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OLIGOARABINOSIDES OF HYDROXYPROLINE ISOLATED FROM POTATO LECTIN

ROGER H. A. MURAY and D. H. NORTHCOTE
Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U.K.

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; lectin; hydroxyproline; arabinose: glycoprotein; cell-wall; membranes.

Abstract—The lectin from potato tubers is a glycoprotein containing 50% sugars and rich in hydroxyproline and arabinose moieties. The nature of the protein–sugar linkage has been compared to that of insoluble potato cell wall protein and the arabinose was shown to exist as short oligosaccharides of 3 or 4 residues attached to hydroxyproline. In the lectin there were no large oligosaccharides attached to hydroxyproline. Lectin activity with the same specificity as that of the tuber lectin was shown to be associated with particulate membrane fractions prepared from cultured potato roots.

INTRODUCTION

In 1950 hydroxyproline was noted in hydrolysates of plant tissue [1] and in 1960 cell fractionation studies on sycamore [2] and tobacco [3] plant tissue cultures showed that hydroxyprolines was a significant cell wall constituent. Some radioautographic experiments in the 1960s failed to confirm this localization in the cell wall [4, 5] but the results were not relevant since the [3,4^{3}H]L-proline used loses a large proportion to its tritium in the enzymatic transformation of peptidyl proline to peptidyl hydroxyproline [6, 7]. Experiments using randomly tritiated L-proline and in which cytoplasm was plasmolysed away from cell walls after labelling, indicated that both in carrot phloem explants [8] and in plant tissue cultures [9] the hydroxyproline was mainly in the walls.

Alkaline degradation of the cell walls of several plant tissue cultures demonstrated that most of the hydroxyproline is glycosylated, most frequently by arabinose-containing oligosaccharides [10]. Glycosylated hydroxyproline was detected in alkaline hydrolysates of a very wide range of higher and lower plant tissues [11] so that hydroxyproline-rich glycoprotein is probably a component of all higher plant and many lower plant cell walls.

It has become clear that a glycoprotein in which hydroxyproline is glycosylated usually by oligosaccharides containing 3 or 4 arabinosyl moieties [10, 12, 13] and serine by galactosyl moieties [14] is the most common hydroxyproline-rich glycoprotein that occurs in plants. It has not been possible to extract this glycoprotein from the cell walls of plants without using methods which break either peptide or glycosidic bonds. Other hydroxyproline-rich glycoproteins with different structures do exist in plants. These include glycoproteins of the extracellular secretions of plant tissue cultures [15–17], glycopeptide material from wheat germ [18] and a widespread class of glycoside-binding mucopolysaccharides found in seeds [19]. These glycoproteins contain a high proportion of carbohydrate (80–95%) and the hydroxyproline is glycosidically linked to glucose or galactose that connects a large arabinogalactan to the peptide [16, 17, 20]. There are thus at least two main chemical classes of hydroxyproline-rich glycoprotein.

A lectin that binds *N*-acetylglucosamine oligomers has been isolated and purified from potato tubers. It has been shown to be a glycoprotein with about 50% carbohydrate and it is rich in hydroxyproline and arabinose [21, 22], unlike any other phytohaemagglutinin yet investigated. Jermyn and Yeow [19] suggested that its structure might be similar to that of the 'all-β lectins' that they found in plant seeds; despite the name 'lectin' these compounds probably have only one sugar binding site per molecule and they have not been shown to have any cell-agglutinating activity, although they will bind a range of β-glycosides.

In this paper the nature of the glycosidic linkage in the potato lectin is compared with that found in the insoluble wall glycoprotein of potato suspension callus cells.

RESULTS

Amino acid and neutral sugar content of potato lectin

The amino acid content of potato lectin prepared by the method of Allen and Neuberger [21] is shown in Table 1 and the neutral sugar composition in Table 2. Comparison of the sugar and amino acid analysis results with internal standards showed that the lectin contained almost equal amounts of sugars and amino acids. The molar ratio of arabinose to hydroxyproline was 4.7:1.

Preparation of hydroxypropyl arabinosides from potato callus cell walls

Walls, prepared from potato suspension callus (265 g wet weight) were extracted to remove pectin and freeze-dried (yield 2.7 g). On analysis glucose and arabinose were the major sugars, 6.8% w/w was amino acid of which 11.8 mol/100 mol amino acid was hydroxyproline. The walls (2.0 g) were stirred for 8 hr at 95° with saturated $Ba(OH)_2$ (0.2 M) in a stoppered flask. The cooled suspension was neutralized with 30% H_2SO_4 and then

Table 1. Amino acid analyses of potato lectin and of hydroxyprolyl arabinosides prepared from potato cellus cell walls and from potato lectin. Concentrations are expressed as mol amino acid/100 mol amino acid

