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Utility of pentose colorimetric assay for the purification of potato lectin, an arabinose-rich glycoprotein

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Abstract Potato lectin (*Solanum tuberosum* agglutinin, STA) is an unusual glycoprotein containing approximately 50% carbohydrates by weight. Of the total carbohydrates, 92% is contributed by L-arabinose, which are *O*-linked to hydroxyproline residues. The ferric chloride-orcinol assay (Bial's test), which is specific for pentoses has so far been used only for the determination of free pentoses in biological samples. However, this colorimetric assay has not been used for the detection of pentoses in bound form as it occurs in Solanaceae lectins (potato, tomato, and Datura lectins). Utilizing the pentose colorimetric assay for monitoring the presence of potato lectin, a simpler and shorter procedure for the purification of this lectin from potato tubers has been developed. The yield of potato lectin (1.73 mg per 100 g potato tuber) is twice compared to the yields reported in earlier procedures. Although potato lectin is well known for its specificity to free trimers and tetramers of *N*-acetyl-D-glucosamine (GlcNAc), it possesses a similar specificity to the core $(GlcNAc)_2$ of N-linked glycoproteins. The utilization of the pentose assay in the purification of arabinose-rich lectins/agglutinins obviates the necessity for the use of agglutination assay in the various purification steps. The pentose assay appears to be a simple and convenient colorimetric assay for detecting any pentose-rich glycoprotein in plant extracts. The utility of the pentose assay appears to have a significant potential in the detection of hydroxyproline-rich glycoproteins (HRGPs), which are generally *O*-arabinosylated.

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Abbreviations

FCO	ferric chloride-orcinol
GlcN	D-glucosamine
GlcNAc	*N*-acetyl-D-glucosamine
HA	hemagglutination
HRGP	hydroxyproline-rich glycoprotein
HRP	horseradish peroxidase
Hyp	hydroxyproline
STA	*Solanum tuberosum* agglutinin

Introduction

Potato lectin, also known as *Solanum tuberosum* agglutinin (STA) [1], is a blood group-nonspecific lectin (pan-agglutinin) present in potato tubers, and is specific for chitin (poly *N*-acetyl-D-glucosamine) [1–3]; the highest specificity is for trimer and tetramer of GlcNAc. The lectin also binds poly-*N*-acetyllactosamine structures [3]. It is an unusual glycoprotein with high contents of half-cystine and hydroxyproline residues; almost all hydroxyproline residues are substituted with oligo-L-arabinose (mostly trimers and tetramers in the furanose form with β-linkage) and a few serine residues are substituted with D-galactose [4]. Potato lectin is dimeric comprising two identical monomers of ~55 kD, fifty percent of its mass being carbohydrate.

The method of Allen and Neuberger [5] is widely used for the purification of STA. Following ammonium sulfate precipitation step, this method involves 5 chromatographic steps in the order: DEAE-cellulose, CM-cellulose, Sephadex G-100 (twice), and SP-Sephadex. Apart from this, other methods

of purification have been described: affinity chromatography using *N, N′, N″*-triacetyl chitotriose-Sepharose matrix [6] or fetuin-Sepharose matrix [7], and chromatofocusing involving two steps [8]. Although affinity chromatography and chromatofocusing techniques are very powerful, they require specialized and expensive reagents. During the course of our investigation on the modulatory effects of purified potato lectin on the cells of the immune system, we felt the necessity for a simpler and shorter procedure for purifying potato lectin compared to the five lengthy chromatographic steps described in the classical procedure [5]. Here, we describe the purification of potato lectin using only two chromatographic steps beyond the ammonium sulfate precipitation step of Allen and Neuberger [5].

