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Single Step Purification of Polysaccharides using Immobilized Jackfruit Lectin as Affinity Adsorbent*

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An α-D-galactosyl-binding lectin from *Artocarpus integrifolia* (jackfruit) seeds has been coupled to cyanogen bromide-activated Sepharose 4B. Purification of three galactomannans from fenugreek, guar and *Poinciana pulcherrima* seeds, a galactoglucomannan from *Crotalaria saltiana* seed and a polysaccharide from the albumin gland of the snail *Littorina littorea* has been achieved by affinity chromatography on a lectin-Sepharose column. The recovery of the polysaccharides absolutely devoid of protein is about 40%.

Polysaccharides are one of the most important classes of naturally occurring macromolecular components of animal and plant tissue and are usually intimately admixed or chemically bound with various tissue components such as proteins, nucleic acids, other polysaccharides, or materials of lower molecular weight as impurities.

Isolation of a polysaccharide may be achieved by its slow precipitation from an aqueous solution of crude material with organic solvents, e.g., ethanol, acetone, or pyridine, which often results in co-precipitation of extraneous material. A number of substances such as Fehling's solution and various copper salts [1-3] or barium hydroxide [4] are used to precipitate certain polysaccharides from solution in the form of insoluble salts or complexes. Acidic polysaccharides, such as the mucopolysaccharides, pectin, or alginic acid can be separated from neutral ones by precipitating the former with cetyltrimethylammonium bromide and cetylpyridinium chloride [5]. In many instances, polysaccharides closely associated with protein can be deproteinized by digestion of the crude product with trypsin or Pronase and the resulting amino acids and peptides can be separated from the polysaccharide by dialysis, ion exchange chromatography, or by alcohol precipitation. The removal of smaller proportions of protein is often carried out by shaking an aqueous solution of the crude preparation with chloroform, and butyl or amyl alcohol [6] and the resulting denatured protein accumulated at the chloroform water interface as precipitate, is removed. Polysaccharides may also be purified by fractionation on ion exchangers such as DEAE-cellulose, ECTEOLA-cellulose or resins [7].

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Polysaccharides may also be separated from each other, according to their molecular weights, by gel filtration through various types of Sephadex or polyacrylamide columns. Electrophoretic procedures are also useful for separation and purification of polysaccharides.

In recent years affinity chromatography using immobilized lectins has been widely employed for the purification of polysaccharides. Lectins are multivalent proteins of non-immune origin possessing the ability to bind reversibly with specific sugar residues. This sugar-binding specificity enables them to bind polysaccharides and glycoproteins and to agglutinate erythrocytes and various types of cells including tumor cells. Immobilized lectins, which are broadly selective group-specific adsorbents prepared by chemical coupling to insoluble matrices such as agarose, are invaluable tools for isolating and separating glycoproteins [8-12], glycolipids [13], polysaccharides [14-17], subcellular particles and cells [18-21], and for purifying detergent-solubilized cell membrane components [22]. Substances bound to the immobilized lectin are resolved by using a gradient of ionic strength or of a competitive binding substance.

The presence of hemagglutinin in the seeds of *Artocarpus integrifolia* (jackfruit) has first been reported by Chatterjee *et al.* [23] and its specificity for α-D-galactose has been demonstrated by precipitin reactions in agar gel with various galactomannans [24]. The purification, characterization and the carbohydrate specificity of jackfruit lectin, jacalin, have been described by several workers in detail [25-33]. Jacalin has been reported to possess bio-specific binding properties for serum glycoproteins including IGA from both serum and free secretory component [23]. This property has been utilized for facile preparation of IgA free of contaminating IgG using immobilized jacalin as an affinity adsorbent [28]. This affinity system has recently been applied by us for the isolation of a potent allergen from cotton extract, glycoproteins from ant egg and saliva of insects such as honey-bee and wasp (unpublished results).

The present paper describes the purification of polysaccharides using a jacalin-Sepharose 4B adsorbent.

Materials and Methods

Materials

The seeds of guar (*Cyamopsis tetragonolobus*) and *Crotalaria saltiana* were obtained from a local supplier. The seeds of fenugreek (*Trigonella faenugraecum*) were purchased from a local market and the seeds of *Poinciana pulcherrima* were collected from the garden of our Institute. *Littorina littorea* snails were collected from the coastal region of the Bay of Bengal by courtesy of Dr. Biplab Banerjee, Department of Zoology, University of Calcutta.

