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Screening for carbohydrate-binding proteins in extracts of Uruguayan plants

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Abstract

The presence of carbohydrate-binding proteins, namely lectins, ß-galactosidases and amylases, was determined in aqueous extracts of plants collected in Uruguay. Twenty-six extracts were prepared from 15 Uruguayan plants belonging to 12 Phanerogam families. Among them, 18 extracts caused hemagglutination (HAG) that was inhibited by mono- and disaccharides in 13 cases, indicating the presence of lectins. The other 8 extracts did not cause any HAG with the four systems used to detect HAG activity (rabbit and mouse red cells, trypsin-treated rabbit and mouse red cells). For the extracts prepared from *Solanum commersonii*, HAG activity and HAG inhibition were similar for those prepared from tubers, leaves and fruits, with the chitocompounds being responsible for all the inhibitions. Purification of the *S. commersonii* tuber lectin was carried out by affinity chromatography on asialofetuin-Sepharose, and SDS-PAGE under reducing conditions gave a single band of M_r of approximately 80 kDa. The monomer N-acetylglucosamine did not inhibit HAG induced by the purified lectin, but chitobiose inhibited HAG at 24 mM and chitotriose inhibited it at 1 mM. ß-Galactosidase activity was detected in leaves and stems of *Cayaponia martiana*, and in seeds from *Datura ferox*. Only traces of amylase activity were detected in some of the extracts analyzed. The present screening increases knowledge about the occurrence of carbohydrate-binding proteins present in regional plants.

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- Amylases

Introduction

Because of the essential role of protein-carbohydrate interactions in a wide variety of biological recognition processes, these interactions have been and still are a subject of permanent interest. Proteins that bind carbohydrates are present in large numbers in all living cells and are involved in a myriad of important biological functions. Carbohydrate-binding proteins include lectins and enzymes that bind carbohydrates. Lectins are a large and heterogeneous group of proteins that reversibly bind mono- and oligosaccharides (1). Although they have been studied extensively and are useful tools in glycobiology, their biological roles are still a matter of debate. Rüdiger and Rougé (2), when reviewing the structure and functions of plant lectins, mentioned that lectins have been thought of as protective agents against microbial attack, but this is not a general rule. In seeds and other storage organs, lectins often represent a major fraction of the

soluble proteins and it has been suggested that they might function simply as storage proteins, although this postulate cannot answer the question of why lectins possess carbohydrate-binding sites of high specificity (2). Furthermore, due to their functional biological relevance, the design of high-affinity reagents to occupy their carbohydrate recognition domains offers the prospect of a source of new drugs.

The enzymes ß-galactosidases and amylases are widely used in diagnostic techniques. ß-Galactosidases (EC 3.2.1.23) are widely distributed in the plant kingdom, although their precise role is not well understood. The interest in these enzymes has been focused on their *in vivo* functions concerning the degradation of such galactose-containing cell wall polysaccharides as galactan-pectin polymers and xyloglucan in relation to cell growth, fruit ripening and seed germination (3). Amylases (EC 3.2.1.1) are enzymes catalyzing the hydrolysis of glycosidic α-1,4 links in polysaccharides. There are a number of industrial and biotechnological applications of amylases, the largest volume being used for thinning starch and for the liquefaction process in the sugar, alcohol and brewing industries. Some of them are used in pharmaceuticals, in sewage treatment and in animal feed (4). Due to the great economic potential of these proteins and their biological activities, the search for new sources may reveal interesting new proteins with improved properties.

Uruguay possesses a phanerogamic flora consisting of approximately 2600 species belonging to 850 genera and 150 families (5) spontaneously distributed country-wide. This flora includes species which are widespread in the world and some others with a more restricted distribution area, but there are also a few other species which are endemic only to our region. In the present study we have explored for the first time the presence of carbohydrate-binding proteins, namely lectins, ß-galactosidases and amylases, in aqueous extracts prepared from plants belonging to the Phanerogam families, whose distribution area is our country, Uruguay. As a result of this first screening, we purified a lectin from an endemic species of the region, *Solanum commersonii*.

Material and Methods

Pre-packed PD-10 columns (Sephadex G-25) and CNBr-activated Sepharose 4B were from Pharmacia (Uppsala, Sweden). Polyvinylpolypyrrolidone (PVPP), dinitrosalicylic acid, o-nitrophenyl-ß-D-galactopyranoside (ONPG) and all the sugars were from Sigma (St. Louis, MO, USA). The bicinchoninic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL, USA).