Since STA is very rich in arabinose (92% of carbohydrate) [1–5], we attempted the detection of potato lectin by using a colorimetric assay for pentoses (Bial's test) [9–11], which involves the use of ferric chloride-orcinol (FCO) reagent. Although hexoses do react under the conditions of the assay, they give only a faint, yellow color that is masked by the blue-green color given by pentoses [9,10]. The pentose assay has been used earlier to quantitate free pentoses like xylose, ribose, or arabinose in biological samples [9,10], and for the specific determination of RNA [12]. However, the FCO assay has, so far, not been used for the detection of glycoproteins containing pentoses. In this paper, we have incorporated the use of pentose assay to develop a modified purification procedure for detecting STA in each step, and describe its advantages.

Materials and methods

Potato tubers were procured from the local grocery. Sephadex G-75, SP-Sepharose (fast flow) cation-exchanger (wet bead size: 45–165 μ), gum arabic (Acacia gum), avidin, soybean agglutinin, tomato lectin (*Lycopersicon esculentum* agglutinin), and *Dolichos biflorus* agglutinin were products of Sigma-Aldrich Co., St. Louis, MO, USA. Ovalbumin, bovine serum albumin (BSA), lysozyme, concanavalin A and horseradish peroxidase (HRP) were purchased from Bangalore Genei, Bangalore, India. Patatin (major storage protein of potato) obtained during the purification of potato lectin in this study was used. Flat-bottom 96-well microtiter plates (MICROLON) were bought from Greiner Bio-One GmbH, Frickenhausen, Germany. All other chemicals and reagents used in this study were of analytical grade.

Ferric-orcinol assay (Bial's test)

This is a colorimetric assay for the detection of free pentoses in biological samples [11]. In this assay, 200 μL of the sample was taken, then 200 μL of reagent A (10% w/v TCA) was added, the samples were heated at 100°C for 15 min. The tubes were cooled rapidly at 25°C and 1.2 mL of reagent B (1.15% w/v ferric ammonium sulfate and 0.2% w/v orcinol in 9.6 M HCl) was added and mixed well. The samples were again heated at 100°C for 20 min, and then cooled to room temperature. The absorbance of the blue-green color was measured at 660 nm. The calibration curve for D-arabinose was prepared in the 0–40 μg range (volume range: 0–200 μL). Glycoproteins such as patatin, avidin, ovalbumin, horseradish peroxidase, *Dolichos biflorus* agglutinin, and soybean agglutinin were used as control glycoproteins devoid of pentoses. Non-glycoproteins such as BSA, lysozyme, and concanavalin A were used as control proteins in this assay.

Hemagglutination (HA) activity and sugar inhibition assays

HA activity of the purified lectin and the sugar inhibition assays were carried out using trypsinized rabbit or human erythrocyte suspension as described by Burger [13]. Briefly, a 2% suspension of rabbit or human erythrocytes (0.2 mL) were added to a serially diluted lectin, gently mixed and incubated at 37°C for 1 h and the agglutination was visualized. The amount of protein present at the highest dilution represents the minimum quantity of protein necessary for agglutination and is taken as the titer. One unit of HA activity is the concentration of the protein at the titer. The specific activity is given as the number of HA units per mg of protein.

For sugar inhibition, different concentrations of the sugar solutions (in a volume of 0.1 mL) in each of the wells were preincubated with 0.1 mL lectin solution at 37°C for 1 h before the addition of 0.2 mL 2% trypsinized erythrocytes. Agglutination was visualized as described above. The extent of inhibition by different sugars or glycoproteins was assessed by their ability to inhibit the hemagglutination activity of lectin [13].

HRP-binding assay

Microtiter wells were coated with 30 μg of protein (100 μL volume) in 0.1 M carbonate-bicarbonate buffer, pH 9.6 at 4°C overnight. After blocking the wells using 3% gelatin in PBS and subsequent washings, the microtiter plate was incubated with 20 μg of horseradish peroxidase (100 μL volume) at 37°C for 2 h. Color development was carried out using *o*-phenylenediamine/H_2O_2 substrate system and absorbance was read at 492 nm.

