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Isolectins from *Solanum tuberosum* with Different Detailed Carbohydrate Binding Specificities: Unexpected Recognition of Lactosylceramide by *N*-Acetyllactosamine–Binding Lectins[1]

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Glycosphingolipid recognition by two isolectins from *Solanum tuberosum* was compared by the chromatogram binding assay. One lectin (PL-I) was isolated from potato tubers by affinity chromatography, and identified by MALDI-TOF mass spectrometry as a homodimer with a subunit molecular mass of 63,000. The other (PL-II) was a commercial lectin, characterized as two homodimeric isolectins with subunit molecular masses of 52,000 and 55,000, respectively. Both lectins recognized *N*-acetyllactosamine–containing glycosphingolipids, but the fine details of their carbohydrate binding specificities differed. PL-II preferentially bound to glycosphingolipids with *N*-acetyllactosamine branches, as Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer. PL-I also recognized this glycosphingolipid, but bound equally well to the linear glycosphingolipid Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer. Neolactotetraosylceramide and the B5 pentaglycosylceramide were also bound by PL-I, while other glycosphingolipids with only one *N*-acetyllactosamine unit were non-binding. Surprisingly, both lectins also bound to lactosylceramide, with an absolute requirement for sphingosine and non-hydroxy fatty acids. The inhibition of binding to both lactosylceramide and *N*-acetyllactosamine–containing glycosphingolipids by *N*-acetylchitotetraose suggests that lactosylceramide is also accomodated within the *N*-acetylchitotetraose/*N*-acetyllactosamine–binding sites of the lectins. Through docking of glycosphingolipids onto a three-dimensional model of the PL-I hevein binding domain, a Galβ4GlcNAcβ3Galβ4 binding epitope was defined. Furthermore, direct involvement of the ceramide in the binding of lactosylceramide was suggested.

Key words: *N*-acetyllactosamine–binding, glycosphingolipid recognition, lactosylceramide, molecular modeling, *Solanum tuberosum* lectins.

Lectins are carbohydrate-binding (glyco)proteins of non-immune origin that have one or several carbohydrate binding domains (*1*). Lectins have been found in plants, viruses, bacteria, insects, fungi, and animal cells (*2*). Although their biological function is still undefined (*3*), lectins are frequently used for the detection, isolation and characterization of glycoconjugates in biomedical research (*4*, *5*).

Potato lectins, *i.e.* the lectins from tubers of *Solanum tuberosum*, are glycoproteins with two identical subunits. Each subunit of the lectin contains three regions (*6*). The N-terminal domain has several proline residues, and is not glycosylated. The next domain has a high content of hydroxyproline, all of which is substituted with L-arabinose, and also has some serine residues substituted with galactose. The third domain is not glycosylated. This domain has a high content of cysteine and carries the carbohydrate binding site.

By gel filtration, the molecular mass of each subunit of the potato lectin was previously estimated to be approximately 46,000 (*7*). One recent investigation by MALDI-TOF mass spectrometry demonstrated a subunit molecular mass of 65,500 (*6*). However, another recent analysis by MALDI-TOF mass spectrometry gave a subunit molecular mass of 55,010 (*8*).

The carbohydrate binding domain of potato lectin has

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Abbreviations: Ac-AMP2, antimicrobial peptide 2 from *Amaranthus caudatus*; MALDI-TOF mass spectrometry, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PBS, phosphate-buffered saline; PTH, phenylhydantoin; SDS-PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; UDA, *Urtica dioica* lectin, WGA, wheat germ agglutinin. The glycosphingolipid nomenclature follows the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature [CBN for Lipids: *Eur. J. Biochem.* (1977) **79**, 11–21; *J. Biol. Chem.* (1982) **257**, 3347–3351; and *J. Biol. Chem.* (1987) **262**, 13–18]. It is assumed that Gal, Glc, GlcNAc, GalNAc, NeuAc, and NeuGc are in the D-configuration, Fuc in the L-configuration, and all sugars present in the pyranose form.

several features in common with other chitin-binding proteins for which the three-dimensional structures are known. These include *e.g.* wheat germ agglutinin isolectin 2 from *Triticum vulgare* (*9*), the antimicrobial peptide 2 (Ac-AMP2) from *Amaranthus caudatus* (*10*) and hevein from the latex of *Hevea brasiliensis* (*11*). The presence of a cysteine-rich binding domain in the potato lectin, as well as the involvement of aromatic residues in chitin binding (*12*, *13*), is highly suggestive of a binding domain fold similar to that of the three proteins mentioned above. Sequence alignment of peptides obtained by chymotryptic digestion confirmed, furthermore, that the potato lectin contains a hevein binding domain for chitin (*14*).

By hemagglutination inhibition experiments, the carbohydrate binding specificity of potato lectin has been defined as GlcNAc(β4GlcNAc)$_2$β4GlcNAc > GlcNAcβ4GlcNAcβ4-GlcNAc > GlcNAcβ4GlcNAc ≫ GlcNAc (*4*). An extended carbohydrate binding site was suggested since the inhibitory power of chitin oligosaccharides increases with increasing chain length up to the tetraose. *N*-Acetyllactosamine is a relatively weak inhibitor of hemagglutination induced by the potato lectin. Still, this lectin is sometimes used in immunohistochemical studies for the detection of *N*-acetyllactosamine–containing glycoconjugates (*15*, *16*). However, some reports have indicated that there are specific structural features required for the interaction of potato lectin with *N*-acetyllactosamine (*17*, *18*).

In the present study the detailed structural requirements for binding of potato lectins were determined by binding radiolabeled lectins to a large number of glycosphingolipids in a chromatogram binding assay. Two potato lectin preparations were used. One was a lectin isolated from potato tubers by affinity chromatography (designated PL-I), and the other was a commercial potato lectin (designated PL-II). SDS-PAGE and MALDI-TOF mass spectrometry demonstrated that the former lectin was a homodimer with a subunit molecular mass of 63,000, while the commercial lectin preparation contained two homodimeric isolectins with subunit molecular masses of 52,000 and 55,000, respectively. The binding preferences of PL-I and PL-II for *N*-acetyllactosamine–containing glycosphingolipids was determined. The docking of glycosphingolipids onto a three-dimensional model of the PL-I binding site, based on the known partial amino acid sequence of PL-I and the NMR structure of the homologous chitin-binding protein hevein, revealed that a Galβ4GlcNAcβ3Galβ4 epitope is responsible for the binding. The unexpected binding of lactosylceramide with sphingosine and non-hydroxy fatty acids is also rationalized by the PL-I model showing the direct involvement of the ceramide in the binding.

MATERIALS AND METHODS

Solanum tuberosum Lectins—Two preparations of potato lectin were used. In the first, the lectin was isolated from potato tubers by affinity chromatography (*19*). In brief, potato tubers (2 kg, cultivar Desiree, purchased from the Institute of Potato Culture, Brasov, Romania) were extracted at 4°C in 3 M acetic acid for 24 h. The extract was filtered through cheese cloth and centrifuged at 10,000 ×*g* for 30 min. Solid ammonium sulfate was added to the supernatant to 60% saturation, and the resultant precipitate was dissolved in 0.1 M acetate buffer, pH 3.8, containing 5 mM thiourea, 0.9% NaCl (w/v), and 0.02% sodium azide (w/v) (buffer I), followed by dialysis against the same buffer. The solution was then centrifuged at 10,000 ×*g* for 30 min to remove insoluble materials, and the supernatant was applied to a column of chitin, which had been equilibrated with buffer I. The column was washed with buffer I until the absorbance at 280 nm reached approximately 0.01, and then eluted with 0.2 M acetic acid. The hemagglutinating activity of the fraction obtained was confirmed using a 4% (v/v) rabbit erythrocyte suspension. The pH of the solution was adjusted to 7.0, and the solution was concentrated on Centriprep 10, dialyzed against phosphate-buffered saline, pH 7.3 (PBS), and applied to a fetuin-Sepharose column [prepared as described by Young and Leon (*20*)], which had been equilibrated with PBS. After washing the column with PBS, elution was performed with 0.2 M acetic acid. The hemagglutinating activity of the fractions was determined, and hemagglutinating fractions were pooled, dialyzed against PBS, and concentrated on Centriprep 10, giving 3 mg of lectin. The purity of the lectin was checked by SDS-PAGE (*21*) in 12.5% acrylamide gels. This preparation of potato lectin was designated PL-I.

The second preparation of potato lectin (designated PL-II) was purchased from Vector (Vector Laboratories, Burlingame, CA).

Molecular Mass Determinations—Molecular masses were estimated from SDS-PAGE using the Pharmacia PhastSystem™ (Amersham Pharmacia Biotech) according to the protocols of the manufacturer. Briefly, samples were dissolved in 20 mM Tris-HCl (pH 8.0) buffer containing 2.5% SDS, 5% 2-mercaptoethanol, and 1 mM EDTA. Before electrophoresis, the samples were heated to 95°C for 5 min and centrifuged at 10,000 ×*g* for 2 min. A homogeneous gel of 7.5% was used and 0.5–1 μg protein was applied to each lane. After electrophoresis, the gel was stained with GelCode® Blue Stain Reagent (Pierce, Rockford, IL) according to the manual.

Molecular masses were further investigated by MALDI-TOF mass spectrometry. The measurements were performed on a TofSpec-E time-of-flight mass spectrometer (Micromass, Manchester, UK) equipped with a pulsed nitrogen laser (337 nm; 3ns), and with a delayed extraction unit. Aqueous solutions of lectins (16 nmol/ml) were diluted 1:1 in aqueous 50% acetonitrile containing sinapinic acid (11 mg/ml). One microliter of the mixture was deposited on the target and left to air dry. The MALDI was run in linear mode with an acceleration voltage of 25 kV and the lag pulse set to 3,200 V. Carbonic anhydrase II, monomer and dimer, was used for external calibration.

Amino Acid Sequence Analyses of PL-I and PL-II—Sequential Edman degradation was performed on a Procise 492 protein sequencer (Perkin Elmer). Samples (20–100 pmol) were applied to polybrene-coated TFA-resistant filters after two rounds of precycling. The sequencing was performed in the pulsed-liquid mode using the standard program supplied by the manufacturer. All PTH–amino acids were identified by their retention times compared with standards, except PTH-hydroxyproline which was identified by the reported elution of two peaks on each side of PTH-alanine (*22*).

Radiolabeling—Aliquots of 100 μg (in PBS containing 100 mM *N*-acetylchitotetraose; Glycorex, Lund, Sweden) of each potato lectin preparation were labeled with ^{125}I by the

Iodogen method (*23*), giving in average 2×10^3 cpm/μg.

