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The Purification and Properties of the Lectin from Potato Tubers, a Hydroxyproline-Containing Glycoprotein

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1. Potato lectin has been purified and shown to be a glycoprotein containing about 50% of carbohydrate. Most of the sugar residues (92%) are arabinose; small amounts of galactose, glucose and glucosamine are also present. 2. The most abundant amino acid is hydroxyproline (16% of the residues), 11.5% of the residues are half-cystine and phenylalanine is absent. The lectin also contains about one residue/molecule of a basic amino acid, not usually found in proteins, which has been tentatively identified as ornithine. There is indirect evidence that the components of the glycoprotein are linked through hydroxyproline and arabinose. 3. By gel filtration in 6M-guanidine–HCl on Sepharose 4B, it was found that both the native glycoprotein and its *S*-carboxymethylated derivative had subunit molecular weights of 46000 (±5000). In a non-denaturing solution, two of these units appear to be associated. 4. The lectin is specifically inhibited in its agglutination reaction by oligosaccharides that contain *N*-acetylglucosamine. Its specificity is similar to, but not identical with, that of wheat-germ agglutinin.

Lectins are proteins that interact with cell surfaces and cause cells to agglutinate. Some lectins have been shown to have a greater capacity to agglutinate transformed cells than normal cells, and are being used to investigate the differences between the surfaces of these cells. Further knowledge of this interaction may lead to a better understanding of the development and growth of cancers (see reviews by Sharon & Lis, 1972; Lis & Sharon, 1973). The agglutination of cells by lectins is in many cases inhibited by specific sugars and it is concluded that these proteins bind to specific sugar residues on the surfaces of cells. Examples of these are concanavalin A, which is inhibited by α-D-mannose and α-D-glucose (Poretz & Goldstein, 1970), soya-bean agglutinin by *N*-acetylgalactosamine (Lis *et al.*, 1970) and wheat-germ agglutinin by *N*-acetylglucosamine and β-1,4-linked oligosaccharides containing this sugar (Burger & Goldberg, 1967; Allen *et al.*, 1973).

Recently, wheat-germ agglutinin has been purified and characterized by three separate groups of workers including ourselves (Nagata & Burger, 1972; LeVine *et al.*, 1972; Allen *et al.*, 1973). It is an atypical lectin, apparently existing as a monomer with a low molecular weight (18000–23000). It is also unusual in having a very high content of half-cystine residues and we showed that, unlike most lectins, wheat-germ agglutinin is not a glycoprotein. The lectin present in the potato tuber has a specificity with respect to its inhibition by sugars similar to wheat-germ agglutinin (Pardoe *et al.*, 1969; Masumoto & Osawa, 1971). We therefore decided to see if potato lectin also resembles wheat-germ agglutinin in composition and molecular weight. On finding that the procedure of Marinkovich (1964) did not yield a pure product, we developed a procedure for the purification of potato lectin to homogeneity. The purified lectin was found to be an unusual glycoprotein with a high content of half-cystine and hydroxyproline residues, and with arabinose as the major sugar component.

Experimental

Materials

DEAE-cellulose (Whatman grade DE23) and CM-cellulose (Whatman grade CM23) were obtained from H. Reeve Angel and Co., London E.C.4., U.K. SP(sulphopropyl)-Sephadex (C-50), QAE (quaternary amino-ethyl)-Sephadex (A-50), Sephadex G-100, Sephadex G-150 and Sepharose 4B were from Pharmacia, Uppsala, Sweden. The standard amino acid mixture used for amino acid analysis was supplied by Beckman–Spinco, Palo Alto, Calif., U.S.A., *allo*-4-hydroxy-L-proline by Calbiochem, Los Angeles, Calif., U.S.A.; L-1-methylhistidine, L-3-methylhistidine and *N*-ε-methyl-L-lysine by Sigma Chemical Company, St. Louis, Mo., U.S.A.; DL-5-hydroxylysine by Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. and the other amino acids used as standards by BDH Chemicals Ltd., Poole, Dorset, U.K., as were the neutral sugars used as standards for g.l.c. The methods of synthesis or sources of the sugars used to investigate the specificity of the lectin have been given elsewhere (Allen *et al.*,