Amino acid	Potato lectin	From cell walls of suspension callus		From potato lectin	
	mpl/100 mol amino acid	hyp-ara$_4$ mol/100 mol amino acid	hyp-ara$_3$ mol/100 mol amino acid	hyp-ara$_4$ mol/100 mol amino acid	hyp-ara$_3$ mol/100 mol amino acid
Hyp	14 2	72 5	57 8	73 9	59.8
Asp	6 3	3 2	2 3	2 3	3 8
Thr	7 3	1.4	1 3	1.2	1.9
Ser	13 6	3.3	4 7	3.6	6 3
Glu	8 9	3.0	4.8	4.0	5.9
Pro	6 5	0 0	1.6	2.0	3.2
Gly	15.9	7.8	9.2	5.0	8.3
Ala	6.1	5.1	6 1	3 3	5 5
Cys	5 9	0 0	0 0	0.0	0 0
Val	1 4	0.8	1.4	0 8	1 3
Met	0.3	0.0	0.0	0 0	0 0
Ile	0.9	0.6	0.7	0 4	0 6
Leu	1.3	1.4	1 8	0 0	1.3
Tyr	1 9	0.5	0 9	0 5	0 0
Phe	0 0	0 5	0.5	0 4	0 0
His	1.3	0 0	3 1	0 0	0 0
Orn	0 3	0 0	0 0	0 0	0 0
Lys	4 2	0 0	2 1	1 4	2 0
Arg	1 0	0 0	1 6	0 0	0 0
Trp	2.5 (from alkaline hydrolysis data)	not determined	not determined	not determined	not determined

centrifuged three times at 2000 *g* for 20 min to remove $BaSO_4$ and cell wall residue. The supernatant was concentrated to 10 ml, filtered and then applied to a column of Sephadex G15 (133 × 3.5 cm, total volume 1280 ml, void volume *ca* 430 ml). The column was eluted with distilled water at a flow rate of 60 ml/hr. Fractions (12 ml) were collected and assayed for total carbohydrate and hydroxyproline and the absorbance at 280 nm was measured. The elution profile is shown in Fig. 1. The large peak of material (E_{280}) at 1.81 void volume (fractions 66–71) indicates that alkaline hydrolysis liberated large quantities of free amino acids. Very little neutral sugar was detected in this molecular weight range. The small peak of material (E_{280}) at 2.18 void volume (fractions 76–80) must represent an aromatic amino acid or phenolic compound which had interacted with the Sephadex. Of the hydroxyproline detected 75% was eluted between 1.42 and 1.65 void volume and free hydroxyproline (14%) was eluted between 1.79 and 1.96 void volume. No hydroxyproline was detected near the void volume of the column.

Fractions 50–60 (1.39–1.70 void volume) were pooled, concentrated and re-run on the same Sephadex G15 column. Fractions (6 ml) were collected and assayed for hydroxyproline and arabinose. The elution profile showed a large coincident peak of pentose and arabinose

Table 2. Neutral sugar content of potato lectin

Sugar	% (w/w) of neutral sugars
Arabinose	82.5
Mannose	7.0
Galactose	7.8
Glucose	2.7

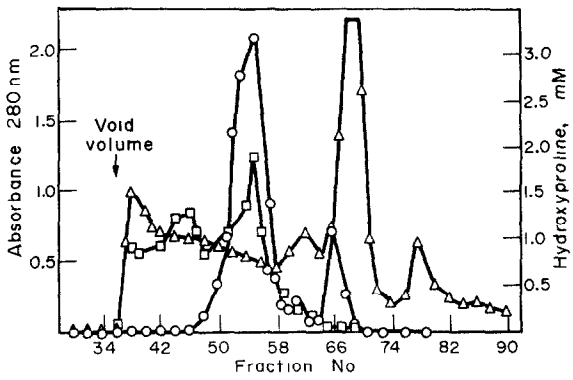

Fig. 1. Chromatography of an alkaline hydrolysate of potato cell walls on Sephadex G15. Pectin-extracted potato cell walls (2 g) were hydrolysed with $Ba(OH)_2$ (0.2 M, 6 hr, 90°) and the neutralized hydrolysate concd to 15 ml and chromatographed on a column of Sephadex G15 (133 × 3.5 cm). For each fraction (12 ml) the hydroxyproline concn (○) and neutral sugar content (□) arbitrary units was determined and *A* at 208 nm (△) was measured.

with a molar ratio of pentose (as arabinose) to hydroxyproline of about 4 at the leading edge of the peak (1.42 void volume) and about 2.5 at the trailing edge (1.60 void volume). When a small quantity of material isolated from the trailing edge of the peak (1.52–1.64 void volume) was chromatographed on a column (100 × 2.3 cm) of the sulphonic acid resin Zeocarb 225 equilibrated in 0.4 M HCl and eluted with a gradient from 0.4–1.0 M