Thin-Layer Chromatography—Thin-layer chromatography was performed on glass- or aluminum-backed silica gel 60 HPTLC plates (Merck, Darmstadt, Germany), using chloroform/methanol/water (60:35:8, by volume) as a solvent system. For separation of gangliosides, a solvent system composed of chloroform/methanol/water with 0.25% KCl (50:40:10, by volume) was used. Chemical detection was done with anisaldehyde (*24*).

Chromatogram Binding Assay—The binding of radiolabeled lectins to glycosphingolipids separated on thin-layer plates was done as previously described (*25*). Mixtures of glycosphingolipids (20–40 μg/lane) or pure compounds (0.01–4 μg/lane) were separated on aluminum-backed silica gel plates. After drying, the chromatograms were treated with 0.5% (w/v) polyisobutylmethacrylate (Aldrich Chem., Milwaukee, WI) in diethylether for 1 min. After drying, the chromatograms were soaked in PBS containing 2% bovine serum albumin (w/v), 0.1% NaN_3 (w/v), and 0.1% Tween 20 (w/v) (Solution 1) for 2 h at room temperature. Thereafter, suspensions of ^{125}I-labeled lectins (approximately 2×10^3 cpm/μl), diluted in PBS containing 2% bovine serum albumin (w/v) and 0.1% NaN_3 (w/v) (Solution 2) were gently sprinkled over the plates and the plates were incubated for 2 h at room temperature, then washed 6 times with PBS. Autoradiography was performed for 12–24 h using XAR-5 X-ray film (Eastman Kodak, Rochester, NY) with an intensifying screen.

Microtiter Well Assay—The microtiter well binding assay was performed as previously described (*25*). In short, 50 μl of serial dilutions (each dilution in triplicate) of pure glycosphingolipids in methanol were applied to microtiter wells (Falcon3911, Becton Dickinson Labware, Oxnard, CA). When the solvent had evaporated, the wells were blocked for 2 h with 200 μl of Sol. 1. Thereafter, 50 μl of radiolabeled potato lectin, diluted in Sol. 2 (approximately 2×10^3 cpm/μl), was added to each well, and the plates were incubated for 4 h at room temperature. After washing 6 times with Sol. 2, the wells were cut out and the radioactivity counted in a gamma counter.

Inhibition Studies—The ability of lactose and *N*-acetylchitotetraose to inhibit the binding of potato lectins to glycosphingolipids on thin-layer plates was tested by incubating the ^{125}I-labeled lectins with lactose (J.T. Baker Chem., Phillipsburg, NJ) or *N*-acetylchitotetraose (Seikagaku, Tokyo, and Glycorex, Lund, Sweden) in Sol. 2 (1 mg/ml) for 1 h at room temperature. Thereafter the lectin suspensions were utilized in the chromatogram binding assay.

Reference Glycosphingolipids—Total acid and non-acid glycosphingolipid fractions, from the sources given in Table II, were obtained by standard procedures (*26*). The individual glycosphingolipids were isolated by repeated chromatography on silicic acid columns of the native glycosphingolipid fractions, or their acetylated derivatives.

A number of modified glycosphingolipids were also utilized. Lactosylceramide, with the ceramide modified by a trifluoroacetimide moiety (No. 9 in Table II), was produced as described (*27*). Hydrolysis with β-galactosidase from *Streptococcus pneumoniae* (Oxford Glycosystems, Abingdon, UK) of glycosphingolipids Nos. 21 and 36 (producing Nos. 14 and 37) was done according to the manufacturer's instructions. De-*N*-acylation of glycosphingolipids Nos. 21 and 26 (yielding Nos. 22 and 27) was performed by treatment with anhydrous hydrazine, as described (*28*). For the removal of fucose residues glycosphingolipids Nos. 33 and 43 were incubated in 0.05 M HCl at 80°C for 2 h, followed by Folch partitioning (*29*). The glycosphingolipids in the lower phases thus obtained were subsequentely purified on silicic acid columns, yielding glycosphingolipids Nos. 29 and 41. Desialylation of glycosphingolipid No. 56 (yielding No. 39) was conducted by mild acid hydrolysis (1% acetic acid at 100°C for 1 h), while glycosphingolipids Nos. 30 and 38 were prepared from No. 51 by mild acid hydrolysis (No. 38), followed by treatment with β-galactosidase (No. 30).

The identity of the purified glycosphingolipids was confirmed by mass spectrometry (*30*), proton NMR spectroscopy (*31–34*), and degradation studies (*35, 36*).

Molecular Modeling—Minimum energy conformers for several of the glycosphingolipids listed in Table II and chitins of different length were constructed within the Biograf molecular modeling program and then transferred to the Quanta97/CHARMm22 modeling package (both from Molecular Simulations), the latter residing on a Silicon Graphics Indigo2 Extreme workstation. Glycosidic dihedral angles for minimum energy conformers of constituent disaccharides were taken from the literature (*37–39*) and are defined as follows: Φ = H1-C1-O1-C′X and Ψ = C1-O1-C′X-HX for 2-, 3-, or 4-linked residues; Φ = H1-C1-O1-C′6, Ψ = C1-O1-C′6-C′5, and ω = O1-C′6-C′5-O′5 for 6-linked residues. For the Glcβ1Cer linkage the angles are defined as Φ = H1-C1-O1-C′1, Ψ = C1-O1-C′1-C′2, and θ = O1-C′1-C′2-C′3 (*40*).

Since PL-I had the broadest range of binding glycosphingolipid structures it was chosen for conformational studies. The three-dimensional NMR structure of hevein (PDB entry 1HEV, structure 1) was used as a template for the construction of the corresponding binding domain of PL-I by mutating all hevein residues for which the corresponding PL-I residues are known. As seen in Table IV, a gap of three residues in the PL-I sequence occurs at positions 21–23 (hevein numbering), which for other known hevein domain sequences (*10*, *14*) contain an aromatic residue always followed by glycine and a second aromatic residue.

Since chemical modification studies indicate that a single tryptophan residue and several tyrosine residues are involved in chitin binding (*12*, *13*), two different PL-I models were constructed in which the sequence gap was filled with either Trp-Gly-Tyr or Tyr-Gly-Trp. Residues 1–13 and 36–43 from the original hevein sequence were kept to ensure the structural integrity of the chitin binding domain. The only minor concern here is on the N-terminal side, which for PL-I must continue beyond the N-terminus of hevein. This in turn might affect the orientation of the Trp23 side chain to some degree. However, superposition of the hevein, WGA, and Ac-AMP2 binding sites reveals very similar orientations of the residue at this position, despite elongation on the N-terminal side of WGA and a four-residue deletion for Ac-AMP2 (*10*). The side chains of these two structures were energy minimized with the protein backbone fixed, after which the entire structure was allowed to relax. The r.m.s. distance deviations as compared with the similarly relaxed hevein structure were 0.79 Å for both models when considering the protein backbones, whereas the r.m.s. value for relaxed *vs.* unrelaxed hevein as a whole was found to be 1.17 Å and for the backbone 0.79 Å. *N*-Acetylchitobiose was docked onto the binding surface of the three structures (*i.e.*

hevein and the two versions of the PL-I binding domain) and energy minimized. The complexes were subsequently subjected to a brief molecular dynamics simulation with their backbones harmonically constrained, after which a representative frame from the dynamics trajectories was chosen, followed by energy re-minimization of the structures. Regarding the hevein–*N*-acetylchitobiose complex, all the salient features of the sugar–protein interactions reported earlier (*11*, *38*) were reproduced. In the ensuing docking of different oligosaccharides of glycosphingolipid origin, the two PL-I complexes thus produced were used as templates, after which similar protocols were applied to some of the various oligosaccharide–PL-I complexes.

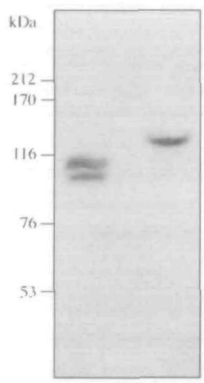

Fig. 1. **SD *lanum tuberosum*, PL-II (left lane), and PL-I (right lane).** Experimental conditions were as described in "MATERIALS AND METHODS."

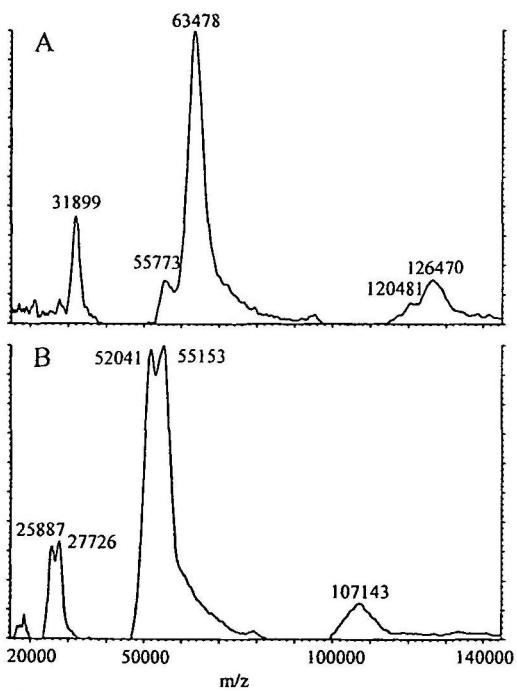

Fig. 2. **Comparison of MALDI-TOF mass spectra of the two lectins from *Solanum tuberosum*, PL-I (A), and PL-II (B).** Mass spectra were obtained on a TofSpec-E time-of-flight mass spectrometer using a sinapinic acid matrix, as detailed in "MATERIALS AND METHODS."

RESULTS

Characterization of Potato Lectins (PL-I and PL-II)—On SDS-PAGE gels (Fig. 1) PL-I migrated as a single band with an apparent relative molecular mass of 130,000, while PL-II gave two bands corresponding to molecular masses of 104,000 and 112,000.

The molecular masses were further determined by MALDI-TOF mass spectrometry. The spectrum of PL-I (Fig. 2A) contained peaks corresponding to the doubly $(M+2H)^{2+}$ and singly charged $(M+H)^{+}$ molecular ions of the monomeric lectin at m/z 32,000 and 63,000, respectively, while the peak at m/z 126,000 corresponded to the lectin dimer.

The spectrum of PL-II (Fig. 2B) had two doubly charged monomer molecular ions at m/z 25,000 and 27,000, and the corresponding singly charged molecular ions were found at m/z 52,000 and 55,000. A peak corresponding to the dimeric lectin was found at m/z 107,000.