Purification of potato lectin

Peeled potato tubers (100 g) were grated, and transferred to 2 volumes of 0.1 M sodium acetate buffer, pH 3.8, containing 2 mM sodium metabisulfite as an antioxidant to prevent

browning. After keeping at 4°C for 2 h, the extract was filtered through a muslin cloth, and the filtrate was centrifuged at 5000 × *g* at 4°C for 15 min. The supernatant was subjected to sequential 20% and 60% ammonium sulfate saturation as described in the method of Allen and Neuberger [5]. The precipitate obtained upon 60% ammonium sulfate saturation was resuspended in distilled water, dialyzed extensively, and concentrated by ultrafiltration using Amicon stirred-cell fitted with Millipore YM-10 membrane (mol. wt. cut-off = 10 kD).

The concentrated material obtained from ultrafiltration (3 mL) was subjected to gel filtration on Sephadex G-75 (2.5 × 110 cm), pre-equilibrated with Tris-buffered saline (TBS). Elution was carried out using TBS, and fractions containing pentose-positive material were pooled (component I), dialyzed against 50 mM sodium acetate buffer, pH 4.0.

Component I was applied on the cation-exchanger SP-Sepharose FF (1 × 13 cm; bed volume, 10 mL) equilibrated in 50 mM sodium acetate buffer, pH 4, and then washed with 5 column volumes of the same buffer. Bound proteins were eluted by step-wise elution with different NaCl concentrations (steps of 0.1, 0.25, 0.5, 0.75, 1, and 1.5 M) in the starting buffer. Volume of buffer used for each step was 30 mL.

SDS-PAGE and protein assay

Selected pools from the above two chromatographic steps were analyzed by 12% SDS-PAGE (reducing), as per the procedure of Laemmli [14]. The protein bands were visualized either by coomassie blue or silver staining. Protein assay was carried out as per the procedure of Bradford [15], using BSA as the standard.

Periodic acid-Schiff (PAS) staining

PAS staining for glycoprotein [16] was used to confirm the glycoprotein nature of purified potato lectin. Purified potato lectin and potato extracts at various stages of purification, were run on 10% SDS-PAGE under reducing conditions; following fixation in 12.5% TCA solution for 1 h, the gel was incubated in the dark with 1% periodic acid solution for 1 h. The gel was thoroughly washed and the bands were developed by incubation with Schiff reagent (Schiff's Fuchsin-sulfite reagent) at 4°C for 1 h in the dark. After the development of colored bands, the reaction was stopped by transferring the gel into a solution of 7% glacial acetic acid.

Reverse-phase HPLC

Reverse-phase HPLC analysis was carried out using a C_{18} column (4.5 × 250 mm; particle size 5 μm) in a Shimadzu LC-10A HPLC system (Shimadzu Corp., Kyoto, Japan). The sample was eluted using a binary gradient of solvent A (0.1% TFA) and B (70% acetonitrile in 0.05% TFA) at a flow rate of 0.8 mL/min. Protein detection was monitored at 230 nm.

Results

The resolubilized precipitate obtained from 60% ammonium sulfate saturation of potato extract was concentrated by ultrafiltration using YM-10 membrane, and gel filtered on Sephadex G-75. The elution profile (Figure 1) shows the resolution of protein components (>10 kD) into three components. Among the three components seen, only fractions representing component I were positive in the FCO assay; HA activity of component I was found to be ∼165 units/mg protein. Pooled component I containing active STA was subjected to SDS-PAGE (reducing), and the gel pattern shows the presence of three bands all having molecular mass >40 kD (Figure 3A, lane 4). The major intense band at ∼43 kD represents the abundant storage protein of potato, namely, patatin [17]. Component II which was found to be negative in both the FCO and HA assays, contained several proteins all having a molecular mass of <35 kD (data not shown).

