Purification of the Lectin from *Datura stramonium*

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The lectin from *Datura stramonium* can be inhibited by oligomers of *N*-acetylglucosamine. This property was exploited to purify the lectin by affinity chromatography on Sepharose-fetuin. The purified lectin is a glycoprotein having subunits of 40000 and 45000 mol.wt.

Lectins are carbohydrate-binding proteins with the ability to agglutinate erythrocytes and other cells (Sharon & Lis, 1972). Although largely of plant origin, lectins have been used mainly to study animal cell surfaces (Nicolson, 1974), particularly with regard to their ability selectively to agglutinate tumour or virally transformed cells. In addition, lectins have been used to stimulate cell proliferation, to monitor cell-surface changes during development, and to study the organization of the cell surface and its control (Nicolson, 1974).

Recently, a lectin partially purified from seed extracts of *Datura stramonium* has been described (Kilpatrick *et al.*, 1978). This lectin is of particular interest as *D. stramonium* is a member of the family Solanaceae. The majority of known lectins are from the family Leguminosae, and only one lectin, that of the potato tuber, has been purified from a solanaceous plant (Allen & Neuberger, 1973).

The *Datura* lectin can be purified by adsorption on glutaraldehyde-fixed erythrocytes followed by incubation of the cells at 56°C (Yeoman *et al.*, 1978). The recovery of the adsorbed lectin, however, was less than 1%. The development of a more satisfactory means of purification has been hampered by two considerations. The lectin is not inhibited by any of the common monosaccharides (Kilpatrick *et al.*, 1978), so affinity adsorption or affinity chromatography could not readily be carried out. Furthermore, the lectin displays a tendency to aggregate, frustrating attempts to purify it by conventional protein-separation methods. Variable results are obtained when lectin-containing extracts are eluted from gel-filtration columns, and little increase in specific activity results (D. C. Kilpatrick, unpublished observations).

We now report that the *Datura* lectin can be inhibited specifically by oligomers of *N*-acetylglucosamine, and this property has been exploited to allow its purification by affinity chromatography on immobilized fetuin.

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Materials and Methods

Seeds of *Datura stramonium* were obtained from mature fruits of plants grown in the greenhouse of this department. Samples of purified *N*-acetylglucosamine oligomers (dimer, trimer and tetramer) were kindly given by Miss H. Marcan, University of Hull, Hull, U.K. Celite, periodic acid, *N*-acetylglucosamine and fetuin covalently attached to Sepharose 4B were from Sigma (London) Chemical Co., Poole, Dorset BD17 7NH, U.K. Other sugars and Schiff reagent were from BDH Chemicals, Poole, Dorset, U.K.

A crude mixture of oligomers of *N*-acetylglucosamine was prepared as follows. Chitin (5g) from crab shells (Sigma, practical grade) was hydrolysed for 2.5 h at 40°C in conc. HCl, then applied to a column (35 cm × 2.6 cm diam.) of charcoal/Celite as described by Stirling (1974). After a washing with water (1 litre), bound material was eluted with 200 ml of 30% (v/v) ethanol. After evaporating to dryness at 65°C using a rotary evaporator, the residue was redissolved in about 50 ml of water. The mixture of *N*-acetylglucosamine oligomers obtained by this means consists mainly of chitobiose (dimer) and chitotriose (trimer), with traces of higher saccharides (Rupley, 1964). It was found to have reducing activity equivalent to 13 mM-glucose, estimated as described by Ashwell (1957). This preparation was used in the purification of the lectin described below.

