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A SIMPLE PROCEDURE FOR THE ISOLATION OF L-FUCOSE-BINDING LECTINS FROM *Ulex europaeus* AND *Lotus tetragonolobus**

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ABSTRACT

L-Fucose-binding lectins from *Ulex europaeus* and *Lotus tetragonolobus* were isolated by affinity chromatography on columns of L-fucose–Sepharose 6B L-Fucose was coupled to Sepharose 6B after divinyl sulfone-activation of the gel to give an affinity adsorbent capable of binding more than 1 2 mg of *Ulex* lectin/ml of gel, which could then be eluted with 0 1M or 0 05M L-fucose Analysis of the isolated lectins by hemagglutination assay, by gel filtration, and by polyacrylamide disc-electrophoresis revealed the presence of isolectins, or aggregated species, or both The apparent mol wt of the major lectin fraction from *Lotus* was 35 000 when determined on Sephadex G-200 or Ultrogel AcA 34 In contrast, the apparent mol wt of the major lectin fraction from *Ulex* was 68 000 when chromatographed on Sephadex G-200 and 45 000 when chromatographed on Ultrogel AcA 34 The yields of lectins were 4 5 mg/100 g of *Ulex* seeds and 394 mg/100 g of *Lotus* seeds

INTRODUCTION

In recent years, lectins have come into wide use as a means of studying the carbohydrate constituents of cell surfaces and of glycoproteins They are also widely used for the affinity-chromatographic isolation of glycoproteins The properties and uses of lectins have been reviewed[1–3]

One class of lectins of particular interest is that of the L-fucose-binding lectins of *Ulex europaeus* and *Lotus tetragonolobus* These lectins are generally considered O-blood group-specific[4,5] The *Ulex* lectin has been isolated by other investigators using conventional[6] and affinity-chromatographic methods[7,8] The *Lotus* lectins have been isolated by affinity methods employing precipitation procedures[9] and column-chromatography procedures[10,11]

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As is true for many affinity-chromatographic methods of isolation, the most serious obstacle is the synthesis of an appropriate ligand suitable for coupling to an insoluble matrix We recently reported an improved and simple procedure for the isolation of soybean lectin and other lectins on acid-treated Sepharose[1,2]. We describe herein an affinity adsorbent that can be easily prepared, is quite stable, has an acceptable capacity, and can be used for the isolation of the L-fucose-binding lectins of *U europaeus* and *L tetragonolobus*

EXPERIMENTAL

Materials — Seeds from *Ulex europaeus* were obtained from F W Schumacher Co , Sandwich, MA 02563, and seeds from *Lotus tetragonolobus* from Thompson and Morgan, Ltd , Ipswich, England Divinyl sulfone was obtained from Polysciences, Inc , Warrington, PA 18976, Sepharose 6B and Sephadex G-200 from Pharmacia Fine Chemicals, Inc , Piscataway, NJ 08854, and Ultrogel AcA 34 from LKB Instruments, Inc , Rockville, MD 20852

Extraction — *Ulex* seeds were ground in a grist mill and extracted, in the cold, overnight, with a buffer solution of PBS (0 15M NaCl–15mM Na_3PO_4–0 05% NaN_3, pH 7 0) at a ratio of 1 kg of seeds/8 liters of PBS The suspension was filtered, and the residue was re-extracted with 4 liters of PBS The suspension was filtered, and the filtrate was combined with the first extract The liquid extract was centrifuged at 27 000 *g* for 60 min, and the supernatant solution fractionated with ammonium sulfate to separate the L-fucose-binding lectin from the *N,N'*-diacetylchitobiose-binding lectin[6] The precipitates were dialyzed against de-ionized water and lyophilized The lyophilized crude lectin was stored in the dry state at −20° until used

Lotus seeds were ground in a mortar and were then extracted, in the cold, overnight, with PBS at a ratio of 1 g of seeds per 10 ml of PBS After centrifugation of the extract as just described, the residue was re-extracted and centrifuged The supernatant solutions were combined to give a viscous solution that was used directly for affinity chromatography

Hemagglutination assays — These assays were carried out in microtiter "U" plates with rabbit and with human type A, B, and O (H) erythrocytes Blood was collected in tubes containing ethylenediamine tetraacetate, and the red blood-cells were washed and suspended in PBS to give a concentration of 2% One drop of red blood-cells suspension, one drop of PBS or inhibitor, and one drop of lectin were added to the wells, and the mixture was incubated at room temperature for 15 min to 24 h Hemagglutination was read as positive or negative When quantitative titers were determined by two-fold serial dilution, 50 µl of each reagent were added to the wells and hemagglutination was scored from − to +3

