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The production and properties of an antiserum to potato (*Solanum tuberosum*) lectin

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Precipitation of potato (*Solanum tuberosum*) lectin by antisera was not affected by treatments that abolish lectin activity. An antiserum precipitated glycosylated derivatives of the lectin but not a deglycosylated peptide. The haemagglutination inhibition titre of this antiserum was not affected by removing anti-glycopeptide antibodies. This evidence suggests that the antiserum contains two populations of antibodies, specific for different domains of the lectin.

Plant lectins have long been used as tools in immunology (Lis & Sharon, 1977), but it is only relatively recently that they themselves have been studied by immunological techniques (Nachbar & Oppenheim, 1973; Talbot & Etzler, 1978; Howard *et al.*, 1979; Hankins *et al.*, 1979; Kilpatrick *et al.*, 1980; Borrebaeck & Etzler, 1981). In the present paper we describe the production of antisera to potato (*Solanum tuberosum*) lectin and investigations of the interactions of one of these sera with the lectin molecule.

Potato lectin is a doubly unusual glycoprotein; firstly, it contains large amounts of hydroxyproline and arabinose, and secondly, these constituents are localized in a single area of the molecule (Allen *et al.*, 1978). The arabinose is mainly present as tri- and tetra-arabinofuranosides linked *O*-glycosidically to hydroxyproline (Allen *et al.*, 1978; Muray & Northcote, 1978). These hydroxyprolyl oligo-arabinosides are also found in the cell wall of plants (for reviews, see Lamport, 1977, 1980). The usefulness of potato lectin as a soluble model of insoluble plant cell-wall glycoproteins, discussed by Ashford & Neuberger (1980), has been greatly increased by two recent findings. Firstly, we have found (Ashford *et al.*, 1982) that the carbohydrate moieties of potato lectin are identical with those obtained from plant cell-wall glycoproteins (Akiyama *et al.*, 1980). Secondly, Owens & Northcote (1981) have shown that deglycosylated potato lectin is able to act as an acceptor of arabinose in studies *in vitro* of the biosynthesis of arabinose-containing proteins, such as those found in the plant cell wall. A preliminary communication containing some of the data presented here has been published (Ashford *et al.*, 1980).

Experimental

Materials

Potato lectin and *NN'*-diacetylchitobiose were prepared as described previously (Desai & Allen, 1979). Modified potato lectin and defined glycopeptides were prepared by the procedure of Allen *et al.* (1978). Wheat-germ agglutinin and marrow (*Cucurbita pepo*) lectin were prepared as described by Allen *et al.* (1973) and Allen (1979) respectively. Thorn-apple (*Datura stramonium*) lectin (Desai *et al.*, 1981) was a gift of Dr. N. N. Desai of this laboratory. Tomato (*Lycopersicon esculentum*) lectin (Nachbar *et al.*, 1980) was a gift of Dr. M. S. Nachbar of the Department of Microbiology, School of Medicine, New York University Medical Center, New York, NY, U.S.A. Arabinogalactan, gum arabic, polyhydroxyproline and agarose Type II were obtained from Sigma (London) Chemical Co. Ltd. (Poole, Dorset, U.K.). Arabinan was from Koch–Light Laboratories Ltd. (Colnbrook, Bucks, U.K.). Specified varieties of potato were gifts of Dr. M. Adams of Rothamsted Experimental Station, Harpenden, Herts., U.K.

Methods

Preparation of antiserum to potato lectin. New Zealand White rabbits were immunized by multiple-site intradermal injection of potato (variety 'King Edward') lectin emulsified with Freund's complete adjuvant for the first dose and subsequently with incomplete adjuvant. Antibody production was monitored by immunodiffusion tests.

Immunodiffusion tests. Double immunodiffusion was carried out in 1% (w/v) agarose gel, containing 0.15 M-NaCl, cast to a depth of 1.5 mm on glass

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slides. Wells of 3mm diameter were cut in the gel for antigen and wells of 4 or 5mm diameter for antiserum. Diffusion was allowed to take place for 24–36h at 4°C in humidity chambers. The gels were washed with 0.15M-NaCl and distilled water, dried and stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in aq. 45% (v/v) ethanol containing 10% (v/v) acetic acid.

Micro complement-fixation tests. The procedure used was that of Levine (1973), except that half volumes were used in all cases to give a final volume of 3.5ml. Haemolysis was determined spectrophotometrically at 413nm. Complement-fixation titres are expressed as the reciprocal of the dilution of antiserum giving 50% complement fixation in the presence of 0.05μg of potato lectin, after taking into account the fixation of the serum in the absence of lectin. Potato lectin alone fixed very little complement (3.5%) compared with the sera being tested.