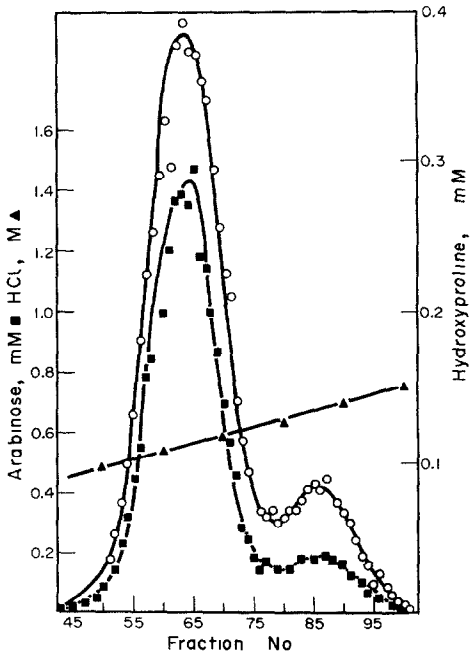

Fig. 2. Chromatography of hydroxyprolyl-arabinose from potato cell walls on Zeocarb 225. Material from an alkaline hydrolysate of potato cell walls which chromatographed between 1.39 and 1.70 void vols on Sephadex G15 was chromatographed on a column of Zeocarb 225 (100 × 2.3 cm), eluting with a gradient from 0.4–1.0 M HCl. Fractions (12 ml) were collected and the concns of arabinose (■) and hydroxyproline (○) were determined.

HCl (*ca* 800 ml), it gave 2 well resolved peaks of equal size eluting at 0.63 and 0.75 M HCl. The spectrophotometric assays showed that these contained hydroxyproline tetra-arabinoside (hyp-ara$_4$) and hydroxyproline triarabinoside (hyp-ara$_3$) respectively. The remainder of the material eluting from the Sephadex G15 column between 1.40 and 1.64 void volume was rotary evaporated to dryness and dissolved in 0.4 M HCl (10 ml) and applied to the Zeocarb 225 column which was equilibrated in 0.4 M HCl. The column was eluted with a gradient of 0.4–1.0 M HCl. Fractions (12 ml) were collected and assayed for hydroxyproline and pentose. The elution profile is shown in Fig. 2. There were two peaks of material containing hydroxyproline and arabinose; the larger had a molar ratio of arabinose to hydroxyproline of approximately 4:1, the smaller one had a ratio of approximately 3:1. The material from the column, fractions 51–70 (hyp-ara$_4$) (Fig. 2) were pooled together as were fractions 76–92 (hyp-ara$_3$). They were neutralized with basic lead carbonate and silver carbonate and then chromatographed separately on a Sephadex G15 column from which they emerged as symmetrical peaks at 1.48 void volume for hyp-ara$_4$ and at 1.54 void volume for hyp-ara$_3$. Significant amounts of free hydroxyproline and arabinose were not detected at the totally included volume of the column indicating that the chromatographic procedure with the sulphonic acid resin did not cause hydrolysis of the hydroxyproline–arabinose linkages.

Samples of the hyp-ara$_4$ and hyp-ara$_3$ preparations were hydrolysed and analysed by GLC for neutral sugars. In both cases more than 95% of the neutral sugar was arabinose. The results of amino acid analysis (Table 1) suggested that the preparation might be contaminated to some degree by peptides rich in hydroxy- and acidic amino acids although the majority of the amino acids was hydroxyproline. Two peaks for hydroxyproline appeared indicating that the alkaline hydrolysis caused racemization of the hydroxyprolyl moiety of the glycopeptides [10].

Alkaline hydrolysis of potato lectin

Potato lectin (4.0 mg) was hydrolysed in saturated Ba(OH)$_2$ solution (1.5 ml) in a sealed tube for 18 hr at 100°. After neutralization the hydrolysate, concentrated to 0.5 ml, was applied to a column of Sephadex G15 (1.0 × 136 cm) and eluted with water at a flow rate of 15 ml/hr. Fractions (1.67 ml) were collected and assayed for pentose and hydroxyproline. The elution profile is shown in Fig. 3. Three peaks were obtained, a small peak (I) containing pentose but not free hydroxyproline or hydroxyproline glycoside; a large peak (II) containing hydroxyproline and pentose in a molar ratio of 1:3.8 emerging at 1.48 void volume and a small peak (III) at the included volume (1.92 void volume) with free hydroxyproline, but no pentose. Less than 0.3% of the hydroxyproline detected emerged between 0.97 and 1.32 void volume (peak I), 86% eluted between 1.32 and 1.60 void volume (peak II), the hyp-ara$_3$ and hyp-ara$_4$ region and 9% as free hydroxyproline (peak III) 8% of the arabinose eluted in peak I and 91% in peak II.

