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Amino acid sequence, glycan structure, and proteolytic processing of the lectin of *Vatairea macrocarpa* seeds

Juan J. Calvete[a,*], Cláudia F. Santos[b], Karlheinz Mann[c], Thalles B. Grangeiro[b], Manfred Nimtz[d], Claus Urbanke[e], Benildo Sousa-Cavada[b]

[a] *Institut für Reproduktionsmedizin, Tierärztliche Hochschule Hannover, Bünteweg 15, 30559 Hannover-Kirchrode, Germany*
[b] *Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, C.P. 6020, 60451-970 Fortaleza, Brazil*
[c] *Max-Planck-Institut für Biochemie, 82152 Martinsried, Germany*
[d] *Gesellschaft für biotechnologische Forschung (GBF) mbH, Mascheroder Weg 1, 38124 Braunschweig, Germany*
[e] *Biophysikalisch-biochemische Verfahren, Medizinische Hochschule, Carl-Neuberg-Strasse 1, 30623 Hannover, Germany*

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Abstract VML is a galactose-binding lectin isolated from *Vatairea macrocarpa* seeds. By SDS-polyacrylamide gel electrophoresis, VML is a glycoprotein composed of a major 32–34 kDa double band (alpha-chain) and minor 22 kDa and 13 kDa bands. N-terminal sequencing of electroblotted samples showed that the 22 and 13 kDa bands corresponded to C-(beta) and N-(gamma) terminal fragments of the alpha-chain, respectively. The primary structure of VML displays similarity with other leguminous lectins, particularly with *Erythrina variegata*, *Robinia pseudoacacia* and *Sophora japonica* lectins. VML is *N*-glycosylated at asparagine residues at positions 111 and 183 with one major glycan structure. Tandem mass spectrometry and methylation analysis indicated the presence of Manα1-6[(Manα1-3)(Xylβ1-2)]Manβ1-4-GlcNAcβ1-4(Fucα1-3)GlcNAc, a typical plant *N*-glycan. Equilibrium sedimentation analysis by analytical centrifugation showed that VML had a mass of 122–130 kDa, which did not change within the pH range 2.5–8.5. These data indicated that VML is a pH-independent homotetrameric protein and that a small proportion of the alpha-subunits is cleaved into noncovalently associated N- and C-terminal fragments. Mass spectrometric analysis suggested a mechanism for the proteolytic processing of VML. *V. macrocarpa* lectin contains a mixture of doubly (28 525 Da) and singly (27 354 Da) glycosylated alpha-chains. Deglycosylation of Asn-111 correlates with proteolytic cleavage of the Asn-114-Lys-115 bond yielding glycosylated gamma (residues 1–114, 12 304 Da) and nonglycosylated beta-(residues 115–239, 14 957 Da) chains. Some beta-chain molecules are further deglycosylated and N-terminally processed yielding products of molecular masses of 13 783 Da and 13 670 Da.

Key words: *Vatairea macrocarpa* seed; Galactose-binding leguminous lectin; Primary structure; Carbohydrate structure; Mass spectrometry; Posttranslational processing

1. Introduction

Lectins are a structurally very diverse class of proteins which bind reversibly and with high specificity mono- and oligosaccharides without enzymatically modifying them [1,2]. Lectins were discovered more than hundred years ago in castor bean, and since then hundreds of different lectins have been characterized from plants, animals, bacteria, and viruses. Lectins of the same protein family exhibit specificity for different saccharides or recognize different determinants of the same complex carbohydrate structure [3]. Hence, lectins are well suited for acting in a large number of biological processes (including lymphocyte function, cell communication and signal transduction, host defense, fertilization, development, etc.) by deciphering the glycocodes encoded by the tremendous variety of glycans attached to soluble and integral membrane glycoconjugates.

The most thoroughly studied lectins are those isolated from leguminous plants, such as peas and beans. Leguminous plant lectins, although differing in carbohydrate-binding specificity and quaternary structures, have similar amino acid sequences [4,5] and their subunits have a common three-dimensional conformational motif, the so-called 'lectin fold' [6]. This demonstrates that this class of proteins has been conserved throughout evolution and argues that they must have important functions rather than represent 'flotsam of evolution' as once postulated [7]. However, the function of plant lectins remains elusive [8]. Proposed functions include a storage or transport role for carbohydrates in seeds, binding of nitrogen-fixing bacteria to root hairs, and inhibition of fungal growth or insect feeding [8]. The existence of hydrophobic sites within the structure of leguminous lectins which bind phytohormones suggests a possible role in certain aspects of hormonally regulated plant growth and development (reviewed in [5]).