1973), with the exception of cellobiose which was obtained from Koch–Light Laboratories Ltd. and *N*-acetyl lactosamine which was a gift of Professor Winifred Watkins, F.R.S., Lister Institute of Preventive Medicine, London S.W.1., U.K. Rabbit γ-globulin and hen ovalbumin were gifts of Dr. R. D. Marshall of this Department. Human Tamm–Horsfall urinary glycoprotein was prepared in this Department by Dr. Wendy A. Ratcliffe (Fletcher *et al.*, 1970*a*,*b*). Hen ovomucoid was obtained from Worthington Biochemical Corp., Freehold, N.J., U.S.A., bovine serum albumin from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K., cytochrome *c* (bovine) was from BDH Chemicals Ltd., Miracloth was supplied by Calbiochem. All other products used were BDH AnalaR grade or the highest purity available.

Methods

Amino acid and amino sugar analyses. Analyses were done on a Locarte Mini analyser fitted with an automatic-loading attachment. The standard system for analyses involved successive elution of the 23cm column with the sodium citrate buffers (Moore & Stein, 1963), pH3.25 (0.2M) for 55min, pH4.25 (0.2M) for 100min and pH6.65 (1.0M) for 140min at 50°C at a flow rate of 30ml/h, with a $SnCl_2$-ninhydrin system for colour development. This system, however, did not resolve hydroxyproline from aspartic acid or glucosamine from leucine. To separate hydroxyproline from aspartic acid, the analyser column was eluted first with a buffer of pH2.80 (0.2M) for 120min, followed by pH4.25 (0.2M) buffer for 100min. Glucosamine, galactosamine and tryptophan were separated from each other and from the other amino acids, including the breakdown products of tryptophan, by a buffer system published by Liu (1972) which, on this analyser, involved successive elution of the 23cm column by pH4.25 (0.2M buffer for 66min followed by pH5.28 (0.35M) buffer for 200min.

Samples of protein were hydrolysed for 24, 48 and 72h in constant-boiling HCl at 110°C *in vacuo* and correction factors were obtained for the destruction of serine and threonine residues. Half-cystine values were obtained from the analysis of cysteic acid residues resulting from performic acid oxidation (Hirs, 1956) and subsequent hydrolysis of the protein for 24h at 110°C in constant-boiling HCl. Tryptophan was determined spectrophotometrically in 6M-guanidine–HCl (Edelhoch, 1967).

Liu (1972) showed that hydrolysis of a protein in *p*-toluene sulphonic acid gave high yields of tryptophan for subsequent estimation on the amino acid analyser. He also showed that high yields of tryptophan were also obtained from hydrolysates which contained up to 35% of hexose. We found that hydrolysis of potato lectin, or a mixture of equal amounts of lysozyme and arabinose gave low (about 25%) yields of tryptophan. This may indicate that the presence of pentoses in the hydrolysate causes greater destruction of this amino acid than does the presence of hexoses. Glucosamine was determined on the analyser from a sample of protein that had been hydrolysed in 3M-*p*-toluene sulphonic acid *in vacuo* at 100°C for 24h. When this procedure was carried out on well-characterized glycoproteins, the recovery of glucosamine was found to be over 90%.

Neutral sugar analyses. These compounds were estimated by g.l.c. on a Pye series 104, model 24 gas chromatograph after methanolysis and trimethylsilylation of the glycoprotein (Chambers & Clamp, 1971). Sugar analyses were related quantitatively to the amino acid analyses by adding internal standards (mannitol or mannose for sugars and *p*-fluorophenylalanine for amino acids) to samples taken from the same stock solution of glycoprotein.

Reduction and alkylation of proteins. The method used was that of Konigsberg (1972) which involved reduction by dithiothreitol in 6M-guanidine–HCl at 50°C, followed by alkylation with iodoacetic acid and subsequent dialysis. Amino acid analysis showed that there had been a complete conversion of half-cystine into *S*-carboxymethylcysteine residues. To determine the content of free thiol groups in the potato lectin the procedure for alkylation was carried out in the absence of dithiothreitol followed by amino acid analysis.