Plant material

All plants were collected in rural places in Uruguay and identified, and a sample was registered and deposited at the Herbarium of the Facultad de Química, and the Herbarium of Eduardo Alonso Paz, General Flores 2124, Montevideo, Uruguay. The plants selected for study are indicated in Table 1.

Preparation of plant extracts

The fruits and flowers were washed with distilled water, processed with a blender and extracted with PBS buffer for 2 h at 4ºC. The suspensions were filtered through cheese cloth and then centrifuged at 9200 *g* for 30 min at 4ºC. The supernatant solutions were adjusted to 50% saturation with ammonium sulfate. The mixture was left to stand overnight and then centrifuged at 9200 *g* for 20 min at 4ºC. The precipitates were collected and resuspended in PBS buffer.

Tubers were washed with distilled water, peeled and processed with a blender. The mixture was filtered through cheese cloth and then centrifuged at 9200 *g* for 30 min at

4ºC. The solution was precipitated with ammonium sulfate at 50% saturation, left to stand overnight and centrifuged as before. The precipitate was collected and resuspended with 50 mM Tris-HCl buffer, pH 7.4.

The leaves were washed with distilled water, treated in a mortar by abrasion with sand (acid and heat treated and washed with distilled water) and extracted with PBS buffer. The mixture was filtered through cheese cloth and then centrifuged at 9200 *g* for 30 min at 4ºC. The dark solutions were treated overnight with PVPP (15 mg/ml) and the mixtures were centrifuged as indicated above. The clear supernatants were precipitated with ammonium sulfate at 50% saturation and processed as above with PBS buffer.

The stems were washed with distilled water and processed with a food processor and the extract was prepared as described for the leaves.

The seeds were dried at room temperature, washed with distilled water and ground to meal with a mill. The meal was suspended in 50 mM acetate buffer, pH 6.0, processed with a blender and extracted for 2 h at 4ºC. The suspension was treated as indicated above. The dark solution was treated with PVPP, the mixture was centrifuged as before and the treatment was repeated. The clear supernatant was then concentrated by precipitation with ammonium sulfate at 50% saturation. The mixture was left to stand overnight and then centrifuged at 9200 *g* for 20 min at 4ºC; the precipitate was collected and resuspended in acetate buffer.

Protein determination

Protein was determined by the BCA method (6). An appropriate dilution of the sample (100 µl) was incubated with 2 ml BCA reagent for 15 min at 60ºC and the absorbance was read at 562 nm. A calibration curve was prepared using pure bovine serum albumin (0.02-0.2 mg/ml) as standard.

Amylase activity

Amylase activity was determined by the method of Bernfeld (7) using 1% soluble starch in 0.05 M acetate buffer, pH 5.0, as substrate. A calibration curve was constructed using glucose solution (0-1 mg/ml) as standard. One enzyme unit (EU) was defined as the amount of enzyme catalyzing the production of 1 µmol of glucose per minute under the assay conditions. The specific amylase activity, EU/mg protein, is the ratio of the EU/ml to the protein concentration (mg/ml).

ß-Galactosidase activity

ß-Galactosidase activity was determined essentially by the method of Giacomini et al. (8) using ONPG as substrate and a molar extinction coefficient of 3.5×10^3 M^{-1} cm^{-1} for the free o-nitrophenol. The EU was defined as the amount of enzyme hydrolyzing 1 µmol of ONPG per min under the assay conditions. Specific ß-galactosidase activity, EU/mg protein, is the ratio of the EU/ml to the protein concentration (mg/ml).

Hemagglutination activity

The lectin activity was determined by measuring hemagglutination (HAG) by the method of Nowak et al. (9) using rabbit and mouse red cells and estimated by the two-fold serial dilution assay. The erythrocytes were obtained from fresh blood collected in Alsever's medium, washed four times with PBS buffer by centrifugation for 3 min at 1500 *g* and diluted to give a suspension of 4% red cells.