Haemagglutination and haemagglutination inhibition. Haemagglutination was estimated visually after 1h at room temperature with rabbit erythrocytes (Allen *et al.*, 1976). Inhibition of agglutination was determined by using 10 haemagglutination units (1μg) of the lectin. Haemagglutination units and specific activity of the lectin are as defined by Allen & Neuberger (1973). The reciprocal of the dilution of serum required to decrease haemagglutination by 50% was taken as the inhibition titre of that serum.

Results

Characterization of antisera

After a single dose of potato lectin (0.5mg), three out of four rabbits had produced antibodies that gave a positive reaction in immunodiffusion tests with potato lectin. Serum from rabbit 238 was able to fix more complement in the presence of potato lectin than in its absence. Sera from rabbits 238, D-1 and D-2 were also able to inhibit agglutination of rabbit erythrocytes by the lectin (see Table 1). Rabbit-237 serum gave no significant reaction in any of the tests and neither did control sera taken before immunization from rabbits 238, D-1 and D-2. Repeated injection of antigen into rabbit 237 did not elicit an immunological response. Re-immunization of rabbits 238, D-1 and D-2 gave sera with stronger precipitation and greater inhibition of agglutination for all three sera and greater complement-fixing activity for serum 238 (see Table 1). Precipitation of the lectin was not abolished by treatments that totally remove haemagglutinating activity, such as heating the lectin at 100°C for 10min, reduction of the lectin with dithiothreitol and subsequent carboxymethylation of thiol groups, nor is precipitation prevented by the presence in the gel (5mM) of *NN'*-diacetylchitobiose (an inhibitor of the lectin). Immunodiffusion tests showed that the antibodies to potato lectin in serum 238 did not cross-react with any proteins other than the lectins that were present in aqueous extracts of potato tubers. In addition, if potato lectin was removed from the extracts by affinity chromatography (Desai & Allen, 1979), no precipitation line was seen. Antiserum 238 originally raised against 'King Edward'-potato lectin gave a line of identity with potato lectin in tuber extracts of all varieties of potato tested and with lectin purified from tubers of the variety 'Ulster Sceptre'. Also, the intensity of immunoprecipitation with the extract of a particular variety of potato gave a good correlation with the

Table 1. *Haemagglutination inhibition and complement-fixation titres of rabbit sera before and after immunization with potato lectin*

Sera from four rabbits were tested for their ability to inhibit agglutination of rabbit erythrocytes by potato lectin and to fix complement in the presence of potato lectin. Details of methods and definitions of end points of titrations are given in the text under 'Methods'. Abbreviation used: n.d., not determined.

Rabbit no.	No. of doses of potato lectin	Haemagglutination-inhibition titre	Complement-fixation titre
237	0	0*	30
	1	0	120
	3	1	100
238	0	0	60
	1	2	240
	2	8	400
D-1	0	0	n.d.
	1	8	n.d.
	2	32	n.d.
D-2	0	0	n.d.
	1	4	n.d.
	3	8	n.d.

* No inhibition of agglutination even with undiluted serum.

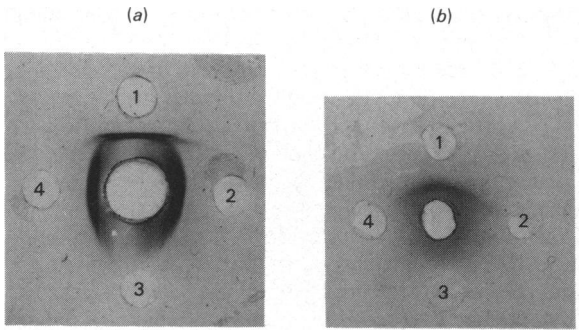

Fig. 1. *Immunodiffusion of potato lectin and its derivatives against anti-(potato lectin) serum*

(*a*) Centre well, rabbit serum 238, 20 μl; well 1, potato lectin (1 μg); well 2, potato lectin glycopeptide (10 μg); 3, de-arabinosylated potato lectin glycopeptide (20 μg); 4, *S*-carboxymethylcysteinyl potato lectin (10 μg). (*b*) Centre well, rabbit serum 238 from which antibodies reacting with the glycopeptide portion of potato lectin have been removed by precipitation with excess (2 mg/ml of serum) potato-lectin glycopeptide (15 μl); wells 1–4 as in (*a*). The reactants were allowed to diffuse for 36 h at 4°C and then washed and stained as described in the Experimental section.