If less severe hydrolysis conditions were used (6 hr at 100° in saturated Ba(OH)$_2$) peak (I) of pentose was larger although hydroxyproline was still not detectable in this region by the Kivirikko method [23]. GLC analysis of the acid hydrolysate of this fraction showed that the predominant sugar was arabinose and amino acid analysis showed that hydroxyproline was the major amino acid component representing 37% (mol/100 mol of amino acid) of the amino acids. The molar ratio of arabinose to hydroxyproline was 3.4:1. This material was therefore glycopeptides in which peptide bonds involving hydroxyproline had not been broken.

Material from peak (II) was chromatographed on Zeocarb 225 and eluted with a 0.4–1.0 M gradient of HCl. Fractions were analysed for hydroxyproline (Fig. 4). Two peaks were obtained corresponding in position to hyp-ara$_4$ and hyp-ara$_3$ comparable to that obtained from callus wall material. Fractions 42–55 (hyp-ara$_4$) and 61–77 (hyp-ara$_3$) were separately combined and hydrolysed. The neutral sugar present in each case was arabinose (>95%). Hydroxyproline was the major amino acid present (Table 1).

High voltage paper electrophoresis of the alkaline hydrolysate of potato lectin

Potato lectin (5 mg) was hydrolysed with saturated Ba(OH)$_2$ (0.5 ml) in a sealed tube at 100° for 6 hr. The neutralized hydrolysate was electrophoresed for 1 hr at 10.0 kV/m and the electrophoretogram stained with

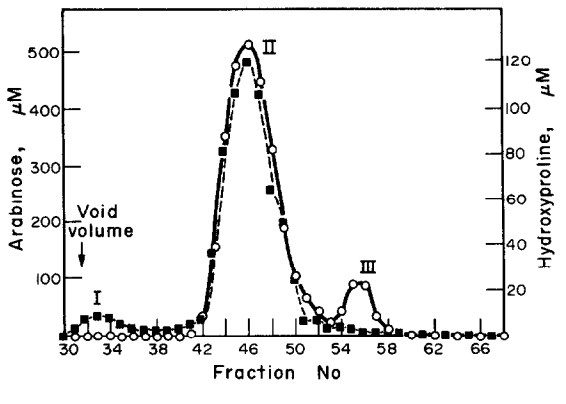

Fig. 3. Chromatography of an alkaline hydroxysate of potato lectin on Sephadex G15. Potato lectin (4 mg) was hydrolysed with Ba(OH)$_2$ (0.2 M, 18 hr, 100°) and the neutralized hydrolysate was chromatographed on a column of Sephadex G15 (136 × 1 cm). For each fraction (1.67 ml) the concns of hydroxyproline (○) and arabinose (■) were determined. Three main peaks of eluted material I, II, III were isolated. The HCl gradient is shown (▲).

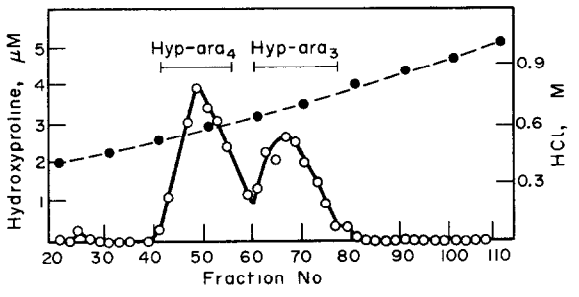

Fig. 4. Chromatography of hydroxyprolyl arabinosides from potato lectin on Zeocarb 225. Material from an alkaline hydrolysate of potato lectin which chromatographed on Sephadex G15 between 1.35 and 1.65 void vols was chromatographed on Zeocarb 225 and eluted with a gradient from 0.4–1.0 M HCl. Fractions (12 ml) were collected and concns of HCl (●) and hydroxyproline (○) were determined.

ninhydrin-isatin reagent [24]. A hydroxyproline marker run in parallel migrated 35.5 cm towards the cathode. The lectin preparation produced three major red spots (R_{hyp} 0.46, 0.52 and 0.61), two fainter spots (R_{hyp} 0.68 and 0.73) and a very faint spot in the position of free hydroxyproline. A large number of purple spots corresponding to the other amino acids which had migrated further than hydroxyproline were present. According to Lamport [10], the major spots would correspond to trans hyp-ara$_4$ (R_{hyp} 0.46) *cis*-hyp-ara$_4$ and *trans*-hyp-ara$_3$ (R_{hyp} 0.52) and *cis*-hyp-ara$_3$ (R_{hyp} 0.61).

SDS-polyacrylamide gel electrophoresis of potato lectin

On gel electrophoresis in the presence of SDS and dithiothreitol potato lectin showed one intense band staining with Coumassie blue with two extremely weak bands at higher molecular weight.