Trypsinization of red cells

Trypsinization of red cells was carried out by the method of Nowak et al. (9). Lectin unit (LU) was defined as the reciprocal of the highest dilution giving visible agglutination of the erythrocytes after 30 min of incubation. Specific HAG activity, LU/mg protein, is the

ratio of LU/ml to protein concentration (mg/ml). Red cells from rabbits and mice (with and without trypsin treatment) were used to test HAG. The purification factor is the ratio of specific activity after to specific activity before affinity chromatography.

Sugar specificity

The saccharide specificity of lectin binding to erythrocytes was determined by inhibiting agglutination with 100 mM sugar solutions in 0.15 M NaCl. The lectin dilution used for the end point was the highest dilution able to cause 50% HAG. The sugars used are given in Table 2 and/or mentioned in the Results section. Fetuin and asialofetuin were also tested at 8 mg/ml concentration. To determine the minimum concentrations required for HAG inhibition by these different carbohydrates, a two-fold serial dilution of the saccharide solutions was performed. The contents of the wells were mixed by gentle shaking and covered with plastic wrap, and the extent of HAG was detected visually after 30 min of incubation. These inhibition studies were performed with the extracts that were able to cause visible HAG under the conditions described.

Preparation of asialofetuin-Sepharose

Fetuin (300 mg) was dissolved in 25 ml of 0.2 N HCl and heated at 80ºC for 1 h. The solution was then cooled to 25ºC, neutralized with NaOH and dialyzed overnight against 0.2 M $NaHCO_3$ buffer, pH 7.9, to remove the free sialic acid. The resulting asialofetuin solution (31 ml) was adjusted to 0.1 M $NaHCO_3$, pH 8.3, containing 0.5 M NaCl and coupled to CNBr-activated Sepharose 4B (15 g of the freeze-dried powder was suspended in 1 mM HCl and washed for 15 min with 1 mM HCl on a sintered glass filter). The suspension was agitated slowly at room temperature for 2 h. The remaining active CNBr groups were blocked with 1 M ethanolamine, pH 9.0, for 2 h at room temperature. The product was washed with three cycles of alternating pH. The adsorbent thus prepared contained 120.2 mg asialofetuin per gram of dry gel, as determined by total amino acid analysis.

Purification of the *Solanum commersonii* lectin

The precipitate obtained with ammonium sulfate at 50% saturation was resuspended in 50 mM Tris/HCl buffer, pH 7.4 (4.0 ml), and applied to a column (2.0 ml) packed with asialofetuin-Sepharose gel equilibrated with the same buffer. The column was eluted with 0.1 M Glc-NAc in the same buffer. The eluted fractions were pooled and concentrated with an Amicon-10 ultrafilter. The concentrate was used for SDS-PAGE and to study HAG activity and HAG inhibition. Electrophoretic analysis was carried out with the PhastSystem equipment (Pharmacia, Uppsala, Sweden) using 12.5% homogeneous Phast gels and 8-25 gradient Phast gels. SDS-PAGE was performed under reducing and nonreducing conditions and proteins were stained with silver according to manufacturer instructions.

Results and Discussion

The botanical data, as well as the common names of the Uruguayan plants used in this screening are reported in Table 1. We included the references where these species have been mentioned or described, indicating their traditional uses by the local population. We used these materials to determine HAG, ß-galactosidase and α-amylase activity. By far the most common of the three tested activities found in the aqueous extracts was HAG activity (Table 2). Of the 26 extracts assayed, 18 caused HAG, while the remaining 8 extracts were negative at concentrations up to 6.68 mg/ml against rabbit and mouse red cells and trypsin-treated rab-

Table 1. Plant species studied in the present investigation.

Scientific name (Family)	Herbarium number	Common names	References
Bidens laevis (Asteraceae)	EAP N/N MVFQ 4164	–	
Cayaponia martiana (Cucurbitaceae)	EAP 2872	Tayuyá	10
Sebastiania brasiliensis (Euphorbiaceae)	EAP 2877	Blanquillo	
Sebastiania schottiana (Euphorbiaceae)	EAP 2878	Sarandí negro	
Sesbania virgata (Fabaceae)	EAP 2968	Acacia mansa	
Vigna luteola (Fabaceae)	EAP N/N MVFQ 4166	Porotillo	
Xylosma venosum (Flacourtiaceae)	EAP 2879	Espina corona	11
Pavonia sepium (Malvaceae)	EAP 2874	Malvavisco de cerco	12,13
Myrsine coriacea (Sw.) R. Br. Ex Roem & Schult (Myrsinaceae)	EAP 2875	Canelón	
Oxalis articulata (Oxalidaceae)	Plá N/N MVFQ 4165	Macachín	
Salix humboldtiana (Salicaceae)	EAP 2876	Sauce criollo	14
Allophylus edulis (Sapindaceae)	EAP 2870	Chal chal	11,14
Datura ferox (Solanaceae)	EAP N/N MVFQ 4163	Chamico	11,14,15
Solanum commersonii (Solanaceae)	EAP N/N MVFQ 4218	Batatilla purgante	15
Daphnopsis racemosa (Thymelaeaceae)	EAP 2873	Envira	