Alkali treatment of the glycoprotein. The lectin (1mg) was dissolved in 0.5ml of 0.5M-NaOH and dialysed against 0.5M-NaOH at 4°C for 2 days, followed by dialysis against several changes of water. The lectin was then analysed for amino acids and sugars.

Polyacrylamide-gel disc electrophoresis. This was done for us in the laboratory of Dr. M. J. Crumpton at the National Institute for Medical Research, London N.W.7., U.K. The procedure, which is basically that described by Allan & Crumpton (1971), involved electrophoresis in columns (10cm × 0.8cm) of 7.5% (w/v) polyacrylamide gel in 50mM-sodium phosphate buffer (pH7.2) containing 0.1% sodium dodecyl sulphate and 5M-urea run for 16h at a current of 4.5mA/gel. Before electrophoresis the glycoprotein was heated in a boiling-water bath for 1min in 2% (w/v) sodium dodecyl sulphate–2.5% 2-mercaptoethanol–8M-urea. Reo virus proteins were used as markers for molecular weight. The lectin stained well with Coomassie Brilliant Blue, but not with Naphthalene Black.

Ultracentrifugation studies. Dr. P. A. Charlwood (National Institute for Medical Research, Mill Hill, London N.W.7, U.K.) investigated the sedimentation rate of potato lectin (10mg/ml) dissolved in 0.02M-sodium phosphate buffer (pH7.1) containing

0.2M-NaCl by the use of a Spinco Model E analytical ultracentrifuge.

Assay of agglutination. Full details of the procedure are given in our paper on wheat-germ agglutinin (Allen *et al.*, 1973). A serial dilution of the lectin was made in 100μl of phosphate-buffered saline (Herbert, 1967) and 200μl of a 1.5% suspension of trypsin-treated rabbit blood cells were added. The tubes were shaken at 15min intervals and after 2h the degree of agglutination was assessed on the serological scale (0 to ++++). The amount of lectin required to cause half-maximal (++) agglutination of the cells was taken at 1 unit. This assay is only semi-quantitative and should be regarded as being liable to an error of ±20%. With this method an activity of 10000 units/mg was obtained for pure potato lectin, which compares with 4000 units/mg for wheat-germ agglutinin (Allen *et al.*, 1973).

To determine the 50% inhibition values for various sugars in this system, the method of Lis *et al.* (1970) for soya-bean agglutinin was used, except that the degree of agglutination was determined on the visual serological scale rather than spectrophotometrically.

Results

Purification of potato lectin

The purification scheme is shown in Table 1. All operations were at 0–4°C unless otherwise stated. Potato tubers (4500g) were peeled, washed, cut into small pieces and homogenized in 2vol. of 0.1M-sodium acetate buffer (pH3.8) containing 2mM-sodium metabisulphite as an anti-oxidant (Galliard, 1971). The homogenate was centrifuged at 600*g* for 15min and the supernatant was filtered through Miracloth. The precipitate was resuspended in the same buffer and the procedure repeated twice. $(NH_4)_2SO_4$ (10.6g/l) was added to the combined supernatants to produce 20% saturation at 4°C. The precipitate was collected by centrifugation at 9000*g* for 20min and discarded. $(NH_4)_2SO_4$ (17.5g/l) was then added to the supernatant to produce 50% saturation at 4°C, the precipitate was left to settle overnight and then collected by centrifugation at 9000*g* for 20min. The precipitate was suspended in water, dialysed extensively against water, and the suspension centrifuged at 9000*g* for 20min.

DEAE-cellulose column. The pH of the supernatant from the previous step was adjusted to 8.6 with 0.5M-Tris solution and the material was applied to a column of DEAE-cellulose (30cm×3.5cm) equilibrated with 0.05M-Tris–HCl buffer (pH8.6) and washed with the same buffer. Fractions (25ml) were collected. About 25% of the protein passed through the column with the wash and this included all the lectin activity.

CM-cellulose column. The active material that had passed through the previous column was applied directly without concentration or dialysis to a column (30cm×1.8cm) of CM-cellulose equilibrated with 0.05M-Tris–HCl buffer (pH8.6) and washed with the same buffer. Fractions (10ml) were collected. Inactive protein (55%) was washed through the column with the initial buffer and the active material (45% of the protein) was eluted with 0.05M-Tris–HCl buffer (pH8.6) containing 0.1M-NaCl applied in a step. The application of a gradient of NaCl concentration did not result in any substantially better purification.