Lectin activity associated with potato root membranes

Particulate fractions from cultured potato roots were prepared, extracted and assayed for lectin activity and protein content. Results are shown in Table 3. Supernatants from membrane preparations showed no lectin activity. In membrane fractions prepared from potato roots by the method of Brett and Northcote [40] there was no significant difference in total or specific activities of lectin between the various membrane fractions obtained. Agglutination by both the potassium phosphate and the Triton-EDTA extracts prepared from the fraction which pellets at 100000 g was inhibited by 4 dilution steps by tri-*N*-acetyl chitotriose (1.8 mM) but not by *N*-acetylglucosamine, cellobiose, D-glucose, D-mannose and D-galactose at 40 mM.

DISCUSSION

The amino acid composition determined for our preparation of potato lectin was very similar to that of Allen and Neuberger [21]. We similarly found a trace of an amino acid running in the same position as ornithine on the amino acid analyser. Glycine and hydroxyproline were the most abundant amino acids and in general hydrophilic amino acids predominated. The lack of phenylalanine also reported by Allen and Neuberger [21] suggested a high degree of purity which was confirmed by the single predominant line on SDS gel electrophoresis. The only significant difference between our results and those of Allen and Neuberger [21] was a lower, though still high half-cystine content; this was almost certainly a result of hydrolysis losses.

Table 3. Lectin activities of particulate fractions from potato roots

Fraction	Extractant	Lectin activity titre (duplicates)	Protein (μg/ml)	Specific activity units/μg
19000 *g* 20 min	Potassium phosphate	2,4	75	0 04
19000 *g* 20 min	Triton/EDTA	256, 512	22	16 5
100000 *g* 90 min	Potassium phosphate	16, 32	65	0 35
100000 *g* 90 min	Triton/EDTA	512, 512	25	20.5

Particulate fractions sedimenting at 19000 g (20 min) and 100000 g (1.5 hr) were extracted sequentially with potassium phosphate (0.5 M pH 7.1) and with Triton X-100 (0.06%)/EDTA (50 mM) pH 6.8. Agglutination titres against trypsinized erythrocytes were determined.

During purification the lectin did not bind to DEAE-cellulose equilibrated in Tris–HCl buffer (pH 8.6, 50 mM) indicating that it has a high isoelectric point, so it is possible that most of the aspartic and glutamic acids in the hydrolysate were derived from the amides. Marinkovitch [25] suggested that in her impure preparation the carbohydrate might have been attached to glutamic or aspartic acids. However the isolation of hydroxyprolyl arabinosides, demonstrated in this paper, shows that this view is untenable.

The predominant sugar in the lectin was arabinose although small amounts of galactose, mannose and glucose were also present. The amino acid to sugar ratio was close to 1:1 and this is consistent with the values reported previously [21, 22]. The molar ratio of arabinose to hydroxyproline was 4.7:1 (Allen and Neuberger [21] found 4.3:1). The use of the amino acid analyser is not the most accurate method of determining hydroxyproline since the colour yield of the reaction product with ninhydrin is low. The ratio therefore is probably not more accurate than $\pm 20\%$, and is thus not inconsistent with a value a little below 4:1 as suggested by the isolation of hyp-ara$_4$ and hyp-ara$_3$.

From a bulk growth of potato suspension callus it has proved possible to prepared enough hydroxypropyl arabinosides to allow further chemical studies. Pectin extraction of the cell walls was designed particularly to remove associated arabinogalactans so that as much as possible of the remaining arabinose was associated with cell wall glycoprotein. After alkaline hydrolysis of residual material considerable quantities of free amino acids were liberated but very little free sugar was produced. This confirms that the $Ba(OH)_2$ hydrolysis procedure cleaves peptide but not glycoside bonds.

The hydroxyproline assay used [23] will only detect hydroxyproline with free imino and carboxylic acid groups but is not affected by substituents on the C_4 hydroxyl group. On Sephadex G15 chromatography of alkali-hydrolysed cell wall material no hydroxyproline-glycosides were detected near the void volume of the column, so the insoluble wall glycoprotein did not contain significant quantities of high MW polysaccharide linked to hydroxyproline [17].

The ion exchange chromatography on Zeocarb 225 was unusual in that the gradient of HCl titrated the charged groups on the sulphonic acid resin rather than those of the bound glycoptotides and peptides. This effected a separation according to charge to molecular weight ratio and hyp-ara$_4$ and hyp-ara$_3$ were isolated. These glycopeptides were contaminated with peptides rich in acidic and hydrophilic amino acids, but arabinose was the only sugar present. The ratio of hyp-ara$_4$ to hyp-ara$_3$ was approximately 5:1 similar to that found in sycamore callus cell wall glycoprotein [17].