EAP, MVFQ and Plá: Eduardo Alonso Paz, Montevideo Faculdad de Química and A. Plá Herbaria, respectively. N/N, not numbered.

Table 2. Hemagglutination activity of extracts prepared from native and trypsin-treated red cells from Uruguayan plants.

Plant name and part used	Protein (mg/ml)	HAG				Lectin activity		Sugars that inhibit $HAG_{50\%}$
		R	M	T_R	T_M	LU/ml	SHA	
A. edulis fruits	2.74	+	+	+	+	640	234	–
A. edulis leaves	3.28	–	–	–	–	–		–
C. martiana leaves	0.62	+	–	–	–	160	258	Man, Gal
C. martiana fruits	4.50	+	–	–	–	2560	569	–
C. martiana stems	0.22	–	–	–	–	–		–
X. venosum fruits	5.56	–	–	+	–	160	29	Gal-NH_2
V. luteola beans	12.33	+	–	–	–	160	13	Gal, Gal-NH_2
S. commersonii tubers	5.17	+	+	+	+	20480	3961	Chitobiose, chitotriose
S. commersonii leaves	1.85	+	+	+	+	320	173	Chitobiose, chitotriose
S. commersonii fruits	8.22	+	+	+	+	320	39	Chitobiose, chitotriose
S. brasiliensis fruits	2.16	+	–	+	–	2560	1185	Glc, Gal-NH_2, Lac
S. humboldtiana flowers	0.31	+	–	+	–	640	2064	–
S. humboldtiana leaves	41.70	–	+	–	+	1280	31	Mellibiose
M. coriacea leaves	2.39	–	–	–	–	–		–
M. coriacea fruits	4.01	–	–	+	–	160	40	Glc, Mal, Lac
P. sepium leaves	0.87	+	–	–	–	1280	1471	Glc, Gal-NH_2, Lac
D. racemosa fruits	4.50	+	–	+	–	10240	2276	Mal
D. racemosa leaves	1.19	–	–	–	–	–		–
D. ferox fruits	1.74	+	–	–	–	1280	736	Chitobiose
D. ferox seeds	0.98	+	–	–	–	10240	10449	–
S. virgata beans	2.62	+	–	–	–	80	30	–
O. articulata tubers	2.71	–	+	–	+	80	29	–
B. laevis flowers	0.37	–	–	–	–	–		–
B. laevis leaves	0.41	–	–	–	–	–		–
S. schottiana fruits	4.90	–	–	–	–	–		–
S. schottiana leaves	6.68	–	–	–	–	–		–

HAG: hemagglutination; $HAG_{50\%}$: the extract dilution one below the highest dilution not able to agglutinate red blood cells; LU: lectin units, as defined in Material and Methods; M: mouse red cells; R: rabbit red cells; SHA: specific hemagglutination activity (LU/mg protein); T_M: trypsin-treated mouse red cells; T_R: trypsin-treated rabbit red cells. + or –: positive or negative for the HAG activities assayed.

bit and mouse red cells.

In some cases, the activities were determined in different parts of the same plant. Only in one case (*Solanum commersonii*) did we detect the same type of HAG activity and the same inhibitory behavior in extracts from tubers, fruits and leaves. The results of HAG inhibition by some of the sugars used in the present study are indicated in Table 2. ß-Galactosidase activity was found in some cases, and only traces of amylase activity were detected under the assay conditions used (Table 3).

Table 3. Distribution of the enzymatic activities analyzed in extracts from Uruguayan plants.