The purification of this lectin was carried out as follows. Seeds of *D. stramonium* (5g) were ground in a seed mill and extracted with 100 ml of phosphate-buffered saline (50 mM-sodium phosphate, pH 7.2, containing 0.15 M-NaCl). After extraction by constant stirring overnight at 4°C, the material was strained through a layer of Miracloth (Calbiochem, Bishop's Stortford, Herts., U.K.) and made up to 100 ml with phosphate-buffered saline. The crude cell-free extract obtained was centrifuged at 30000g for 1 h in an MSE 18 centrifuge using an 8 × 50 rotor. The resulting supernatant was incubated at 70°C for 15 min, and, after cooling to 4°C, centrifuged (10000g; 15 min)

again. The supernatant from this second centrifugation step (92ml) was then cooled in an ice bath to 0°C. Acetone (23ml) was slowly added, the mixture being maintained at 0°C. After standing for 10min, the precipitate that formed was collected by centrifugation (10000g; 10min), discarded, and a further 161ml of acetone was added to the supernatant. After centrifugation, the supernatant was discarded, and the precipitate redissolved in 75 ml of phosphate-buffered saline. The solution was dialysed overnight against 5 litres of phosphate-buffered saline at 4°C and then warmed to room temperature. The solution containing lectin was then applied to 5ml of Sepharose-fetuin packed into a 5ml disposable syringe barrel which had been equilibrated with phosphate-buffered saline at room temperature. The flow rate was maintained at 10ml/h. After application of the lectin solution, the column was washed at the same flow rate with phosphate-buffered saline until the A_{278} of the eluate was below 0.02. Finally, the lectin was eluted with 25ml of the mixture of *N*-acetylglucosamine oligomers prepared as described above. The preparation was assayed for protein and lectin activity after dialysis against phosphate-buffered saline (2 litre) for 3 days with buffer changes each morning and evening.

Electrophoresis was carried out on 10% polyacrylamide gels containing 0.1% sodium dodecyl sulphate as described by Weber & Osborn (1969). The gels were stained for protein with Coomassie Brilliant Blue (Weber & Osborn, 1969) and destained with 7% acetic acid. Molecular weights were estimated by reference to a calibration curve constructed by using the following markers (all from Sigma; mol.wts. in parentheses): catalase (60000), ovalbumin (43000), yeast alcohol dehydrogenase (37000), trypsin (23000) and cytochrome *c* (12000). Parallel gels were stained for carbohydrate with the periodic acid/Schiff reagent by the method of Glossman & Neville (1971).

Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma, type V) as standard. Lectin activity was estimated as described by Kilpatrick *et al.* (1978). Activity is taken to be the reciprocal of the titre. Since the volume of lectin solution added to each well of the agglutination trays is 25 μ l, a titre of 1/16, for example, would be 16 units/25 μ l or 640 units/ml. Specific lectin activity is defined as the activity in such units per mg of protein.

Results

The *Datura* lectin is not inhibited by any of the component monosaccharides of erythrocyte glycoconjugates at concentrations up to 30mM (Kilpatrick *et al.*, 1978). The search for saccharide inhibitors was therefore extended to available disaccharides and

higher saccharides (Table 1). Oligomers of *N*-acetylglucosamine were strongly inhibitory, and the potency of the inhibition increased with chain length, the tetramer producing the same degree of inhibition as the dimer at a concentration one-tenth as great. Sucrose, cellobiose and raffinose had no effect on the lectin's activity.

A crude mixture of *N*-acetylglucosamine oligomers was prepared by controlled hydrolysis of chitin. This preparation was found to be highly inhibitory to the *Datura* lectin. At a concentration with the reducing ability equivalent to 1mM-glucose determined by the Nelson method (Ashwell, 1957), lectin activity could be decreased from a titre of 1/128 to 1 (i.e. >99% inhibition).

This specific inhibition was exploited in the purification of the lectin using fetuin covalently attached to Sepharose 4B as an affinity resin (Table 2). A crude extract of *D. stramonium* seeds was first partially purified by centrifugation, heat clarification and fractional acetone precipitation. The lectin-containing solution was then applied to a column of immobilized fetuin and washed extensively with phosphate-buffered saline. Finally, the lectin was eluted from the column using the crude preparation of *N*-acetylglucosamine oligomers. An overall increase in the purification ratio of over 30-fold was obtained with a 50% recovery of the starting material.