Chromatography of pooled component I was carried out on SP-Sepharose cation-exchanger at pH 4. The elution profile was monitored using step-wise NaCl elution, and is shown in Figure 2. Based on FCO assay, almost all the fractions eluting at 100 mM NaCl concentration (component Ia in step elution A, Figure 2) were found to be pentose-positive. The HA activity was remarkably higher compared to the 60% ammonium sulfate precipitate of potato extract, and also component I of Sephadex G-75. The HA activity was found to be ∼3891 units/mg protein. Component Ib obtained upon elution with 0.25 M NaCl (step elution B, Figure 2) was found to have a higher amount of protein than component Ia. Only a small amount of protein was found to elute in

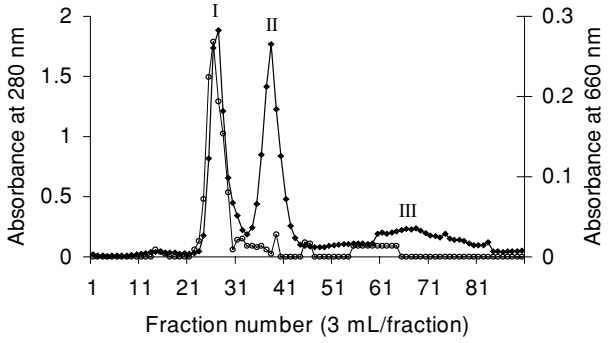

Fig. 1 Gel filtration of concentrated resolubilized precipitate from 60% ammonium sulfate saturation of crude potato extract on Sephadex G-75 (2.5 × 110 cm) using 10 mM Tris-HCl, pH 7.4 containing 140 mM NaCl as eluant at 25°C. Flow rate: 15 mL/h. Protein detection: absorbance at 280 nm (-●-). Pentose detection (FCO assay): absorbance at 660 nm (-○-).

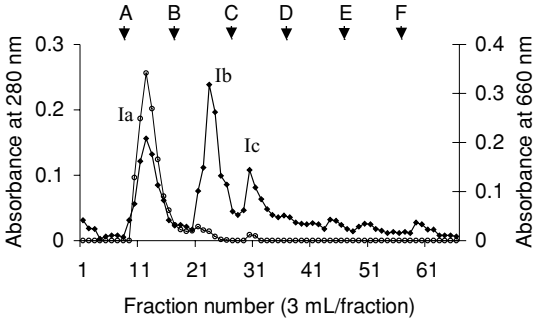

Fig. 2 SP-Sepharose (Fast Flow) chromatography of component I (from Sephadex G-75 purification step) by step-wise elution using different concentrations of NaCl in 50 mM sodium acetate buffer, pH 4. Arrows indicate the point of application of buffer containing the indicated NaCl concentration (M): A, 0.1; B, 0.25; C, 0.5; D, 0.75; E, 1.0; F, 1.5. Protein detection: absorbance at 280 nm (-●-). Pentose detection (FCO assay): absorbance at 660 nm (-○-).

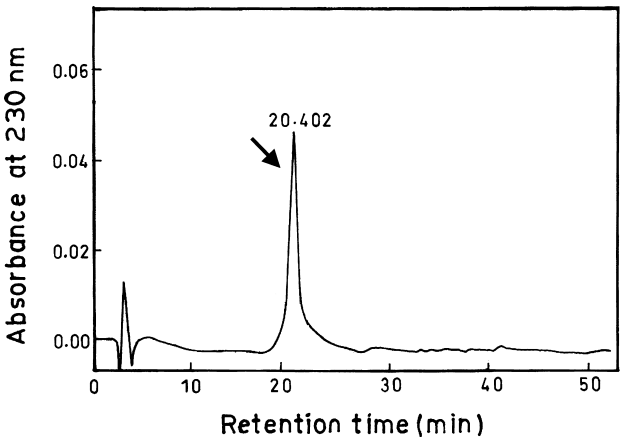

Fig. 4 Reverse-phase HPLC (C_{18}) profile of purified potato lectin (component Ia of Figure 2). Protein load: 1.6 μg in a volume of 20 μL. Protein detection: 230 nm. The arrow represents the major peak with a retention time of 20.4 min.

component Ic (step elution C using 0.5 M NaCl, Figure 2). Both components Ib and Ic were found to be negative in the FCO and hemagglutination assays.