Plant name and part used	ß-Galactosidase activity		Amylase activity (EU/ml)
	EU/ml	SA	
A. edulis fruits	3.2	1.2	0.93
A. edulis leaves	–	–	–
C. martiana leaves	120.0	193.5	–
C. martiana fruits	25.0	5.5	–
C. martiana stems	46.0	209.0	–
X. venosum fruits	–	–	0.80
V. luteola beans	–	–	–
S. commersonii tubers	–	–	0.07
S. commersonii leaves	–	–	–
S. commersonii fruits	–	–	–
S. brasiliensis fruits	3.6	1.7	–
S. humboldtiana flowers	–	–	–
S. humboldtiana leaves	–	–	–
M. coriacea leaves	2.7	1.1	–
M. coriacea fruits	2.7	0.7	–
P. sepium leaves	–	–	–
D. racemosa fruits	4.9	1.1	0.67
D. racemosa leaves	–	–	–
D. ferox fruits	–	–	–
D. ferox seeds	37.0	37.8	–
S. virgata beans	–	–	–
O. articulata tubers	–	–	–
B. laevis flowers	–	–	0.13
B. laevis leaves	–	–	–
S. schottiana fruits	–	–	–
S. schottiana leaves	–	–	–

SA: specific activity, defined as enzyme unit per mg of soluble extractable protein (EU/mg).

Sebastiania brasiliensis (Euphorbiaceae)

The fruit extract was positive for HAG activity. The extract agglutinated untreated and trypsin-treated rabbit red cells but did not cause agglutination of untreated or trypsin-treated mouse red cells. HAG was inhibited by glucose (Table 2) but not by the other glucose-related sugars tested (2-amino-2-deoxy-D-glucose, Glc-NH_2 and N-acetyl-D-glucosamine, Glc-NAc). The 1-amino-1-deoxy-ß-D-galactose (Gal_1-NH_2) inhibited HAG. Lactose and maltose were the only two disaccharides tested that caused HAG inhibition. Traces of ß-galactosidase activity were detected but no amylase activity was observed.

Solanum commersonii (Solanaceae)

The tuber, fruit and leaf extracts were all positive for HAG activity. The specific HAG activity was 23-fold higher in the tubers (3961 LU/mg) than in the leaves (173 LU/mg) (Table 2). The extracts agglutinated untreated and trypsin-treated rabbit and mouse red cells and HAG was inhibited by N,N'-diacetylchitobiose (chitobiose) and N,N',N"-triacetylchitotriose (chitotriose). The monomer Glc-NAc did not inhibit HAG even at a concentration of 500 mM.

Cayaponia martiana (Cucurbitaceae)

The leaf extract was positive for HAG activity. The extract agglutinated only rabbit red cells and the activity was inhibited by different sugars including mannose, galactose, trehalose and raffinose. The glycoprotein Fetuin also inhibited HAG. The fruit extract was positive for HAG activity, agglutinating only the rabbit red cells and this activity was inhibited by Fetuin. The specific HAG activity found in the fruit extract was 20-fold higher than the specific HAG activity found in the leaf extract.

ß-Galactosidase activity was detected in all the plant parts analyzed, i.e., leaves, stems and fruits. The specific activities for the leaf and stem extracts were similar (193.5 and

209.0 EU/mg, respectively) and much higher than for the fruit extract (5.5 EU/mg).

***Salix humboldtiana* (Salicaceae)**

The leaf and flower extracts were positive for HAG activity. The leaf extract agglutinated untreated and trypsin-treated mouse red cells but did not agglutinate untreated or trypsin-treated rabbit red cells. HAG was inhibited only by mellibiose. In the flower extract, HAG was positive only for the untreated rabbit red cells. The specific HAG activity for the leaf extract was 67-fold lower than the specific HAG activity for the flower extract and was not inhibited by any of the sugars assayed, indicating that the HAG, in this case, was nonspecific. Neither amylase nor ß-galactosidase activity was detected in the extracts.

***Allophylus edulis* (Sapindaceae)**

The fruit extract was positive for HAG activity but was not inhibited by any of the sugars assayed, indicating that the HAG, in this case, was nonspecific and possibly due to extract components other than lectins. Traces of ß-galactosidase activity as well as amylase activity were detected in the fruit extract. The leaf extract was negative for the activities assayed.

