CROSS-LINKED GUARAN: A VERSATILE IMMUNOSORBENT FOR D-GALACTOPYRANOSYL BINDING LECTINS

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1. Introduction

Affinity chromatography has become the standard procedure for isolation of lectins [1]. In its most general form, a ligand which binds to the lectin is linked covalently to a solid matrix. However, an insoluble or cross-linked polysaccharide for which the lectin exhibits affinity can also be used as the adsorbent. Notable examples of this approach include the isolation of concanavalin A using Sephadex G-50 (cross-linked dextran, [2,3]) the isolation of the *Ricinus communis* lectins using agarose [4] and the isolation of wheat germ agglutinin on chitin [5]. The documented specificity of the lectins from the seeds of *Bandeiraea simplicifolia* [6] and *Ricinus communis* [7] for D-galactopyranosyl end units suggested that these as well as other lectins that bind these sugar units could be isolated on cross-linked guaran (guar gum). Guaran is a galactomannan composed of a chain of β-(1→4)-linked D-mannopyranosyl residues having side stubs of single α-D-galactopyranosyl units linked α-(1→6) to approximately one half of the β-D-mannosyl residues [8]. The present report describes the preparation and evaluation of this new adsorbent for lectin isolation.

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2. Materials and methods

Protein concentrations were determined by the microbiuret assay [9] or by estimation of the absorbance at 280 nm using $E^{1\%}_{280} = 14.1$ for the purified *B. simplicifolia* lectin [6]. Gel electrophoresis (7.5% polyacrylamide) was performed at pH 4.3 using β-alanine–acetic acid buffer as described earlier [10]. The gels were stained with Coomassie Brilliant Blue R250. Extraction of *B. simplicifolia* seeds was carried out as described previously [6]. Extracts from *R. communis* and *S. japonica* seeds were the generous gifts of Drs M. E. Etzler, University of California, Davis, CA. and Dr R. D. Poretz, Rutgers University, New Brunswick, N. J., respectively. Purified *Helix pomatia* agglutinin was a gift of Dr S. Hammarström, University of Stockholm, Stockholm, Sweden and soy bean agglutinin a gift of Dr N. Sharon, Weizmann Institute, Israel. Purified lima bean *(Phaseolus lunatus)* lectin was kindly supplied by Mr L. Murphy of this University and the *D. bilflorus* lectin obtained from Dr M. E. Etzler of the University of California, Davis, CA. Guaran was purified from crude guar gum (Meer Corp., New York) by repeated alcohol precipitation from water. Guaran beads were prepared by cross-linking a 2% solution of purified guaran with epichlorohydrin [11]. The particle size of the beads was in the range of 40–250 μ. The gel was contained in columns eluted with phosphate-buffered saline.

In a typical experiment, a column (1 × 18 cm) of guaran beads was equilibrated at 4°C with 0.01 M phosphate-buffered saline (0.15 NaCl M), pH 7.2 at a flow rate of 3.5 ml/h. An ammonium sulfate fraction (55–75% saturation) [6] of an extract of *B. simplicifolia* seeds containing 41 mg protein in 8 ml buffer was applied to the column and fractions (5 ml) collected. The column was washed until A_{280} was < 0.02 when a solution of D-galactose (300 mg in 5.0 ml phosphate–buffered saline) was added. The protein-containing fractions were pooled and dialyzed exhaustively against phosphate buffered saline. The resulting solution contained a total of 7.5 mg pure lectin.

Purification of the *R. communis* lectins as well as the attempted absorption of the *S. japonica, P. lunatus* and *D. biflorus* lectins was performed as above. A solution of the *Helix pomatia* agglutinin (100 μl, 3 mg) and the soy bean lectin (400 μl, 1.5 mg) were independently passed over the column and eluted with D-galactose as above.

The wild cucumber (*Echinocystis lobata*) lectin was isolated by extracting ground seeds (14 g, collected in October 1973 at the Matthaei Botanical Gardens of The University of Michigan) with phosphate-buffered saline (2 × 40 ml). The combined extracts were made 80% saturated with respect to ammonium sulfate and the precipitate collected by centrifugation. Dialysis of the dissolved precipitate against phosphate-buffered saline gave 320 mg protein.

Affinity chromatography was performed as described above. Crude protein mixture (40 mg) was applied to the column containing 7.5 ml guaran beads. For hemagglutination and inhibition tests, 2% suspensions of A_1, B and O human erythrocytes were used.

3. Results and discussion

The *B. simplicifolia* lectin shows a high specificity for α-D-galactopyranosyl groups and the lectin can be precipitated quantitatively by guaran [6]. Earlier this lectin was isolated using a melibionate-aminoethyl-Biogel column [6]. However, the preparation of this column required three separate operations starting from commercially available materials.

When a crude extract of *B. simplicifolia* seeds (55–75% ammonium sulfate fraction) was applied to the guaran column, all proteins, except the lectin were eluted with the void volume. The lectin was specifically eluted by D-galactose. After dialysis to remove