Electrophoresis — Analytical disc-electrophoresis was performed with 7 5% poly(acrylamide) gels (5 × 75 mm) The lectin (up to 100 µg) in 10% sucrose—2 5mM Tris—19mM glycine (pH 8 3, 50 µl) was deposited on the gels which contained 18 9mM Tris (pH 8 9) An electrical current at 3 mA/gel was applied at 10° for ~1 h

until the tracking dye reached the end of the gel The buffer in the reservoir was 5mM Tris–38mM glycine (pH 8 3) The gels were stained with Coomassie Blue for protein and with the periodic acid–Schiff reagent for carbohydrate

Electrophoresis at pH 4 5 and in the presence of dodecyl sodium sulfate at pH 7.0 was carried out as previously described[12 13]

Affinity chromatography — L-Fucose was coupled to Sepharose 6B, according to the method of Fornstedt and Poráth[14] for coupling D-mannose to Sepharose 6B, as follows Sepharose 6B (20 ml) was washed with de-ionized water, and then with 0 5M Na_2CO_3 (pH 11) The gel was drained under mild suction and was suspended in 0 5M Na_2CO_3 (20 ml, pH 11) at room temperature Divinyl sulfone (2 ml) was added, and the gel was stirred for 70 min at room temperature The gel was then washed with de-ionized water, drained under mild suction, and suspended in 20% L-fucose in 0 5M Na_2CO_3 (pH 10) (20 ml) The gel was stirred in the cold for 24–72 h, washed with de-ionized water at room temperature, and suspended in 20 ml of 0 5M Na_2CO_3 (pH 8 5) containing 0 4 ml of 2-mercaptoethanol The gel was stirred for 3 h at room temperature, washed with de-ionized water, equilibrated with PBS, and stored in the cold until used

Columns containing 8–32 ml of affinity gel equilibrated at 6° with PBS were used for affinity chromatography with flow rates of 2–9 ml/h After being loaded with the sample, the columns were washed with 4–10 bed-volumes of PBS, and then eluted with 0 1M or 0 05M L-fucose in PBS (both concentrations of L-fucose were effective) All fractions were assayed for hemagglutinating activity with type O (H) red blood-cells The nonadsorbed fractions and the L-fucose-eluted fractions were pooled separately, dialyzed against PBS to remove L-fucose, and concentrated to the initial load volume of the affinity column by vacuum dialysis The hemagglutinating activity was quantitatively determined by the two-fold serial dilution technique Occasionally, isolated lectin fractions were concentrated in the presence of L-fucose to determine the effect of L-fucose on the gel filtration and electrophoresis results, no effect was observed

Standards for calibration of gel-filtration columns were lyzozyme (mol wt 14 400), chymotrypsinogen (mol wt 25 000), ovalbumin (mol. wt 44 000), bovine serum albumin (mol wt 68 000), and Blue Dextran 2 000

RESULTS

Isolation of Ulex europaeus *lectin.* — The seed-extract fraction that precipitated at 0–40% saturation of $(NH_4)_2SO_4$ was essentially devoid of the *N,N'*-diacetyl-chitobiose-binding lectin[6], and that fraction was used for the affinity chromatographic isolation of the L-fucose-binding lectin The lyophilized, crude lectin was suspended in PBS at 20–40 mg/ml The suspension was centrifuged at 27 000 *g* for 30 min The hemagglutinating activity and the absorbance at 280 nm were determined on an aliquot of the supernatant solution, and the remainder was used for affinity chromatography The results for one experiment are shown in Fig 1, with details given in

the legend The complete hemagglutinating activity was consistently recovered in the L-fucose-eluted protein This eluted fraction was analyzed by gel filtration on Sephadex G-200 and Ultrogel AcA 34, and by analytical disc-electrophoresis