***Myrsine coriacea* (Myrsinaceae)**

The fruit extract was positive for HAG activity only for the trypsin-treated rabbit red cells and the activity was inhibited by glucose, maltose and lactose. The leaf extract was negative for HAG. Traces of ß-galactosidase activity were detected in both fruit and leaf extracts, while no amylase activity was detected in either extract.

***Datura ferox* (Solanaceae)**

The fruit extract was positive for HAG activity, which was inhibited by chitobiose and also by Fetuin. The seed extract was positive for HAG activity but none of the sugars tested were able to inhibit HAG. ß-Galactosidase activity was detected (38 EU/mg of soluble extractable proteins) but no amylase activity was found in the seed extract.

Other plant extracts

The seed extract from *Vigna luteola* was positive for HAG with untreated rabbit red cells and the activity was inhibited by galactose and Gal_1-NH_2. The seed extract from *Sesbania virgata* was also positive for HAG with untreated rabbit red cells but in this case the activity was not inhibited by the tested sugars. The *Oxalis articulata* tubers were positive for HAG activity when using untreated and trypsin-treated mouse red cells, although the HAG was not inhibited by the sugars tested. The leaf extract from *Pavonia sepium* was positive for HAG with untreated rabbit red cells and the activity was inhibited by glucose, Glc-NH_2, 2-amino-2-deoxy-D-galactose (Gal_2-NH_2), maltose and lactose. The fruit extract from *Daphnopsis racemosa* was positive for HAG with untreated and trypsin-treated rabbit red cells and the activity was inhibited only by the disaccharide maltose. The fruit extract from *Xylosma venosum* exhibited low HAG activity and only for trypsin-treated rabbit red cells (160 LU/ml) and the activity was inhibited by Gal_1-NH_2. Traces of amylase activity were detected. The extracts from *Sebastiania schottiana* (Euphorbiaceae) were negative for all of the activities assayed.

The literature describes several lectins purified and characterized from the Solanaceae, Fabaceae and Cucurbitaceae families (1), local species of which have been included in this screening. For some of the other families used in the present study there are only a few reports about the presence and purification of new lectins. Thus, the present

screening contributes to increasing the number of potential new lectins to be purified and characterized in the future. Based on the present results, we attempted the purification of the lectin from *S. commersonii* tubers.

Purification of the *Solanum commersonii* tuber lectin

S. commersonii is one of the two Uruguayan tuberous species belonging to the Solanum genus. It grows wild country-wide and it is endemic in Uruguay, southern Brazil and the provinces of Entre Rios and Buenos Aires in Argentina. This local species has received special attention due to its resistance to frost and to some local pests (16,17). Furthermore, it is known that potato (*S. tuberosum*) tubers contain a lectin that has been well characterized (18-20) and has been suggested to be possibly involved in the defense mechanism of the plant. Several affinity adsorbents have been used to attempt the purification of this lectin, including Fetuin-Sepharose (18). When analyzing the soluble extract from *S. commersonii* tubers, we found some behaviors similar to those of the *S. tuberosum* tuber extract. For instance, the HAG activity present in the extracts was not inhibited by the monomer Glc-NAc but was inhibited by chitobiose and chitotriose, a behavior described for the potato (*S. tuberosum*) tuber extracts. A comparative analysis performed with both extracts showed a 7-fold higher specific HAG activity (3961 LU/mg protein) for *S. commersonii* than for *S. tuberosum* (611 LU/mg protein). The soluble extractable protein content represents 2.6% (w/w) for the *S. commersonii* tubers and the material precipitable with ammonium sulfate at 50% saturation represents 9% of the total soluble proteins. Due to the information reported about the potato lectin and due to the potential importance of this lectin in plant defense, we attempted a preliminary purification of the *S. commersonii* lectin. Results for the purification of the lectin from the tuber extract using asialofetuin-Sepharose are shown in Table 4 and Figure 1. It should be noted that only about 50% of the total proteins applied were recovered from the column in the washing and elution steps. The purified fraction gave a single protein band corresponding to an M_r of approximately 80 kDa, as shown by silver staining after SDS-PAGE under reducing conditions (Figure 2). The tuber lectin isolated from the cultivated species *S.*

Table 4. Purification of the *Solanum commersonii* lectin by affinity chromatography on asialofetuin-Sepharose.