In summary, the molecular mass determinations by SDS-PAGE and MALDI-TOF mass spectrometry showed that PL-I is a homodimer, with a molecular mass of 126,000 (2 × 63,000). For PL-II MALDI-TOF mass spectrometry demonstrated two subunits with molecular masses of 52,000 and 55,000. Since PL-II migrated as a double band on SDS-PAGE, this preparation presumably contains two homodimeric isolectins.

Amino Acid Sequence Analyses of PL-I and PL-II—The

TABLE I. **N-terminal amino acid sequences of lectins from *Solanum tuberosum*.**

	Ref. 6[a]	PL-I	PL-II
1.	A		
2.	A		
3.	S		
4.	T	T	
5.	O	O	**N**
6.	S	S	**A**
7.	P	P	**S**
8.	P	P	**O**
9.	P	P	P
10.	L	L	L
11.	P	P	P
12.	Y	Y	Y
13.	P	**L**	P
14.	Q	Q	**Q**
15.	X	Y	
16.	G	G	
17.	L	L	
18.	K	K	
19.	K	K	
20.	P	**G**	
21.	G	X	
22.	G	X	
23.		X	
24.		X	
25.		I	
26.		K	
27.		G	
28.		X	
29.		E	

[a]The N-terminal amino acid sequences of PL-I and PL-II have been aligned against the N-terminal potato lectin sequence published by Allen *et al.* (*6*). Residues differing from the published sequence are in bold. O = hydroxyproline, X denotes undetermined amino acids.

results of N-terminal amino acid sequencing of PL-I and PL-II are summarized in Table I. Thus the N-terminal amino acids of both lectins differ from the potato lectin reported by Allen *et al.* (*6*). However, after alignment, it was seen that 14 out of 17 residues of PL-I are identical with the reported sequence, while six out of ten determined amino acids of PL-II could be aligned with the reported sequence.

Chromatogram Binding Assays—The binding of the two ^{125}I-labeled potato lectin preparations (PL-I and PL-II) to glycosphingolipids separated on thin-layer chromatograms was investigated using a broad panel of reference glycosphingolipids, as exemplified in Figs. 3 and 4. The results are summarized in Table II.

The minimal *N*-acetyllactosamine–containing glycosphingolipid recognized was neolactotetraosylceramide (No. 21 in Table II, Fig. 3, lane 4, and Fig. 4, lanes 1–4). The absence of binding to lactotriaosylceramide (No. 14, and Fig. 3, lane 3) or lactotetraosylceramide (No. 40) indicated an absolute requirement for the Galβ4GlcNAcβ sequence. Both lectins also bound to terminal *N*-acetyllactosamine substituted with an αGal at the 3-position of the terminal Gal (B5 pentaglycosylceramide, No. 26, Fig. 3, lane 5, and Fig. 4, lanes 1–4). However, while the detection limit of PL-I for neolactotetraosylceramide and B5 pentaglycosylceramide was 0.3 nmol, PL-II binding was only occasionally obtained when 3 nmol of these two compounds were applied to thin-layer plates (see Fig. 4, lane 1). Conversion of the acetamido groups of the *N*-acetylglucosamines of neolactotetraosylceramide and the B5 pentaglycosylceramide to amines (Nos. 22 and 27) abrogated the binding of both lectins. Other substitutions at the 3-position of the terminal Gal of neolactotetraosylceramide, as GalNAcβ (No. 28), GalNAcα (No. 29), or GlcNAcβ (No. 30, and Fig. 3, lane 7), were not tolerated. In addition, no binding of the lectins to glycosphingolipids with substitutions such as Fucα2 (No. 23, Fig. 3, lane 6), Galα4 (No. 25), NeuAcα3 (No. 48), or NeuAcα6 (No. 49) at the terminal Gal of neolactotetraosyl-

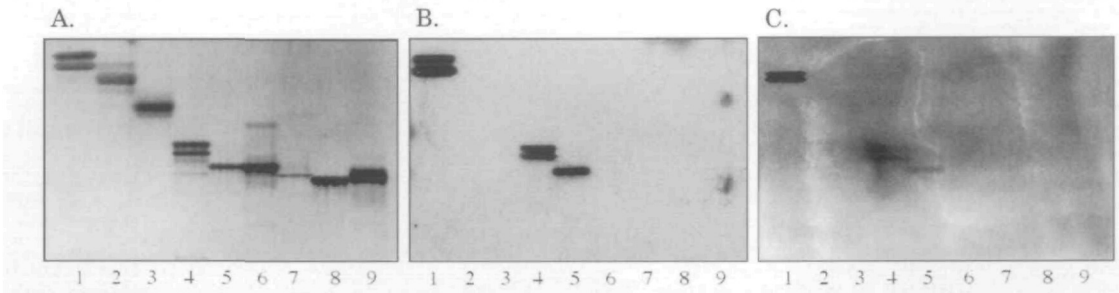

Fig. 3. **Binding of ^{125}I-labeled potato lectins to glycosphingolipids separated on thin-layer plates.** Glycosphingolipids (4 μg/lane) were separated on aluminum-backed thin-layer plates, using chloroform/methanol/water (60:35:8, by volume) as the solvent system. The chromatogram in (A) was stained with anisaldehyde, while (B) and (C) are autoradiograms obtained by the binding of radiolabeled PL-I (B) and PL-II (C), as described in the "MATERIALS AND METHODS." The lanes are: (1) Galβ4Glcβ1Cer (lactosylceramide) with sphingosine and non-hydroxy 16:0 and 24:1 fatty acids; (2) Galβ4Glcβ1Cer (lactosylceramide) with sphingosine and hydroxy 16:0 fatty acid; (3) GlcNAcβ3Galβ4Glcβ1Cer (lactotriaosylceramide) with sphingosine and non-hydroxy 16:0 and 24:1 fatty acids; (4) Galβ4GlcNAcβ3Galβ4Glcβ1Cer (neolactotetraosylceramide); (5) Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer (B5 pentaglycosylceramide); (6) Fucα2Galβ4GlcNAcβ3Galβ4Glcβ1Cer (H5 type 2 pentaglycosylceramide); (7) GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer; (8) Galα3(Fucα2)Galβ4GlcNAcβ3Galβ4Glcβ1Cer (B6 type 2 hexaglycosylceramide); (9) Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer (X-5 pentaglycosylceramide). The glycosphingolipids were from the sources given in Table II. Autoradiography was for 12 h.

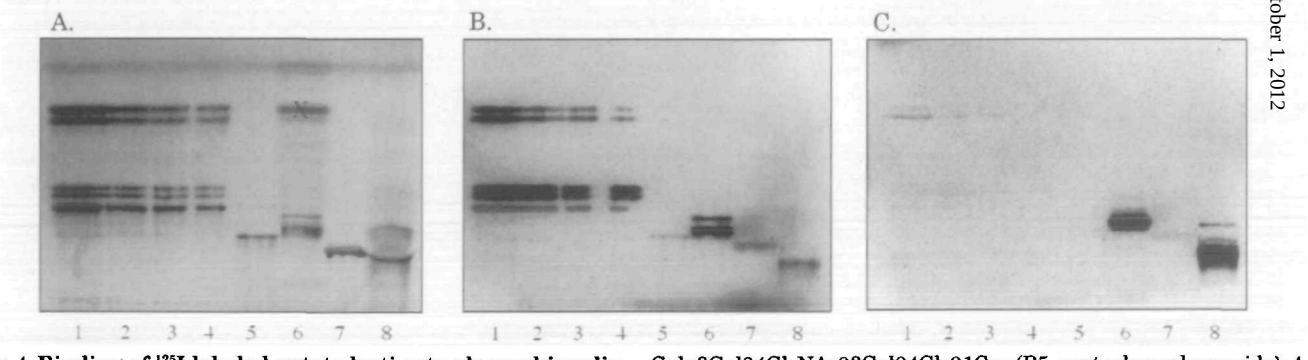

Fig. 4. **Binding of ^{125}I-labeled potato lectins to glycosphingolipids on thin-layer chromatograms.** The glycosphingolipids were chromatographed on aluminum-backed silica gel plates, and vizualized with anisaldehyde (A). Duplicate chromatograms were incubated with ^{125}I-labeled PL-I (B) and PL-II (C), followed by autoradiography for 12 h, as described under "MATERIALS AND METHODS." The solvent system used was chloroform/methanol/water (60:35:8, by volume). The lanes are: (1) serial dilutions of Galβ4Glcβ1Cer (lactosylceramide) with sphingosine and non-hydroxy 16:0 and 24:1 fatty acids, Galβ4GlcNAcβ3Galβ4Glcβ1Cer (neolactotetraosylceramide), and Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer (B5 pentaglycosylceramide); 4 μg of each compound; (2) 2 μg of each compound; (3) 1 μg of each compound; (4) 0.5 μg of each compound; (5) Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer, 4 μg; (6) Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer, 4 μg; (7) NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer, 4 μg; (8) Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4Glcβ1Cer, 4 μg. The glycosphingolipids were from the sources listed in Table II. The band marked X is a non-glycosphingolipid contaminant.

TABLE II. **Binding of ^{125}I-labeled lectins from *Solanum tuberosum* to glycosphingolipids on thin-layer chromatograms.**