Besides their physiological roles, studies on lectins have made important contributions to our understanding of protein-carbohydrate interactions. In addition, lectins have been extensively used as tools for glycoconjugate purification and characterization as well as specific reagents for biomedical research [9,10]. The large majority of leguminous lectins that have been isolated and characterized belong to the *Phaseoleae*, and *Vicieae* tribes of the *Papilionoideae* subfamily of *Leguminosae*. The genus *Vatairea* of the tribe *Dalbergieae* of the same family and subfamily of plants comprises seven species of leguminous trees that are widespread in Brazil, Guaina, and the atlantic coastal regions of tropical Central America. Here we report the first determination of the covalent structure and posttranslational mechanism of a lectin isolated from seeds of a species of the genus *Vatairea*, *Vatairea macrocarpa* Duke, a tree growing in the northeastern of Brazil.

*Corresponding authors: Juan J. Calvete, Fax: +49 (511) 953 7172.
E-mail: jcalvete@repro.tiho-hannover.de and Benildo Sousa-Cavada, Fax: +55 (85) 288 9818.
E-mail: bscavada@ufc.br

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2. Materials and methods

2.1. Isolation of V. macrocarpa lectin

V. macrocarpa lectin was extracted from deflated (with *n*-hexane), air-dried ground of *V. macrocarpa* Duke seeds collected from trees growing at the Campus of the Federal University of Ceará (Fortaleza) in the northeastern of Brazil with 10 volumes of 0.15 M NaCl at room temperature for 3 h. After centrifugation at 16 000×*g* for 20 min at 4°C, the lectin, identified by its hemagglutination activity using 2-fold serial dilutions of rabbit erythrocytes in 100 mM Tris/HCl, 150 mM NaCl, pH 7.6, was recovered in the supernatant of 60% ammonium sulfate saturation. Following dialysis against 0.15 M NaCl, VML was purified by affinity chromatography on a 26×2.6 cm Guar gum (Sigma) column [11] equilibrated with the same solution. After washing out the nonbinding material, VML was eluted with 100 mM D-galactose in 0.15 M NaCl, dialyzed against distilled water, and freeze-dried.

2.2. Compositional analyses

Amino acid and amino sugar analyses of purified *V. macrocarpa* seed lectin were carried out with an AlphaPlus (Pharmacia) amino acid analyzer after sample hydrolysis in sealed, evacuated ampoules at 110°C with 6 M HCl for 24 h and with 4 M HCl for 4 h, respectively.

2.3. Electrophoresis and blotting

SDS (15%)-polyacrylamide gel electrophoresis was done as described [12]. Gels were blotted onto polyvinylidene fluoride (PVDF) membranes for 4 h at 1 mÅ/cm^2 [13] and blots were stained with Ponceau S Red [14]. Protein bands were excised, destained with water, and subjected to N-terminal sequence analysis on an Applied Biosystems Procise instrument following the manufacturer's instructions.

2.4. Protein cleavages and isolation of peptides

Samples of purified VML (2–5 mg in 100 mM ammonium bicarbonate, pH 8.6) were degraded with endoproteinases Lys-C, Asp-N, Glu-C, and Arg-C (Boehringer Mannheim) overnight at 37°C at an enzyme:substrate ratio of 1:100 (w/w). For cleavage of the protein at methionine residues, VML (10 mg/ml in 70% formic acid) was incubated with CNBr (100 mg/ml final concentration) for 8 h at room temperature in the dark and under nitrogen. The reaction mixtures were centrifuged at 13 000×*g* for 10 min, supernatants and pellets were separately dried using a Speed-Vac, and peptides were isolated by reversed-phase HPLC using a Lichrospher RP100 (Merck) 4.6×250 mm C18 (5 μm particle size) column eluting at 1 ml/min with a gradient of 0.1% trifluoroacetic acid in water (solution A) and acetonitrile (solution B), isocratically (10% B) for 5 min, followed by 10–40% B for 120 min, and 40–70% B for 30 min. Fractions containing several peptides (revealed by N-terminal sequence analysis) were subjected to size-exclusion chromatography on a Superdex Peptide HR 10/30 FPLC column (Pharmacia) eluted with 0.1% trifluoroacetic acid at a flow rate of 0.3 ml/min.

2.5. Analytical ultracentrifugation

Determination of molecular masses by analytical ultracentrifugation was performed at 20°C using a Beckman XL-A centrifuge with absorption optics. For equilibrium measurements, six-channel cells were used allowing the simultaneous analysis of nine samples containing initially around 1 mg/ml VML in the following buffers supplemented with 1 mM of $CaCl_2$ and $MgCl_2$: 20 mM Tris/HCl, 0.1 M NaCl, (a) pH 8.5 and (b) pH 7.5; 20 mM sodium citrate, 0.1 M NaCl at pHs 6.5, 5.5, 4.5, 3.5, and 2.5.