Sephadex G-100 columns. The active fractions from the previous column were combined and dialysed against water, freeze-dried and redissolved in 8ml of phosphate-buffered saline (pH7.4). The material was then applied to a column (60cm×2.5cm) of Sephadex G-100 and eluted with the same buffer. Fractions (3.4ml) were collected (Fig. 1). The fractions containing the active material, which had moved ahead of the bulk of the protein close to the V_0 of the column, were combined, dialysed, concen-

Table 1. *Purification of potato lectin*

This procedure started with 4.5kg of potato tubers. Because of the inaccuracy of the haemagglutination assay the activities are liable to an error of ±20%. The protein content was estimated photometrically in the first three steps by assuming an $E^{1\%}$ of 1.1; for later steps the freeze-dried weight of the protein is given.

Procedure	10^{-3} × Total activity (agglutinating units)	Total protein content (g)	Specific activity (units/mg)	Yield (%)
Aqueous extraction	1600	80	20	100
20–50%-$(NH_4)_2SO_4$ precipitate after dialysis and centrifugation	1600	60	27	100
DEAE-cellulose column	1200	2.7	440	75
CM-cellulose column	1000	1.6	620	62
1st G-100 Sephadex column	600	0.147	4000	37
2nd G-100 Sephadex column	600	0.091	7000	37
SP-Sephadex column	400	0.038	10000	25

trated by freeze-drying, redissolved in 5ml of the same buffer and chromatographed on the same Sephadex G-100 column under the same conditions as before. The active fractions were once more combined, dialysed and freeze-dried.

SP-Sephadex column (*Fig.* 2). A column (21cm × 1.8cm) of SP-Sephadex C-50 was prepared in 0.1M-sodium acetate buffer (pH3.8) and the active material from the previous step which had been dissolved in the same buffer, was applied. Fractions (10ml) were collected. The column was first washed with 200ml of 0.1M-sodium acetate buffer (pH3.8), then a linear gradient was applied from the same buffer (300ml) to 0.1M-sodium acetate buffer containing 0.2M-NaCl. The lectin was eluted as a single symmetrical peak by 0.12M-NaCl. Inactive protein was eluted from the column by stepwise elution with 0.1M-sodium acetate buffer (pH3.8) containing 1.0M-NaCl. The fractions which contained the active protein were combined, dialysed and freeze-dried.

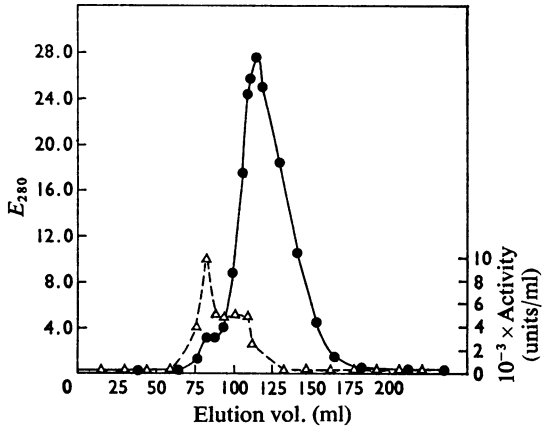

Fig. 1. *Elution profile of potato lectin from Sephadex G-100*

The column (60cm × 4cm) was eluted with phosphate-buffered saline. ●, E_{280}; △, agglutinating activity (units/ml). The V_0 was 75ml.

Molecular-weight estimations

Polyacrylamide-gel estimations. The active material eluted from the SP-Sephadex column was found to be homogeneous by electrophoresis in 7.5% (w/v) polyacrylamide gel in the presence of sodium dodecyl sulphate and mercaptoethanol. It migrated at the rate expected for a protein with a molecular weight of 105000 (±10000). However, these conditions are likely to give a considerable overestimate of the molecular weight of a glycoprotein (Segrest & Jackson, 1972).