No.	Trivial name	Structure	PL-I	PL-II	Source
Simple compounds					
1.	Cerebroside d18:1-16:0-24:0[a]	Galβ1Cer	–[b]	–	Pig kidney
2.	Cerebroside d18:1-16:0-24:0	Glcβ1Cer	–	–	Pig kidney
3.	Sulfatide	SO_3-Galβ1Cer	–	–	Human meconium
4.	LacCer d18:1-16:0 and 24:1	Galβ4Glcβ1Cer	++	+	Human granulocytes
5.	LacCer d18:1-h16:0	Galβ4Glcβ1Cer	–	–	Rabbit small intestine
6.	LacCer t18:0-24:0	Galβ4Glcβ1Cer	–	ND[c]	Rabbit small intestine
7.	LacCer t18:0-h16:0-h24:0	Galβ4Glcβ1Cer	–	–	Rabbit small intestine
8.	LacCer-disulfon	Galβ4Glcβ-O-$(SO_2C_{10}H_{33})_2$	–	–	[d]
9.	LacCer-TFI-d18:1-16:0-24:0	Galβ4Glcβ1-TFI-Cer	–	–	Human erythrocytes[e]
10.		Galβ4Glcβ-O-OTE	–	–	[f]
11.	Galabiosyl d18:1-14:0-1 8:0	Galα4GalCer	–	–	[g]
12.	Isoglobotri	Galα3Galβ4Glcβ1Cer	–	–	Dog intestine
13.	Globotri d18:1-16:0 and 24:0	Galα4Galβ4Glcβ1Cer	–	–	Human erythrocytes
14.	Lactotri d18:1-16:0 and 24:1	GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human granulocytes[h]
Ganglioseries					
15.	Gangliotri d18:1-16:0 and 24:0	GalNAcβ4Galβ4Glcβ1Cer	–	–	Guinea pig erythrocytes
16.	Gangliotetra	Galβ3GalNAcβ4Galβ4Glcβ1Cer	–	–	Mouse feces
17.	Fuc-gangliotetra	Fucα2Galβ3GalNAcβ4Galβ4Glcβ1Cer	–	–	Mouse small intestine
Globoseries					
18.	Globotetra	GalNAcβ3Galα4Galβ4Glcβ1Cer	–	–	Human erythrocytes
19.	Isoglobotetra	GalNAcβ3Galα3Galβ4Glcβ1Cer	–	–	Rat colon carcinoma
20.	Forssman	GalNAcα3GalNAcβ3Galα4Galβ4Glcβ1Cer	–	–	Dog intestine
Neolactoseries					
21.	Neolactotetra	Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	(+)	Human granulocytes
22.		Galβ4GlcNH2β3Galβ4Glcβ1Cer	–	–	Human erythrocytes[i]
23.	H5-2	Fucα2Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human erythrocytes
24.	X-5	Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	–	–	Dog intestine
25.	P1	Galα4Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human erythrocytes
26.	B5	Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	(+)	Rabbit erythrocytes
27.		Galα3Galβ4GlcNH$_2$β3Galβ4Glcβ1Cer	–	–	Rabbit erythrocytes[i]
28.	x_2	GalNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	ND	Human erythrocytes
29.		GalNAcα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	ND	Human erythrocytes[j]
30.		GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	–	Rabbit thymus[k]
31.	Y-6	Fucα2Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	–	–	Dog intestine
32.	B6-2	Galα3(Fucα2)Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human erythrocytes
33.	A6-2	GalNAcα3(Fucα2)Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human erythrocytes
34.	A7-2	GalNAcα3(Fucα2)Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human erythrocytes
35.		GalNAcβ3Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	ND	Rat colon carcinoma
36.		Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer	++	++	Bovine buttermilk
37.		GlcNAcβ6(GlcNAcβ3)Galβ4Glcβ1Cer	–	–	Bovine buttermilk[h]
38.		Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	++	(+)	Rabbit thymus[k]
39.		Galα3Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	+	Bovine erythrocytes[l]
Lactoseries					
40.	Lactotetra	Galβ3GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human meconium
41.		Galα3Galβ3GlcNAcβ3Galβ4Glcβ1Cer	–	ND	Monkey intestine[j]
42.	Le^a-5	Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human meconium
43.	B6-1	Galα3(Fucα2)Galβ3GlcNAcβ3Galβ4Glcβ1Cer	–	ND	Monkey intestine
44.	Le^b-6	Fucα2Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human meconium
Gangliosides					
45.	GM3 d18:1/d20:1-18:0	NeuAcα3Galβ4Glcβ1Cer	–	–	Human brain
46.	GM1	Galβ3GalNAcβ4(NeuAcα3)Galβ4Glcβ1Cer	–	–	Human brain
47.	GD1a	NeuAcα3Galβ3GalNAcβ4(NeuAcα3)Galβ4Glcβ1Cer	–	–	Human brain
48.	NeuAcα3SPG	NeuAcα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human erythrocytes
49.	NeuAcα6SPG	NeuAcα6Galβ4GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human meconium
50.	NeuAcα3Le^x	NeuAcα3Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	–	–	Human bilebladder tumor
51.		NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	++	(+)	Rabbit thymus
52.		Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4Glcβ1Cer	+	++	Bovine buttermilk
53.		NeuAcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	+	Human erythrocytes
54.		NeuAcα3Galβ4GlcNAcβ6(NeuAcα3Galβ4GlcNAcβ3)Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	+	Human placenta

55.	Galα3Galβ4GlcNAcβ6(NeuAcα3Galβ4GlcNAcβ3)Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	+	Bovine erythrocytes
56.	Fucα2Galβ4GlcNAcβ6(NeuAcα3Galβ4GlcNAcβ3)Galβ4GlcNAcβ3Galβ4Glcβ1Cr	+	+	Human erythrocytes
57. NeuAcα3-dimeric-Le^x	NeuAcα3Galβ4(Fucα3)GlcNAcβ3Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	–	ND	Human bilebladder tumor

[a]In the shorthand nomenclature for fatty acids and bases, the number before the colon refers to the carbon chain length and the number after the colon gives the total number of double bonds in the molecule. Fatty acids with a 2-hydroxy group are denoted by the prefix h before the abbreviation *e.g.* h16:0. For long chain bases, d denotes dihydroxy and t trihydroxy. Thus d18:1 designates sphingosine (1,3-dihydroxy-2-aminooctadecene) and t18:0 phytosphingosine (1,3,4-trihydroxy-2-aminooctadecene). [b]Binding is defined as follows: A significant darkening on the autoradiogram when 0.05 μg of the glycosphingolipid was applied on the thin-layer plate is denoted by ++, and 0.5 μg by +. (+) denotes an occasional binding at 4 μg, while – denotes no binding even at 4 μg of glycosphingolipid. [c]Not determined. [d]Glycosphingolipid No. 8 was a kind gift from Dr. Göran Magnusson, Lund University, Sweden. [e]Glycosphingolipid No. 9, having the ceramide modified by a trifluoroacetimide (TFI) moiety was produced as described (27). [f]Glycosphingolipid No. 10 [2-(octadecylthio)ethyl-*O*-α-D-galactopyranosyl(1-4)-β-D-galactopyranoside] was purchased from Svenska Sockerbolaget, Arlöv, Sweden. [g]Glycosphingolipid No. 11 was a kind gift from Dr. K. Stenvall, Symbicom AB, Lund, Sweden. [h]Glycosphingolipids Nos. 14 and 37 were prepared from Nos. 21 and 36, respectively, by treatment with β-galactosidase. [i]Glycosphingolipids Nos. 22 and 27 were prepared from Nos. 21 and 26, respectively, by treatment with anhydrous hydrazine. [j]Glycosphingolipids Nos. 29 and 41 were prepared from Nos. 33 and 43, respectively, by incubation in 0.05 M HCl at 80°C for 2 h. [k]Glycosphingolipids Nos. 30 and 38 were prepared from No. 51 by mild acid hydrolysis (No. 38) and subsequent treatment with β-galactosidase (No. 30). [l]Glycosphingolipid No. 39 was prepared from No. 55 by mild acid hydrolysis.

TABLE III. **Minimum amounts of glycosphingolipids required for the binding of ^{125}I-labeled lectins from *Solanum tuberosum* on thin-layer chromatograms.**

No. Trivial name	Structure	PL-I	PL-II
1. LacCer d18:0-16:0 and 24:1	Galβ4Glcβ1Cer	0.05 μg (0.05 nmol)	0.5 μg (0.5 nmol)
2. Neolactotetra	Galβ4GlcNAcβ3Galβ4Glcβ1Cer	0.5 μg (0.38 nmol)	4 μg (3 nmol)
3. B5	Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	0.5 μg (0.34 nmol)	4 μg (2.7 nmol)
4.	Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	0.05 μg (0.03 nmol)	4 μg (2.4 nmol)
5.	NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	0.05 μg (0.026 nmol)	4 μg (2.1 nmol)
6.	Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer	0.05 μg (0.03 nmol)	0.05 μg (0.03 nmol)
7.	Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4Glcβ1Cer	0.5 μg (0.26 nmol)	0.05 μg (0.026 nmol)

ceramide, or Fucα3 (No. 24, and Fig. 3, lane 9) of the GlcNAc, was obtained. There was no binding to blood group B (No. 32, and Fig. 3, lane 8) or blood group A (No. 33) determinants on type 2 chains.

Both PL-I and PL-II bound to a number of glycosphingolipids having two or more *N*-acetyllactosamine moieties (Nos. 36, 38, 39, and 51–57 in Table II). A clear preference of PL-II for glycosphingolipids with *N*-acetyllactosamine branches over linear *N*-acetyllactosamine sequences was detected. This is best illustrated by comparison of the binding to Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer (No. 36, and Fig. 4, lane 5) and Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer (No. 38, and Fig. 4, lane 6), where the detection limit of PL-II for the branched neolactohexaosylceramide was 0.03 nmol, while the detection limit for the linear equivalent was 3 nmol. In contrast, the detection limit of PL-I for both types of neolactohexaosylceramide was 0.03 nmol.

The detection limits for selected binding-active glycosphingolipids on thin-layer chromatograms are summarized in Table III. It should, however, be noted that binding of the lectins to the amounts given as detection limits were obtained under optimal conditions, while occasionally higher amounts of glycosphingolipids were required for binding.

Although no binding of the lectins to NeuAcα3-neolactotetraosylceramide (No. 48 in Table II), or NeuAcα6-neolactotetraosylceramide (No. 49) was obtained, the presence of a terminal sialic acid was tolerated with retained binding activity when the glycosphingolipids contained two or more *N*-acetyllactosamine units (Nos. 51–53, 55–58). Thus, the terminal α6-linked sialic acid on the 3-linked branch of Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4Glcβ1Cer (No. 7 in Table III) was tolerated by PL-II, since the detection limit for this compound (0.03 nmol) was the same as for Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer (No. 6). Comparison of the detection limits of PL-I for Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer (No. 6; 0.03 nmol) and Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4Glcβ1Cer (No. 7; 0.26 nmol) indicated that the binding of PL-I was abrogated to some extent by the terminal α6-linked sialic acid. However, the detection limit of PL-I for Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer (No. 4 in Table III) was the same as for NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer (No. 5), indicating that the α3-linked terminal sialic acid was accepted by this lectin.

Surprisingly, lactosylceramide, *i.e.* a glycosphingolipid devoid of *N*-acetyllactosamine, was also recognized by both lectins. However, only lactosylceramide with sphingosine and non-hydroxy fatty acids (No. 4 in Table II, Fig. 3, lane 1, and Fig. 4, lanes 1–4) was bound by the lectins, while all other variants of lactosylceramide tested (Nos. 5–10, exemplified in Fig. 3, lane 2) failed to show any binding activity. The detection limit of PL-I for lactosylceramide with sphingosine and non-hydroxy fatty acids was 0.05 nmol, while 0.5 nmol was required for the binding of PL-II. No binding to other related glycosphingolipids with sphingosine and non-hydroxy fatty acids, as glucosylceramide (No. 3), galabiosylceramide (No. 11), globotriaosylceramide (No. 13), lactotriaosylceramide (No. 14), gangliotriaosylceramide (No. 15), or the GM3 ganglioside (No. 45), was obtained.

