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# 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. 

## 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 biological 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*.

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## 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.

| Fraction | Total protein (mg) | Total agglutination activity (titre $g^{-1}$ flour)* | Specific agglutination activity (titre $mg^{-1}$ protein) | Extent of purification (times) |
|---|---|---|---|---|
| Crude extract | 28 100 | 81 900 | 2 920 | 1.0 |
| PII (guar gum column) | 2.6 | 2 480 | 7 880 | 2.7 |

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

*rocarpa* lectin (VML), quantitatively extracted with 0.15 M NaCl from de-fatted 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 D–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 D–galactose residues (Table 3). Among the carbohydrates tested, D–galactose and lac-

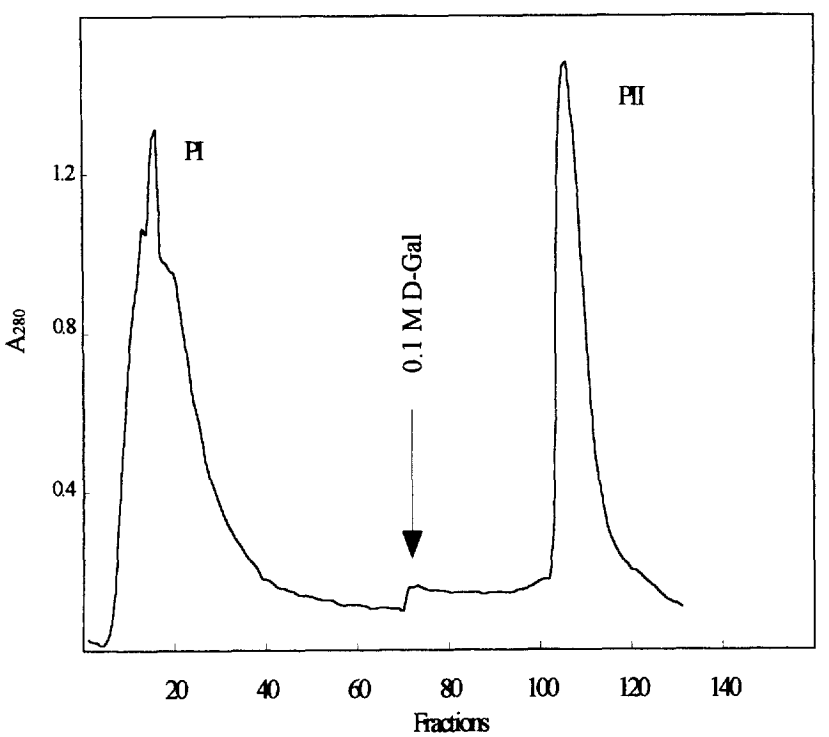


Fig. 1. Purification of the lectin from *Vatairea macrocarpa* seeds by affinity chromatography on guar gum column. The column was equilibrated and first eluted with 0.15 M NaCl to remove the unbound proteins (PI). The lectin (PII) was recovered with 0.1 M D–galactose in the equilibrium solution.

Table 2. Erythrocyte specificity of *Vatairea macrocarpa* lectin.

| Erythrocytes (2%) | HU $mg^{-1}$ protein* |
|---|---|
| Cow | 2.21 |
| Goat | NA† |
| Pig | 1 130 |
| Sheep | 2.21 |
| Rabbit | 565 |
| Human A | 110 |
| Human B | 55 |
| Human O | 110 |

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

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

| Sugar | Minimum inhibitory concentration* |
|---|---|
| D(+)-Galactose | 6.25 |
| Raffinose | 12.50 |
| D(+)-Xylose | N.I.† |
| D(+)-Glucuronic acid | N.I. |
| *N*-Acetyl-D-glucosamine | N.I. |
| L-Rhamnose | N.I. |
| D(−)-Fructose | N.I. |
| Melibiose | 3.13 |
| Salicine | N.I. |
| D(+)-Arabinose | 25.00 |
| Cellobiose | N.I. |
| Melizitose | N.I. |
| D(+)-Glucose | N.I. |
| Methyl α-D-glucopyranoside | N.I. |
| Methyl α-D-mannopyranoside | N.I. |
| Lactose | 0.98 |
| Trehalose | N.I. |

* 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.

