Isolation of a lectin from the pericarp of potato (*Solanum tuberosum*) fruits

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A lectin has been isolated from potato (*Solanum tuberosum*) fruits by affinity adsorption on to glutaraldehyde-fixed erythrocytes and elution with oligomers of *N*-acetylglucosamine. The characteristics of the lectin resemble those of the potato tuber lectin and other closely related lectins from solanaceous plants.

Lectins are carbohydrate-binding cell-agglutinating proteins occurring widely in both plant and animal kingdoms (Goldstein *et al.*, 1980). The lectin in potato (*Solanum tuberosum*) tubers was first described by Marcusson-Begun (1926) and has since been the subject of detailed studies (Marinkovich, 1964; Allen & Neuberger, 1973; Allen *et al.*, 1978). Recently, two lectins differing in saccharide specificity have been detected in crude homogenates of potato fruits, one occurring in the seeds, the other in the pericarp and juice within the pericarp (Kilpatrick *et al.*, 1980). Here the isolation of the potato pericarp lectin is reported and some properties of the purified glycoprotein are described.

Materials and methods

Potato fruits were obtained from the Scottish Plant Breeding Station, Roslin, Midlothian, Scotland, U.K. *NN'*-Diacetylchitobiose and *NN'N''*-triacylchitotriose were prepared by the method of Rupley (1964). *N*-Acetylglucosamine and chitin were obtained from Sigma (London) Chemical Co., and cellobiose from BDH Chemicals.

The potato pericarp lectin was purified as follows, all operations being carried out at 4°C unless otherwise stated. Potato fruits (250 g) were chopped into small pieces with scalpel blades. The seeds were separated out by repeated gentle centrifugation combined with removal of contaminating non-seed tissue with forceps. The seedless fruit material was then mixed with 300 ml of 10 mM-sodium phosphate, pH 7.2, containing 0.9% NaCl and 0.05% Na₂S₂O₅ (sodium metabisulphite). The mixture was pounded with a pestle in a plastic beaker, then left overnight with stirring. The homogenate obtained was strained through Miracloth, then centrifuged at 40 000 g for 1 h. The soluble fraction of the homogenate obtained was then added to 15 ml (packed

volume) of glutaraldehyde-fixed (Kilpatrick *et al.*, 1979) human blood-group-B erythrocytes. The cells were gently suspended in the homogenate and maintained in suspension by very gentle stirring every 5 min. After 30 min, the cells were collected by centrifugation (1000 g, 5 min) and washed four times with a large excess of 0.9% NaCl/10 mM-sodium phosphate, pH 7.2. The lectin was eluted by suspending the washed cells in 30 ml of a solution of mixed *N*-acetylglucosamine oligomers prepared as previously described (Kilpatrick & Yeoman, 1978) for 15 min at room temperature. After removal of erythrocytes by centrifugation, the lectin preparation was dialysed against 4 litres of 0.9% NaCl (adjusted to pH 7 with Na₂HPO₄) for 48 h, with a buffer change after 24 h. It was further dialysed against 4 litres of 10 mM-NaCl, pH 7, for 24 h, and finally against 50 mM-NaCl, pH 7, for a further 24 h. Insoluble material was removed from the preparation by centrifugation at 30 000 g for 15 min.

Electrophoresis was carried out by the method of Weber & Osborn (1969). The following proteins were used as molecular-weight markers (assumed subunit molecular weight in parentheses): thyroglobulin (330 000), transferrin (77 000), bovine serum albumin (67 000), immunoglobulin G (50 000 and 23 500), ovalbumin (43 000) and cytochrome *c* (12 000). The gels were stained for protein with Coomassie Brilliant Blue (Weber & Osborn, 1969) or for carbohydrate as described by Glossman & Neville (1971), the method being modified by the use of Basic Fuchsin in acid ethanol (Borzynski *et al.*, 1972) instead of Schiff's reagent.

Chitinase activity was assayed by incubating material to be tested (0.2 ml) with a suspension of crab-shell chitin (20 mg) in 0.8 ml of either 0.15 M-NaCl/50 mM-sodium phosphate, pH 7.2, or 0.1 M-sodium acetate adjusted to pH 5.0 with acetic acid, for 40 h at room temperature on a rotating shaker.