Microtiter Well Assays—The relative binding activities of ^{125}I-labeled PL-I and PL-II for selected glycosphingolipids were further examined by binding to glycosphingolipids adsorbed in microtiter wells. In accordance with the results described above, PL-I binding to lactosylceramide with sphingosine and non-hydroxy fatty acids was obtained, while

lactosylceramide with phytosphingosine and hydroxy fatty acids was non-binding (Fig. 5A). Half maximal binding occurred at a concentration of approximately 0.02 nmol per well.

The binding of PL-II to lactosylceramide with sphingosine and non-hydroxy fatty acids was half maximal at a concentration of approximately 0.15 nmol per well (Fig. 5B). The preferential binding of PL-II to glycosphingolipids with *N*-acetyllactosamine branches compared to linear *N*-acetyllactosamine sequences was also obtained in microtiter wells. The binding of PL-II to Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer and Galβ4GlcNAcβ6(NeuAcα6-Galβ4GlcNAcβ3)Galβ4Glcβ1Cer was half maximal at a concentration of approximately 0.08 nmol per well, while no binding to Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1-Cer or NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc-β1Cer occurred.

Inhibition Studies—Radiolabeled PL-I and PL-II were incubated with *N*-acetylchitotetraose or lactose (1 mg/ml) for 1 h at room temperature, and the suspensions were thereafter incubated on thin-layer chromatograms with serial dilutions of lactosylceramide with sphingosine and non-hydroxy fatty acids (No. 4 in Table I), the B5 pentaglycosylceramide (No. 26) and Galβ4GlcNAcβ6(Galβ4GlcNAc-β3)Galβ4Glcβ1Cer (No. 36). As shown in Fig. 6D preincubation of PL-I with *N*-acetylchitotetraose abolished the binding to all three glycosphingolipids. The binding of PL-II to lactosylceramide and the B5 pentaglycosylceramide was also inhibited by *N*-acetylchitotetraose, while the binding to Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer was diminished (Fig. 6E). Preincubation with lactose had no inhibitory effect on either lectin (not shown).

Conformational Aspects—The following analysis is restricted to PL-I, for which the largest range of binding glycosphingolipid structures was observed (Table II). A partial amino acid sequence covering the crucial parts of the carbohydrate binding domain is available (Table IV), except for an undetermined three-residue stretch (residues 21–23, hevein numbering), which for other hevein domains invariably consists of an aromatic residue followed by Gly and another aromatic residue. It has been shown, furthermore, that one tryptophan and four tyrosines per binding site in PL-I are protected from chemical modification in the presence of *N*-acetylchitotriose (*12*, *13*), suggesting that either a Tyr-Gly-Trp or Trp-Gly-Tyr sequence should fill this gap. However, due to the lack of a three-dimensional structure for PL-I, related chitin-binding structures carrying similar cysteine-rich binding domains, such as hevein (*11*, *38*), Ac-AMP2 (*10*), and WGA (*9*, *41*), must be relied on for structural information. Thus, using the NMR-derived hevein structure as a template, two different models of the PL-I carbohydrate binding domain, including either of the two tripeptides mentioned above, was built as outlined in "MATERIALS AND METHODS." As will be evident below, the model containing the Tyr-Gly-Trp sequence (Fig. 7) appears consistent with the glycosphingolipid binding pattern.

The structure of the complex between hevein and methyl-β-*N*-acetylchitobiose (*11*, *38*) reveals that this ligand is accomodated in a surface-located site involving three aromatic residues (Trp21, Trp23, and Tyr30) providing extensive hydrophobic contacts with the two GlcNAc residues. Importantly, the acetamido moiety of the GlcNAc at the non-reducing end is found to be partly buried in a hydrophobic cleft forming hydrogen bonds to the side chain of the conserved Ser19, whereas the acetamido moiety of the reducing GlcNAc is more exposed to the solvent. Furthermore, the glycosidic dihedral angles of the disaccharide assume values of an extended conformation (Φ, Ψ ≈ 45°, 0°). Given the conserved overall structure of hevein binding domains, it was not unexpected that almost identical interactions were observed for *N*-acetylchitobiose complexed to PL-I when compared with the corresponding hevein complex, excepting of course the replacement of Trp21 by tyrosine (not shown).

Since the acetamido moiety of the penultimate GlcNAc of neolactotetraosylceramide is crucial for the binding of this glycosphingolipid (*cf.* Nos. 21 and 22 in Table II), docking was performed by placing the GlcNAc residue in a position analogous to the nonreducing GlcNAc of chitobiose, which interacts with Ser19 in the hevein complex. In order to avoid serious steric interference between the internal Galβ4 and Tyr21 the GlcNAcβ3Gal glycosidic bonds must assume dihedral angles corresponding to one of the minor minimum energy conformers (*37*) at Φ, Ψ = 31°, 152°. In

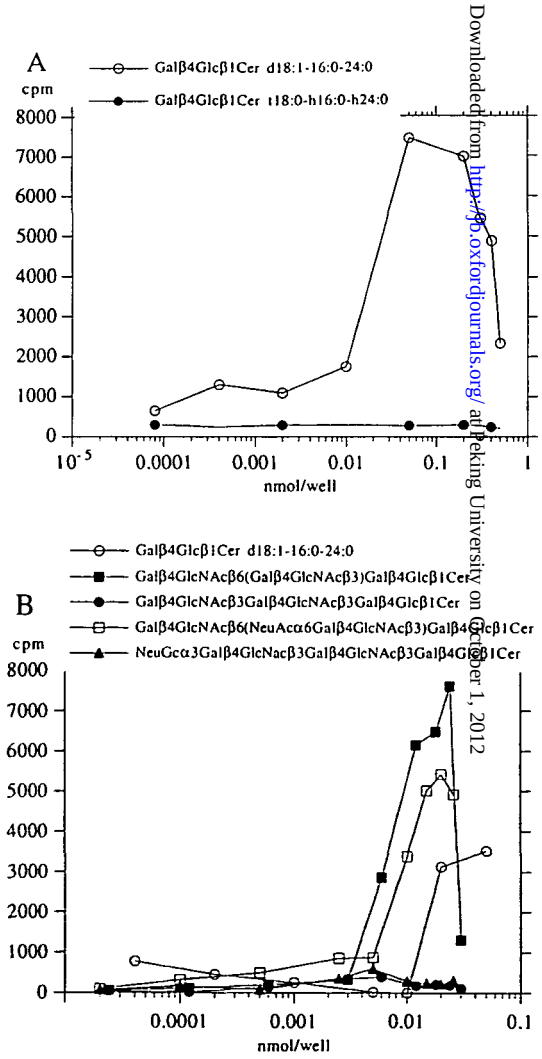

Fig. 5. **Binding of ^{125}I-labeled PL-I (A) and PL-II (B) to serial dilutions of glycosphingolipids adsorbed in microtiter wells.** The assay was done as described in "MATERIALS AND METHODS." Data are expressed as mean values of triplicate determinations.

this configuration the Galβ4 H3, H4, and H5 ring protons interface favorably with the tyrosine ring plane (Fig. 8). As for the terminal Galβ4, the plane formed by the H1, H3, and H5 ring protons positions itself coplanarly over the benzyl ring of the Trp23 indole moiety, providing the further hydrophobic interactions necessary to explain why neolactotetraosylceramide is binding whereas lactotriaosylceramide (No. 14) is nonbinding. Thus, the binding epitope corresponds to the Galβ4GlcNAcβ3Gal sequence. Furthermore, the presence of tyrosine instead of tryptophan at position 23 can most likely be ruled out since the favorable interactions just described for the terminal Galβ4 would be substantially reduced if this was the case.

The present model explains in most cases the binding or nonbinding of neolactotetraosylceramide extended by different sugars. For example, the non-binding of the H5-2 glycosphingolipid (Fucα2 extension, No. 23) is most likely due to the fact that the Fuc methyl group forces the hydroxymethyl group of the GlcNAc to assume the *gg* conformation (–60°), resulting in very unfavorable interactions with Trp23. As for the NeuAcα6 extended sialylneolactotetraosylceramide (No. 49), direct interference with the peptide chain explains why this glycosphingolipid is non-binding for all allowed NeuAcα6Gal glycosidic dihedral angles (see further below). Regarding the NeuAcα3 extension (No. 48), the non-binding of sialylneolactotetraosylceramide with this sugar residue in the synclinal conformation results from steric interference between the glycerol tail and Trp23, whereas for the anticlinal conformation it must be postulated that the fold of the peptide chain from residue 12 in the direction of the N-terminal is such that probably both the acetamido and glycerol moieties clash with the protein. Furthermore, the addition of Fucα3, yielding sialyl-Le^x (No. 50), makes the situation even worse due to severe steric clashes between this sugar and Tyr30. The non-binding of the Galα4-extended P1 glycosphingolipid (No. 25) may also be explained on similar grounds. Furthermore, neolactotetraosylceramide extended by either GalNAcβ3 (No. 28), GalNAcα3 (No. 29), or GlcNAcβ3 (No. 30), which may adopt very similar conformations (*42*), is not tolerated by the protein. This is in contrast to Galα3-extended B5 glycosphingolipid (No. 26) whose naked terminal sugar is tolerated but does not enhance the binding affinity. Thus, a rather restricted access to the protein surface for a fifth sugar is at hand.