Analytical ultracentrifugation. The glycoprotein sedimented in a single symmetrical boundary with no detectable heterogeneity. A sedimentation coefficient of 3.37S was obtained which corresponds

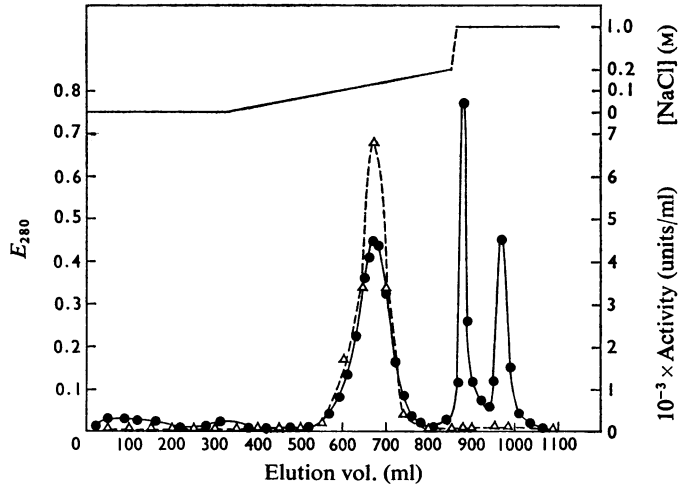

Fig. 2. *Elution profile of potato lectin from SP-Sephadex* (*C*-50)

The column (40cm × 2.5cm) was eluted with 0.1M-sodium acetate buffer (pH3.8) and the protein was eluted by a linear gradient of NaCl. Inactive protein was eluted by a step to 1.0M-NaCl. ●, E_{280}; △, agglutinating activity (units/ml); ——, NaCl concn. (M).

to a minimum molecular weight of 24000 (assuming that $f/f_0 = 1.0$).

Gel filtration in a non-denaturing buffer. A column of Sephadex G-150 (60cm×2cm), equilibrated with phosphate-buffered saline (pH7.4) at 4°C was calibrated by applying proteins of known molecular weights (Andrews, 1965). A linear relationship was obtained between the elution volume and the logarithm of the molecular weight for the following proteins: cytochrome *c* (12400), chymotrypsinogen (25700), ovalbumin (45000) and its dimer, bovine serum albumin (68000) and rabbit γ-globulin (150000). The latter protein was reported by Andrews (1965) to behave as if it had a molecular weight of 200000 and we have used it as a marker for this molecular weight. The potato lectin was eluted as a symmetrical peak in a position expected for a globular protein of molecular weight 120000 (±10000). The *S*-carboxymethyl derivative of the lectin was eluted in the position expected for a protein of molecular weight of 200000.

Gel filtration in 6M-guanidine–HCl. Sepharose 4B was equilibrated with a solution of 6M-guanidine–HCl containing 0.1M-Tris–HCl buffer (pH8.6), packed in a column (60cm×2cm) and eluted at 4°C with the same buffer at a flow rate of 3ml/h. The column was calibrated with the following unreduced (but presumably completely disaggregated) proteins: human Tamm–Horsfall urinary glycoprotein (79000), bovine serum albumin (68000), ovalbumin (45000), chymotrypsinogen A (25700) and cytochrome *c* (12400). The potato lectin was eluted in the position of a protein of molecular weight of 46000 (±5000). After removal of the guanidine–HCl by dialysis the lectin was fully active.

The Sepharose 4B column was then calibrated with reduced and *S*-carboxymethylated derivatives of the proteins and glycoproteins used in the previous calibration. In addition, reduced and *S*-carboxymethylated derivatives of hen ovomucoid (28000) and rabbit γ-globulin light (23500) and heavy chains (50000) were used. These derivatives were eluted earlier from the column than the native proteins, but showed the linear relationship between the logarithm of the molecular weight and elution volume reported by Mann & Fish (1972). The *S*-carboxymethylated potato lectin gave a molecular weight of 46000 (±3000) with this calibration.

