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Immobilized mucin : an affinity matrix for the isolation of winged bean acidic and basic lectins

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Lectins, sugar binding and cell agglutinating proteins, are predominantly present in the seeds of legumes. They show a wide diversity in their sugar specificities and have proved to be useful for probing the structural features of cell surface glycoconjugates. As a result, lectins have widespread applications in various fields of research in biochemistry, medicine and cell biology[17–20].

A lectin with a molecular: weight (M_r) of 46 000 and a p*I* of 5.5 was first isolated from winged bean (*Psophocarpus tetragonolobus* LDC) seeds by Pueppke[21]. The lectin agglutinated only trypsinized and desialysed human erythrocytes of A, B and O types. Appukuttan and Basu[22] reported the isolation of a lectin from winged beans by affinity chromatography on Sepharose–N-caproyl-D-galactosamine, which has an M_r of 38 000 and agglutinated untreated human A, B and O erythrocytes. Recently, two types of lectins with different p*I*s and erythrocyte-agglutinating specificities have been reported. Kortt[3,4] reported the isolation of three acidic lectins (p$I \approx 5.5$)[4] and three basic lectins (p$I > 9.5$)[3]. Higuchi *et al.*[12] reported the isolation of a single acidic lectin (p$I \approx 5.5$)[14] and a single basic lectin (p$I \approx 8.6$)[13] from winged beans. Acidic lectin(s) agglutinate human A, B and O erythrocytes strongly but not rabbit, whereas the basic lectin(s) agglutinate rabbit and human A and B but not O erythrocytes. The specificity of the acidic lectins was directed towards β-D-galactosides, whereas that of the basic lectins was directed towards α-D-galactosides.

Here, we describe a simple, one-step preparative method for the isolation of acidic and basic lectins from seeds of winged beans by affinity chromatography.

EXPERIMENTAL

Porcine gastric mucin and glutaraldehyde were obtained from Sigma. All other chemicals were of analytical-reagent grade. Winged bean seeds (*Psophocarpus tetragonolobus*) were obtained locally.

Protein concentrations were determined by absorbance measurements at 280 nm using bovine serum albumin as standard ($A_{1\,cm}^{1\%} = 7.45\ cm^{-1}$, 1%). Haemagglutination assays were carried out as reported earlier[1]. Centrifugation was carried out in Sorwall SS-3 automatic centrifuge using an SS-34 rotor (capacity 8 × 50 ml, 12 080 *g*)

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at room temperature (26–28°C) and in a refrigerated centrifuge (capacity 2 × 180 ml, 2412 *g*) at 4°C. Saline solutions I (0.145 *M* sodium chloride) and II (1.0 *M* sodium chloride) were used.

The preparation of delipidated acetone dried powder from dry winged bean seeds, the extraction of acetone dried powder with saline solution I and ammonium sulphate fractionation were carried out as described previously[1]. The protein fraction precipitating between 30 and 80% ammonium sulphate saturation was dissolved in the minimum amount of water and extensively dialysed against water and finally against saline solution II. On dialysis the solution containing lectin activity was clarified by centrifugation at 12 080 *g* and then stored at −20°C. This solution (fraction A) was used to isolate lectin by affinity chromatography.

Preparation of affinity matrix

Porcine gastric mucin was partially desialylated (by gentle acid treatment) according to the procedure of Svennerholm[2]. Desialomucin was then immobilized by entrapment in glutaraldehyde-cross-linked gelatin gel granules as follows. Desialomucin (1.0 g) was suspended in borate buffer (pH 8.0, 0.2 *M*, 30 ml) to give a uniform suspension. Gelatin solution was prepared by suspending skin gelatin (6.0 g) in 70 ml of borate buffer (pH 8.0, 0.2 *M*) and warming on a hot waterbath (70–80°C), to give a clear solution. It was then cooled to room temperature, mixed with mucin suspension and the mixture was allowed to set in the form of a gel (5 mm thick) in a tray at 4°C. Next day, the gel was cut into pieces (1 × 1 cm), suspended in glutaraldehyde solution [8% (v/v), 250 ml] and the pH was adjusted to 9.0 with 1.0 *M* sodium hydroxide solution. After allowing it to stand at 4°C overnight, the cross-linked gel was blended in a blender and the gel granules were collected by centrifugation (2418 *g*). It was then washed free of glutaraldehyde by repeated washing with distilled water (4°C). The fines, removed by decantation, were discarded. The gel granules were then suspended in saline solution I containing glycine (0.1 *M*) overnight, to block the unreacted aldehyde groups. After washing with borate buffer (pH 8.0, 0.2 *M*), the gel was preserved in saline solution I containing sodium azide (0.02%, w/v) at 4°C. The amount of sedimented gel obtained was 150 ml.