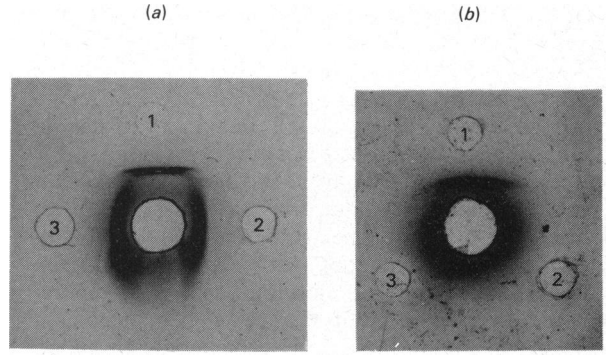

Fig. 2. *Cross-reaction of anti-(potato lectin) serum with other solanaceous lectins*

(*a*) Centre well, rabbit serum 238, 20 μl; well 1, potato lectin (1 μg); well 2, *Datura stramonium* lectin (10 μg); well 3, tomato lectin (10 μg). (*b*) Centre well, rabbit serum 238 from which antibodies reacting with the glycopeptide portion of potato lectin have been removed by precipitation with excess (2 mg/ml of serum) potato-lectin glycopeptide (20 μl); wells 1–3 as in (*a*). The reactants were allowed to diffuse for 24 h at 4°C and then washed and stained as described in the Experimental section.

lectin content determined by haemagglutination assays.

Interaction of anti-(potato lectin) serum 238 with modified potato lectin and its glycopeptides

As mentioned above, potato lectin antiserum 238 cross-reacted with *S*-carboxymethylcysteinyl-potato lectin, as did sera D-1 and D-2. Serum 238 also reacted with the glycopeptide derived from potato lectin by Pronase digestion (Allen *et al.*, 1978), but not with the glycopeptide from which all the arabinose had been removed by acid treatment (Allen *et al.*, 1978). As Fig. 1(*a*) shows, both reacting molecules give precipitation lines that fuse with one of the potato lectin lines, indicating partial identity. When antibodies reacting with the glycopeptide were removed from serum 238 by precipitation with excess glycopeptide (2 mg/ml of serum) the remaining antibodies were still able to react with the whole lectin, but no longer reacted with the glycopeptide or with *S*-carboxymethylated lectin (Fig. 1*b*). It is also worth noting that the antibodies remaining in the supernatant after the antiserum had been precipitated with glycopeptide inhibited haemagglutination by the lectin and that no change in the inhibition titre of the serum was observed.

Cross-reaction of serum 238 with other molecules

Potato lectin antiserum 238 gave a positive reaction in immunodiffusion tests with the lectins from both *Datura stramonium* seeds and tomato fruit juice (see Fig. 2*a*), but the serum did not inhibit agglutination of erythrocytes by *Datura* lectin and gave only a slight inhibition of haemagglutination with tomato lectin. The cross-reaction in immunodiffusion disappeared when these lectins were tested with serum 238 from which antibodies that precipitated the glycopeptide had been removed (Fig. 2*b*). The whole antiserum gave no reaction with other proteins with similar carbohydrate-binding specificity, such as wheat-germ agglutinin, marrow lectin and hen's-egg-white lysozyme. Neither did it react with polyhydroxyproline, nor with the arabinose-containing polysaccharides arabinan, arabinogalactan and gum arabic.

Discussion

Sera from three out of four rabbits immunized with potato lectin interacted specifically with this glycoprotein. The possibility that this interaction was a lectin–glycoprotein interaction rather than an antibody–antigen reaction was considered, but was rejected for the following reasons. Firstly, the lectin does not give any reaction with control sera taken before immunization. In addition, serum from rabbit 237, which was injected with potato lectin, did not react, even though raised levels of immunoglobulins could be demonstrated by immunoelectrophoresis (results not shown). Secondly, the presence of a

strong inhibitor of the lectin activity, *NN'*-diacetylchitobiose, did not inhibit the reaction between the sera and the lectin. Thirdly, treatments that remove lectin activity, such as heat-denaturation or reduction of disulphide bonds followed by *S*-alkylation, did not abolish the precipitation with the sera.

In aqueous extracts of potato tubers, only potato lectin reacted with the antisera; when the lectin was removed from the extracts by affinity chromatography, no precipitation with the sera was seen. This indicates that no molecules with similar structures to potato lectin are present in significant amounts among the soluble proteins of the potato tuber. There seem to be no obvious varietal differences in the lectin, and antibodies raised to 'King Edward'-potato lectin react in the same manner with lectin purified from the 'Ulster Sceptre' variety. The intensity of the precipitation with antiserum 238 of an extract of each variety paralleled the haemagglutinating activity of that extract.

