1.** Purification of the lectin from *Vatairea macrocarpa* seeds by affinity chromatography on guar gum column. The column was equilibrated and first eluted with 0.15 M NaCl to remove the unbound proteins (PI). The lectin (PII) was recovered with 0.1 M D-galactose in the equilibrium solution.
Table 2. Erythrocyte specificity of *Vatairea macrocarpa* lectin.

<table>
<thead>
<tr>
<th>Erythrocytes (2%)</th>
<th>HU mg⁻¹ protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>2.21</td>
</tr>
<tr>
<td>Goat</td>
<td>NA†</td>
</tr>
<tr>
<td>Pig</td>
<td>1.130</td>
</tr>
<tr>
<td>Sheep</td>
<td>2.21</td>
</tr>
<tr>
<td>Rabbit</td>
<td>565</td>
</tr>
<tr>
<td>Human A</td>
<td>110</td>
</tr>
<tr>
<td>Human B</td>
<td>55</td>
</tr>
<tr>
<td>Human O</td>
<td>110</td>
</tr>
</tbody>
</table>

*Specific activity
†N.A., no haemagglutinating activity

Table 3. Inhibition of *Vatairea macrocarpa* lectin by mono- and oligosaccharides.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Minimum inhibitory concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D(+-)-Galactose</td>
<td>6.25</td>
</tr>
<tr>
<td>Raffinose</td>
<td>12.50</td>
</tr>
<tr>
<td>D(+-)-Xylose</td>
<td>N.I.†</td>
</tr>
<tr>
<td>D(+-)-Glucuronic acid</td>
<td>N.I.</td>
</tr>
<tr>
<td>N-Acetyl-d-glucosamine</td>
<td>N.I.</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>N.I.</td>
</tr>
<tr>
<td>D(-)-Fructose</td>
<td>N.I.</td>
</tr>
<tr>
<td>Melibiose</td>
<td>3.13</td>
</tr>
<tr>
<td>Salicine</td>
<td>N.I.</td>
</tr>
<tr>
<td>D(+)-Arabinose</td>
<td>25.00</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>N.I.</td>
</tr>
<tr>
<td>Melizitose</td>
<td>N.I.</td>
</tr>
<tr>
<td>D(+)-Glucose</td>
<td>N.I.</td>
</tr>
<tr>
<td>Methyl α-D-glucopyranoside</td>
<td>N.I.</td>
</tr>
<tr>
<td>Methyl α-D-mannopyranoside</td>
<td>N.I.</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.98</td>
</tr>
<tr>
<td>Trehalose</td>
<td>N.I.</td>
</tr>
</tbody>
</table>

*Inhibition is expressed as the minimum concentration (mM) of the sugar necessary for total inhibition of 2 HU ml⁻¹.
†N.I., no inhibition even at a sugar concentration of 100 mM.

tose were the most potent inhibitors (minimal inhibitory concentration of 12.5 mM), followed by raffinose (25.0 mM). These results clearly support the classification of VML as a D-Gal-specific lectin.

By SDS-polyacrylamide gel electrophoresis, affinity-purified VML displays a four-band pattern: two major bands of Mr of 34 and 32 kDa and two minor components of 22 and 13 kDa (Fig. 2). Edman degradation analysis of electroblotted samples showed that the 34, 32, and 13 kDa bands possess identical N-terminal sequence: SEVVSFSFSTKF. This sequence shows the greatest similarity with those of the galactose-specific isolecins isolated from *Erythrina variegata* (Ev) seeds: VETJSFSFSEF [8], Sophora japonica (Sj) seeds, leaves, and barks: AE(I/V)LSFSFPKF [9–11], and *Robinia pseudoacacia* (Rp) seeds and barks: TGSLSFSFPKF [12, 13]. On the other hand, the N-terminal sequence of *V. macrocarpa* 22 kDa band was: KS(I/V)QTVAVEFDT, which can be aligned with an internal polypeptide stretch of the above galactose-specific lectins located around the middle of the polypeptide chains (Table 4). This sequence, in turn, is homologous to the N-terminal amino acid sequence of the α- and β-polypeptide of concanavalin A (ADTIVAVELDT) and related leguminous lectins. Contrary to ConA, which display a mixture of full-length, 237-residue α-chain and non-covalently associated fragments?1–118 (β-chain) and 119–237 (γ-chain) [14], Ev, Sj, and Rp lectins have been reported to be single chain 30–35 kDa molecules [8–10, 12]. Our results, however, clearly show that *V. macrocarpa* seed lectin is a mixture of both single-chain and two-chain molecules.

Estimation of the Mr of the native lectin by gel
| V. macrocarpa lectin is relatively heat stable retaining about 55% of its original haemagglutinating activity after 5 min exposure at 100°C. The activation energy of denaturation (ΔG') was estimated to be 26 kcal mol⁻¹ which is similar to the values found for other legume lectins such as *Erythrina variegata* and *Robinia pseudoacacia* lectins [8, 15].