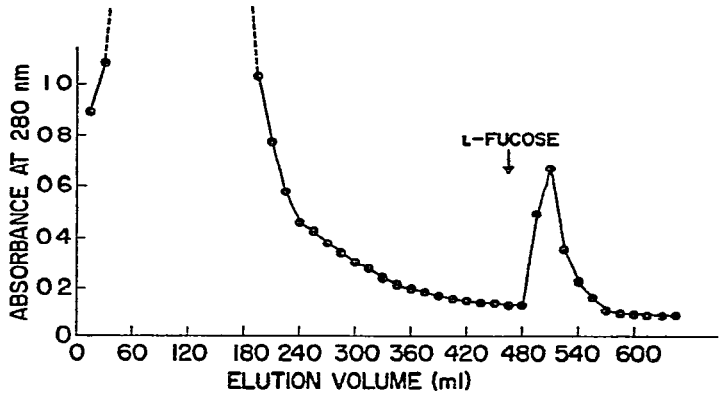

Fig 1 Affinity chromatography of *Ulex* hemagglutinin I on L-fucose–Sepharose 6B The crude lectin (2 4 g) in PBS (106 ml) was applied to a column (1 6 × 16 cm) at 6° The column was washed with PBS and, at the point indicated, elution was started with PBS–0 1M L-fucose The flow rate was 7 3 ml/h

Gel filtration on Sephadex G-200 and on Ultrogel AcA 34 gave similar elution profiles (see Figs 2 and 3, respectively), except for a shift in the apparent molecular weights of the retarded lectin fractions The amount of material in Peak 1 and Peak 2 varied somewhat from lot to lot of L-fucose-eluted protein The apparent mol wt of Peak 3 of the Sephadex G-200 column was 68 000, whereas that of Peak 3 of the Ultrogel AcA 34 column was 45 000

The pooled protein peaks from the Ultrogel AcA 34 chromatography were assayed for hemagglutinating activity The minimum concentrations giving a positive hemagglutination (+1) with type O (H) red blood-cells was 10 1 μg/ml for Peak 1, 1 1 μg/ml for Peak 2, and 2 1 μg/ml for Peak 3, compared to 2 8 μg/ml for the material before fractionation on the Ultrogel AcA 34 column

The L-fucose-eluted protein and the gel-filtration Peaks 1, 2, and 3 were analyzed by electrophoresis in poly(acrylamide) gels The L-fucose-eluted protein (see Fig 4) showed one, somewhat diffuse, band at pH 4 5, whereas in Tris buffer, at least 3 bands were observed with some protein not entering the gel Electrophoresis in the presence of dodecyl sodium sulfate revealed the presence of two closely migrating polypeptide subunits which migrated between chymotrypsinogen and ovalbumin These subunits gave a positive reactior for carbohydrate with the periodic acid–Schiff reagent When subjected to electrophoresis in the presence of dodecyl sodium sulfate, gel-filtration Peaks 1, 2, and 3, gave results identical to those for the L-fucose-eluted protein from the affinity column However, the material of Peak 1 apparently did not dissociate well, even in the presence of dodecyl sodium sulfate, since poly-

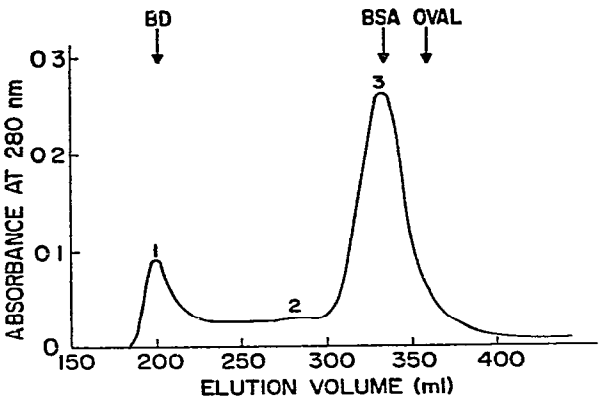

Fig 2 Sephadex G-200 chromatography of isolated *Ulex* hemagglutinin I The purified lectin (~14 mg) in PBS (5 1 ml) was applied to a column (2 6 × 93 1 cm) at 18° The column was equilibrated and eluted with PBS The flow rate was 19 7 ml/h The elution of the protein was scanned with a Gilson UV monitor at 280 nm with a full-scale sensitivity of 0 5 absorbance unit The elution positions of Blue Dextran 2000 (BD), bovine serum albumin (BSA), and ovalbumin (OVAL) are indicated

