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Extraction and Purification of Lectin from Florunner Peanut Seeds

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ABSTRACT

Large quantities of lectin were extracted from Florunner peanut seeds and purified by affinity chromatography. The purity and biochemical properties of this lectin were comparable to commercially prepared peanut lectin (Sigma Chem. Co.). These were evidenced by monitoring hemagglutinating activity, UV absorption spectra, absorbance ratio at 280 nm and 260 nm, extinction coefficient, disc gel electrophoresis and subunit molecular weight determinations.

Key Words: Lectin extraction, lectin purification, peanut seed lectin, lectin biochemical properties, Florunner peanut seeds.

In comparison with other legumes, the peanut has received considerably less attention with respect to its contents of antinutritional factors (e.g., trypsin inhibitors and lectins) and their impact on the growth of rats and physiological disorders in humans. Recently, it was demonstrated that there are differences in liver and pancreatic functions as well as several blood chemistries in rats fed raw and thermally processed peanut flour, even though protein efficiency ratios of the diets were similar (1, 12).

Animal feeding studies using peanut flour (12) and soybean flour (6, 15) demonstrated that raw and dry heated flours have adverse effects due primarily to lectins and protease inhibitors. Other antinutritional factors present or other constituents of the flour may have masked the effect of lectin per se. Such an interference could be obviated by feeding purified lectin extracted from the native legume. Such a study, however, requires the availability of relatively large quantities of the lectin which (10-20 g) can be procured by affinity chromatography separation. Commercially available lectin is an expensive chemical; $7.00 for one mg (Sigma Catalog, 1988).

The objectives of this study were to extract and purify a large quantity of lectin from Florunner peanut seeds and determine its purity and biochemical properties in comparison with a commercially available peanut lectin. This product will be utilized in rat studies to determine *in vivo* effects of lectin independent of other antinutritional factors.

Materials and Methods

Defatting of Peanuts Seeds

Florunner peanut seeds were ground with a commercial Waring blender and defatted for 4 hr with petroleum ether in a Soxhlet extractor. The defatted peanuts were allowed to dry in air.

Ammonium Sulfate Precipitation

A total of 4.4 kg of defatted peanuts, which were used in fractions of 400 g, were suspended in 0.9% NaCl, phosphate buffered at pH 7.2 (400 g/L), and stirred overnight at room temperature. The mixture was centrifuged at 10,000 x g for 25 min and the supernatant was retained and stored at 3.5 C with 0.02% (w/v) sodium azide added as a preservative. The precipitate was resuspended in the same volume of phosphate buffered NaCl and the procedure was repeated. Ammonium sulfate was added to the supernantant to 60% saturation (39 g/100 mL), and the precipitate which formed was allowed to settle overnight at 3.5 C and collected by centrifugation at 15,000 x g for 30 min (9). The precipitate was dissolved in distilled water and dialyzed extensively for 48 hr at room temperature against distilled water. Dialysis tubing (Fisher Scientific G. E, Catalog No. 8-667E) was first sterilized according to McPhie (10) and stored at 3.5 C in a 0.02% solution of sodium azide (w/v). After dialysis, the insoluble material in the dialysis bag was removed by centrifugation at 25,000 x g for 20 min combined, freeze dried and stored at 4 C for further analysis.

Affinity Chromatography

Preparation of the column packing of the conjugate of Sepharose and N-E-aminocaproyl-B-galactopyranosylamine (SAG) was generally similar to the method described by Gordon *et al.* (2) with some modification. Cyanogen bromide activated Sepharose 4 G (30 g) was washed with 5 volumes of cold water on a Buchner funnel. The wet Sepharose was then added to a solution of N-E-aminocaproyl-B-galactopyranosylamine (1.6 mmoles in 40 mL of 0.5 M $NaHCO_3$). The mixture was gently stirred for 16 hr at 3.5 C. The conjugated SAG was filtered and washed with 0.1 M $NaHCO_3$ (1L) and water (1L). A slurry was formed and poured into a glass column (32 cm x 2.5 cm). On settling, the height of the column bed was 21 cm. The SAG column was washed with 0.9% saline and kept at 3.5 C. Five hundreds mg of the freeze-dried ammonium sulfate fraction prepared previously was dissolved in 20 mL of 0.9% NaCl and applied to the column. The column was eluted at a rate of 1.5 mL/min with 0.9% NaCl until there was no effluent absorbing above 0.05 at 280 nm. (A280 < 0.05). The column was then eluted with a solution of galactose, 0.05 M in 0.9% NaCl, (9). Fractions (10 mL) eluting with galactose and absorbing at 280 nm were pooled, dialyzed against water for 48 hr at room temperature, and then lyophilized.