RESULTS AND DISCUSSION

Winged bean seeds contain two distinct groups of D-galactose/N-acetyl-D-galactosamine-specific lectins, the acidic and the basic, which differ in their erythrocyte agglutination specificities and isoelectric points. The acidic lectins agglutinate human A, B and O erythrocytes, but not rabbit erythrocytes, and their sugar specificity is directed towards β-D-galactosides. The basic lectins, specific for α-D-galactopyranosides, agglutinate human A and B erythrocytes and rabbit erythrocytes, but not O erythrocytes[3–5,12–14].

Our preliminary results indicated the presence of both acidic and basic lectins in the local varieties of winged bean seeds.

When fraction A was loaded on a column packed with immobilized desialomucin, both lectins were retained on the column (as judged from the haemagglutinating assay). Fig. 1 shows the elution profile of the winged bean lectins on the desialomucin column, and Fig. 2 shows the polyacrylamide gel electrophoresis (PAGE) pattern at

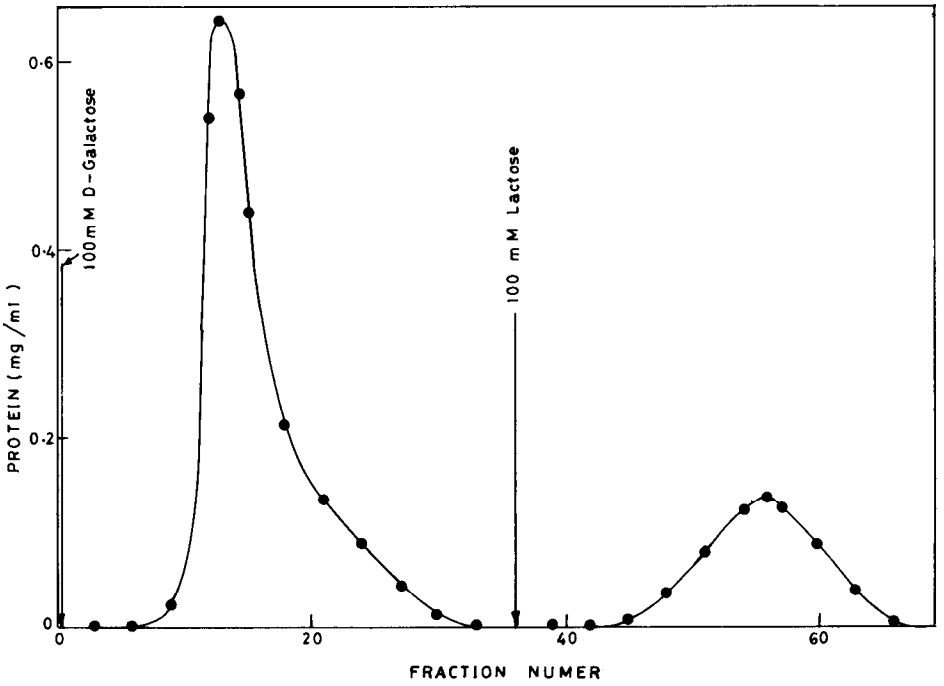

Fig. 1. Elution profile of winged bean seed basic and acidic lectins on an immobilized desialomucin column. Fraction A of winged bean seeds was applied on the immobilized desialomucin column (18.0 × 3.0 cm I.D., capacity 90 ml), equilibrated with saline solution II (1.0 *M* sodium chloride containing 0.02% sodium azide). The column was washed with saline solution II until free of unadsorbed proteins. The adsorbed lectins were first eluted with 100 m*M* D-galactose followed by elution with 100 m*M* lactose in saline solution II.