The purified STA (pooled component Ia) shows a single diffuse band on 12% SDS-PAGE (reducing) in the molecular mass range of 90 to 100 kD (Figure 3B, lanes 2 and 3), which is in good agreement with the anomalous behavior of this glycoprotein reported previously [1–3,5]. On periodic acid-Schiff (PAS) staining for glycoproteins, purified potato lectin showed up as a single, slightly diffused pink band against a clear background (Figure 3C, lanes 1 and 2). As a control non-glycoprotein, BSA was used which gave a negative PAS staining (Figure 3C, lane 3). Analytical reverse-phase HPLC profile, shown in Figure 4, revealed a major peak for the purified potato lectin with a retention time of 20.4 min and was found to be approximately 95% pure.

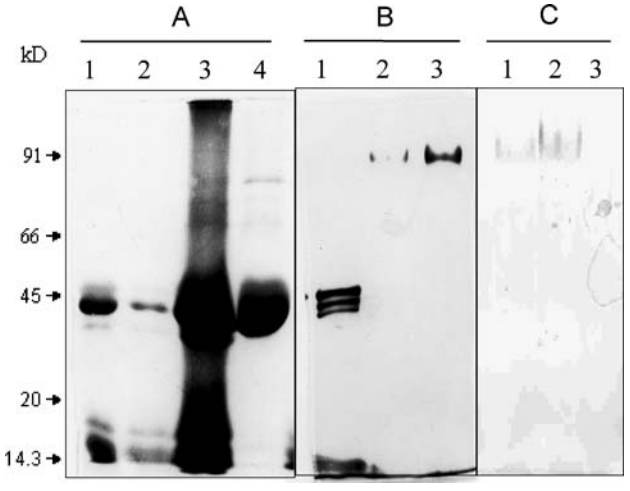

Fig. 3 SDS-PAGE (12%, reducing) of potato extract and certain chromatographic components from Sephadex G-75 gel filtration (panel A: coomassie staining) and SP-Sepharose cation-exchange chromatography (panel B: silver staining). The mol. wt. of marker proteins are indicated in kD on the left. **A**: Coomassie-stained gel. Lane 1, 60% ammonium sulfate precipitate of potato extract, 15 μg; lane 2, 50% (w/v) potato tuber extract, 10 μg; lane 3, ultrafiltration retentate of resolubilized ammonium sulphate precipitate, 25 μg; lane 4, component I of Sephadex G-75 (Figure 1), 15 μg. The major band near 45 kD in this panel represents the major storage protein, patatin. **B**: Silver-stained gel. Lane 1, component Ib, 10 μg; lane 2, component Ia, 4 μg; lane 3, component Ia, 10 μg. The components refer to the elution pattern of SP-Sepharose cation-exchange chromatography in Figure 2. **C**: Periodic acid-Schiff (PAS) staining of purified potato lectin. Lane 1, component Ia, 10 μg; lane 2, component Ia, 20 μg; lane 3, bovine serum albumin (BSA), 20 μg. Component Ia refers to the first component of SP-Sepharose chromatography in Figure 2.

The specific activity of potato lectin as measured by hemagglutination, and the resultant fold-purification at various steps are summarized in Table 1. The specific HA activity of potato lectin increased from 59 units/mg in crude extract to 3891 units/mg in the final step of purification (component Ia of the second chromatographic step). This translates to a 66-fold purification for potato lectin after only two chromatographic steps. The yield of potato lectin was approximately 1.73 mg from 100 g potato tubers. The detection of potato lectin in various purification steps was followed by FCO assay in parallel, and the results are summarized in Table 2. A 16-fold increase in absorbance at 660 nm is seen in the case of component Ia (purified STA) compared to the crude extract of potato, for a constant amount of protein (50 μg).