2.6. Mass spectrometry

The molecular masses of alpha-, beta-, and gamma-chains of VML were determined by matrix-assisted laser-desorption ionization (MALDI) mass spectrometry using a Bruker REFLEX time-of-flight (TOF) instrument equipped with a reflectron system and an N_2 laser (337 nm) operating with 3 ns pulse width and 107–108 W/cm^2 irradiance at the surface of 0.2 mm^2 spots. One μl of sample (containing 5–25 pmol/μl) was mixed with equal volume of sinnapinic acid (10 mg/ml in 10% ethanol), spotted onto the stainless steel tip and dried at room temperature. Spectra were recorded under an acceleration voltage of 30 kV.

2.7. Carbohydrate composition and methylation analysis

N-glycans were released from VML (approximately 250 μg) by automated hydrazinolysis using a Glycoprep 1000 instrument (N mode, Oxford Glycosystems). After methanolysis, re-*N*-acetylation and trimethylsilylation, monosaccharides were analyzed as the corresponding methyl glycosides by gas chromatography on a Carlo Erba Mega Series instrument using a 30 m DB1 capillary column [15]. For methylation analysis, oligosaccharides were permethylated [16], purified, hydrolyzed, reduced, and peracetylated [17]. Separation and identification of partially methylated alditol acetates were performed with a Finnigan MAT gas chromatograph equipped with a 30 m DB5 capillary column and connected to a Finnigan GCQ ion-trap mass spectrometer running in the electron-impact (EI) mode.

2.8. Structural characterization of oligosaccharides by electrospray ionization tandem mass spectrometry

A Finnigan MAT TSQ 700 triple quadrupole mass spectrometer equipped with an electrospray ion source (Finnigan MAT corp., San Jose, CA) was used for ESI-MS. Reduced and permethylated

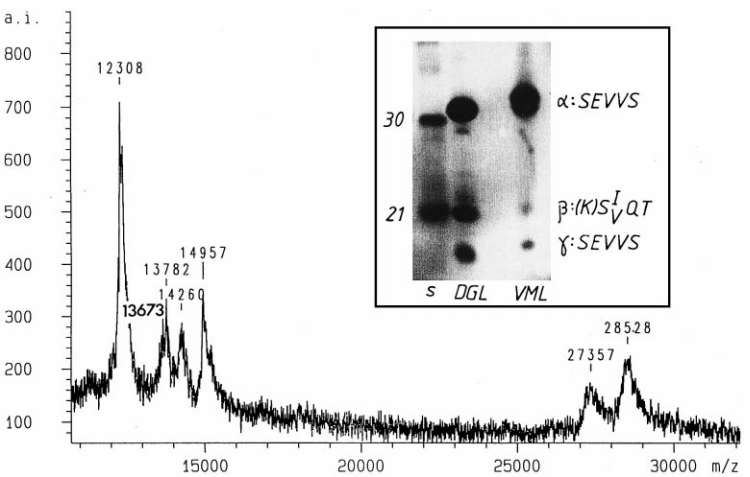

Fig. 1. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometric analysis of purified *Vatairea macrocarpa* lectin. Molecular masses are in Da. Insert, lane VML, SDS-(15%) polyacrylamide gel electrophoresis of *Vatairea macrocarpa* lectin. N-terminal sequences of the α- (32–34 kDa), β- (22 kDa), and γ- (13 kDa) chains are shown at the right. For comparison, the lectin of *Dioclea grandiflora*, a Concanavalin A-like lectin made up of a full-length 30 kDa α-chain and its noncovalently associated β- and γ-fragments (apparent molecular masses of 21 and 12 kDa, respectively), is shown in lane DGL. Lane s, molecular mass markers, from top to bottom, carbonic anhydrase and soybean trypsin inhibitor, whose molecular masses (in kDa) are indicated at the left.

samples were dissolved in acetonitrile, saturated with NaCl (concentrations approximately 10 pmol/μl) and injected at a flow rate of 1 μl/min into the electrospray chamber. A voltage of +5.5 kV was applied to the electrospray needle. For collision-induced dissociation (CID) experiments, parent ions were selectively transmitted by the first mass analyzer and directed into the collision cell. Argon with a kinetic energy set at around −60 eV was used as the collision gas.