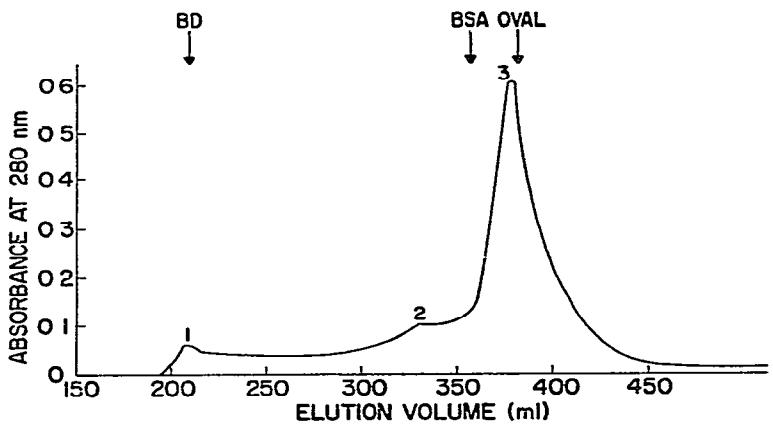

Fig 3 Ultrogel AcA 34 chromatography of isolated *Ulex* hemagglutinin I Purified lectin (~25 mg) in PBS (4 5 ml) was applied to a column (2 6 × 95 8 cm) at 18° The column was equilibrated and eluted with PBS and monitored, as described for Fig 2, with a full-scale sensitivity of 1 0 absorbance unit Flow rate was 17 6 ml/h The elution positions of Blue Dextran 2000 (BD), bovine serum albumin (BSA), and ovalbumin (OVAL) are indicated

peptide bands staining very weakly were observed, indicating that most of the material did not enter the poly(acrylamide) gel

The average yield of L-fucose-eluted lectin from the affinity column was ~4 5 mg/100 g of seeds It has been possible to obtain over 32 mg of lectin from a single affinity-column containing 29 ml of gel

Isolation of Lotus tetragonolobus *lectin* — In the affinity chromatography

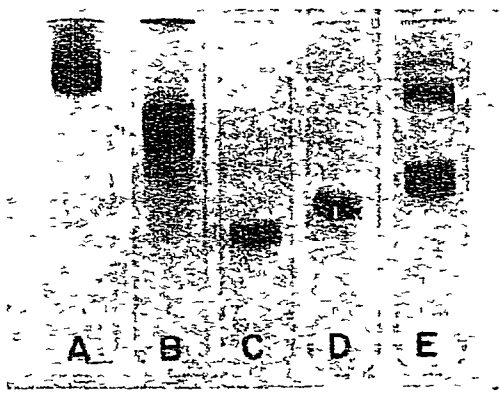

Fig 4 Poly(acrylamide) gel electrophoresis of isolated *Ulex* hemagglutinin I performed as described in Methods The gels were stained with Coomassie Blue for protein lectin examined by electrophoresis at pH 4 5 (A), by electrophoresis in Tris buffer (B), chymotrypsinogen examined by electrophoresis in dodecyl sodium sulfate (C), lectin examined by electrophoresis in dodecyl sodium sulfate (D), and ovalbumin examined by electrophoresis in dodecyl sodium sulfate (E)

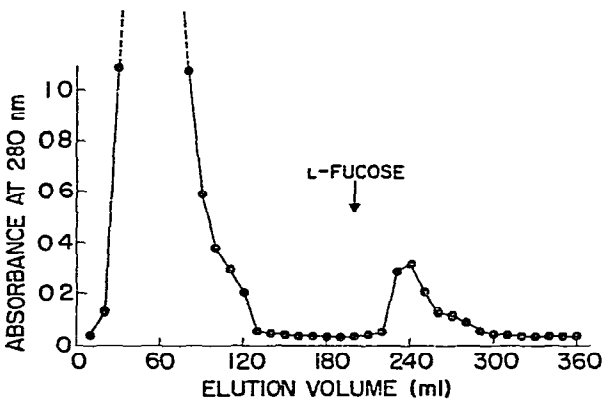

Fig 5 Affinity chromatography of *Lotus* extract on L-fucose–Sepharose 6B Whole extract (49 ml) in PBS was applied to a column (1 6 × 16 cm) at 6° The column was washed with PBS and, at the point indicated, elution was started with PBS–0 1M L-fucose Flow rate was 6 4 ml/h

of *Lotus* extract on L-fucose–Sepharose 6B (see Fig 5), the total hemagglutinating activity was usually recovered in the L-fucose-eluted protein Occasionally, a trace of hemagglutinating activity (less than 7% of the activity of the applied material) was observed in the nonadsorbed fractions that wer washed off the column with PBS Chromatography of the L-fucose-eluted protein on Sephadex G-200 (see Fig 6) and on Ultrogel AcA 34 gave identical results Four protein components were observed The apparent mol wt of the major component (Peak 3) was 35 000. The pooled protein peaks from the Sephadex G-200 chromatography were assayed for hemagglutinating activity The minimum concentrations giving positive (+1) hemag-

glutination with type O (H) red blood-cells was 500 μg/ml for Peak 1, 15 6 μg/ml for Peak 2 and 6 9 μg/ml for Peak 3, compared to 4 1 μg/ml for the material before fractionation The material present in Peak 4 was not active at a concentration of 500 μg/ml An extinction coefficient[9] of $E^{1\%}_{1\,cm}$ 17 5 at 280 nm was used to determine the lectin concentrations in the materials of Peaks 1, 2, and 3