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Hemagglutination Assay

Agglutination of human type A red blood cells (RBCs) was the criterion used to compare the activity of commercial peanut lectin (Sigma Chem. Co., catalog no. L-0881, Lot no. 41F-9500) to that of the sample purified by affinity chromatography. The method used was a modification of the methods (6,7,9) as follows: Plasma was separated from the RBCs by centrifugation at 117 x g for 5 min and removed by aspiration. Two mL of packed RBCs were suspended in 50 mL. of phosphate buffered 0.85% saline (pH 7.2). Approximately 8 mL of the RBCs suspension were added to each of 6 tubes and centrifuged at 117 x g for 5 min. The supernatant was then aspirated and the erythrocytes were washed three times with the buffered saline and suspended to give a 4% (v/v) RBCs suspension. Neuraminidase (11.7 mg/2 mL) was added to 50 mL of the 4% RBC suspension and gently mixed for one min. The mixture was placed in a water bath (37 C) for 70 min. The enzyme treated RBCs were then washed four times with four volumes of 0.85% buffered saline, and made up to a suspension of 1.5% (v/v). The absorbance at 620 nm of this suspension was then adjusted by addition of either RBCs or buffered saline to give an absorbance value ranging between 0.5 and 0.6.

Two-tenths mg each of commercial peanut lectin (Sigma Chem. Co.) and peanut lectin obtained in this study by affinity chromatography was dissolved in 200 mL of phosphate buffered saline, pH 7.2. A series of twelve tubes was used within each lectin source covering a serial dilution range of 1 mL of undiluted lectin in tube 1 to 1:1024 in tube 12, which left a final volume of 1 mL in each tube. To each tube was added 1.0 mL of the neuraminidase treated RBCs, with mixing by inversion immediately following addition of the blood. The tubes were set aside in a vertical position at room temperature and absorbance readings at 620 nm were taken for each dilution at 0, 15, 30, 45, 60, and 150 min using a Coleman Junior Spectrophotometer. The difference in absorbance values between 0 and 150 min was considered as the index of hemagglutinating activity. The tubes were also tested for visual agglutination after 150 min by pouring most of the fluid off except approximately 0.5 mL and shaking to observe clumping, an indication of agglutination. A control was made using 1 mL of 0.85% NaCl and 1 mL of the enzyme treated and diluted RBCs.

Electrophoresis

Electrophoretic studies were performed with Pharmacia equipment (Piscataway, NJ): Gel electrophoresis apparatus GE-4, Gel Destainer GD-4, Electrophoresis Power Supply EPS 500/400 and Destainer Power Supply DPS.

Electrophoretic analyses of the commercial lectin (Sigma Chem. Co.) and the lectin sample eluted from the affinity chramatography column were carried out using a discontinous sodium dodecyl sulfate - polyacrylamide disc gel electrophoresis (SDS-PDGE) system (4, 13) with some modifications. Acrylamide and N, N_1 methylene-bis-acrylamide (Sigma Chem. Co.) were dissolved in distilled water in the ratio of 30% to 1.6% (w/v), respectively to prepare the gel stock solution. A 7.7% running gel without a spacer was prepared by mixing 6.0 mL of the gel stock solution, 6.0 mL distilled water, 4 mL of 0 .38 M tris-HCl buffer, pH 8.9, containing 0.1% SDS and 16 mL 0.10% ammonium persulfate. N, N, N_1-tetramethylethylenediamine (Temed) was added to the gel solution at the rate of 0.23% (v/v). Temed initiated polymerization of the gel and ammonium persulfate was the catalyst. The gel mixture was poured into 7.5 x 0.5 cm glass cylinders and was allowed to solidify for 30 min at room temperature. The gels were placed in the electrophoretic chamber containing 0.046 M tris-glycine buffer, pH 8.3 with 0.1% SDS. Lectin samples and protein standards were dissolved in 0.062 M Tris-HCl buffer, pH 6.7, containing 0.1% SDS. The quantities of lectin samples added were 100, 200 or 300 μg and each of the protein standards used was 50 μg. All samples and standard solutions contained bromophenol blue tracking dye. An electric current of 2 mAmp per gel was applied and the electrophoresis was stopped after 2 hours when the dye front reached the bottom end of each gel. The gels were stained with 0.25% Coomasie Blue, R-250, (Eastman Chemical Co.) in 50% methanol and 10% acetic acid (w/v) and destained electrolytically for 24 hr in 7.5% acetic acid: 5% methanol solution (v/v). Electrophoresis by the SDS-PDGE method separates proteins according to their molecular weights. Distance of migration of the bands of lectin samples and protein standards in the gels were measured and results are expressed as Rf values. The standards used were bovine serum albumin (68.0 KDa), oval albumin (45.0 KDa), soybean trypsin inhibitor (21.5 KDa) and egg lysozyme (14.3 KDa).