Alkaline hydrolysis of potato lectin also released hyp-ara$_4$ and hyp-ara$_3$. When severe alkaline hydrolysis conditions were used only 8% of the total arabinose was present in high MW material. The rest appeared as hyp-ara$_4$ and hyp-ara$_3$. Less than 0.3% of the hydroxyproline was detected in the high MW material. If the polypeptide chain of the potato lectin subunit is assumed to have a MW of 23500 [21] there are 30 residues of hydroxyproline per chain. If only one of these hydroxyproline residues bore a large oligosaccharide unit then it

would be expected that 3% of the hydroxyproline detected would be in this high MW fraction after alkaline hydrolysis. Since less than 0.3% was detected in this fraction not even one hydroxyproline of the chain can carry a large carbohydrate group. A high proportion of the detected hydroxyproline (86%) could be isolated as hyp-ara$_3$ and hyp-ara$_4$ and 9% as the free amino acid. With less severe alkaline hydrolysis conditions a higher proportion of the arabinose appeared in the high molecular weight fraction but again no free hydroxyprolyl glycoside was detected in this fraction. Nevertheless this material was enriched in peptide bound hydroxyproline which accounted for 37% of the total amino acids present in this fraction. The arabinose to hydroxyproline ratio was 3.4:1. Thus this material contained fragments of the lectin containing hydroxyprolyl arabinosides still peptide bound. If potato lectin were to contain short sections of peptide with a sequence of consectutive hydroxyprolyl moieties similar to those in tomato callus cell wall glycoprotein [13] these results from the alkaline hydrolysis would be expected. The electrophoresis confirmed the presence of hydroxyprolyl arabinosides. Our results therefore suggest that in potato lectin all the arabinose is present as short oligosaccharides of 3 or 4 arabinose residues and that no large polysaccharide is attached to hydroxyproline.

Recently it has been recognized that there are at least two main classes of hydroxyproline-rich glycoprotein in higher plant cells. Both classes are produced by sycamore suspension callus cells [17]. In the insoluble wall glycoprotein there might be traces of polysaccharide linked to hydroxyproline, but hyp-ara$_4$ and hyp-ara$_3$ predominate [17]. Similar glycoproteins are probably present in a wide variety of plant cell walls [10, 11]. In addition to the hydroxyproline arabinosides a serine–galactoside linkage has been discovered [14]. Covalent cross-linking of this glycoprotein to polysaccharide components of the cell wall has been postulated but despite great efforts never demonstrated [10, 15, 26, 27]. Since the glycoprotein material has not been solubilized as a discrete unit a complete amino acid sequence has not been determined, but fragments show conspicuous sequences with up to four consecutive hydroxyprolyl residues [13]. Potato lectin is, so far as we are aware, the only example of a soluble glycoprotein shown to contain only short oligosaccharide side chains attached to hydroxyproline.

In sycamore extracellular mucopolysaccharide released by the callus tissue into the culture medium polysaccharides are attached to about 50% of the hydroxyproline residues [17]. This linkage is probably via a hydroxyprolyl glucoside or galactoside bond which has also been found in algal glycoproteins [28]. The polysaccharide is an arabinogalactan, about 85% of the residues being arabinose and galactose present in approximately equal amounts. In these glycoproteins approximately 20% of the hydroxyproline is not glycosylated and the remaining 30% carries short arabinose oligosaccharides with hyp-ara$_3$ predominating [17]. This type of glycoprotein has been isolated from the growth medium of tobacco tissue culture cells and in this case it is characteristically rich in alanine [41].

In sycamore suspension callus there is also a heterogeneous membrane associated fraction which is possibly a precursor of both extracellular mucopolysaccharide and of cell wall glycoprotein [17].

In wheat germ there are soluble glycopeptides with polysaccharide chains possibly linked by hydroxyprolyl–galactoside bonds [18, 20]. Barium hydroxide treatment does not release any low MW glycopeptides from this material and the high sugar to amino acid ratio found is similar to that of the sycamore extracellular mucopolysaccharide. A similar mucopolysaccharide is found in maize pericarp [29]. The 'all-β lectins' found by Jermyn and Yeow [19] are similar in structure to these mucopolysaccharides and are either cell wall or extracellular components. They are mucopolysaccharides that bind non-specifically to β-glycosides and can be isolated from plant seed extracts by means of their interaction with β-glycosides covalently attached to phoroglucinol. Mucopolysaccharide released into the culture medium by rye-grass (*Lolium multiflorum*) tissue culture has also been purified by this method [16]. In these polymers arabinofuranose residues are attached to a galactan backbone which is in turn linked to the peptide chain. These compounds, especially those most enriched in hydroxyproline. appear to have been subjected to proteolysis [19] and the petide chain can be as short as 20 residues [20]. It has been suggested that potato lectin may have a similar structure to these polymers [16, 19]. However this cannot be correct since hydroxyproline tri- and tetra-arabinosides can readily be isolated from potato lectin but large oligosaccharides attached to hydroxyproline are not present. It has also been suggested that the amino acid composition of potato lectin is similar to that of the all-β lectins. However among the 19 amino acids the proportions of 10, both in our preparations and in that of Allen and Neuberger [21], lie outside the extreme values obtained for 'all-β lectins' from a range of 17 plant tissues. In addition the carbohydrate specificity of potato lectin is very different from that of all-β lectins; the latter are probably monovalent and bind a wide range of β-glycosides whereas the potato lectin is a true agglutinin with more than one carbohydrate-binding site per molecule and a stringent specificity for oligonomers of *N*-acetyl-D-glucosamine and their close analogues [21, 22].