FCO assay was carried out using free sugars (pentoses and hexoses), various non-glycoproteins and glycoproteins. The results are shown in Table 3. The non-glycoproteins tested (BSA, lysozyme, and concanavalin A) at 50 μg were all found to be negative in this assay. The glycoproteins containing the N-linked glycans (patatin, avidin, ovalbumin, HRP, soybean agglutinin, and *Dolichos biflorus* agglutinin) were also found to be negative. With the exception of soybean agglutinin, the other glycoproteins exhibited only slight

Table 1 Purification of potato lectin from potato tubers (100 g)

Purification step	Volume (mL)	Total HA activity (Units)	Total protein (mg)	Specific HA activity (Units/mg)	Recovery (%)	Fold purification
Potato crude extract (50% w/v)	200	8000	136.0	58.8	100.0	1.0
Ammonium sulfate precipitate (60% saturation)	25	7468	121.5	61.7	93.4	1.0
Post-dialysis and ultrafiltration	3	7202	43.9	164.2	90.0	2.8
Sephadex G-75, pooled peak I	28	6750	10.8	625.0	84.4	10.6
SP-Sepharose FF, pooled peak Ia	21	6692	1.7	3891.0	83.7	66.2

Table 2 Ferric chloride-orcinol (FCO) assay for the detection of potato lectin at various steps of its purification

Purification step	Protein (μg) for ferric-orcinol assay	A_{660}	Fold-increase in absorbance*
Potato crude extract (50% w/v)	50	0.036	1.00
Ammonium sulfate precipitate (60% saturation)	50	0.063	1.75
Post-dialysis and ultrafiltration	50	0.078	2.20
Sephadex G-75, pooled			
Peak I	50	**0.147**	**4.10**
Peak II	50	0.019	—
SP-Sepharose FF, pooled			
Peak Ia	50	**0.573**	**15.90**
Peak Ib	50	0.012	—
Peak Ic	50	0.008	—

*for a constant amount of protein (50 μg).

Table 3 Ferric-orcinol assay for saccharides, and various purified proteins

Sample	Amount taken for assay (μg)	A_{660}[a]
Saccharide		
L-Arabinose	20	**0.743**
D-Xylose	20	**0.617**
D-Ribose	20	**0.769**
D-Glucose	20	0.021
D-Mannose	20	0.034
D-Galactose	20	0.043
Gum arabic	20	**0.234**
Protein		
Concanavalin A	50	0.002
Bovine serum albumin	50	0.005
Lysozyme	50	0.007
Glycoprotein		
Soybean agglutinin	50	0.007
Patatin	50	0.009
Avidin	50	0.023
Ovalbumin	50	0.029
Dolichos biflorus agglutinin	50	0.042
Horseradish peroxidase	50	0.067
Potato lectin	50	**0.573**
Tomato lectin	50	**0.585**

[a]Mean of triplicate anlaysis.

positivity compared to non-glycoproteins. Only potato and tomato lectins were positive in this assay.

The inhibition in HA activity of potato lectin by GlcNAc, chitosan and its oligomers is shown in Table 4. Among these, chitosan oligomers were found to be the most potent inhibitor of agglutination. This sample of chitosan oligomers obtained by non-specific enzymatic hydrolysis using pepsin [18] was found to contain 78.2% of chitotetraose, 1.6% of chitotriose, 0.9% of chitobiose, 18.2% of GlcNAc, and 1.1% of GlcN as analyzed by HPLC on an aminopropyl column (data not shown). Among the proteins/glycoproteins tested at 1 mg/mL concentration, only HRP was found to be inhibitory at a concentration of 0.19 μg. This appears to be due to the binding of potato lectin to the core $(GlcNAc)_2$ of HRP which is rich in N-glycans (eight N-glycans per molecule) [19].

In order to confirm the binding of potato lectin to the core $(GlcNAc)_2$ of HRP, we used HRP-binding assay in a microtiter plate format, and the results are shown in Table 5. Both potato and tomato lectins were found to be positive in this assay, and the binding was substantially inhibited in the presence of chitosan oligomers. Based on its specificity for mannose, Con A was used as a positive control for HRP binding [20].