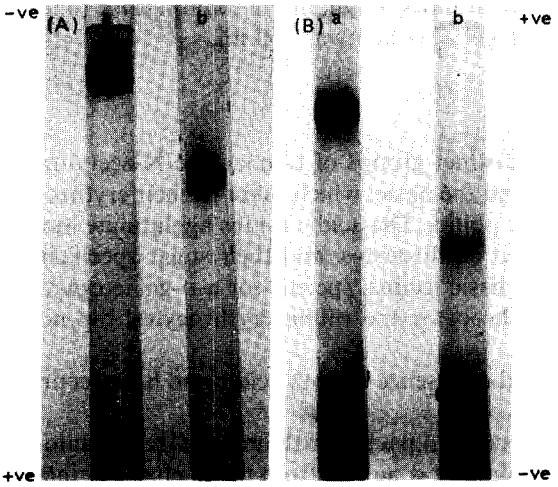

Fig. 2. PAGE pattern of winged bean lectins at (A) pH 8.3 and (B) pH 4.5. (a) D-Galactose-eluted peak protein (fraction 13); (b) lactose-eluted peak protein (fraction 56).

pH 4.5[9] and 8.3[6] of the D-galactose- and lactose-eluted protein peaks. Lectin eluted with D-galactose agglutinated normal human A and B erythrocytes in addition to normal and papain-treated rabbit erythrocytes, but not O erythrocytes, the property characteristic of the basic lectin. The protein eluted with lactose agglutinated human A, B and O erythrocytes but not rabbit erythrocytes, the property characteristic of acidic lectin. Hence a clear cut gross separation of acidic and basic lectins has been achieved. The yield of the lectins (acidic + basic) was 36 mg per 10 g of seeds with 80-fold purification and the ratio of acidic to basic lectins was 1:4.

Lectins are usually isolated by affinity chromatography using matrix-coupled monosaccharides, glycosides or water-insoluble polysaccharide derivatives[7]. The isolation of winged bean basic and acidic lectins has been reported by a number of workers. Kortt[3,4] reported the separation of acidic and basic lectins from the seeds and tuberous roots of *Phosphocarpus tetragonolobus*[3,4,8,10] and *Phosphocarpus scandens*[11] seeds, using a combination of gel filtration on Ultrogel AcA44, ion-exchange chromatography on SP-Sephadex C-25 and/or affinity chromatography. A single-step purification of winged bean acidic and basic lectins from tuber extracts by affinity chromatography on lactose–Sepharose 4B and melibiose–Bio-Gel P-150, respectively, has also been reported[13]. Higuchi *et al.*[12] reported the isolation of a single acidic[14] (pI = 5.5) and a single basic lectin (pI = 8.6)[13] by initial ion-exchange chromatography of the seed extract on DEAE-Sephadex A-50 followed by affinity chromatography on N-acetylgalactosamine–agarose and *p*-aminophenyl-β-D-galactopyranoside-bound Sepharose 4B, respectively. Khan *et al.*[5] isolated winged bean basic and acidic lectins by affinity chromatography on Sepharose–6-aminocaproyl-D-galactosamine and lactosaminyl–Bio-Gel respectively. All the above methods involved either at least two steps of purification and/or use of separate affinity media for the basic and acidic lectins.

Many lectins bind only to complex oligosaccharide determinants on cell surfaces or naturally occurring glycoproteins, and special procedures have to be developed for the isolation of such lectins[15,16]. Freier *et al.*[16] reported the use of two easily available glycoproteins, *viz.*, hog gastric mucin and ovomucoid, by coupling to cyanogen bromide-activated Sepharose 4B as general affinity adsorbents for lectins. They isolated 30 lectins from 27 different plants.

In this work, desialyated porcine gastric mucin immobilized by entrapment in glutaraldehyde-cross-linked gelatin gel granules was used as an affinity matrix for the isolation of winged bean seed lectins. Both acidic and basic lectins from winged bean seeds were absorbed on the immobilized desialomucin column. Elution of the adsorbed lectins was carried out first with D-galactose, when only the basic lectin was eluted. The acidic lectin was eluted only when lactose was employed as an eluent. Hence gross separation of winged bean basic and acidic lectins from the crude seed extract can be achieved in one run on the same affinity matrix by simply changing the desorption conditions. The method is simple, rapid and reproducible.

Many plants are known to contain more than one lectin with only minor differences in their sugar specificity[16]. The use of an immobilized mucin column and elution of the adsorbent lectins with different sugars can be employed as a general method for the isolation of such lectins.

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