Potato lectin is the only lectin known so far which is rich in hydroxyproline and arabinose. Its binding specificity is for saccharide structures which are not typical cell wall components, so it is not reasonable to suggest potato lectin as a cell-wall glycoprotein which cross-links the cell wall by non-covalent interactions with sugars, a role which has been proposed for lectins [30]. Our investigations into the subcellular location of the lectin suggest that it is associated with intracellular membranes since a lectin activity with similar specificity to that of the tuber lectin was extracted from potato root particulate fractions. Similar locations have been reported for other lectins [31, 32]. Potato lectin is rich in hydrophilic sugars and amino acids so this localization at membranes was unexpected.

Potato lectin might carry sequences of consecutive hydroxyprolyl residues as found in cell wall glycoprotein and perhaps it is a sequence of consecutive prolyl residues which acts as a recognition site for hydroxylation and glycosylation enzymes. A different sequence of amino acids might then be the recognition site for hydroxylation and subsequent attachment of large oligosaccharides to the other glycoproteins containing hydroxyproline, typified by the sycamore extracellular mucopolysaccaride.

EXPERIMENTAL

Suspension cultures of potato callus (*Solanum tuberosum*, var. King Edward) were routinely grown in *ca* 150 ml PRL4 medium [33] supplemented by 2,4-dichlorophenoxyacetic acid (6 mg/l.) in 500 ml conical flasks in an orbital shaker (100 rev/min) in the dark at 26°. Aliquots (30 ml) were subcultured every 10 days. For bulk prepns of cell walls 300 ml suspension was added to 1200 ml medium in a 5 l. round bottomed flask and rotated on a diagonal axis in the dark at 26°. Cells were harvested after 3 weeks. Potato roots were cultured in 500 ml flasks containing PRL4 medium [33] (300 ml) not supplemented by any hormone in an orbital shaker under similar conditions and *ca* one quarter of the roots were subcultured every 3 weeks. To prepare cell walls, suspension callus (265 g) was frozen in liquid N_2 and ground with a pestle and mortar before sonication in 20 ml aliquots for 5 min using a 3 mm probe on a Dawe 7532A soniprobe converter at 80 W. Cell breakage was checked by light microscopy and cell contents were removed by washing × 10 with H_2O, centrifuging for 10 min at 1000 *g*. The walls were suspended in Na hexametaphosphate (pH 3.7, 20 g/l. 600 ml) and stirred for 2 hr at 95° in a stoppered flask. The extracted walls were washed × 5 with H_2O by centrifugation and then extensively washed again on a sintered glass filter before freeze-drying. Potato lectin was purified by the method of ref. [21]. Na dodecyl sulphate-polyacrylamide gel electrophoresis was performed on slab gels [34] using the system of ref. [35]. Lectin (20 μg) was dissolved in 50 μl Tris buffer (pH 6 8, 1M) containing dithiothreitol (15.4 g/l.) Na dodecyl sulphate (20 g/l.) glycerol (10%) and bromophenol blue (6 ml/l. of a 0.2% soln in absolute EtOH), heated at 70° for 5 min, and applied to the gel. Gels (15%) were run for *ca* 15 hr at 15 V. The gels were fixed in HOAc–MeOH–H_2O (2:9:9), stained with 0.125% Coomassie blue in HOAc–MeOH–H_2O (2:9:9) and destained in HOAc–MeOH–H_2 (7:10:83). To samples for amino acid and sugar analysis int. stands norleucine (100 nmol) and *meso*-inositol (50 μg) were added and the soln divided in two. For amino acid analysis one part was hydrolysed in HCl (Aristar grade, 3 M, 0.5 ml) in a sealed tube *in vacuo* for 24 hr at 105°. Amino acids in the hydrolysate were analysed on a Rank-Hilger Chromaspek J180 Mark I amino acid analyser. The other part was hydrolysed in H_2SO_4 (0.4 M, 2 ml) in an autoclave at 120° (103 kPa) for 1 hr. The hydrolysate was cooled, neutralized with a slight excess of $BaCO_3$ and filtered. The soln was concd to dryness by rotary evapn, dissolved in H_2O (1 ml) and ammonia (20 M, 50 μl) and $NaBH_4$ (*ca* 10 mg) were added. After 2 hr at room temp. surplus $NaBH_4$ was destroyed by dropwise addition of HOAc. The mixture was rotary evapd to dryness and washed by 5 evapns of MeOH (*ca* 7 ml) to remove borate. Ac_2O (1 ml) and H_2SO_4 (98%, 0.1 ml) were added and the mixture was heated for 1 hr at 60° in a stoppered flask. The soln was cooled and H_2O (5 ml) added carefully. The suspension was extracted twice with dichloromethane (redistilled) and the extracts combined, concd and washed by evapn of 10 ml H_2O. The alditol acetates produced were taken up in dichloromethane (1 ml) and concd to 50 μl. GLC of 1 μl samples was carried out isothermally at 183° on a column (1.5 m × 3.2 mm) packed with polyethylene glycol succinate (LAC 4R 886, 0.4%) 2-cyanoethylsilicone (XE60, 0·4%) and polyethylene glycol adipate (0.2%) at an Ar carrier gas flow rate of 25 ml/min. Detection was by FID. Peak areas were calculated by triangulation or a Pye PP80 digital integrator and compared to the meso-inositol standard. Hydroxyproline was assayed by the method of ref. [23] reduced in scale so that the sample was contained in 0.4 ml. The method will only detect hydroxyproline and hydroxyproline glycosides which are not peptide bound. Pentose was assayed by heating a sample (0.7 ml) with 0.7 ml orcinol–$FeCl_3$–HCl reagent for 90 min at 90° and reading *A* at 667 nm, with an arabinose standard [36]. Total carbohydrate was estimated using the α-naphthol–H_2SO_4 reagent [37]. HCl was determined by titration of 1 ml aliquots against Na_2CO_3 (50 mM).