General Assay Procedures

The amount of neutral carbohydrate was determined by the phenol-sulfuric acid assay of Dubois *et al.* [34] using galactose as standard. For identification and estimation of in-

dividual neutral sugars, each bound and unbound polysaccharide from the affinity column was hydrolyzed separately with 0.5 M H_2SO_4 for 8 h at 100°C. The hydrolyzates, after neutralizing with barium carbonate and de-cationizing with Dowex 50W-X8 (H^+) were analyzed by gas-liquid chromatography (Hewlett-Packard, model 5730A) as their alditol acetates using a 3% ECNSS-M column as previously described [35]. For detection of amino sugar, hydrolysis was effected by 3 M HCl at 100°C for 4 h. The acid was removed by co-distillation with methanol under reduced pressure and finally over KOH pellets under vacuum. The alditol acetate of the neutral hydrolyzate was injected on a 3% Poly A-103 GLC column. Protein was determined by measuring the absorbance at 280 nm and by the Lowry method [36] using bovine serum albumin as the standard.

Preparation of Jackfruit Lectin-conjugated Sepharose 4B Adsorbent

Jackfruit (*Artocarpus integrifolia*) lectin was prepared according to the method of Ahmed and Chatterjee [33]. It was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the manufacturer's direction, with a modification. Jackfruit lectin (60 mg) was dissolved in 0.1 M sodium hydrogen carbonate containing 0.5 M sodium chloride, drained into the activated gel suspension (10 ml) and stirred gently for 2 h at room temperature. Absorbance readings at 280 nm and the protein measurement of the initial and final solutions [36] showed that about 95% of the lectin had been coupled to the gel. The lectin-gel was washed several times with buffers alternately at pH 3 and 8 containing 0.5 M sodium chloride, suspended in phosphate buffered saline (PBS; 0.1 M sodium phosphate pH 7.0, containing 0.15 M NaCl) in the presence of 0.01% sodium azide, and stored at 4°C.

Extraction of the Polysaccharides

Seeds of fenugreek, guar, *C. saltiana* and *P. pulcherrima*, 20 g each, were finely ground in a coffee grinder and after removal of seed coating, each meal was separately extracted with water (200 ml) for 6 h at 4°C. The major portion of the viscous supernatant solution was decanted and the remainder centrifuged in a Sorvall RC-5B refrigerated centrifuge at 18 000 × *g* for 30 min. The combined viscous supernatant and centrifugate were passed through a Millipore filter (pore size 0.45 μm) (Millipore Corp, Bedford, MA, USA). The albumin gland of the snail after dissection was homogenised in 0.85% sodium chloride. The extract after centrifugation and dialysis against water was treated with an equal vol of 90% aqueous phenol at 65°C as described by Chatterjee *et al.* [37]. The hydrophilic part of the mixture was lyophilized after exhaustive dialysis against water.

Affinity Chromatography on a Column of Jackfruit Lectin-Sepharose 4B

A Pharmacia C 10/20 column (1.0 × 20 cm) was filled with 10 ml jackfruit lectin-Sepharose 4B gel previously equilibrated and washed thoroughly with PBS. Lyophilized crude extracts (30 mg each in 9.0 ml PBS) were layered on the column and allowed to absorb for 4 h. The column was eluted with the same buffer and the fractions (3.0 ml, 6 ml/h) being collected on an automatic fraction collector were monitored for carbohydrate [34] and protein [36]. After the eluent had attained protein absorbance less than 0.01, a solution of 0.3 M galactose in the equilibrating buffer was added to the column to elute any specifically-bound carbohydrate. The eluted fractions were dialyzed exhaustively against PBS and monitored as already described.

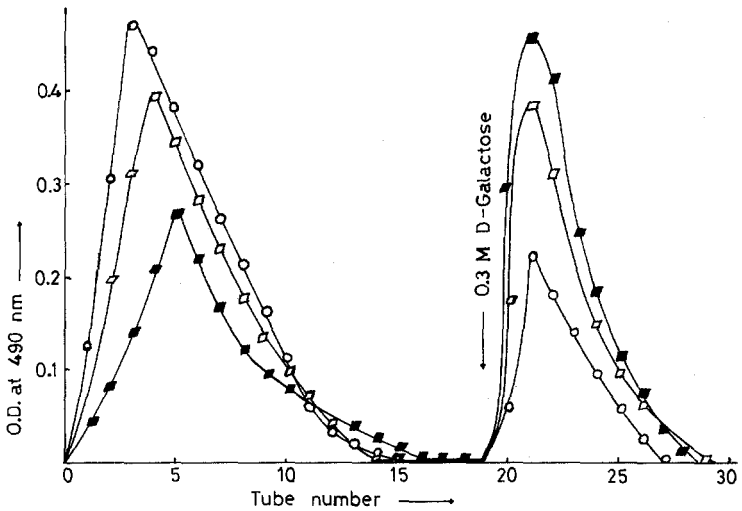

Figure 1. Elution profile of galactomannans on a jacalin-Sepharose 4B column. Absorbance at 490 nm for carbohydrates. ○, *P. pulcherrima*; □, guar (*Cyamopsis tetragonolobus*); ■, fenugreek (*Trigonella faenugraecum*).