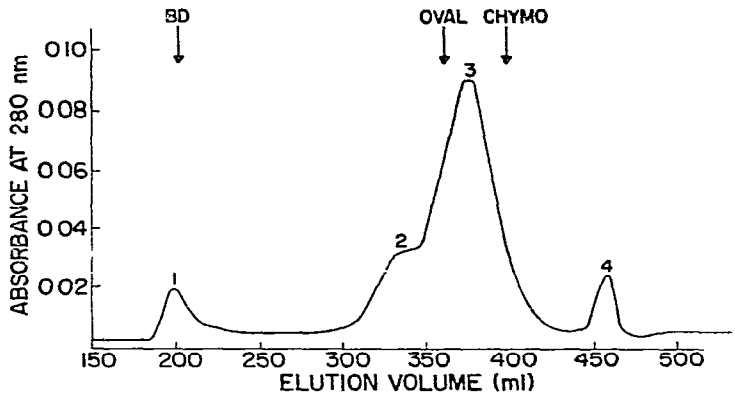

Fig 6 Sephadex G-200 chromatography of isolated *Lotus* lectin Purified lectin (7 1 mg) in PBS (5 0 ml) was applied to the same column as described in Fig 2, eluted and scanned, as described for Fig 2, with a full-scale sensitivity of 0 2 absorbance unit Flow rate was 19 8 ml/h The elution positions of Blue Dextran 2000 (BD), ovalbumin (OVAL), and chymotrypsinogen (CHYMO) are indicated

The L-fucose-eluted protein and the gel-filtration peaks from the Sephadex G-200 column were analyzed by electrophoresis in poly(acrylamide) gels (see Figs 7, 8, and 9)[16] Material from Peak 1, after dialysis and lyophilization, was quite insoluble even in the presence of dodecyl sulfate The material from Peak 4 was acid-soluble and could not be fixed in the electrophoresis gels This material is probably a proteolytic fragment, since it was absent from a lectin fraction prepared immediately from a single seed-extraction, whereas it was present in lectin fractions prepared by the more lengthy, double-extraction procedure

The results of electrophoresis of materials from Peaks 2 and 3 showed the presence of apparent isolectins comprised of different subunits Band C (Fig 7) shows that the material from Peak 2 is composed of polypeptide subunits having a higher mol wt than that of the polypeptide subunits found in the material from Peak 3 All polypeptide subunits of the L-fucose-eluted protein were observed (see Fig 7, A), and they migrated between chymotrypsinogen and ovalbumin Electrophoresis, at pH 4 5 (Fig 8), indicated the presence of isolectins, or aggregated species, or both At least three protein bands were observed for both the L-fucose-eluted protein and for the material of Peak 3, whereas one protein band was observed for the material of Peak 2 On electrophoresis in Tris buffer (see Fig 9), the material from Peak 2 showed the presence of two diffuse protein-bands (Fig 9, A) The

material from Peak 3, which had been previously dialyzed and lyophilized, showed the presence of four protein bands, the fastest-moving component being very diffuse (Fig 9, B), when the material was concentrated by vacuum dialysis without lyophilization (Fig 9, C), essentially the same results shown for the lyophilized sample were observed

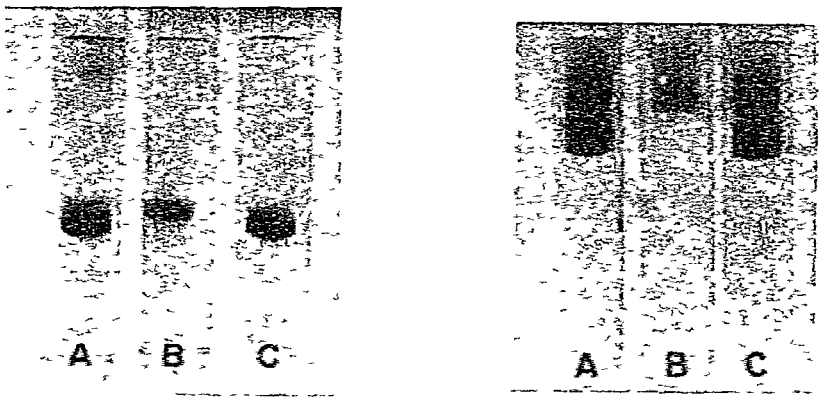

Fig 7 Poly(acrylamide) gel electrophoresis of *Lotus* lectin in dodecyl sodium sulfate was performed as described in Methods The gels were stained with Coomassie Blue for protein A, L-fucose-eluted protein, B, Peak 2 material from Sephadex G-200 column, and C, Peak 3 material from Sephadex G-200 column