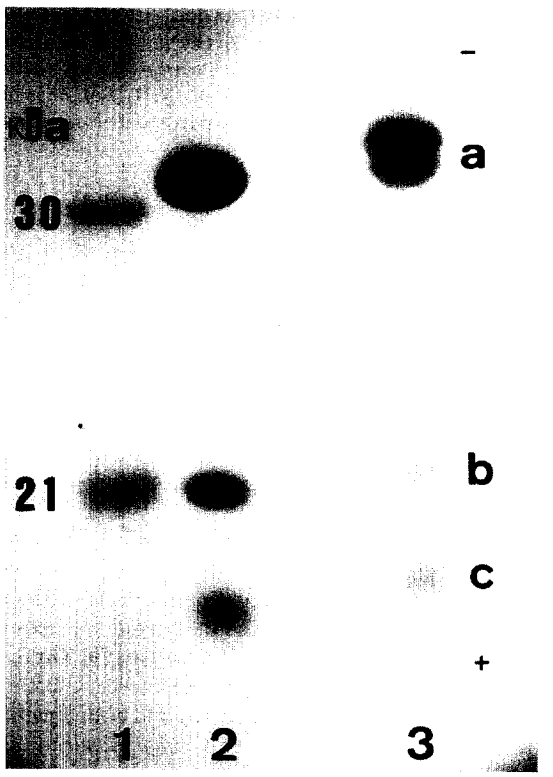


Fig. 2. SDS–polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol of the affinity chromatography-purified *Vatairea macrocarpa* lectin. Lane 1, marker proteins: carbonic anhydrase (30 kDa), and soybean trypsin inhibitor (21 kDa); Lane 2: *Dioclea grandiflora* lectin, a ConA-like lectin made up of a full-length α-chain (30 kDa) and its non-covalently associated β and γ fragments was included for comparison; Lane 3: *Vatairea macrocarpa* lectin showing the one-chain glycoforms (a) and the two fragments which correspond to the C- (b) and N-terminal (c) halves of the full-length chain.

tose were the most potent inhibitors (minimal inhibitory concentration of 12.5 mM), followed by raffinose (25.0 mM). These results clearly supports 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: SEVVSFSFTKF. This sequence shows the greatest similarity with those of the galactose-specific isolectins isolated from *Erythrina variegata* (Ev) seeds: VETISFSFSEF [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 framents? 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

Table 4. Comparison of the N-terminal sequence of the 13 kDa band of *Vatairea macrocarpa* lectin (Vm 13 K) with internal polypeptides of *Erythrina variegata* seed lectin (EvSL), *Sophora japonica* seed lectin (SjSD) and *Robinia pseudoacacia* seed and bark lectins (RpSL and RpBL, respectively).

| Vm13K | 1 | K | S | (I/V) | Q | T | V | A | V | E | F | D | T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EvSL | 119 | N | S | Y | Q | T | L | A | V | E | F | D | T |
| SjSL | 155 | S | S | Y | Q | I | I | A | V | D | F | D | T |
| RpSL | 149 | K | S | N | Q | I | V | A | V | E | F | D | T |
| RpBL | 149 | K | S | N | Q | I | V | A | V | E | F | D | T |

filtration on a Superose–6HR column equilibrated at pH 7.6 yielded two major peaks with *Mr*s around 294 and 70 kDa and a minor peak eluting at 115 k. At this pH, the 70 kDa component, which might correspond to a VML dimer predominates in relation to the tetrameric (115 kDa) and aggregated (294 kDa) forms. Galactose-specific isolectin isolated from the genus *Erythrina* also displays a dimeric structure [8]. On the other hand, *Sophora japonica* leaves and bark lectins display a tetrameric structure [9, 10], and both dimeric (RPA1) and tetrameric (RPA3) *Robinia pseudoacacia* lectins have been reported [15].

Amino acid analysis of the lectin showed it to be particularly rich in asparagine/aspartic acid, alanine, serine, threonine and glycine, with low methionine content and no cysteine (data not shown). Although the tryptophan content was not measured, we determined an extinction coefficient ($E_{1\,cm,\,1\%}$) of 5.6, suggesting that VML may contain few aromatic residues. Most legume lectins show a similar profile of amino acid composition [14, 16]. Sugar analysis revealed that the VM lectin is a glycoprotein containing 7.9% covalently bound carbohydrate. Monosaccharide analysis showed that the carbohydrate chain(s) of VML are composed of GlcNAc, Man, Xyl, and Fuc in approximate molar proportions of 2:3:1:1. Taken together, these results suggest that VML may contain 2 or 3 oligosaccharide chains similar to the carbohydrate structures found in *Erythrina variegata* and *Robinia pseudoacacia* lectins [8, 15].

*V. macrocarpa* lectin is relatively heat stable retaining about 55% of its original haemagglutinating activity after 5 min exposure at 100°. The activation energy of denaturation ($\Delta G'$) was estimated to be 26 kcal mol$^{-1}$ which is similar to the values found for other legume lectins such as *Erythrina velutina* forma *aurantiaca* [7], *Phaseolus vulgaris* [17] and *Glycine max* [6].

## EXPERIMENTAL

### *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 Prisco 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.

### *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° for 30 min and were left 30 min at room temp. The haemagglutination titre (HU ml$^{-1}$) 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° followed by another 30 min interval at room temp. The lowest concn (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, deffated 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°) and the resulting clear supernatant was applied to a guar gum column (26 × 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

150 mM NaCl. The purified lectin was dialysed against $H_2O$ 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° 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, destained with $H_2O$ 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, evacuated ampoules at 110° 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°. 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° 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 ($\Delta G'$) was calculated using the equation: $\Delta G' = (\ln kT/h - \ln k_1)/RT$, where $\Delta G'$ is the standard free energy change, k is the Boltzmann's constant, T is the absolute temperature (K), h is Planck's constant, and $k_1$ is the velocity of reaction given by $k_1 = -(\ln A/A_o)/t$. $A_o$ and A are initial and residual hemagglutinating activities, respectively, and t is time in sec.

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