Fraction	A_{280}	Protein (mg/ml)	Volume (ml)	LU/ml	SHA	Purification (-fold)
Applied	11.95	5.17	4.0	20480	3961	–
Eluted and concentrated	0.24	0.105	0.5	51520	490667	124

LU: lectin units; SHA: specific hemagglutination activity (LU/mg protein).

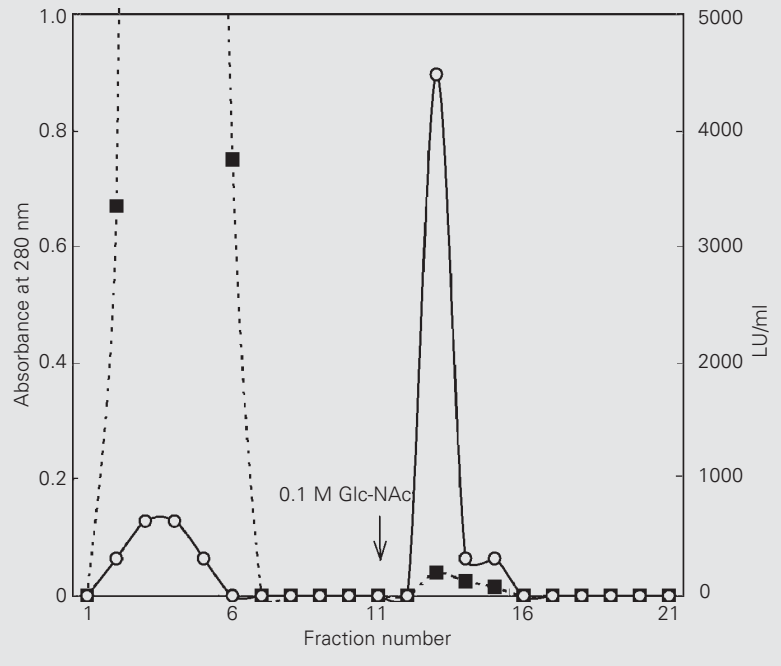

Figure 1. Affinity chromatography of the *Solanum commersonii* tuber lectin on asialofetuin-Sepharose. Sample: 50% saturated ammonium sulfate precipitate dissolved in 50 mM Tris-HCl buffer, pH 7.4 (Total amount of protein applied: 20.7 mg). Elution: 0.1 M Glc-NAc in 50 mM Tris-HCl, pH 7.4. $A_{280\ nm}$ (squares); hemagglutination activity (circles). LU: lectin units.

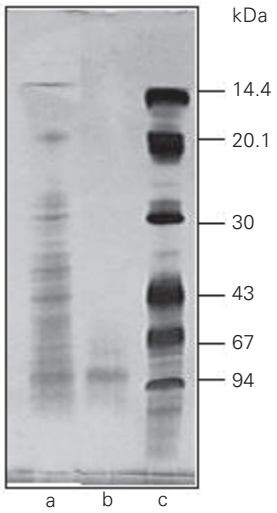

Figure 2. SDS-PAGE of the fractions from the purification of the lectin. The gel (homogeneous, 12.5%) was stained with silver. *Lane a*, Extract from *Solanum commersonii* tubers; *lane b*, material eluted from asialofetuin-Sepharose; *lane c*, low M_r markers.

tuberosum is reported to be a dimeric protein composed of two identical (or very similar) subunits of 50 kDa (19) and 65.5 kDa by MALDI mass spectrometry, which contain up to 40-50% covalently linked carbohydrate (1). We performed SDS-PAGE analysis under both reducing and nonreducing conditions on 8-25 gradient gels and the proteins were stained with silver. The band corresponding to the apparent molecular mass of 80 kDa was obtained only under reducing conditions, while the material run under nonreducing conditions gave a clearly retarded and diffuse band (data not shown). The expected specificity of the lectin was confirmed by inhibition of HAG with Glc-NAc, chitobiose and chitotriose. No inhibition was observed with Glc-NAc even at 500 mM. Chitobiose inhibited at 24 mM and chitotriose at 1 mM (data not shown). A more complete characterization of this lectin is in progress.

The high percentage of positive results detected here will permit us to focus on the isolation, purification and characterization of the lectins in these plants. Detailed information about the biochemistry and biology of these proteins is a prerequisite for understanding their biological role and activity, as well as increasing knowledge about our regional plants.

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