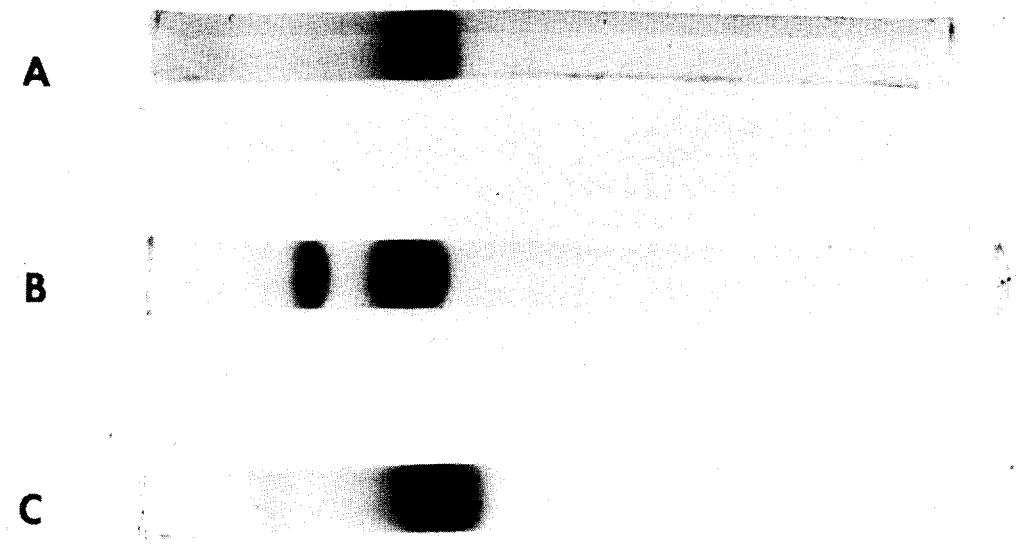

Fig.1. Polyacrylamide gel electrophoresis at pH 4.3 of lectins purified on guaran column. A, *B. simplicifolia* lectin; B, *R. communis* lectins; C, *Echinocystis lobata* lectin. Approximately 20 μg protein applied to each gel. Conditions are described in text.

D-galactose, the lectin showed the expected capacity to precipitate guaran. The purity of the isolated material was established by polyacrylamide gel electrophoresis (fig.1). The protein migrated as a single band, with a mobility identical with an authentic specimen. Yield: 5.8 mg pure lectin from 30 mg crude protein. In a separate experiment the capacity of the guaran gel was determined by the addition of an excess of purified lectin to the column. Approximately 6.5 mg of *B. simplicifolia* lectin was absorbed per 10 ml of swollen guaran beads.

The ability of the guaran gel to bind the lectins from the castor bean was also investigated. It was anticipated that these lectins would be adsorbed, since they also are precipitated by guaran and other galactomannans [12]. The *R. communis* lectins (RCA_I and RCA_{II}) both show specificity for D-galactopyranosyl groups without pronounced anomeric specificity [7]. A crude extract of the seeds was applied to the guaran gel column. After washing the gel free of unbound material, the addition of a D-galactose-containing buffer resulted in the elution of the *R. communis* lectins. Yield: 6.1 mg pure lectins from 14 mg crude protein. On polyacrylamide gel electrophoresis (fig.1). the material showed two strong bands corresponding to the RCA_I and RCA_{II} lectins.

It was also demonstrated that the purified agglutinins from the edible snail (*Helix pomatia*) and the soy bean (*Glycine max*) were specifically adsorbed on the guaran gel and quantitatively eluted by D-galactose. In contrast, the lima bean lectin (both the crude extract and purified agglutinin) and the *Dolichos biflorus* lectin (purified preparation) failed to bind the guaran column. These results are in agreement with the observations that the former two lectins are precipitated from solution by guaran whereas the latter two agglutinins do not react with this galactomannan [13].

In another experiment a crude extract of *Sophora japonica* seeds was applied to the column. Elution of the column with D-galactose failed to displace any protein. Inasmuch as this lectin does not display a high affinity for α-D-galactopyranosyl groups these results are not surprising [14].

The occurrence of a hemagglutinin in extracts of *Echinocytis lobata* seeds was first observed in this laboratory several years ago [15]. An extract of these seeds was fractionated with ammonium sulfate, dialyzed against phosphate-buffered saline and applied

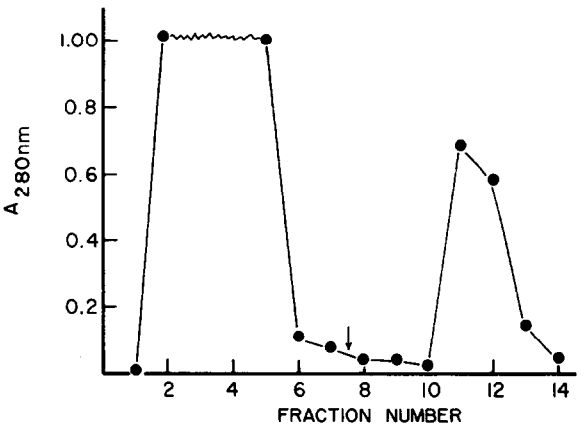

Fig.2. Elution profile of wild cucumber lectin on guaran column. Arrow indicates point at which 0.1 M D-galactose was added to the column.

to a guaran gel column. After elution of unbound protein, D-galactose was added to the column whereupon protein was eluted (fig.2). Yield: 1.2 mg lectin from 40 mg crude protein. Following dialysis of the D-galactose the agglutinin was subjected to polyacrylamide gel electrophoresis. A major band and a second faint band of lower mobility (fig.1) were stained with Coomassie blue. The lectin gave a specific hemagglutination titer of 80 toward A_1, B and O human erythrocytes. Hemagglutination was inhibited by D-galactose and *N*-acetyl-D-galactosamine but not by D-glucose.

These studies demonstrate that cross-linked guaran appears to be a versatile immunosorbent for the isolation of D-galactopyranosyl binding lectins, and, in some cases, for *N*-acetyl-D-galactosaminyl binding agglutinins which also react with D-galactose, e.g. the agglutinins from *H. pomatia* and *G. max*. Furthermore, cross-linked guaran gel could also be employed in studying carbohydrate-binding specificity by the solid phase absorption assay recently described by Poretz and coworkers [16].

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