Fig 8 Poly(acrylamide) gel electrophoresis of *Lotus* lectin at pH 4 5 was performed as described in Methods The gels were stained with Coomassie Blue for protein A, L-fucose-eluted protein, B Peak 2 material from Sephadex G-200 column, and C, Peak 3 material from Sephadex G-200 column

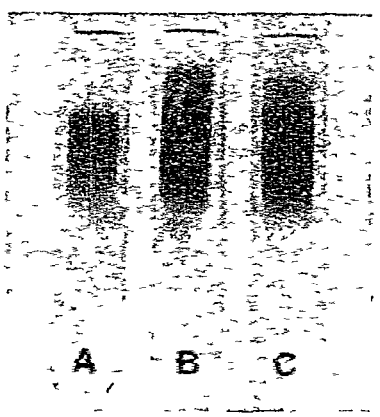

Fig 9 Poly(acrylamide) gel electrophoresis of *Lotus* lectin in Tris buffer was performed as described in Methods The gels were stained with Coomassie Blue for protein A, Peak 2 material from Sephadex G-200 column, B, Peak 3 lyophilized material from Sephadex G-200 column, C, Peak 3 concentrated material, not lyophilized, from Sephadex G-200 column

The average yield of L-fucose-eluted *Lotus* lectin from the affinity column was ~4 mg/g of seeds Over 80 mg of L-fucose-eluted lectin has been obtained from a single experiment with an affinity column having a gel bed of 25 ml

DISCUSSION

A variety of methods have been reported for the isolation of lectins by affinity chromatography[3] In many cases, multi-step procedures are required for the synthesis of appropriate ligands that can be coupled to insoluble matrices In order to circumvent the inconvenience of organic syntheses required for the preparation of many different, carbohydrate-containing ligands suitable for coupling to insoluble matrices, we have previously developed[12] simple procedures for the isolation of several D-galactose- and 2-acetamido-2-deoxy-D-galactose-binding lectins by affinity chromatography on acid-treated Sepharose 6B We report herein the development of an affinity adsorbent that utilizes divinyl sulfone to crosslink L-fucose to Sepharose 6B for the isolation of L-fucose-binding lectins by affinity chromatography Divinyl sulfone reacts with hydroxyl, amino, and thiol groups[15], it has been used previously to stabilize agarose-gel beads[15 16], to couple[17] hormones to Sepharose 6B, and to couple[14] D-mannose to Sepharose 6B The D-mannose–Sepharose 6B gel was used for the affinity chromatographic isolation of a D-mannose-binding lectin[14]

The L-fucose-binding lectin from *Ulex* (hemagglutinin I)[6] was previously isolated by conventional[6] and by affinity-chromatography procedures[7 8,18] After using ion-exchange chromatography and gel-filtration techniques, Matsumoto and Osawa[6] reported the isolation of hemagglutinin I with a yield of 20 mg/100 g of seeds Analysis by analytical ultracentrifugation gave a mol wt of 170 000 for the purified lectin which had a minimum hemagglutinating concentration of 15 μg/ml when tested against O erythrocytes These investigators[19] also found that L-fucose crosslinked to starch *via* epichlorohydrin did not give a satisfactory affinity adsorbent for hemagglutinin I

Reitherman *et al* [18] reported the isolation of hemagglutinin I by affinity chromatography on formaldehyde-treated erythrocytes They reported the presence, in the lectin, of two different polypeptide subunits having a mol wt of 43 000 and 45 000, respectively This procedure did not lend itself to the large-scale preparation of lectin, however