Extinction Coefficient

The commercial lectin and the lyophilized lectin that was eluted from the affinity chromatography column were dissolved in 0.9% saline to prepare 0.2% (w/v) solutions. Dilutions of these solutions ranging from 0.02% to 0.2% were made and absorbance at 280 nm was determined. There were two replications and average values were calculated. The absorbance of a 1% solution was calculated by using the appropriate factor from the absorbance values at 0.16% solutions. Ratios of absorbance at 280 nm and 260 nm for the 0.16% solutions were also calculated.

Absorption Spectra

Both lectin preparations (commercial and eluted) were dissolved in 1 M NaCl and absorption at 240 to 320 nm was recorded using a Perkin-Elmer Lambda 3 UV/Vis spectrophotometer.

Results and Discussion

A total of 4.4 kg of defatted Florunner peanut seeds yielded 17.968 g of ammonium sulfate fractionated protein (freeze-dried). After passing the ammonium sulfate fractionated protein through the affinity column, the galactose eluate was freeze-dried to produce 3.620 g of peanut lectin. This corresponds to a yield of 0.0823%, in contrast to Novogrodsky *et al.* (11) who obtained a yield of 0.150% from the Israeli Shulamit peanut seeds. An example of the elution pattern of bound protein using 0.5 M D-galactose in 0.9% NaCl at a flow rate of 1.5 mL/min is shown in Fig. 1.

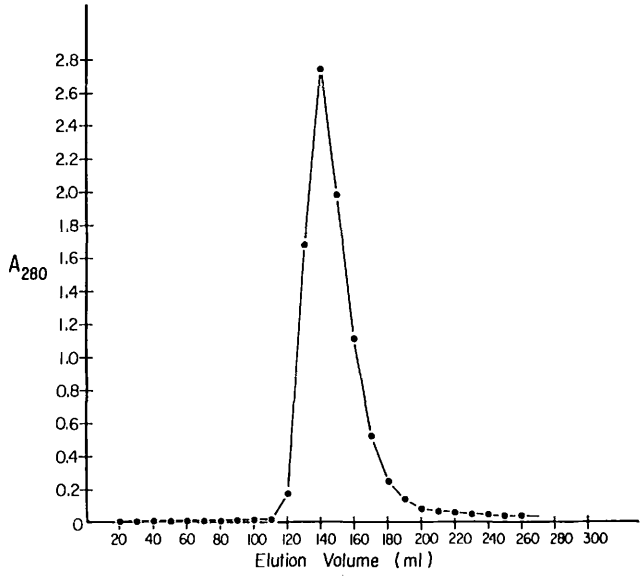

Fig. 1. Elution pattern of bound protein on the affinity column using 0.5M D-galactose in 0.9% NaCl. Flow rate 1.5 mL/min, 10 mL fractions.

The fraction eluted with D-galactose was lyophilized after dialysis. This sample was compared to a commercial preparation of the lectin (Sigma Chem. Co.) by hemagglutination assay, SDS-PDGE, UV absorption spectra extinction coefficients and absorption ratio at 280 nm/260 nm. The hemagglutination assay was carried out on the commercial lectin and the purified sample, using the same lectin concentration (0.2 mg/200 mL). After 150 min, absorbance values decreased in a similar manner (Table 1). Each preparation also showed visible agglutination up to a serial dilution of 1:8, indi-

cating that the preparation (affinity chromatography) was approximately the same purity as the commercial sample.

Table 1. Hemagglutinating activity ($A_{0\ min}$ - $A_{150\ min}$) of eluted Peanut lectin and commercial peanut lectin as influenced by lectin concentration.

Dilution	(A_{0} min - A_{150} mintues)		Visual agglutination	
	Eluted	Commercial	Eluted	Commercial
Undiluted (1.0 mg/ml)	0.140	0.146	Yes	Yes
1:1	0.129	0.125	Yes	Yes
1:2	0.120	0.115	Yes	Yes
1:4	0.110	0.095	Yes	Yes
1:8	0.095	0.080	Yes	Yes
1:16	0.040	0.052	No	No
1:1024	0.020	0.030	No	No
Control	0.005	0.010	No	No

Discontinuous electrophoresis at pH 8.9, in the presence of 0.1% SDS, of the purified lectin and the commercial sample, revealed a single band of Rf values 0.63 and 0.64 for the 100 μg and 200 μg samples, respectively. Electrophoretic analysis of the purified lectin sample also showed a single band for all concentrations used with an Rf value of 0.63 for the 100 μg sample size and 0.64 for the 200 and 300 μg sample sizes. The similarity in Rf values and the presence of only one band indicate that the eluted lectin was of the same degree of purity to that of the commercial preparation. A determination of the molecular weight as compared with standards showed that the lectin samples had a MW of 26.3 KDa (Fig. 2). London *et al.* (8) and Terao *et al.* (14) stated that peanut lectin is a pure tetramer and in SDS-PDGE system it presents as a single band of 27 KDa MW which approximate the value obtained for the eluted and commercial lectins in this study. The peanut lectin MW value (26.3 KDa) also corresponds to that of the subunits found by Lotan *et al.* (9), who have indicated that peanut lectin has a MW of 110 + 10 KDa and