Controls consisted of test material preincubated at 100°C for 10 min and subjected to identical conditions. Chitin and any other insoluble material was removed by centrifugation (10 000g, 20 min) and the supernatants were tested for *N*-acetylglucosamine by the Morgan–Elson (1934) method or for oligomers of *N*-acetylglucosamine by ability to inhibit purified *Datura* (thorn-apple) lectin (Kilpatrick & Yeoman, 1978).

Assays for protein, carbohydrate and lectin activity, and the conditions used for periodate oxidation and immunodiffusion, where the same as previously described (Kilpatrick, 1980).

Results and discussion

Lectin activity sensitive to oligomers of *N*-acetylglucosamine occurs in both the juice and the solid tissue of potato fruits and differs from the seed lectin in antigenic properties and saccharide specificity (Kilpatrick *et al.*, 1980). The seeds were therefore removed from fruits before the latter were homogenized. Particulate matter was first removed by centrifugation before the pericarp lectin was isolated by adsorption on to fixed erythrocytes, followed by elution with oligomers of *N*-acetylglucosamine. From 250 g of potato fruits (from which 1.12 g of soluble protein was extracted), 1.3 mg of purified lectin protein was obtained. The purified preparation had a specific activity of 0.1×10^6 units/mg of protein, representing a 145-fold increase over that of the soluble fraction of the homogenate, with a recovery of 17%. It yielded a single band after electrophoresis in the presence of sodium dodecyl sulphate, whether stained for protein or carbohydrate. It reacted with antiserum raised against *Datura* lectin, forming a line of partial identity with purified *Datura* lectin placed in an adjacent well, but did not react with non-immune rabbit serum.

This essentially one-step procedure was not equally applicable to isolation of the lectin from potato tubers. When grated potato tubers were treated in a manner identical with that used for chopped pericarp tissue, a preparation heterogeneous by electrophoretic analysis was obtained. After electrophoresis in 10% (w/v) polyacrylamide gel containing sodium dodecyl sulphate, a protein band corresponding to an apparent mol.wt. 100 000 [presumed to represent the lectin, since Allen & Neuberger (1973) obtained a similar value] was obtained, but the most intensely stained protein band was of unknown identity (apparent mol.wt. 15 000).

The pericarp lectin was equally active towards human blood-group-O or -B erythrocytes and showed no preference for fresh or glutaraldehyde-fixed cells. All could be agglutinated at a protein concentration of about 0.2 µg/ml under standard

assay conditions. As little as 0.1 µg of lectin protein/ml could be detected, however, when trypsin-treated cells were used.

The pericarp lectin was estimated to contain 47% carbohydrate, based on determinations using glucose as standard. The apparent molecular weight of the lectin in 10% polyacrylamide gels containing sodium dodecyl sulphate was estimated to be 80 000. A similar value was obtained whether or not mercaptoethanol was included in the system, indicating that the species detected was a true monomer and not a combination of subunits linked by disulphide bonds. When the lectin was subjected to electrophoresis in 20% acrylamide, however, the apparent molecular weight was estimated to be only 60 000. A change in mobility relative to protein standards as a function of acrylamide concentration is typical of glycoproteins (Segrest & Jackson, 1972) and is entirely consistent with a high carbohydrate content. A similar high saccharide composition and a relatively low electrophoretic mobility are also characteristics of potato tuber (Allen & Neuberger, 1973) and tomato (*Lycopersicon esculentum*) fruit (Kilpatrick, 1980) lectins. All differ from the *Datura* lectin, however; the latter exhibits three protein or carbohydrate bands after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Horejsi & Kocourek, 1978; Kilpatrick & Yeoman, 1978).