3. Results and discussion

3.1. Amino acid sequence of V. macrocarpa seed lectin

SDS-polyacrylamide gel electrophoresis of VML, the lectin of *V. macrocarpa* seeds purified by affinity chromatography based on its galactose-binding specificity, showed a four-band pattern, two major bands of apparent molecular masses of 34 and 32 kDa, termed α-chains, and two minor components of 22 and 13 kDa (Fig. 1, insert), referred to as β- and γ-chains, respectively. Mass spectrometric analysis also showed that purified VML contained a mixture of molecular ions distributed in broad peaks centered at molecular masses (in Da) of 28 528, 27 357, 14 957, 14 260, 13 782, 13 673, and 12 308 (Fig. 1). Edman degradation analysis of electroblotted samples showed that the 34, 32, and 13 kDa bands possess identical N-terminal amino acid sequences, SEVVSFSFTKF. N-terminal sequence analysis of the 22 kDa band yielded KS-

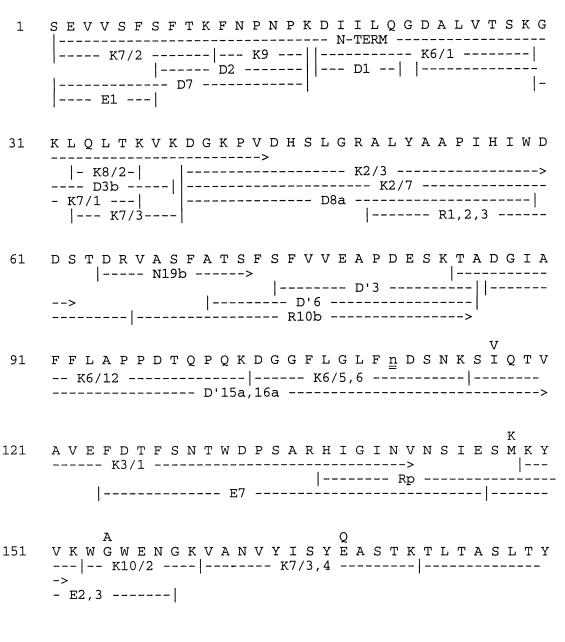

Fig. 2. The amino acid sequence of *Vatairea macrocarpa* lectin alpha-chain. Peptides obtained by reversed-phase HPLC after degradation of purified VML with endoproteinases Lys-C, Asp-N, Glu-C, Arg-C, and CNBr, are denoted K, D and D′, E, R, and C, respectively. A dash followed by a number or a letter indicate peptides purified from heterogeneous K-, D-, and R-fractions by size-exclusion chromatography. Rp, insoluble peptide obtained in the pellet of Arg-C degradation mixture by centrifugation. Heterogeneity was found at positions 117, 148, 154, and 168. n, glycosylated asparagine residue.

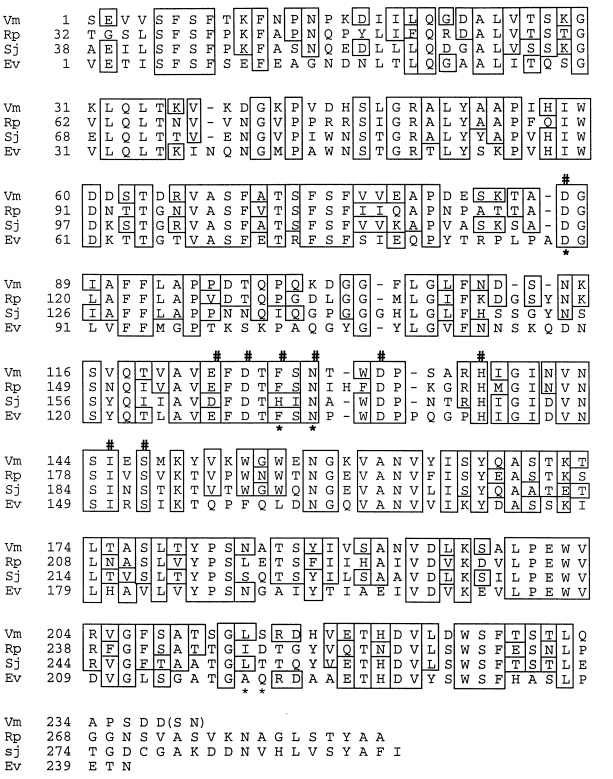

Fig. 3. Alignment of the amino acid sequence of VML with those of the seed lectins of *Robinia pseudoacacia* (Rp), *Sophora japonica* (Sj), and *Erythrina variegata* (Ev). Residues which in other leguminous lectins coordinate metal ions (calcium and manganese) and those forming part of carbohydrate recognition domains are labelled # and *, respectively. Amino acids of VML conserved in the primary structures of Rp, Sj, and Ev are boxed.