**haemagglutination and hapten-inhibition assays**

Lectin-mediated agglutination of red blood cells was determined by the procedure of ref [17]. 200 μl of samples were assayed in small test tubes using 2-fold serial dilution in 100 mM M Tris–HCl pH 7.6 containing 150 mM NaCl. 200 μl of a 2% suspension of erythrocytes was added to each tube and the mixtures were incubated at 37°C for 30 min and were left 30 min at room temp. The haemagglutination titre (HU μl⁻¹) was defined as the reciprocal of the highest dilution giving visible agglutination. This conc is denoted as 1 haemagglutinating unit (HU). The carbohydrate-binding specificity of the purified VM lectin was assessed by the ability of defined sugars to inhibit the agglutination of rabbit erythrocytes. For hapten inhibition tests, 2-fold serial dilutions of each sugar (1 M initial conc) in 200 μl of 150 mM NaCl were mixed with equal vols of a lectin soln displaying a titre of 4, and left for 30 min at room temp. Thereafter, 400 μl of a 2% suspension of rabbit erythrocytes were added to each tube and the mixtures were incubated for 30 min at 37°C followed by another 30 min interval at room temp. The lowest conc (mM) that inhibited haemagglutination was recorded and used to define the inhibitory activity.

**protein determination**

Estimation of protein concn was carried out by the method of ref [18], using BSA as a standard.

**lectin purification**

Seeds of *V. macrocarpa* were finely ground using a coffee mill, defatted with n-hexane at room temp., air-dried and extracted with 10 vol. of 150 mM NaCl at room temp. for 3 hr with continuous stirring. The extract was centrifuged (16,000 g, 20 min, 4°C) and the resulting clear supernatant was applied to a guar gum column (26 x 26 cm) equilibrated with 0.15 M NaCl. The chromatographic medium was prepared by cross-linking guar gum with epichlorohydrin as described [5]. After washing, the lectin was desorbed by eluting with 100 mM D-galactose in equilibrium soln, or with 0.1 M glycine–HCl buffer pH 2.6 containing 0.1 M NaCl.

**Materials**

Seeds of *Vatairea macrocarpa* Duke were obtained from a tree growing at Campus do Pici of the Federal University of Ceará (UFC), Fortaleza, Brazil and identified by Dr. Edson Paula Nunes (Herbarium Princeton Bezerra-UFC, number 15104). Human blood was obtained from healthy donors at the Haematology Centre of the UFC. Rabbit blood was obtained by puncture of the marginal ear vein of healthy animals. Blood from cow, goat, pig, and sheep was obtained from animals reared at the Agronomy School, UFC. Sugars, acrylamide, bis-acrylamide and guar gum were purchased from Sigma. Superose 12 HR 10/30 was from Pharmacia. Other reagents were of analytical grade.
150 mM NaCl. The purified lectin was dialysed against 
H₂O and freeze-dried.

**SDS–PAGE**

SDS–polyacrylamide gel electrophoresis was carried 
out as described [19] using 15% polyacrylamide gels. 
Samples were dissolved in 62.5 mM Tris pH 6.8 
containing 2% SDS and 5% 2-mercaptoethanol, and 
heated at 100°C for 5 min. Gels were stained with 
Coomassie Brilliant Blue R–250.

**N-terminal amino acid analysis**

Proteins separated by SDS–polyacrylamide gel 
electrophoresis were electrotransferred onto PVDF 
membranes (4 hr at 150 mA) [20]. Blots were stained with 
Ponceau S Red [21], and the bands were excised, 
detained with H₂O and subjected to N-terminal amino 
acid analysis (using an Applied Biosystems Procise 
instrument following the manufacturer’s instruction).

**Mr determination**

The Mr of VML was determined by gel filtration 
on a Superose 6HR 10/30 column coupled to an FPLC 
system (Pharmacia) and equilibrated and eluted with 
50 mM Na–Pi buffer, pH 7.5, containing 100 mM D– 
galactose. For calibration, the following Mr standards 
were employed: catalase (232 kDa), aldolase (158 kDa), 
bovine serum albumin (67 kDa), chymotrypsinogen 
(25 kDa), and bovine pancreatic ribonuclease A 
(13.7 kDa).

**Amino acid analysis**

Amino acid analyses of *V. macrocarpa* lectin were 
carried out with an AlphaPlus (Pharmacia) amino 
acid analyser after sample hydrolysis in sealed, evacu-
ated ampoules at 110°C with 6 M HCl for 24 hr.

**Carbohydrate determination**

Total carbohydrate content was estimated by the 
phenol–sulphuric acid method of ref [22], using D– 
glucose as standard. For amino sugar and neutral 
sugar analyses, samples of *V. macrocarpa* lectin were 
hydrolysed with 4 M HCl for 4 hr or 2 M HCl for 2 hr, 
respectively, at 110°C. After drying the hydrolysates in 
a Speed-Vac, the monosaccharides were resolved on a 
CarboPac PA1 column (25 × 0.4 cm) eluting at 1 
ml/min⁻¹ isocratically with 16 mM NaOH using a 
 Dionex DX-300 analyser equipped with pulsed 
amperometric detector and the AI-450 chromatography 
software [23].

**Heat stability**

Heat stability was determined by incubating the 
lectin (2 mg ml⁻¹ in 150 mM NaCl) at 100°C for 5, 10, 
15, 20, 30, 45, and 60 min. The samples were cooled 
to room temp, centrifuged to eliminate precipitated 
material, and evaluated for hemagglutinating activity. 
The activation energy of the denaturing process (ΔG°') 
was calculated using the equation: ΔG°' = (ln kT/h–ln 
k₀)/RT, where ΔG°' is the standard free energy change, 
k is the Boltzmann’s constant, T is the absolute tem-
perature (K), h is Planck’s constant, and k₀ is the 
velocity of reaction given by k₀ = (ln A/A₀)/t. A₀ and 
A are initial and residual hemagglutinating activities, 
respectively, and t is time in sec.

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