DATURA STRAMONIUM LECTIN: ISOLATION AND CHARACTERIZATION OF THE HOMOGENEOUS LECTIN

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Received 20 May 1981

1. Introduction

The seeds of *Datura stramonium* (Jimson Weed) contain a lectin which specifically binds β-(1 → 4)-linked oligomers of *N*-acetyl-D-glucosamine [1,2]. A member of the family Solanaceae, the *Datura* lectin is one of a group of chitin-binding lectins which has commanded extensive attention [3–7]. The lectin has been partially purified by affinity chromatography on fetuin–Sepharose [8], chitin [9], and on an insoluble polysaccharide mixture from *Aspergillus niger* mycelia [10], but has never been obtained free of a contaminating glycoprotein of 32 000 M_r. It has also been isolated on glutaraldehyde-fixed erythrocytes, but recovery of the adsorbed lectin was <1% [11]. In this communication we describe a procedure for the preparation of homogeneous *Datura stramonium* lectin in good yield.

2. Materials and methods

Datura stramonium inermis seeds were obtained from the Botanical Gardens, University of Michigan. Sepharose 4B and Sephadex G-200 superfine were from Pharmacia (Uppsala) and chitin was purchased from Pfanstiehl Labs (Waukegan IL). All chemicals used were of reagent grade or best quality available. Phosphate-buffered saline (PBS) consisted of 0.15 M NaCl, 0.1 mM $CaCl_2$, 0.01 M Na-phosphate (pH 7.0).

2.1. *Hemagglutination procedure*

Hemagglutination assays were conducted using a hemagglutination microtiter plate (Cooke Engineering Co.) and a 3% suspension of type 0 erythrocytes. The degree of agglutination was determined after 1 h at room temperature.

2.2. *Preparation of the affinity absorbant:* p-*Aminobenzyl* N,N′-*diacetyl-β-chitobioside-succinyl-aminohexyl–Sepharose*

Sepharose 4B was activated with cyanogen bromide and coupled to hexanediamine as in [12]. Succinyl-aminohexyl–Sepharose was prepared by treatment of the resin with succinic anhydride in the presence of the water soluble carbodiimide EDC (1-ethyl-3-(3-di-methylaminopropyl)carbodiimide–HCl) [13]. *p*-Nitrophenyl *N,N′*-diacetyl-β-chitobioside, prepared as in [14], was reduced to the corresponding *p*-amino-phenyl-derivative [15], and coupled to the succinyl-aminohexyl–Sepharose linker arm using EDC. Unreacted carboxyl groups were blocked by treatment with ethanolamine in the presence of EDC.

2.3. *Preparation of chitin oligomers*

Oligomers of *N*-acetyl-D-glucosamine were obtained by a modification of the method in [16,17]. The chitin hydrolysate, neutralized with basic lead carbonate, was subjected to charcoal column chromatography. The column was eluted with 5% ethanol to remove *N*-acetyl-D-glucosamine. Subsequent elution with 60% ethanol gave a mixture containing primarily *N,N′*-diacetylchitobiose and *N,N′,N″*-triacetylchitotriose. The mixture of sugars present in this fraction was isolated, and used at varying concentrations to elute *Datura* lectin from the *N,N′*-diacetylchitobioside affinity column.

2.4. *Electrophoresis*

Polyacrylamide gel electrophoresis at pH 4.3 and pH 8.9 was performed as in [18] and SDS gel electrophoresis as in [19]. Lectin subunit M_r was determined by SDS gel electrophoresis as in [20,21], which largely corrects for the irregular behavior exhibited

by carbohydrate containing proteins on SDS gels. Immunoelectrophoresis was performed according to the Gelman instruction manual.

3. Results

3.1. *Lectin extraction*

Finely ground *Datura stramonium inermis* seeds (200 g) were extracted 4 times (1 h) with 500 ml methanol. The orange colored, methanolic solution was decanted and discarded. After the final methanol extraction, the seed meal was filtered on a Buchner funnel, washed with 250 ml dichloromethane, and air dried. All subsequent procedures were carried out at 4°C. Polyvinylpolypyrrolidone (15 g) was added to the dried seeds, and the mixture was extracted by stirring overnight with 700 ml PBS. The extract was centrifuged at 11 000 × *g* for 20 min, and the sediment re-extracted with 500 ml PBS as before. The resulting supernatant solutions, either pooled or worked up separately, were filtered through glass microfiber filter paper and stored until needed.