(I/V)QTVAVEFDT and S(I/V)QTVAVEFDTF, indicating that this band may contain a polypeptide partially processed at the N-terminus. In addition, the two residues at position 3 were present in almost equimolar quantities.

The N-terminal sequence of the 34, 32, and 13 kDa bands displays the greatest similarity with those of precursor proteins of the galactose-specific isolectins isolated from *Robinia pseudoacacia* (Rp) seeds and barks, ^{32}TGSLSFSFPKF [18–20], *Sophora japonica* (Sj) seeds, leaves, and barks, ^{38}AE(I/V)LSFSFPKF [21] and *Erythrina* (*variegata* (Ev) and *corallodendron* (Ecor)) seeds, ^{1}VETISFSFSEF [22,23]. On the other hand, the N-terminal sequence of VML 22 kDa band can be aligned with internal polypeptide stretches of Rp (^{148}KSNQIVEFDT), Sj (^{155}SSYQI(V/I)AV(D/E)FDT), and Ev (^{119}NSYQTLAVEFDT) lectins located around the middle of their polypeptide chains. This sequence is also homologous to the N-terminal amino acid sequence of the α- and β-polypeptides of mature Concanavalin A (^{1}ADTIVAVELDT) and related leguminous lectins [24,25].

Except for the last two C-terminal residues, which were tentatively assigned as SN (see below), the amino acid sequence of *V. macrocarpa* seed lectin was established by Edman degradation of proteolytic and CNBr-derived peptides isolated by reversed-phase HPLC (Fig. 2). We took advantage of the large amino acid sequence similarity between VML and other galactose-binding lectins to initially align the peptide sequences. Except for peptide K7/3,4, overlapping peptides confirmed the similarity-based alignment. Peptide K7/3,4 (res-

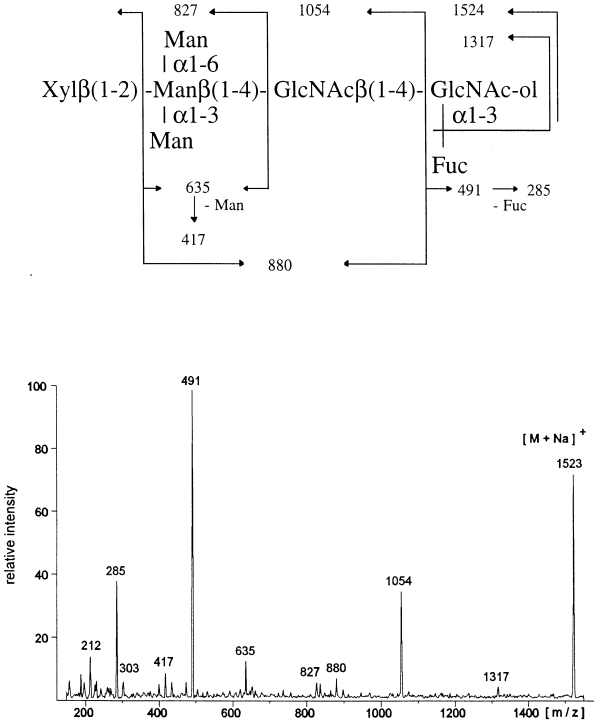

Fig. 4. Electrospray ionization mass spectrum of collision-induced dissociation of the major reduced and permethylated oligosaccharide of *Vatairea macrocarpa* lectin (molecular mass of the parent ion: 1523.5 Da). The structural assignment is shown on top of the mass spectrum.

idues 160–172 of VML) displays a highly conserved sequence in VML homolog lectins (Fig. 3). Residue heterogeneities at positions 117 (I/V), 148 (M/K), 154 (G/A), and 168 (E/Q), suggest that *V. macrocarpa* seeds may contain a mixture of highly related lectins. Analysis of genomic and cDNA clones have revealed the occurrence of multiple lectins or isolectins in many plants [26].

Edman degradation cycles corresponding to residues 111 and 183 showed asparagine in very low yields. These residues are located in Asn-Xaa-Ser/Thr sequences (Fig. 2), which are the typical sequons for *N*-glycosylation. In addition, compositional analyses showed that VML contained approximately 3.2 residues of glucosamine/mol protein, suggesting that the lectin was an *N*-glycoprotein. Together, these data indicated that Asn-111 and Asn-183 were both partially glycosylated.