Discussion

The application of the pentose colorimetric assay for the detection of potato lectin, an arabinose-rich glycoprotein, has been demonstrated in this study. The FCO assay procedure for the determination of free pentoses [9–11] has been applied as such for the determination of protein-bound

Table 4 Inhibition of hemagglutination activity of potato lectin by chitosan, chitosan oligomers, and selected proteins

Inhibitor	Inhibitory concentration (μg)
D-Glucosamine	not inhibitory
N-Acetyl-D-glucosamine	2000
Chitosan	15.80
Chitosan oligomers¶	**4.80**
Horseradish peroxidase	**0.19**
Dolichos biflorus agglutinin	not inhibitory
Ovalbumin	not inhibitory
Bovine serum albumin	not inhibitory
Lysozyme	not inhibitory

¶Chitosan oligomers were obtained from digestion of chitosan (500 mg) with a non-specific enzyme pepsin (5 mg) at pH 5, 45°C for 6 h, followed by neutralization and centrifugation. Lyophilized supernatant represents chitosan oligomers [18]. The composition of chitosan oligomers as analyzed by HPLC is given under 'Results'.

Table 5 HRP-binding assay for potato lectin and inhibition of HRP binding by chitosan oligomers[a]

Protein (30 μg)	A_{492}[b]
Bovine serum albumin	0.020
Concanavalin A	0.474
Potato lectin (STA)	0.419
+ 50 μg chitosan oligomers	0.137
+ 100 μg chitosan oligomers	0.089
Tomato lectin (LEA)	0.413
+ 50 μg chitosan oligomers	0.131
+ 100 μg chitosan oligomers	0.097

[a]chitosan oligomers preparation and composition are described in footnote to Table 4.
[b]mean of triplicates.

pentoses, by taking advantage of the acid-labile nature of hydroxyproline-arabinoside glycosidic linkages. Normally, a typical agglutination assay for the determination of the biological activity of a lectin or agglutinin takes 5 to 6 h. However, the detection of potato lectin by FCO assay can be completed in approximately 1.5 h. Although the FCO assay is not a substitute for the agglutination assay, there appears to be considerable savings in time for the detection of potato lectin during the various stages of the purification. The agglutination assay can then be used to confirm the biological activity of the lectin in question at the final step of the purification. It appears that core $(GlcNAc)_2$ of N-linked glycoproteins are also potent inhibitors of hemagglutination activity of potato lectin (as exemplified by the use of horseradish peroxidase) in addition to free GlcNAc oligomers.

Additionally, a modified purification procedure involving only two chromatographic steps beyond the 60% $(NH_4)_2SO_4$ saturation step of Allen and Neuberger [5] has been developed here. The first step is a gel filtration step wherein potato lectin (molecular mass = 100–110 kD) elutes in the void volume along with the major storage protein, patatin. Preliminary elution pattern of this gel filtration component (component Ia) on cation-exchanger SP-Sepharose by gradient elution indicated to us that almost all of the potato lectin elutes at a NaCl concentration of ~100 mM at pH 4 (data not shown). However, the resolution of potato lectin from other proteins was inadequate. Hence, elution from the cation-exchanger using a step elution of 100 mM NaCl at pH 4 was attempted, and found to be optimal for the purification of potato lectin with respect to its purity and recovery.

Allen and Neuberger [5] obtained an yield of 0.84 mg potato lectin per 100 g tubers. The yield of potato lectin prepared by chromatofocussing [8] was also similar, namely, 0.92 mg per 100 g tubers. Compared to these procedures, we have obtained an yield of 1.73 mg per 100 g tubers, which translates to an increase in yield by twofold.