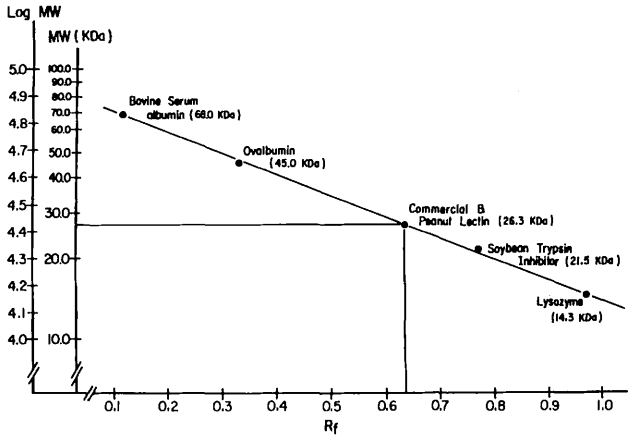

Fig. 2. Migration ratios of protein standards as a function of their Mw plot used to define Mw of peanut lectin samples.

is a tetramer composed of four apparently identical subunits of MW 27 + 1.5 KDa. Absorbance values of the different concentrations of the eluted lectin from the affinity column were slightly higher than those values for the commercial sample as shown in Table 2.

Table 2. Biochemical properties of solutions of eluted peanut lectin and commercial peanut lectin.

	Eluted	Commercial
Absorbance @ 280 nm Concentration (%)		
0.2	1.813	1.800
0.16	1.420	1.353
0.08	0.703	0.670
0.04	0.370	0.178
$E^{1\%}_{280}$	8.875	8.456
A280/A260	1.49	1.39

*0.16% solution.

Extention coefficients $E^{1\%}_{280}$ and absorbance ratio A280 /A260 for the 0.16% (w/v) lectin preparations are shown in Table 2. The values for the eluted lectin from the affinity chromatography column were slightly higher than those for the commercial lectin indicating slightly higher purity of the lectin obtained in this study compared with the commercial lectin.

The absorption spectra for both lectin preparations were similar with maximum absorption occurring at 280 nm (Fig. 3).

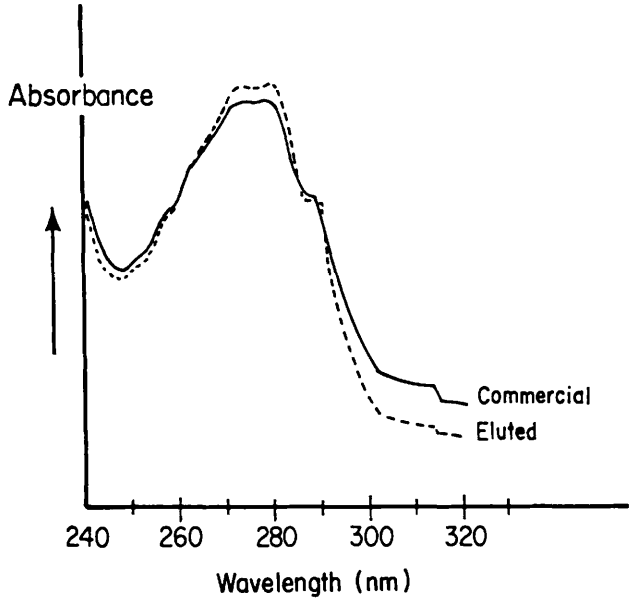

Fig. 3. Absorption spectra of eluted (---) and commercial (---) peanut seed lectin in 1 M NaCl solution.

Summary and Conclusions

The lectin extracted from Florunner peanut seeds was purified by affinity chromatography on Sepharose-N-aminocaproyl-B-glactopyranosylamine and compared to a commercially available peanut lectin.

The biochemical properties of the purified peanut

lectin were similar to the commercial sample according to the following comparisons: 1) Absorbance values of agglutination test of human type A RBCs, 2) Maximum dilution where visual agglutination was observed, 3) Disc gel electrophoresis, 4) Determination of subunit molecular weight, 5) Determination of extinction coefficients, 6) Determination of absorbance ratio at 280 nm and 260 nm, 7) UV absorption spectra.

This similarity in biochemical properties indicates that the extracted and purified Florunner peanut seed lectin was of high purity and thus suitable for subsequent animal feeding studies.

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