The N-terminal sequences of the 22 kDa β-chain and the 13 kDa γ-chain (Fig. 1, insert) start at positions 115 and 1, respectively, of the primary structure of VML (Fig. 2). This clearly showed that, similarly to what has been documented for a number of plant lectins [16] but unlike galactose-specific lectins isolated from *R. pseudoacacia* seeds (RPA1 and RPA3), *S. japonica* leaves and barks (BL1, BL5, SJA, LL1 and LL2), and *E. variegata* seeds (EVLI, EVLII, and EVLIII), which have been reported to be single chain 30–35 kDa molecules [22,27–29], mature VML is composed of major, full-length α-subunits, which are partially cleaved at the 114–115 peptide bond into two-chain (β- and γ-) polypeptides. Similarly to Concanavalin A [30], and other lectins displaying mixtures of intact and cleaved subunits, cleavage of VML α-chain occurs at the carboxyl side of an asparagine residue, suggesting that splicing may be catalyzed by a conserved asparaginyl endopeptidase proteolytic activity. However, unlike most two-chain legume lectins, which are produced by post-translational removal of an internal polypeptide [26], processing of VML only involves the cleavage of a single peptide bond.

The amino acid sequence of VML is most similar (66–68%) to those of the galactose-specific lectins isolated from seeds of the genera *Robinia*, *Sophora* and *Erythrina* (Fig. 3). As expected from its galactose-binding specificity, Asp-118 and Phe-160 of *R. pseudoacacia* lectin precursor, two key carbohydrate-binding residues identified by site-directed mutagenesis [31], as well as the residues forming part of the cation (Ca^{2+} and Mn^{2+}) coordination spheres of a number of leguminous lectins, are conserved in VML (Fig. 3). Noteworthy, VML displays also significant (69.2%) amino acid sequence similarity with CLLRP, a lectin-related protein devoid of carbohydrate-binding activity isolated from barks of *Cladrastis lutea* (*Papilionoideae* subfamily, *Sophoreaea* tribe) [32].

Many leguminous plant lectins display pH-dependent oligomeric structures [25]. The molecular mass of VML in buffers of different pH was investigated by analytical sedimentation equilibrium sedimentation. The apparent molecular mass was 122–130 kDa and this value did not change in the pH range of 2.5–8.5. These results clearly indicated that native VML is a homotetrameric protein and that its quaternary structure is not pH-dependent. Similarly, *S. japonica* leave and bark isolectins possess a tetrameric structure (by gel filtration) at pH 5 and 8.5 [27,28]. However, galactose-specific isolectins isolated from the genus *Erythrina* have been reported to be dimeric proteins [22], and both, dimeric (RPA1) and tetrameric (RPA3) *R. pseudoacacia* lectins have been documented [33].

3.2. Structure of the N-linked oligosaccharides of *V. macrocarpa* seed lectin

Monosaccharide analysis showed that the carbohydrate chain(s) of VML are composed of GlcNAc, Man, Xyl, and Fuc in approximately molar proportions of 2:3:1:1. Electrospray ionization mass spectrometric analysis of reduced (with $NaBD_4$) and permethylated total oligosaccharides released by hydrazinolysis from VML yielded molecular ions at m/z 1522.8 [Hex_3-Pent-HexNAc-dHex-HexNAc-ol+Na] (80%) and 1318.9 [Hex_2-Pent-HexNAc-dHex-HexNAc-ol+Na] (20%) suggesting the presence of a proximally fucosylated trimannosyl or dimannosyl common core structure bearing β1,2-linked xylose at the inner mannose residue. Such carbohydrate structure is characteristic for plant *N*-glycans. These assumptions were confirmed by collision-induced dissociation experiments. Fig. 4 shows the daughter ion spectrum of the dominant oligosaccharide. An intense fragment ion at m/z 491 [dHex-HexNAc-ol-1D+Na] together with the complementary ion [M-(dHex-HexNAc-ol-1D)+Na] at m/z 1054 indicated the presence of proximal Fuc. This residue must be linked to O-3 of the reduced GlcNAc since a secondary fragment ion at m/z 286 is generated by elimination of Fuc from the primary fragment ion. This is characteristic for 3-linked substituents of GlcNAc residues and is not observed with proximal Fuc 1,6-linked. The latter linkage is characteristic of mammalian oligosaccharides. Additional fragment ions of lower intensity can be explained by the cleavage of other glycosidic bonds (Fig. 4). The minor component at m/z 1319 yielded an analogous daughter ion spectrum compatible with a proximally 1–3 fucosylated dimannosyl structure bearing an additional xylose residue. Methylation analysis showing derivatives charac-

teristic for terminal xylose and 2,3,6-trisubstituted mannose confirmed the presence of xylose 1,2-linked to the common core. Similarly, the linkage of fucose to O-3 of the proximal GlcNAc was confirmed. The single terminal mannose residue of the minor dimannosyl component was found to be attached to O-6 of the inner mannose residue, since small amounts of 2,6-disubstituted mannose were detected and O-2 was occupied by the xylose residue.