In immunodiffusion a double line is seen between potato lectin and serum 238 (Fig. 1*a*). This suggests that there are two populations of antibodies of differing concentrations and specificities. This suggestion is confirmed by the finding that if the potato lectin glycopeptide is added to serum 238 and the resulting precipitate removed, the supernatant still forms a precipitation line with native lectin but not with the *S*-carboxymethylated lectin (Fig. 1*b*). This indicates that a separate population of antibodies is recognizing the non-glycosylated regions of the lectin. The conformation of these regions is disrupted by reduction and *S*-alkylation and so they are no longer capable of reacting with these antibodies. The cross-reaction of the modified lectin with the whole serum is therefore due solely to the glycosylated region, the structure of which is identical with that of the glycopeptide (Allen *et al.*, 1978). The whole antiserum also inhibits haemagglutination by the lectin and therefore some antibodies must bind close to, or at, the carbohydrate-binding site of the lectin; which was proposed by Allen *et al.* (1978) to lie in the non-glycosylated domain(s). This is supported by the findings reported in the present paper that, if antibodies to the glycopeptide moiety are removed from the antiserum, it still maintains its ability to inhibit agglutination, with no observed change in the inhibition titre. These findings also support the idea of two populations of antibodies to potato lectin being present in serum 238. Cross-reaction is seen between anti-(potato lectin) serum and *Datura* and tomato lectins (Fig. 2*a*). These lectins have close structural and compositional similarities to potato lectin, particularly in their glycopeptide domains, and a similar, but not identical, carbohydrate-binding specificity (Hořejši & Kocourek, 1978; Nachbar *et al.*, 1980; Desai *et al.*, 1981). *Datura* and *Lycopersicon* are both genera of the family Solanaceae, as is *Solanum*, so these similarities are not altogether surprising. In Fig. 2(*b*) it can be seen that precipitation of serum 238 with potato-lectin glycopeptide removes those antibodies that cross-react with *Datura* and tomato lectins, indicating that their cross-reaction is due to antibodies directed against the glycopeptide portion of these lectins and that their non-glycosylated regions differ sufficiently from that of potato lectin so that no precipitation is seen. This conjecture is supported by the finding that serum 238 was unable to inhibit haemagglutination mediated by *Datura* lectin and only slightly inhibited haemagglutination by tomato lectin. As both *Datura* and tomato lectins have a higher affinity for glycoproteins that potato lectin (Desai *et al.*, 1981; Nachbar *et al.*, 1980), this also demonstrates that the inhibition of potato lectin is due to antibodies binding to the lectin, preventing binding of the lectin to erythrocyte glycoproteins, rather than serum glycoproteins inhibiting haemagglutination.

Kilpatrick *et al.* (1980) have demonstrated the immunological similarities of solanaceous lectins using an anti-(*Datura* lectin) serum, but did not characterize the cross-reacting structures. We would suggest that the glycosylated domains and their arabinose chains are the major structures responsible for the cross-reaction between these lectins Hankins *et al.* (1979) and Howard *et al.* (1979) also report immunological similarities between leguminous lectins. Thus from the present results and those reported previously it would seem that close structural similarities exist among these families of lectins.

Neither polyhydroxyproline nor the acid-treated glycopeptide of potato lectin gave a precipitation line with serum 238, indicating that the population of antibodies that reacts with the intact glycopeptide is reacting with the oligoarabinose chains. However, the antiserum does not react with the arabinose-containing polysaccharides arabinan, arabinogalactan and gum arabic. This suggests that the configuration of the linkages and/or arrangement of the arabinose chains in potato lectin are important in their interaction with the antiserum. These chains have recently been fully characterized (Ashford *et al.*, 1982) and were shown to be exclusively arabinofuranosides, with triasaccharides joined by $\beta 1 \rightarrow 2$ linkages, and tetrasaccharides, where the trisaccharide has been substituted with a terminal $\alpha 1 \rightarrow 3$-linked arabinofuranose. The polysaccharides tested contain almost exclusively α-linked arabinose residues, so the lack of cross reaction is not surprising. The oligoarabinofuranoside chains of potato lectin have also been shown to be identical with those from plant cell-wall glycoproteins (Ashford *et al.*, 1982). Thus if the antibody population directed towards the glycosylated region

is specific for these structures, the anti-glycosyl antibodies can be used to detect oligoarabinosides in plant cell-wall glycoproteins both in the cell wall and as their soluble precursors. They can also be used to monitor the progress of arabinosylation of glycoproteins as they are synthesized.

In conclusion it can be seen that the properties of the antiserum to potato lectin described in the present paper can be explained by the fact that the serum contains two populations of antibodies reacting with distinctly different parts of the molecule. One population reacts with the non-glycosylated portion that contains the carbohydrate-binding site(s) and another reacts with the arabinofuranose chains of the glycopeptide region. These antibodies can be used as unique probes of the structure of potato lectin and of other arabinose-containing glycoproteins.

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