Some further properties of the potato pericarp lectin were studied and found to be very similar to those of the potato tuber, tomato fruit and *Datura* seed lectins. The saccharide specificity was studied in more detail (Table 1) and was found to be similar to the abovementioned lectins; the lectin was twice as susceptible to *NN'*-triacylchitotriose as to *NN'*-diacylchitobiose. The effect of various treatments on the pericarp lectin are summarized in Table 2. Lectin activity was stable at 75°C (and pH 7.2) for at least 30 min, but was rapidly destroyed by boiling. It was decreased on incubation with 0.2% Pronase for 2 h at 37°C, and abolished by overnight incubation with 1% sodium metaperiodate. The latter result might be an indication that the carbohydrate moiety is essential for activity, but it is possible that the periodate inactivates the lectin by

Table 1. *Saccharide specificity of potato pericarp lectin*
N-Acetylglucosamine (GlcNAc) or its dimer [(GlcNAc)₂] or trimer [(GlcNAc)₃] was added to the standard agglutination assay and the concentration required to halve the titre noted. For further details see the Materials and methods section.

Sugar	Concn. for 50% maximal activity (mM)
GlcNAc	>33
(GlcNAc) ₂	1.0
(GlcNAc) ₃	0.4

Table 2. *Effect of various treatments on potato pericarp lectin activity*

A suitable dilution of lectin in 0.15 M-NaCl/0.05 M-sodium phosphate, pH 7.2, was treated under the conditions indicated, then assayed, or assayed in the presence of the chelating agents listed. Results are expressed as a percentage of the appropriate control. For further details, see the Materials and methods section.

Treatment	% of control activity
30 min at 65°C	100
30 min at 75°C	100
30 min at 80°C	18
15 min at 85°C	18
5 min at 100°C	0
2 h at 37°C with Pronase (0.2%)	25
16 h at 4°C with NaIO ₄ (1%)	0
Na ₂ EDTA (2 mM)	100
Trisodium citrate (33 mM)	100

oxidizing amino acid residues on the polypeptide chain (Biroc & Etzler, 1978). Lectin activity was not decreased when assayed in the presence of the chelating agents disodium EDTA (2 mM) or trisodium citrate (33 mM).

It is noteworthy that potato lectins expressed in such morphologically dissimilar tissues as pericarp and tuber are similar, and that they both differ from the lectin found in the seeds of the same species. Both are, in part, storage tissues, and it is possible the lectin functions in some process concerned with the storage, maintenance or utilization of food of food reserves. Another possibility is that the lectin has a defence function. Mirelman *et al.* (1975) have suggested that wheat-germ (*Triticum*) agglutinin might protect plants against chitin-containing phytopathogens. The pericarp and tuber potato lectins have similar saccharide specificity to wheat-germ agglutinin and might also be able to inhibit fungal growth.

The lectin from mung beans (*Phaseolus aureus*) possesses α -mannosidase activity, and it has been suggested that lectins in general might be glycosidic enzymes (Hankins & Shannon, 1978; Hankins *et al.*, 1979). This possibility was investigated by assaying the potato pericarp lectin for chitinase activity. Purified lectin was incubated with chitin for 40 h, and, after removal of particulate material, the supernatant was examined for chitin breakdown products. Neither exochitinase (*N*-acetylglucosamine-monomer-producing) or endochitinase (*N*-acetylglucosamine-oligomer-producing) activity could be detected. The soluble homogenate of pericarp was also assayed in a similar manner before and after adsorption with glutaraldehyde-fixed erythrocytes. Some exochitinase activity (corre-

sponding to the release of 100 μ l of *N*-acetylglucosamine/ml at pH 5 under standard assay conditions) was detected. Over 70% of the original activity was also present in the erythrocyte-treated homogenate, although all of the lectin activity had been removed. No endochitinase activity could be detected before or after treatment with fixed erythrocytes. These observations lend no support to the hypothesis that lectins generally are active hydrolytic enzymes, but are not inconsistent with the speculation that lectins might be inactive precursors of glycosidases. Another example of non-association of glycosidic and lectin activity in solanaceous plants is provided by the stem tissue of *Datura* or tomato. Stems of both are rich sources of chitinase activity (D. C. Kilpatrick, unpublished work), but contain only traces of lectin (Kilpatrick *et al.*, 1979; Kilpatrick, 1980).

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