The major carbohydrate structure of VML is identical to the carbohydrate chain of *R. pseudoacacia* and *E. variegata* seed lectins determined by ^{1}H-NMR spectroscopy [22,33]. N-linked oligosaccharides having a β1,2-linked xylose attached to the β-linked mannose of the core structure appear to be a common characteristic of different allergenic proteins [34]. The xylose units on these glycans may play a critical role in allergenicity as well as in regulating the targeting of these proteins to various organelles such as storage bodies [34].

Zeng and colleagues [34] have purified and studied the specificity of β1,2-xylotransferase, the enzyme that transfers D-xylose from UDP-xylose to the β-linked mannose of plant N-linked oligosaccharides. They show that the enzyme acts on biantennary glycan acceptors having β1,2GlcNAc residues on the Manα1-3 and Manα1-6 arms, whereas oligosaccharides having the core structure of VML glycan-1 are not active as acceptors of xylose. This strongly suggests that the oligosaccharide attached to VML (and *R. pseudoacacia* and *E. variegata* lectins) may be derived from a larger glycan by glycosidase processing.

3.3. Proposed mechanism for the proteolytic processing of *V. macrocarpa* seed lectin

The isotope-averaged molecular mass calculated for the covalent structure of VML assuming V117, M148, G154, E168 and two glycan 1 structures N-linked to asparagine residues 111 and 183 is 28 324 Da. This mass is 204 Da smaller than that measured by MALDI-TOF mass spectrometry (Fig. 1), suggesting that the C-terminus of full-length VML may contain two additional residues (assigned tentatively as SN). The mass difference between molecular ions of 28 528 Da (VML alpha-1) and 27 357 Da (1171 Da) corresponds exactly to the mass calculated for the glycan-1 structure, suggesting that this latter ion may correspond to a monoglycosylated form of VML α-chain (VML alpha-2) (Fig. 5). From mass spectrometric (Fig. 1) and sequence data (Figs. 1 and 2), we propose the mechanism for the proteolytic processing of VML depicted in Fig. 5. Cleavage of VML alpha-2 at the peptide bond N114-K115 may produce a nonglycosylated γ-chain (residues 1–114, M_{calc} = 12 304 Da; M_{meas} = 12 308 Da) and a mixture of C-terminally truncated β-chains (residues 115–239) glycosylated at N183 with glycan-1 (glyco-beta-1, M_{calc} = 14 954 Da; M_{meas} = 14 957 Da) and nonglycosylated (beta-1, M_{calc} = 13 783 Da; M_{meas} = 13 782 Da). This strongly suggests that deglycosylation of N111 is a necessary step before proteolytic cleavage of N114-K115 can occur because the glycan moiety attached to N111 sterically hinders the action of the endopeptidase. The species of 14 260 Da (Fig. 1) correspond to the doubly charged, $(M+2H)^{2+}$, quasimolecular