3.2. *Acetic acid precipitation*

The crude *Datura* extract formed a brown precipitate upon storage for >24 h. This was removed by centrifugation, and the supernatant solution was dialyzed against 3 changes of 0.01 M acetic acid, giving a further brown precipitate. After removal of the precipitate by centrifugation, the lectin extract was dialyzed against PBS.

3.3. *Affinity chromatography*

The *Datura* extract was applied to the *N,N'*-diacetylchitobioside–Sepharose column (1.5 × 7 cm) at 20 ml/h, until the column was saturated. The column was washed with PBS until the absorbance at 280 nm was <0.04. Absorbed lectin was subjected to stepwise elution with *N*-acetyl-D-glucosamine oligomers; single step elution with a high concentration of oligosaccharides resulted in displacement of the lectin from the column in a highly concentrated form, which underwent aggregation. A 1 mg/ml solution of the oligosaccharide mixture in PBS (75 ml) was used as the first eluting buffer. The eluting sugars were increased stepwise to 3 mg/ml and then to 6 mg/ml (75 ml each). A final elution with 0.1 N acetic acid removed any remaining lectin. The results of a typical run are shown in fig.1. Fractions having an absorbance at 280 nm >0.1 were combined and dialyzed against PBS. Generally, trace amounts of the 32 000 M_r contaminant were detected in the lectin fractions eluted with 1 mg/ml chitin oligomers, so these fractions were pooled separately from the latter fractions.

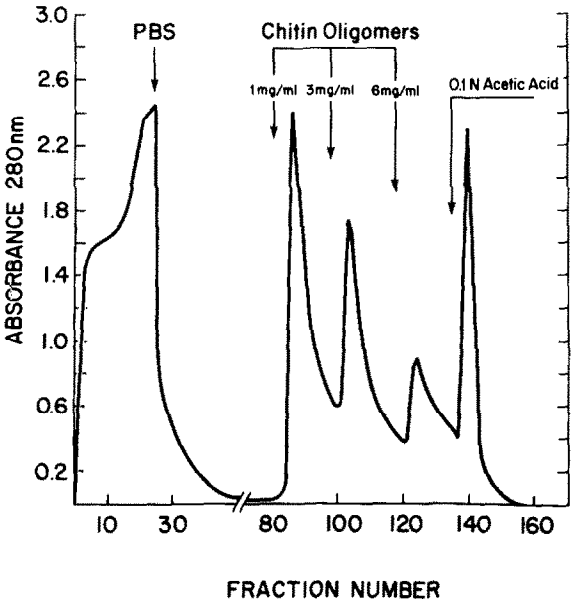

Fig.1. Affinity chromatography of *Datura* extract (300 ml) on *p*-aminobenzyl *N,N'*-diacetylchitobioside–Sepharose column (1.5 × 7 cm). Unbound protein was collected in 12 ml fractions. Lectin was eluted (3 ml fractions) with PBS containing a mixture of chitin oligosaccharides at successive concentrations (→) of 1 mg/ml, 3 mg/ml and 6 mg/ml, and finally with 0.1 N acetic acid (75 ml each). Flow rate: 12 ml/h.

3.4. *Chromatography on Sephadex G-200 superfine*

The trace amount of contaminating glycoprotein present in the affinity-purified lectin fractions was removed by gel filtration on Sephadex G-200 superfine (2.5 cm × 86 cm). The lectin fractions were applied to the G-200 column in 10–12 ml aliquots, at 2.0–2.5 mg/ml. *Datura* lectin, which migrated with app. M_r 120 000, was readily resolved from the contaminating protein. Aggregated lectin, if present, was eluted in the void volume.

The yield of homogeneous lectin was ~200 mg/100 g seeds.