Table 1. Chemical analyses of polysaccharides fractionated on immobilized jacalin.

Polysaccharides	Fractions	Yield (mg)	Neutral sugar (%)	Protein (%)	Sugar[a]
Trigonella faenum graecum	Bound	12.0	93	0	Man(1.2);Gal(1.0)
	Unbound	17.0	55	17	Rha(1.7);Ara(2);Xyl(1); Man(15);Gal(8);Glc(3).
Cyamopsis tetragonolobus	Bound	10.6	90	0	Man(1.92);Gal(1.0)
	Unbound	17.0	60	15	Man(3);Gal(1);Glc(trace)
Poinciana Pulcherrima	Bound	4.0	95	0	Man(3);Gal(1)
	Unbound	24.0	55	20	Man(2.2);Gal(1);Glc(0.6)
Crotalaria saltiana	Bound	4.2	87	0	Man(2.3);Gal(1.0);Glc(1.0)
	Unbound	24.6	57	30	Rha,Ara,Xyl(trace);Man(1.5); Gal(1.0);Glc(0.5)
Littorina littorea	Bound	6.6	51	3	Fuc(0.12);Man(0.21);Gal(1); Glc(0.4);GalNAc (1)
	Unbound	21.0	62	12	Man(0.12);Gal(1);Glc(1.0)

[a] Figures in brackets denote mole ratio.

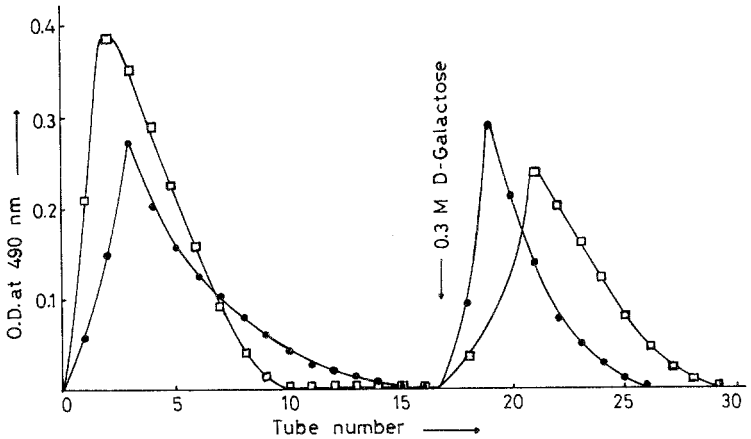

Figure 2. Elution profile of polysaccharides on a jacalin-Sepharose 4B column. Absorbance at 490 nm for carbohydrate. ●, *C. saltiana*, □, *L. littorea* polysaccharides.

Homogeneity

The homogeneity of the polysaccharides selectively eluted from the column was tested by gel filtration on a Sephadex G-100 column using pyridine-acetate buffer (pyridine/HOAc/H_2O, 2/5/500 by vol).

Results

Several polysaccharides, particularly galactomannans, were examined for their capacity to bind to the lectin affinity column and the results are presented in Fig. 1. The three galactomannans, i.e. those from fenugreek, guar, and *P. pulcherrima*, when loaded on the lectin column were retarded and strong binding was observed upon elution with 0.3 M galactose. Table 1 shows that binding of fenugreek and guar galactomannans was 40 and 35%, respectively, while that of *P. pulcherrima* was 13.3%. In a similar manner galactoglucomannan from *C. saltiana* was also retarded and bound 14% specificially to the column (see Fig. 2 and Table 1). The elution profile of the polysaccharide from *L. littorea* snail albumin gland is shown in Fig. 2. The material was significantly (22%) and selectively bound by the lectin column (see Table 1). The polysaccharides were found to be homogenous as judged by gel filtration on a Sephadex column whereby a single peak was obtained in each case.