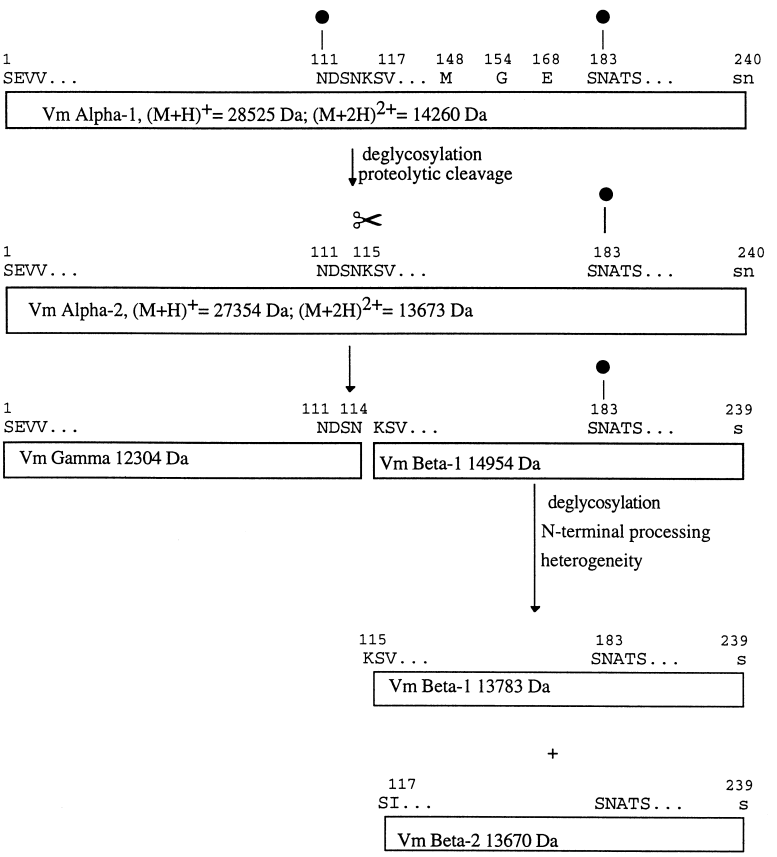

Fig. 5. Proposed pathway leading to proteolytic processing of *Vatairea macrocarpa* lectin. It is hypothesized that deglycosylation of Asn-111 is a necessary step for cleavage of the Asn-114-Lys-115 bond of VML alpha-chain. Subsequent deglycosylation of VML beta-chain, together with N- and C-terminal processing and heterogeneity at position 117, generate four molecular isoforms of the beta-chain.

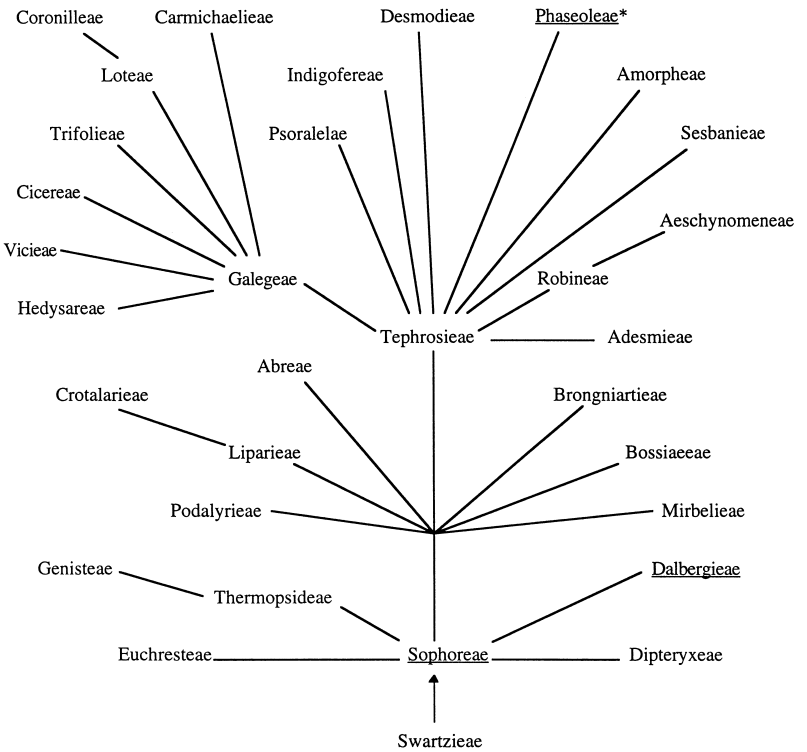

Fig. 6. Supposed phylogenetic relationship of tribes of the *Papilionoideae* subfamily of leguminous plants. Tribes containing two-chain lectins are underlined. *, posttranslational processing of lectins of *Phaseoleae* subtribes include covalent religation of circular permutated polypeptides.

ions of 28 528 Da. Finally, the ion of 13 673 Da (Fig. 1) may correspond to a mixture of doubly charged quasimolecular ion of 27 354 Da (M_{calc} = 13 678 Da) and a deglycosylated beta-chain comprising residues 116–239 with isoleucine at position 117 (M_{calc} = 13 670 Da). This latter assignment is consistent with N-terminal sequencing of electroblotted VML chains (Fig. 1, insert).

Posttranslational processing of Concanavalin A (*Papilionoideae* subfamily, *Phaseoleae* tribe) (Fig. 6) in maturing jackbeans also involves activation by deglycosylation of an inactive precursor (glyco-pro-Con A) [30]. Pro-Con A is then cleaved to produce β- and γ-fragments which are religated in a different order to produce mature Con A [30]. The new junction is a peptide bond between mature 118 and 119 [35]. Our results show that VML processing does not involve such circular permutation. Ueno et al. [36] have shown that the mannose/glucose-specific lectin B-SJA-II isolated from barks of *S. japonica* is, like VML, a two-chain lectin. The N-terminal sequences of the B-SJA-II fragments correspond to amino acid sequences of VML initiating at positions 1 and 115. Since *Sophoreae* (B-SJA-II) and *Dalbergieae* (VML) are at the root of the *Papilionoideae* phylogenetic tree (Fig. 6), these data indicate that posttranslational cleavage evolved early in this branch of legume plant evolution, and support the view that circular permutation originated latter and might be restricted to members of the *Phaseoleae* tribe.

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