3.5. *Purity*

Lectin purification was monitored by SDS gel

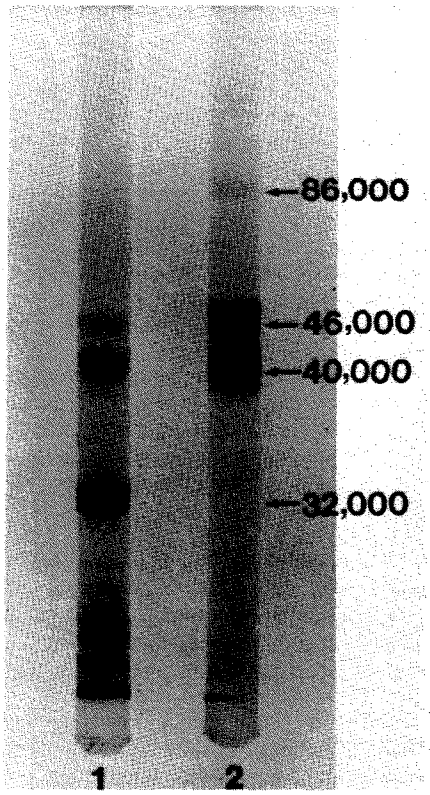

Fig.2. SDS–polyacrylamide gel electrophoresis of *Datura* lectin. The samples were run on 9% gels in the presence of 2-mercaptoethanol: (1) crude extract; (2) purified *Datura* lectin after chromatography on Sephadex G-200.

electrophoresis. In the presence of 2-mercaptoethanol, the *Datura* lectin migrated as 2 bands (M_r 46 000, 40 000), distinct from the contaminating glycoprotein (M_r 32 000). The results of gel electrophoresis of crude *Datura* extract and purified lectin are shown in fig.2. The gel of purified *Datura* was heavily overloaded to demonstrate the complete absence of contaminating protein. The faint band with app. M_r 86 000 corresponds to non-reduced lectin which, when excised from the gel and re-electrophoresed, separated into the same 2 subunit bands. Polyacrylamide gel electrophoresis of *Datura* lectin at pH 4.3 and 8.9 each gave a single protein-staining band. Immunoelectrophoresis with rabbit antibodies raised to the crude seed proteins and the purified lectin provided evidence for a single, homogeneous lectin species.

4. Discussion

Investigators had experienced difficulty in purifying the *Datura* lectin. We have now succeeded in isolating the lectin as a homogeneous glycoprotein in good yield. The success of this purification scheme is due largely to the preferential removal of most of the contaminating 32 000 M_r protein before the affinity chromatographic step. The brown precipitates which formed upon storage of the crude PBS-extract and upon dialysis of the extract against acetic acid were shown to consist primarily of the 32 000 M_r protein. The trace amount of this contaminant which remained in the extract, bound to the chitobiosyl affinity column and was eluted with *Datura* lectin at the lowest concentration of sugar used for lectin displacement. Separation from pure lectin was readily accomplished using gel filtration.

The significance of the 32 000 M_r protein is not yet known. However, based on its amino acid analysis, which is very similar to the purified lectin, we suggest that it may be a fragment of the *Datura* lectin. The 32 000 M_r protein is not mitogenic.

The *Datura* lectin undergoes a concentration-dependent aggregation. For this reason, protein should be kept to $\lesssim$2.5 mg/ml to prevent aggregation. Lyophilization of the lectin also leads to aggregation and results in a partially insoluble preparation. The lectin may be stored in solution at 4°C without loss of activity. The aggregated lectin is active, and its titer against human red blood cells is not significantly different from that of non-aggregated lectin. However, aggregated lectin is a more potent mitogen towards human peripheral blood lymphocytes than the non-aggregated species.

Gel filtration and electrophoresis data indicate that *Datura* lectin is a dimeric protein of M_r 86 000, composed of non-identical subunits (M_r 46 000, 40 000) linked by disulfide bonds. The lectin is composed of ~37% carbohydrate by weight; the predominant constituent sugar is L-arabinose (93%), with smaller amounts of D-galactose (7%). Amino acid analysis reveals a high content of half cystine (16%), glycine (12%), serine (11%) and hydroxyproline (11%) residues. *Datura* lectin shares a sugar binding specificity for β-(1 → 4)-linked oligomers of *N*-acetyl-D-glucosamine with other solanaceous lectins and wheat germ agglutinin, but at least one notable difference has been discovered. *N*-Acetyllactosamine (β-D-Gal-(1 → 4)-D-GlcNAc) is a good inhibitor of *Datura*

lectin, but not of wheat germ agglutinin [22] or potato lectin [23]. The β-(1 → 3) isomer and β-D-Gal-(1 → 4)-D-ManNaC, however, do not bind to the lectin. The *Datura* lectin also reacts to form a specific precipitate with CEA (carcinoembryonic antigen) and asialofetuin.

We are continuing our investigation of the carbohydrate-binding and immunological properties of the purified lectin.

Acknowledgements

We thank Dr Francis Delmotte for assistance in preparing the chitobioside–Sepharose affinity column. This research was supported by NIH research grants AM-10171 and GM 29470.

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