Hořejší and Kocourek[7] reported the successful isolation of *Ulex* hemagglutinin I by affinity chromatography Their adsorbent is somewhat laborious to prepare and requires the synthesis of allyl α-L-fucopyranoside and its subsequent copolymerization with acrylamide and *N,N'*-methylenebis(acrylamide) A yield of lectin of 2 9 mg/100 g of seeds and a minimum hemagglutinating concentration of 1 2 μg/ml against O erythrocytes was reported Two different mol wt were observed 65 000 by thin-layer gel filtration on Sephadex G-200 and 46 000 by analytical ultracentrifugation After poly(acrylamide) gel electrophoresis in the presence of dodecyl sodium sulfate, a single polypeptide of mol wt 40 000 was detected

Recently, Frost *et al*[8] reported the isolation of *Ulex* hemagglutinin I by affinity chromatography Their procedure requires the rather complex synthesis of 6-aminohexyl α-L-fucopyranoside, and its subsequent coupling to CNBr-activated Sepharose 4B Application of unfractionated seed extract to the affinity column gave a yield of 8 7–13 0 mg of hemagglutinin I/100 g of seeds The purified lectin had a minimum hemagglutinating concentration of about 1 μg/ml against O erythrocytes, and two polypeptide subunits of mol wt 31 000 and 32 000 were observed

The method for the isolation of *Ulex* hemagglutinin I described herein has obvious advantages over the conventional procedure[6] and does not require complex organic syntheses The lectin has been prepared from the equivalent of 800 g of seeds in one affinity-column run with no evidence of overloading of the column (bed-volume of 29 ml) A single column has been used repeatedly, with an occasional washing with 0 1M acetic acid, with no loss of efficiency over a seven-month period

In order to simplify the hemagglutination assays, hemagglutinin II (*N,N'*-diacetylchitobiose-binding) was separated from hemagglutinin I by ammonium sulfate fractionation[6] However, ~50% of the total hemagglutinin I activity was found in the fraction containing hemagglutinin II Hence, the yield of 4 5 mg of hemagglutinin I/100 g of seeds reported herein could be doubled by the use of an unfractionated seed extract This yield compares favorably with that reported by Frost *et al*[8] From the present results and those presented by others[6–8 18], it appears that *Ulex* hemagglutinin I may form aggregates at neutral and basic pHs, as well as exhibit an anomalous behavior The hemagglutinating activity against O erythrocytes, of the lectin described here compares favorably with that previously reported by others[7 8] The lower activity reported by Matsumoto and Osawa[6] is comparable to that reported herein for the aggregated species detected by gel filtration The anomalous behavior of the isolated *Ulex* lectin was demonstrated by gel filtration on Sephadex G-200 and Ultrogel AcA 34 The apparent mol wt of the major lectin fraction was determined by G-200 chromatography as 68 000, which is in close agreement with 65 000 reported by Hořejší and Kocourek[7]. determined by thin-layer, gel filtration on Sephadex G-200 Gel filtration on Ultrogel AcA 34 (an agarose–poly(acrylamide) copolymer) gave a mol wt value of 45 000, which is in close agreement with the value of 46 000 determined by analytical ultra-centrifugation[7] Poly(acrylamide) gel electrophoresis in Tris buffer demonstrated the presence of lectin aggregates, this was also suggested by the gel filtration results Aggregation may be a function of pH, however, since electrophoresis at pH 4 5 showed the presence of a single, somewhat diffuse, polypeptide component Electrophoresis of the isolated *Ulex* hemagglutinin I in the presence of dodecyl sodium sulfate, at a high gel-load, revealed the presence of a polypeptide band migrating between ovalbumin (mol wt 44 000) and chymotrypsinogen (mol wt 25 000) but being closer to chymotrypsinogen At lower gel-loads, however, the presence of two closely migrating polypeptide subunits was observed, in agreement with Frost *et al*[8] These subunits were also observed by staining the gels with the periodic acid–Schiff reagent, which indicates the presence of carbohydrate, in agreement with others[6 7]

We have consistently observed, in our *Ulex* lectin fraction, the presence of an aromatic compound which is slowly eluted from the affinity column This contaminant is not removed by simple dialysis, but it can be removed from the lectin by vacuum dialysis or by gel filtration Although this contaminant has no effect on the hemagglutinating activity of *Ulex* hemagglutinin I, its u v absorption at 280 nm does introduce an error into the determination and use of extinction coefficients