At the six-sugar level the affinity of the protein for both branched and linear neolactohexaosylceramide (Nos. 36 and 38) increases tenfold (Table III). Starting with the branched version, it is found that only the β3-linked arm can be accommodated in the binding site proper with the neolactotetra portion assuming the same conformation as neolactotetraosylceramide itself (Fig. 8). The β6-linked arm reveals additional interactions with the protein, mainly van der Waals interactions, but also two probable hydrogen bonds between the Tyr21 hydroxyl and the glycosidic oxygen of the β6-link as well as between the Tyr35 hydroxyl

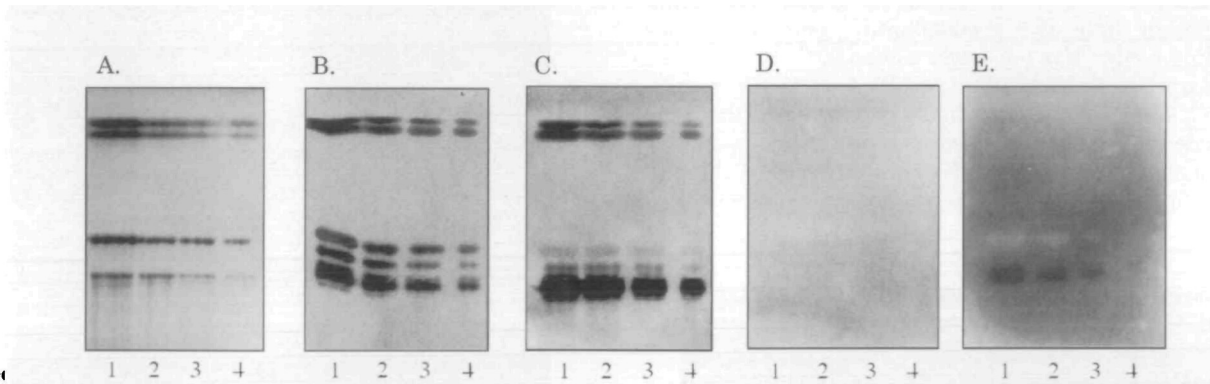

Fig. 6. **Effe**
***N*-acetylchitotetraose.** Radiolabeled PL-I and PL-II were incubated with *N*-acetylchitotetraose (1 mg/ml) for 1 h at room temperature. Thereafter the suspensions were used in the chromatogram binding assay, as described under "MATERIALS AND METHODS." (A) Thin-layer chromatogram stained with anisaldehyde. The binding of PL-I and PL-II is shown in (B) and (C), respectively, while the binding of PL-I preincubated with *N*-acetylchitotetraose is shown in (D), tions of Galβ4Glcβ1Cer with sphingosine and non-hydroxy 16:0 and 24:1 fatty acids, Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer and Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer were applied to the thin-layer plates. The lanes are: (1) 4 μg of each compound; (2) 2 μg of each compound; (3) 1 μg of each compound; (4) 0.5 μg of each compound. The solvent system was chloroform/methanol/water (60:35:8, by volume). Autoradiography was for 12 h.

TABLE IV. **Sequence alignment of hevein binding domains.**

PL-I[a]		T G E C C S I	- - - C G T T S D Y	C S P G Y
Hevein	E Q C G R Q A G G K	L C P N N L C C S Q	W G W C G S T D E Y	C S P D H N C Q
UDA A	H R C G S Q G G G G	T C P A L W C C S K	W G W C G D S E P Y	C G R · · T C E
UDA B	Q R C G A A V G N P	P C G Q D R C C S V	H G W C G G G N D Y	C S G S · K C Q
WGA A	Q R C G E Q G S N M	E C P N N L C C S Q	Y G Y C G M G G D Y	C G K G · · C Q
WGA B	K R C G S Q A G G A	T C P N N H C C S Q	Y G H C G F G A E Y	C G A G · · C Q
WGA C	I K C G S Q S G G K	L C P N N L C C S Q	W G F C G L G S E F	C G G G · · C Q
WGA D	K P C G K D A G G R	V C T N N Y C C S K	W G S C G I G P G Y	C G A G · · C Q
Ac-AMP2	G E C V R · · · · G	R C P S G M C C S Q	F G Y C G K G P K Y	C G R
	5 10	15 20	25 30	35

[a]Sequences were obtained from the following sources: potato lectin PL-I (*14*), hevein (*52*), nettle lectin UDA A, B (*53*), wheat germ agglutinin WGA A, B, C, D (*9*), and Ac-AMP2 (*10*). Numbering refers to the hevein sequence. Conserved residues are shown in bold while aromatic residues implicated in chitin binding are underlined.

and the 6-OH of the terminal Gal, provided this branch is in the energetically most favored extended conformation regarding the β6-link (Φ, Ψ, ω = 64°, 27°, −163° after energy refinement) (*25*). The reduction in affinity by a factor of ten (Table III), found by adding a terminal NeuAcα6 on the β3-linked arm (No. 53), is explained by the loss of interactions between the penultimate Gal and Trp23, since the galactose must assume an energetically less favorable conformation (Φ, Ψ = 49°, 178°) similar to the internal Gal of neolactotetraosylceramide (see above) in order for the sialic acid to be unobstructive. Such behavior also rationalizes the non-binding of NeuAcα6-neolactotetraosylceramide, since a tenfold affinity reduction, relative to neolactotetraosylceramide, most likey is below the detection limit of the chromatogram binding assay. Extension of the β6-linked arm by either NeuAcα3, Galα3, or Fucα2 (Nos. 55–57) does not affect the affinity significantly (Table II), in agreement with the observation that these substitutions point away from the protein surface.

Regarding the linear version of neolactohexaosylceramide, it can be concluded that the core neolactotetra sequence binds similarly to neolactotetraosylceramide since no additional interactions are to be expected if the terminal tetrasaccharide goes into this position. The Gal of the terminal *N*-acetyllactosamine unit must in this case provide substantial interactions with the as yet undetermined N-terminal portion of the protein binding domain, which either compensates for the detrimental effect of penultimate GlcNac or allows this residue to assume an orientation different from the predominating one in the corresponding nonbinding five-sugar compound.

The surprisingly high affinity of PL-I for lactosylceramide with sphingosine and non-hydroxy fatty acids (the same magnitude as neolactohexaosylceramide, Table III) is also in excellent agreement with the present model of the protein binding domain. As was demonstrated above, the interactions provided by stacking a galactose residue onto the Trp23 indole moiety are crucial for binding to occur. This is further corroborated by the non-binding of glucosylceramide (No. 2 i Table II), which argues for a similar stacking in the lactosylceramide case. Of the nine possible conformers of the Glcβ1Cer linkage in lactosylceramide (*40*), only one of the more energetically favored conformations having dihedral angles Φ, Ψ, θ = 53°, −77°, −169° (after energy minimization of the complex) will allow accomodation of this glycosphingolipid in the binding site (Fig. 9). The lack of an acetamido moiety on the glucose is compensated for by hydrophobic interactions between Tyr21 and the ceramide head. Importantly, the introduction of a hydroxy fatty acid renders the lactosylceramide non-binding, a fact that may be rationalized as a combination of two effects. First, the fatty acid C′2-OH would make a very unfavorable contact with the Tyr21 phenol ring and, second,

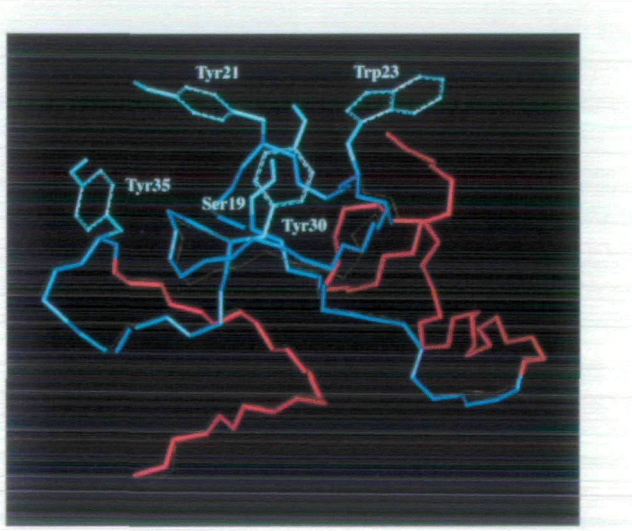

Fig. 7. **Model of potato lectin PL-I based on the NMR-derived structure of hevein.** The portion of the backbone for which the peptide sequence of PL-I is known is shown in blue, with some of the amino acid side chains implicated in sugar binding shown in light blue, whereas the red portions of the backbone stem from the original hevein structure kept for structural support. This energy-relaxed model can be compared to the backbone conformation of the unrelaxed hevein structure, which is shown as thin yellow lines.

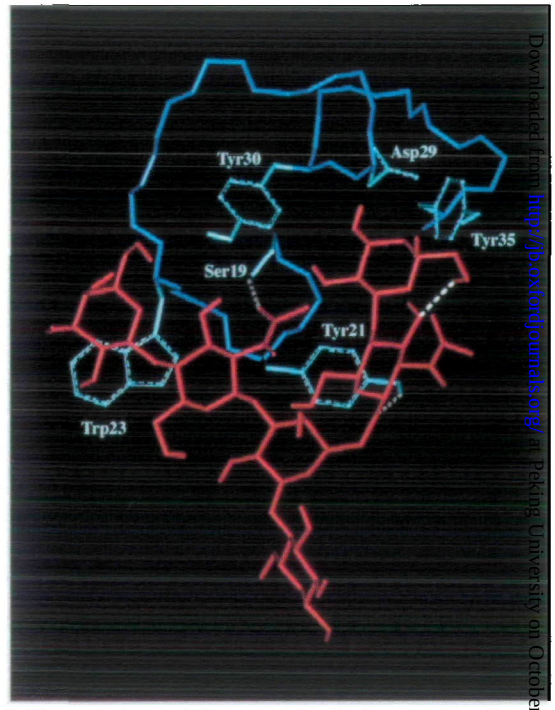

Fig. 8. **Partial view of the complex between the oligosaccharide part of branched neolactohexaosylceramide Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer (red) and PL-I (blue) as found by docking and a brief molecular dynamics simulation followed energy relaxation.** It is to be noted that the conformation and position of the neolactotetraose core containing the β3-linked *N*-acetyllactosamine unit (seen to the left) of the hexasaccharide is practically coincident with that of neolactotetraose itself. The conformation of the β6-linked branch (seen to the right) corresponds to the energetically most favored extended conformation of the isolated oligosaccharide. Hydrogen bonds are indicated by white dashed lines. A probable hydrogen bond between the Tyr35 hydroxyl and the 6-OH of the terminal Gal of the β6-linked branch is not included due to a somewhat unfavorable angle. Glycosidic dihedral angles (Φ, Ψ, ω) areas follows: Galβ4GlcNAcβ3 (47°, −20°), GlcNAcβ3Gal (16°, 171°), Galβ4Glc (62°, −5°), Galβ4GlcNAcβ6 (17°, −49°), and GlcNAcβ6Gal (64°, 127°, −163°) for branched neolactohexaose, and Galβ4GlcNAcβ3 (39°, −24°), GlcNAcβ3Gal (31°, 152°), and Galβ4Glc (56°, 0°) for neolactotetraose, The small differences in the values for the two oligosaccharides arise mainly from different behaviors in the dynamics simulations, but also from the presence of the β6-linked arm in the case of neolactohexaose.