Membrane fractions from potato roots. Cultured potato roots (23 g wet wt) were washed with 8% sucrose medium (40 mM Tris–HCl pH 8, 10. mM EDTA, 0.1 mM $MgCl_2$, sucrose 8% w/w) and homogenized in this medium (20 ml) with an ice cold pestle and mortar for 30 sec. The homogenate was gently squeezed through 2 thicknesses of muslin and centrifuged (1100 *g*, 10 min) to remove cell walls, nuclei and debris. The homogenate (34.5 ml) was centrifuged for 20 min at 19000 *g* in a Beckman SW27 rotor in a Beckman L2 centrifuge at 4°. The supernatant was again centrifuged for 1.5 hr at 100000 *g*. The pellets from these centrifugations were separately suspended in K_2PO_4 (0.5 M, pH 7.1) and sonicated for 30 sec, using a 3 mm horn on a Dawe soniprobe type 7532A at 80 W. The vol. was made up to 5 ml and the suspension centrifuged at 45000 rev/min (150000 *g*) for 0.5 hr in a Beckman SW 50.1 rotor at 4°. The supernatant was dialysed against several changes of Pi buffered saline (Na_2HPO_4, 1.48 g/l.; KH_2PO_4, 0.5 g/l.; NaCl, 7.2 g/l.) at 4° and the pellet was similarly extracted with Triton X-100 (0.06%)/EDTA 50 mM, pH 6.8.

Protein and lectin assay. Protein was estimated with the Folin phenol reagent [38]. Lectin activity was assayed by the agglutination of trypsin-treated erythrocytes. Rabbit (New Zealand White) erythrocytes were collected in heparin coated tubes and stored in an equal vol. of Alsever's soln (glucose 20.5 g/l., Na citrate 8.0 g/l. NaCl 4.2 g/l. adjusted to pH 6.1 with citric acid) for no longer than 2 weeks at 4°. Erythrocytes were washed × 3 by centrifugation for 2 min at 1000 *g* with Pi buffered saline (Na_2HPO_4 1.48 g/l., KH_2PO_4 0.5 g/l., NaCl 7.2 g/l.) and suspended at a concn of 4 ml packed cells/100 ml in Pi buffered saline and treated with trypsin (Difco bactorypsin) 1 mg/ml for 40 min at 37°. Cells were washed × 6 with Pi buffered saline and suspended at 1.5 ml packed cell vol./100 ml Pi buffered saline. The suspension was used within 48 hr of prepn. Serial 2 fold dilutions of 25 μl aliquots of lectin were made in microtiter ® U-plates (Cook Engineering Co. Ltd.) using Titertek ® Microdiluters (Flow Laboratories, UK) and 25 μl of erythrocyte suspension was added to each well. In hapten inhibition studies 25 μl sugar soln or Pi buffered saline in blanks was added to each well 5 min before the erythrocytes. Titres were read after 4 hr at room temp. Tri-*N*-acetylchitotriose was prepared from a partial acid hydrolysate of chitin [39] by gel chromatography on Sephadex G15.

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