The isolation of *Lotus tetragonolobus* lectin was first reported by Yariv *et al* [9], who precipitated the lectin with a trifunctional L-fucosyl dye These investigators reported a yield of 45 mg of lectin/100 g of seeds Their preparation had a mol wt of 107 000 (by sedimentation equilibrium) and a minimum hemagglutinating activity of 38 μg/ml against O erythrocytes Kalb[20] reported the presence of 3 isolectins in the protein isolated by the method of Yariv *et al* [9] These isolectins were separated by DEAE-cellulose ion-exchange chromatography and they had molecular weights of 120 000, 117 000, and 58 000, respectively (by sedimentation equilibrium) The three isolectins were also found to be glycoproteins[20] Blumberg *et al* [10] isolated a *Lotus* lectin fraction by affinity chromatography on a column of *N*-(6-aminohexanoyl)-β-L-fucopyranosylamine coupled to CNBr-activated Sepharose 4B One of the isolectins adsorbed weakly at room temperature and was washed off with buffer The three isolectins were also separated by DEAE-cellulose ion-exchange chromatograpny A yield of lectin of 100 mg/100 g of seeds[22] was reported Pereira and Kabat[11] prepared an affinity adsorbent by insolubilizing hog A+H blood-group substance through copolymerization with the *N*-carboxyanhydride of L-leucine A yield of ~4 mg of lectin per gram of seeds was reported The same investigators[11] separated three isolectins by isoelectric focusing, however, one of the isolectins had very low hemagglutinating-activity (450 μg/ml were required to agglutinate O erythrocytes) The lectin fraction obtained by affinity chromatography was found to have a minimum hemagglutinating activity of 33–50 μg/ml against O erythrocytes The isolectins were reported to have similar specificities but different affinities for various ligands

The method of *Lotus* lectin isolation reported herein is more convenient than the procedures of Yariv *et al* [9] and Blumberg *et al* [10], in that the synthesis of a ligand is not required, and compares favorably with that reported by Pereira and Kabat[11], giving similar yields of lectin On poly(acrylamide) gel electrophoresis at acidic and alkaline pH (see Figs 8 and 9, respectively), the presence of isolectins or aggregated species was observed, in agreement with the electrophoresis results of Pereira and Kabat[11] Gel filtration of the isolated lectin at pH 7 0 on Sephadex G-200 and Ultrogel AcA 34 gave identical results, showing the presence of isolectins or aggregated species Electrophoresis in the presence of dodecyl sodium sulfate revealed the presence, in the material isolated from Sephadex G-200 Peak 2, of two different polypeptide subunits that migrated somewhat slower than the single polypeptide observed in the material isolated from Sephadex G-200 Peak 3 All polypeptides migrated between chymotrypsinogen and ovalbumin, within the mol wt range reported by others for the po'ypeptide subunits[10 11 20] The presence of carbohydrate in the polypeptides was indicated by staining with the periodic acid–Schiff procedure,

in agreement with the results of Kalb[20], Matsumoto and Osawa[6], and Pereita and Kabat[11], who showed the presence of amino and neutral sugars in the lectin

The material isolated from Sephadex G-200 Peak 1 had low hemagglutinating activity and was solubilized with difficulty, even in the presence of dodecyl sodium sulfate This may represent partially denatured and aggregated lectin The material isolated from Sephadex G-200 Peak 2 appeared to be devoid of at least two of the isolectins present in the material isolated from Peak 3 when examined by electrophoresis at pH 4 5 In Tris buffer, the same examination showed the presence of at least three polypeptides present in Peak 3 material but absent from Peak 2 material

The apparent mol wts of the *Lotus* lectin fractions obtained by gel filtration on Sephadex G-200 were not in agreement with values obtained by others with analytical ultracentrifugation methods In the case that the lectin fractions were being somewhat adsorbed by the glucosyl residues present in the Sephadex resin, the isolated lectin fractions were analyzed by gel filtration on Ultrogel AcA 34 which contains galactosyl residues Sephadex and Ultrogel gave identical results for the *Lotus* lectin The discrepancy in apparent mol wt values for the native lectin fractions cannot yet be explained The presence of 0 1M L-fucose in the solutions subjected to gel filtration did not change the protein elution profiles It should be emphasized, however, that we, as well as many others, have observed that various degrees of proteolytic degradation may occur during the isolation of many lectins The extent to which such degradation may influence lectin aggregation, the presence of apparent isolectins, mol wt determinations, and the presence of detectable polypeptide subunits cannot be readily assessed For example, Peak 2 of the *Lotus* lectin was not detected on gel filtration of some samples of the isolated lectin

While this work was in progress, Vretblad[22] reported the isolation of lectins from wheat germ and soybean by affinity chromatography using an adsorbent similar to that reported herein However, Vretblad[22] used commercially available epoxy-activated Sepharose 6B to which 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose were coupled

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