The FCO assay appears to be useful for the detection of glycoproteins which are rich in pentoses. Soybean agglutinin which has 6.2% carbohydrates is negative in the assay since the oligosaccharide is solely composed of $Man_9GlcNAc_2$, and behaves similar to concanavalin A, a non-glycoprotein. Ovalbumin (3.2% glycans; oligosaccharides of hybrid or high-mannose type), *Dolichos biflorus* agglutinin (4% glycans of mannose-rich type), avidin (11% glycans of mannose-rich type), and horseradish peroxidase (21.6% glycans; oligosaccharides of complex high-mannose type with L-fucose and D-xylose characteristic of plant glycoproteins) [4,19] give slightly higher values in the pentose assay compared to non-glycoproteins. This appears to be due to differences in glycan percentages mostly reflecting mannose content, as well as the presence or absence of a small number of xylose residues. It may be pointed out here that phenol-sulfuric acid assay is commonly used for the detection of all neutral sugars in glycoproteins [9], whereas the FCO assay can be used to specifically detect pentose-rich glycoproteins.

Although potato lectin belongs to the family of chitin-binding lectins [3], it also possesses the property of binding to core $(GlcNAc)_2$ of *N*-linked glycoproteins such as ovomucoid, and fetuin [3,7]. This has also been confirmed here by inhibition of agglutination by HRP as well as HRP-binding and its inhibition by chitosan oligomers. Tomato lectin behaves similarly to potato lectin in both FCO assay and HRP-binding assay, based on their identical sugar specificity and glycan compositions [3,21].

The FCO assay for pentoses appears to be a convenient assay for the detection of other Solanaceae (tomato, datura, etc) lectins, and glycoproteins rich in pentoses (arabinose, xylose, or ribose). Glycoproteins rich in either xylose or ribose have not been reported so far [4]. It may be noted here that L-arabinose is one of the few L-sugars that occurs naturally; it is widely distributed in plants in complex carbohydrate gums,

hemicelluloses, and pectin. The ease of application of pentose assay should prove useful for the detection and purification of these complex carbohydrate polymers as well as several hydroxyproline-rich glycoproteins (HRGPs), which are generally *O*-arabinosylated at Hyp residues. HRGPs of the plant extracellular matrix include extensins from plant cell walls [1, 22–24], gum arabic glycoprotein [25], repetitive proline-rich proteins, nodulins, and arabinogalactan-proteins (AGPs) [26]. Recently, glycans comprising arabinose residues have been identified as carbohydrate determinants in plant pollen allergens [27,28]. Two novel types of *O*-glycans, not found in extensins or solanaceous lectins, have been characterized in Art v 1, the major allergen of mugwort (*Artemisia vulgaris* L.) pollen—a type III arabinogalactan characterized by a large Hyp-linked arabinogalactan composed of a short β1,6-galactan core, which is substituted by a variable number (5–28) of α-arabinofuranose residues, and mono-β-arabinosylated Hyp residues [27]; the latter constitutes a new cross-reactive carbohydrate IgE epitope in plant proteins. Structural and immunological properties of arabinogalactan polysaccharides from timothy grass (*Phleum pratense* L.) pollen have revealed an IgG4 reactivity instead of IgE reactivity in humans [28]. In the case of mycobacteria, antibodies to arabinomannan are diverse and heterogeneous with respect to antigen recognition and V_H determinant expression [29]. An important epitope of anti-lipoarabinomannan antibodies has been characterized as the terminal branched hexaarabinofuranosyl motif of mycobacterial arabinans, whether present in lipoarabinomannan or arabinogalactan [30].

Note added in proof After the acceptance of this paper and, while in press, an article on the sugar chain-binding specificity of tomato lectin appeared in the final issue for the year 2005 in Glycoconj. J. [Oguri, S.: Analysis of sugar chain-binding specificity of tomato lectin using lectin blot: recognition of high mannose-type N-glycans produced by plants and yeast. Glycoconj. J. **22**, 453–61 (2005)]. Some of the results reported by us using glycoprotein-binding assay on the saccharide specificity of potato and tomato lectins are very similar to that reported by Oguri using lectin blot. Both papers lead to the same conclusion with respect to the binding of potato and tomato lectins to the chitobiose core of high mannose-type N-glycans.

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