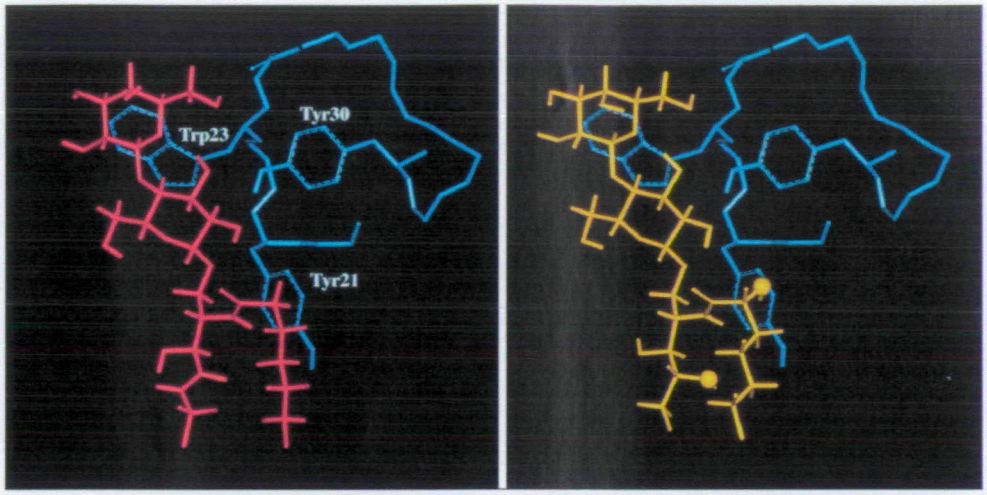

Fig. 9. **View of the complex between lactosylceramide (Galβ4Glcβ1Cer) having a sphingosine base and a non-hydroxy fatty acid (red) and PL-I (blue) (left chart).** The lactose unit (with Galβ4Glc glycosidic dihedral angles Φ, Ψ = 35°, –27°) assumes the same position as the terminal *N*-acetyllactosamine unit of the neolactotetraose complex and the only possible conformation of the Glcβ1Cer linkage corresponds to the energetically favored conformer 6 (Φ, Ψ, θ = 53°, –77°, –169°) of the isolated glycosphingolipid (*40*). The right panel shows the same view as in the left panel, but with the ceramide consisting of phytosphingosine and hydroxy fatty acid. The oxygens of the hydroxyl groups are shown as small spheres. Note especially the unfavorable interaction between the fatty acid hydroxyl group and Tyr21 resulting in this lactosylceramide species being non-binding. For clarity, the ceramide base and fatty acid have been trimmed back to the 7- and 6-carbon levels, respectively, in both panels.

the distribution of Glcβ1Cer conformers would change in favor of non-binding conformers (*40*). However, hydroxylation at the ceramide base, which also renders lactosylceramide non-binding (No. 6), is most likely a different effect since the phytosphingosine C4-OH would be somewhat removed from Tyr21 in the binding site. As can be surmised from Fig. 9, the binding of lactosylceramide probably has to entail an elevation of the molecule above the monolayer plane that is present in, *e.g.,* the microtiter well assay, but in the presence of phytosphingosine lateral hydrogen bond interactions (*43*) between the lactosylceramide molecules are likely to disfavor molecules from rising above the monolayer plane.

DISCUSSION

The occurrence of several isoforms of lectin from tubers of *Solanum tuberosum* have previously been indicated (*6*, *44*), and it has been suggested that different batches of potato tubers may contain different proportions of these isolectins (*44*). The results of the present study show that different preparations of potato lectin not only differ in molecular size and N-terminal amino acid sequences, but also differ with respect to their detailed carbohydrate binding specificities. Isolectins with different molecular masses, amino acid compositions and carbohydrate binding specificities have previously been described for several plant lectins (*45*).

In this study the glycosphingolipid recognition of two preparations of lectins from potato tubers was investigated. One lectin (PL-I) was isolated from potato tubers by affinity chromatography, and was a homodimer with a subunit molecular mass of 63,000, as determined by MALDI-TOF mass spectrometry. The other (PL-II) was a commercial lectin, and molecular mass determinations by SDS-PAGE and MALDI-TOF mass spectrometry showed that this lectin preparation contained two homodimeric lectins with subunit molecular masses of 52,000 and 55,000. With regard to molecular masses, PL-I corresponds most closely to the potato lectin characterized by Allen *et al.* (*6*), while PL-II corresponded to the lectin described by Kieliszewski and Orlando (*8*). However, the N-terminal amino acids of both lectin preparations differ from the sequence described by Allen *et al.* (*6*), although both lectins carry motifs resembling the reported lectin. In addition, the differences in N-terminal amino acids between PL-I and PL-II suggest that the two proteins are separate gene products.

In summary, PL-I bound to several *N*-acetyllactosamine–containing glycosphingolipids. The best ligands were Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer, NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer, and Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer, but the lectin also bound to neolactotetraosylceramide, the B5 pentaglycosylceramide and Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4Glcβ1Cer.

PL-II had a more restricted binding pattern and preferentially recognized the branched glycosphingolipids Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer and Galβ4GlcNAcβ6(NeuAcα6Galβ4GlcNAcβ3)Galβ4Glcβ1Cer.

In addition, specific binding of both potato lectins to lactosylceramide with sphingosine and non-hydroxy fatty acids was detected. Since the binding to these ceramide species of lactosylceramide could be mediated by a site outside the *N*-acetyllactosamine–binding site of the lectin, inhibition studies were conducted. In the presence of the inhibitor *N*-acetylchitotetraose, the binding of the lectins to both lactosylceramide and *N*-acetyllactosamine–containing glycosphingolipids was abrogated, indicating that both types of compounds indeed are accomodated within the *N*-acetylchitotetraose/*N*-acetyllactosamine–binding sites of the lectins.

In the absence of a crystal structure for potato lectin, the NMR structure of the homologous chitin-binding protein hevein was used as a template for the construction of a three-dimensional model of the PL-I binding site, using the known partial amino acid sequence of PL-I. Docking of glycosphingolipids onto the model of the PL-I binding site suggested that a Galβ4GlcNAcβ3Galβ4 epitope is necessary for binding to *N*-acetyllactosamine–containing glycoconjugates to occur. Furthermore, the selective recognition of lactosylceramide with sphingosine and non-hydroxy fatty

acids was explained by the PL-I model, showing a direct involvement of the ceramide in the binding.

It should, however, be noted that the ability to recognize lactosylceramide with sphingosine and non-hydroxy fatty acids is not a common property of all *N*-acetyllactosamine–binding lectins. No binding to lactosylceramide was detected when the glycosphingolipid binding patterns of tomato lectin and nettle lectin was investigated (unpublished data). Not even the lectins from seeds of *Erythrina cristagalli* and *Erythrina corallodendron* bind to lactosylceramide on thin-layer chromatograms (*25*), although lactose is an inhibitor of hemagglutination induced by these two lectins (*46*).

A large number of bacteria also bind to lactosylceramide on thin-layer chromatograms (*47*). However, most of these bacteria require a ceramide with phytosphingosine and/or hydroxy fatty acids for binding to occur. The only exception reported is *Proprionibacterium freudenreichii*, which binds preferentially to lactosylceramide with sphingosine and non-hydroxy fatty acids (*48*). A specific recognition of the same molecular species of lactosylceramide has also been reported for yeasts, as *Cryptococcus neoformans* (*49*).

The differences in the carbohydrate recognition preferences of potato isolectins might be of consequence when potato lectins are used in histochemical studies to detect *N*-acetyllactosamine–containing glycoconjugates. Potato lectins with binding preferences similar to PL-II would preferentially bind to glycoconjugates carrying branched *N*-acetyllactosamine, and the presence of linear sequences might thus be overlooked.

Furthermore, the ability of potato lectins to bind to lactosylceramide might confuse the interpretation of results from histochemical studies, especially if carbohydrate expression of tumor tissues is studied. Treatment with galactose oxidase, followed by reduction with tritiated sodium borohydride, did not label lactosylceramide in human erythrocytes, indicating that in normal cells, lactosylceramide is not accessible (*50*). However, concomitant with malignant transformation, an accumulation of precursor glycosphingolipids, as lactosylceramide, sometimes occurs (*51*).