The purified lectin was subjected to electrophoresis in 10% polyacrylamide gels containing sodium dodecyl sulphate after treatment with mercaptoethanol. Two protein bands were detected with mobilities corresponding to mol.wts. of 40000 and 45000. Parallel gels were examined for carbohydrate by using the periodic acid/Schiff reagent stain.

Table 1. Effect of higher saccharides on *Datura* lectin activity

N-Acetylglucosamine (GlcNAc), oligomers of *N*-acetylglucosamine (GlcNAc)₂₋₄ and the other sugars were added to the standard agglutination assay and the final concentration required to halve the titre was determined. *N*-Acetylglucosamine, cellobiose and sucrose were not tested at final concentration above 30mM, nor raffinose at a final concentration above 15mM. For further details, see the Materials and Methods section.

Sugar	Concentration for 50% inhibition of agglutination (mM)
GlcNAc	>30
(GlcNAc) ₂	2.0
(GlcNAc) ₃	1.0
(GlcNAc) ₄	0.2
Cellobiose	>30
Sucrose	>30
Raffinose	>15

Table 2. *Purification of the lectin from D. stramonium*

The crude extract was the 100ml of homogenate that had been strained through Miracloth. The purification is taken as the ratio of specific activity to that of the crude extract. For further details, see the Materials and Methods section.

Step	$10^{-3} \times$ Activity (units)	$10^{-3} \times$ Specific activity (units/mg of protein)	Purification (fold)
Crude extract	1024	1.20	1
Centrifugation and heat clarification	942.1	3.02	2.52
Acetone precipitation	768	8.50	7.08
Sepharose-fetuin chromatography	512	40.96	34.13

Two periodic acid/Schiff-positive bands were observed with mobilities corresponding to the protein bands. Heavily loaded gels displayed in addition a minor protein band (mol.wt. 32000), which was also periodic acid/Schiff-positive.

The purified lectin preparation was also subjected to double diffusion (Ouchterlony, 1958) against rabbit antiserum to the *Datura* lectin prepared by affinity adsorption on fixed erythrocytes (Yeoman *et al.*, 1978). A single precipitation line was obtained, which formed a line of identity with the lectin preparation used as antigen to prepare the antiserum. Neither lectin preparation reacted with non-immune rabbit serum.

Discussion

A simple and rapid method has been described for purifying the lectin from *D. stramonium*. The recovery is of the order of 100-fold greater than that obtained by affinity adsorption followed by non-specific elution by heating the cells (Yeoman *et al.*, 1978). The lectins obtained by both methods are apparently the same protein, since they have polypeptide subunits of identical mobilities and are not antigenically distinct. It is noteworthy that Sela *et al.* (1975) have suggested that lectins generally can be eluted from fetuin-Sepharose with 0.5M-NaCl/50mM-glycine/HCl, pH 3.0, in substantial yield, but we were able to liberate only 0.25% of the *Datura* lectin bound to fetuin by that means.

The purified *Datura* lectin exhibited two major protein bands after electrophoresis, both of which had associated carbohydrate. In this regard, the *Datura* agglutinin is typical of lectins, which are usually glycoproteins (Sharon & Lis, 1972). When the lectin was prepared from erythrocytes (Yeoman *et al.*, 1978), although two protein bands were obtained after electrophoresis in the presence of

sodium dodecyl sulphate, a single band was obtained if the detergent was omitted from the system. It is therefore assumed that the two major bands reported here also represent subunits, although the possibility that one of the bands represents a non-lectin protein has not been excluded. The presence of a third glycopeptide band may indicate the existence of a minor subunit of the lectin, but more probably is due to an unrelated protein contaminating the preparation.

It is interesting to compare the *Datura* lectin with that of the potato, *Solanum tuberosum*. Both lectins are glycoproteins and are inhibited by *N*-acetylglucosamine oligomers, but not by the monosaccharide itself. It is possible the two proteins are closely related and/or fulfil a similar function.

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