Table 1 shows the chemical analyses of polysaccharides fractionated on immobilized jacalin. The galactomannans from fenugreek, guar and *Poinciana* which were selectively eluted from the lectin column were shown to contain a high amount of sugar (> 90%) and were absolutely free of protein. On the other hand the unbound fractions of guar

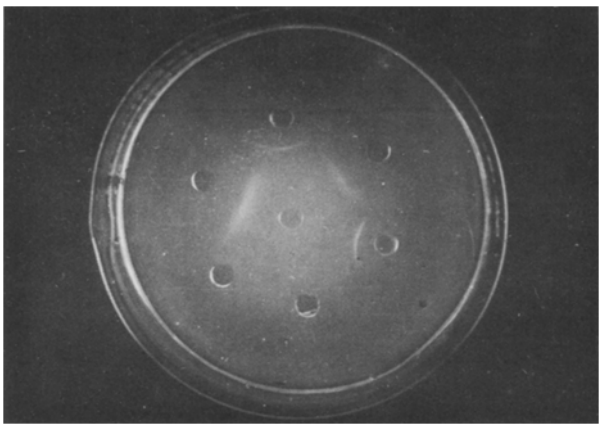

Figure 3. Precipitin reaction of jacalin (in middle well) with different polysaccharides. (1) *C. saltiana* galactoglucomannan; (2) *P. pulcherrima* galactomannan; (3) Guar galactomannan; (4), (5) NaCl; (6) Fenugreek galactomannan.

The plate, was prepared with 1% agarose gel. The wells are numbered clockwise from the top (well 1). All wells were filled with 10 μl of test substances (5 mg/ml) and saline.

and *Poinciana* were found to contain, besides galactose and mannose, a trace amount of glucose. The unbound material of fenugreek galactomannan was a heteropolysaccharide, as shown in Table 1. Similar result was also obtained with galactoglucomannan from *C. saltiana*. The selectively-bound material from *L. littorea* was also a heteropolysaccharide as it contained *N*-acetyl-galactosamine as well as neutral sugars and a small amount of protein, whereas unbound polysaccharide was rather simple in its composition and only contained galactose, glucose, and a small amount of mannose. The bound material from fenugreek, guar, *Poinciana* and *Crotalaria* gave an intense precipitation band in gel diffusion with jacalin (Fig. 3), while unbound material gave only a faint precipitate with the lectin (not shown). The *Littorina littorea* polysaccharide eluted by galactose also precipitated jacalin in agar gel (not shown).

Discussion

The results reported here demonstrate that jackfruit lectin, which has a specificity for Galβ1-3GalNAcα > Galα [33], retains both its activity and carbohydrate-binding specificity when covalently linked to cyanogen bromide-activated Sepharose 4B. Of a series of polysaccharides investigated for their capacity to interact with the immobilized lectin, galactomannans from fenugreek, guar, *P. pulcherrima* and a galactoglucomannan from *C. saltiana* were all bound to the column in appreciable amounts. All of them contain non-reducing, terminal α-D-galactopyranosyl groups. The galactomannans from fenugreek (molar ratio Gal:Man, 1.0 : 1.2) and guar (molar ratio Gal:Man, 1.0 : 1.9) were bound to the column more strongly than that from *P. pulcherrima* (molar ratio Gal:Man, 1:3) and the galactoglucomanan from *C. saltiana* (molar ratio Gal:Glc:Man, 1:1:2) as shown by their recovery. The reason is obviously due to crowding

of non-reducing terminal α-galactose units in fenugreek and guar which facilitate the interaction with the lectin, and such crowding is comparatively less in the polysaccharides from *Poinciana* and *Crotalaria* as observed from the molar ratio of the sugars. The interaction of galactomannans and galactoglucomannan with immobilized jacalin was further substantiated by a precipitation study of these polysaccharides with jacalin in agar gel plates. It is surprising to note that the unbound materials contain, in addition to galactose and mannose, other sugars precipitating faintly with the lectin. This indicates that a small amount of specific polysaccharides was eluted during washing of the column. Such a phenomenon may be due to either overloading of the column or polydispersity with respect to molecular weight and composition.

Littorina littorea snail polysaccharide precipitated too with jacalin in agar gel and the precipitin band showed a line of identity with that produced by a BSA-TF antigen, [β-D-Gal-(1-3)-α-D-GalNAc-*O*-$(CH_2)_8$]$_{30}$-BSA, (kindly given by Prof. G. Uhlenbruck, Medical University Clinic, Cologne, W. Germany). This precipitin reaction is presumably due to the presence of the Thomsen-Friedenreich (TF)-disaccharide, 3-*O*-β-D-galactopyranosyl-*N*-acetyl-D-galactosamine (Gal:GalNAc, 1:1; Table 1). However, such interpretation is speculative, and complete chemical study is needed to prove this immunochemical finding.

By the use of a 10 ml column containing about 60 mg of jacalin, it is possible to isolate 4-12 mg quantities of polysaccharide in a single step by affinity chromatography, thus affording considerable amount of material for chemical characterization. Such a method described herein should be highly useful for the purification of specific carbohydrate-containing biomolecules and is considered to be advantageous over the classical ones as it is easy to adopt, less time consuming, and affords a highly purified product.

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