REFERENCES

1. Barondes, S.H. (1988) Bifunctional properties of lectin: lectins redefined. *Trends Biochem. Sci.* **13**, 480–482
2. Lis, H. and Sharon, N. (1986) Lectins as molecules and as tools. *Annu. Rev. Biochem.* **55**, 35–67
3. Lis, H. and Sharon, N. (1998) Lectins: Carbohydrate-specific proteins that mediate cellular recognition. *Chem. Rev.* **98**, 137–174
4. Goldstein, I.J. and Poretz,. R.D. (1986) Isolation and chemical properties of lectins in *The Lectins. Properties, Functions and Applications in Biology and Medicine* (Liener, I.E., Sharon, N., and Goldstein, I.J., eds.) pp. 35–214, Academic Press, London
5. Endo, T. (1996) Fractionation of glycoprotein-derived oligosaccharides by affinity chromatography using immobilized lectin columns. *J. Chromatogr.* **720**, 251–261
6. Allen, A.K., Bolwell, G.P., Brown, D.S., Sidebottom, C., and Slabas, A.R. (1996) Potato lectin: a three-domain glycoprotein with novel hydroxyproline-containing sequences and sequence similarities to wheat-germ agglutinin. *Int. J. Biochem. Cell Biol.* **28**, 1285–1291
7. Allen, A.K. and Neuberger, A. (1973) The purification and properties of the lectin from potato tubers, a hydroxyproline-containing glycoprotein. *Biochem. J.* **135**, 307–314
8. Kieliszewski, M.J. and Orlando, R. (1997) Matrix assisted laser desorption/ionization time-of-flight mass spectrometry of tomato extensin monomers and potato lectin. *Phytochemistry* **45**, 9–14
9. Wright, C.S. (1987) Refinement of the crystal structure of wheat germ agglutinin isolectin 2 at 1.8 Å resolution. *J. Mol. Biol.* **194**, 501–529
10. Martins, J.C., Maes, D., Loris, R., Pepermans, H.A.M., Wyns, L., Willem, R., and Verheyden, P. (1996) 1H NMR study of the solution structure of Ac-AMP2, a sugar binding antimicrobial protein isolated from *Amaranthus caudatus*. *J. Mol. Biol.* **258**, 322–333
11. Asensio, J.L., Cañada, F.J., Bruix, M., González, C., Khiar, N., Rodríguez-Romero, A., and Jiménez-Barbero, J. (1998) NMR investigations of protein-carbohydrate interactions: refined three-dimensional structure of the complex between hevein and methyl β-chitobioside. *Glycobiology* **8**, 569–577
12. Ashford, D., Menon, R., Allen, A.K., and Neuberger, A. (1981) Studies on the chemical modification of potato (*Solanum tuberosum*) lectin and its effect on haemagglutination activity. *Biochem. J.* **199**, 399–408
13. Jimbo, A., Seno, N., and Matsumoto, I. (1984) Tryptophan residues and sugar binding site of potato lectin. *J. Biochem.* **95**, 267–275
14. Kieliszewski, M.J., Showalter, A.M., and Leykam, J.F. (1994) Potato lectin: a modular protein sharing sequence similarities with the extensin family, the hevein lectin family, and snake venom disintegrins (platelet aggregation inhibitors). *Plant J.* **5**, 849–861
15. Callaghan, J.M., Toh, B.H., Pettitt, J.M., Humphris, D.C., and Gleeson, P.A. (1990) Poly-*N*-acetyllactosamine-specific tomato lectin interacts with gastric parietal cells. Identification of a tomato-lectin binding 60–90 × 10^3 M_r membrane glycoprotein of tubulovesicles. *J. Cell Sci.* **95**, 563–576
16. Ito, N., Yokota, M., Kawahara, S., Nagaike, C., Morimura, Y., Hirota, T., and Matsunaga, T. (1995) Histochemical demonstration of different types of poly-*N*-acetyllactosamine structures in human thyroid neoplasms using lectins and endo-β-galactosidase digestion. *Histochem. J.* **27**, 620–629
17. Debray, H., Decout, D., Strecker, G., Spik, G., and Montreuil, J. (1981) Specificity of twelwe lectins towards oligosaccharides and glycopeptides related to *N*-glycosylproteins. *Eur. J. Biochem.* **117**, 41–55
18. Kawashima, H., Sueyoshi, S., Li, H., Yamamoto, K., and Osawa, T. (1990) Carbohydrate binding specificities of several poly-*N*-acetyllactosamine-binding lectins. *Glycoconj. J.* **7**, 323–334
19. Desai, N.N. and Allen, A.K. (1979) The purification of potato lectin on an *N,N′,N″*-triacetylchitotriose-Sepharose matrix. *Anal. Biochem.* **93**, 88–90
20. Young, N.M. and Leon, M.A. (1978) Preparation of affinity chromatography media from soluble polysaccharides by cross linkage with divinylsulfone. *Carbohydr. Res.* **66**, 299–302
21. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
22. Grant, G.A. and Crankshaw, M.W. (1997) Identification of PTH-amino acids by high-performance liquid chromatography in *Protein Sequencing Protocols, Methods in Molecular Biology* (Smith, B.J., ed.) Vol. 64, pp. 197–215, Human Press, Totowa, NJ
23. Aggarwal, B.B., Eessalu, T.E., and Hass, P.E. (1985) Characterization of receptors for human tumour necrosis factor and their regulation by γ-interferon. *Nature* **318**, 665–667
24. Waldi, D. (1962) Sprühreagentien für die dünnschicht-chromatographie in *Dünnschicht-Chromatographie* (Stahl, E., ed.) pp. 496–515, Springer-Verlag, Berlin
25. Teneberg, S., Ångström, J., Jovall, P.-Å., and Karlsson, K.-A. (1994) Characterization of binding of Galβ4GlcNAc-specific lectins from *Erythrina cristagalli* and *Erythrina corallodendron* to glycosphingolipids. Detection, isolation and characterization of a novel glycosphingolipid of bovine buttermilk. *J. Biol. Chem.* **269**, 8554–8563

26. Karlsson, K.-A. (1987) Preparation of total non-acid glycolipids for overlay analysis of receptors for bacteria and viruses and for other studies. *Methods Enzymol.* **138**, 212–220
27. Lanne, B., Ångström, J., Ölwegård, M., Teneberg, S., Stenhagen, G., Karlsson, K.-A., and Nilsson, E. (1998) Binding of *N*-trifluoroacetyl-derivatized natural glycosphingolipids by uropathogenic *Escherichia coli* and *Neisseria subflava*. *Glycoconj. J.* **15**, 1015–1028
28. Ångström, J., Teneberg, S., Abul Milh, M., Larsson, T., Leonardsson, I., Olsson, B.-M., Ölwegård Halvarsson, M., Danielsson,D., Näslund, I., Ljung, Å., Wadström, T., and Karlsson, K.-A. (1998) The lactosylceramide binding specificity of *Helicobacter pylori*. *Glycobiology* **8**, 297–309
29. Folch-Pi, J., Lees, M., and Sloane Stanley, G.H. (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497–509
30. Samuelsson, B.E., Pimlott, W., and Karlsson, K.-A. (1990) Mass spectrometry of mixtures of intact glycosphingolipids. *Methods Enzymol.* **193**, 623–646
31. Falk, K.-E., Karlsson, K.-A., and Samuelsson, B.E. (1979) Proton nuclear magnetic resonance analysis of anomeric structure of glycosphingolipids. The globo series (one to five sugars). *Arch. Biochem. Biophys.* **192**, 164–176
32. Falk, K.-E., Karlsson, K.-A., and Samuelsson, B.E. (1979) Proton nuclear magnetic resonance analysis of anomeric structure of glycosphingolipids. Blood group ABH-active substances. *Arch. Biochem. Biophys.* **192**, 177–190
33. Falk, K.-E., Karlsson, K.-A., and Samuelsson, B.E. (1979) Proton nuclear magnetic resonance analysis of anomeric structure of glycosphingolipids. Lewis-active and Lewis-like substances. *Arch. Biochem. Biophys.* **192**, 191–202
34. Koerner Jr, T.A.W., Prestegard, J.H., Demou, P.C., and Yu, R.K. (1983) High-resolution proton NMR studies of gangliosides. 1. Use of homonuclear two-dimensional spin-echo *J*-correlated spectroscopy for determination of residue composition and anomeric configurations. *Biochemistry* **22**, 2676–2687
35. Yang, H.-j. and Hakomori, S.-i. (1971) A sphingolipid having a novel ceramide and lacto-*N*-fucopentose III. *J. Biol. Chem.* **246**, 1192–1200
36. Stellner, K., Saito, H., and Hakomori, S.-i. (1973) Determination of aminosugar linkages in glycolipids by methylation. Aminosugar linkages of ceramide pentasaccharides of rabbit erythrocytes and of Forssman antigen. *Arch. Biochem. Biophys.* **155**, 464–472
37. Imberty, A., Delage, M.-M., Bourne, Y., Cambillau, C., and Pérez, S. (1991) Data bank of three-dimensional structures of disaccharides: Part II. *N*-Acetyllactosaminic type *N*-glycans. Comparison with the crystal structure of a biantennary octasaccharide. *Glycoconj. J.* **8**, 456–483
38. Asensio, J.L., Cañada, F.J., Bruix, M., Rodríguez-Romero, A., and Jiménez-Barbero, J. (1995) The interaction of hevein with *N*-acetylglucosamine-containing oligosaccharides. Solution structure of hevein complexed to chitobiose. *Eur. J. Biochem.* **230**, 621–633
39. Bock, K., Breimer, M.E., Brignole, A., Hansson, G.C., Karlsson, K.-A., Larson, G., Leffler, H., Samuelsson, B.E., Strömberg, N., Svanborg-Edén, C., and Thurin, J. (1985) Specificity of binding of a strain of uropathogenic *Escherichia coli* to Galα1-4Gal-containing glycosphingolipids. *J. Biol. Chem.* **260**, 8545–8551
40. Nyholm, P.-G. and Pascher, I. (1993) Orientation of the saccharide chains of glycolipids at the membrane surface. Conformational analysis of the glucose-ceramide and the glucose-glyceride linkages using molecular mechanics (MM3). *Biochemistry* **32**, 1225–1234
41. Wright, C.S. and Kellogg, G.E. (1996) Differences in hydropathic properties of ligand binding at four independent sites in wheat germ agglutinin-oligosaccharide crystal complexes. *Protein Sci.* **4**, 1466–1476
42. Teneberg, S., Lönnroth, I., Torres Lopez, J.F., Galili, U., Ölwegård Halvarsson, M., Ångström, J., and Karlsson, K.-A. (1996) Molecular mimicry in the cross-binding of carbohydrate receptors for *Clostridium difficile* toxin A, human natural anti α-galactosyl IgG and the monoclonal antibody Gal-13: characterization of a binding-active human glycosphingolipid non-identical with the animal receptor. *Glycobiology* **6**, 599–609
43. Boggs, J.M. (1987) Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function. *Biochim. Biophys. Acta* **906**, 353–404
44. Allen, A.K., Desai, N.N., Neuberger, A., and Creeth, M.J. (1978) Properties of potato lectin and the nature of its peptide linkages. *Biochem. J.* **171**, 665–674
45. Sharon, N. and Lis, H. (1989) Molecular properties in *Lectins*, pp. 47–58, Chapman and Hall, London
46. Lis, H., Joubert, F.J., and Sharon, N. (1985) Isolation and properties of *N*-acetyllactosamine-specific lectins from nine *Erythrina* species. *Phytochemistry* **24**, 2803–2809
47. Karlsson, K.-A. (1989) Animal glycosphingolipids as membrane attachment sites for bacteria. *Annu. Rev. Biochem.* **58**, 309–350
48. Strömberg, N., Ryd, M., Lindberg, A.A., and Karlsson, K.-A. (1988) Studies on the binding of bacteria to glycolipids. Two species of *Propionibacterium* apparently recognize separate epitopes on lactose of lactosylceramide. *FEBS Lett.* **232**, 193–198
49. Jimenez-Lucho, V., Ginsburg, V., and Krivan, H.C. (1990) *Cryptococcus neoformans, Candida albicans*, and other fungi bind specifically to the glycosphingolipid lactosylceramide (Galβ1-4Glcβ1-1Cer), a possible adhesion receptor for yeasts. *Infect. Immun.* **58**, 2085–2090
50. Gahmberg, C.G. and Hakomori, S.-i. (1973) External labeling of cell surface galactose and galactosamine in glycolipid and glycoprotein of human erythrocytes. *J. Biol. Chem.* **248**, 4311–4317
51. Hakomori, S.-i. (1983) Chemistry of glycosphingolipids in *Handbook of Lipid Research* (Kanfer, J.N. and Hakomori, S.-i., eds.) pp. 381–436, Plenum Press, New York
52. Broekaert, W.F., Mariën, W., Terras, F.R.G., De Bolle, M.F.C., Prost, P., Van Damme, J., Dillen, L., Claeys, M., Rees, S.B., Vanderleyden, J., and Cammue, B.P.A. (1992) Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine rich domain of chitin-binding proteins. *Biochemistry* **31**, 4308–4314
53. Beintema, J.J. and Peumans, W.J. (1992) The primary structure of stinging nettle (*Urtica dioica*) agglutinin. A two-fold domain member of the hevein family. *Fed. Eur. Biochem. Soc. Lett.* **299**, 131–134