Composition of the lectin

By the use of internal standards for amino acid and carbohydrate analysis (see 'Methods') we concluded that potato lectin was a glycoprotein containing 51% (±3%) by weight of carbohydrate. This high value was confirmed by analyses with the phenol–H_2SO_4 reagent (Dubois *et al.*, 1956), which gave values ranging from 45 to 60% for the carbohydrate content of the glycoprotein. The analyses (Table 2 and see below) are calculated from the assumption that the molecular weight of the subunit of the glycoprotein is 46000, and that the molecular weight of the carbohydrate component and the polypeptide chain are about equal.

The assumption that the total molecular weight of the carbohydrate moiety is 23000, means that there are 164 sugar residues. Arabinose (152 residues) is by far the most abundant sugar, with smaller amounts of galactose (7 residues) and glucose (4 residues). There is probably only one residue of glucosamine per subunit; no trace of galactosamine was found. The sugar analysis was not changed after *S*-carboxymethylation of the lectin or after treatment of the glycoprotein with 0.5M-NaOH at 4°C (see 'Methods').

The most notable feature of the amino acid composition (Table 2) is that there is a very high content

Table 2. *Amino acid analysis of potato lectin*

The values were calculated as molar proportions by assuming that the molecular weight of the protein moiety is 23000. The values for the nearest integer were derived from an average of the three hydrolyses, with the exception of threonine and serine, which were extrapolated to zero time and half-cystine and tryptophan (see the footnotes). For further details see 'Methods'.

	Amino acid content (mol/mol)			
Time of hydrolysis ...	24h	48h	72h	Nearest integer
Hyp	35.9	36.1	33.3	35
Asp	10.6	10.4	10.4	10
Thr	12.6	12.5	12.3	13
Ser	24.8	20.8	19.0	28
Glu	16.0	16.0	16.3	16
Pro	15.7	16.3	15.7	16
Gly	28.0	28.0	28.0	28
Ala	9.3	10.3	9.8	10
Cys	18.1	18.1	17.3	25*
Val	1.2	1.1	1.0	1
Met	1.1	0.9	1.1	1
Ile	3.6	3.7	3.9	4
Leu	3.0	3.0	2.7	3
Tyr	6.0	6.7	6.6	7
Phe	0.0	0.0	0.0	0
His	0.8	—	1.2	1
Orn	1.1	1.0	0.9	1
Lys	9.0	9.4	9.2	9
Arg	1.3	1.5	1.4	1
Trp	—	—	—	8†
Total				217

* Determined separately as cysteic acid.
† Determined by a spectrophotometric method.

of hydroxyproline residues (16%). There is no doubt that it is the usual 4-*trans*-hydroxyproline since it coincides in elution time on the amino acid analyser with authentic samples of 4-*trans*-hydroxyproline with both pH2.80 and 3.25 analyser buffers; a sample of allo-4-*cis*-hydroxyproline was found to run more slowly in both these buffers. The 3-hydroxyproline (*cis* and *trans*) isomers are also reported to be well-separated from 4-hydroxyproline on the amino acid analyser (Hamilton, 1968). In addition, an unusual amino acid was detected on the amino acid analyser trace moving slightly faster than lysine. In three different buffer systems it was eluted in the same position as an ornithine marker. On the amino acid analyser, its behaviour differed from 5-hydroxylysine, *N*-ε-methyl-lysine, 1-methylhistidine and 3-methylhistidine; its chromatographic behaviour also differed from that expected for a di- or tri-methyl-lysine or for a substituted arginine. We have therefore tentatively identified the amino acid as ornithine. As far as we are aware, the only reported occurrence of ornithine in a protein is in a human urate-binding α-globulin (Sletten *et al.*, 1971). Other noteworthy features of the composition are that 11.5% of the residues are half-cystine, that there are no free thiol groups and that there is no phenylalanine; the absence of this amino acid on analysis is a useful criterion of purity of a preparation.

Inhibition of agglutination and the specificity of the binding site

Our inhibition experiments (Table 3) confirm the observations of Pardoe *et al.* (1969) and Masumoto & Osawa (1971) that potato lectin is specifically inhibited by oligosaccharides containing *N*-acetylglucosamine, but is not inhibited by the monosaccharide unless it is attached to an aromatic aglycone. The inhibitory power of oligosaccharides increases with increasing chain length up to the tetrasaccharide, which suggests that there could be an extended binding site similar to that which we proposed for wheat-germ agglutinin (Allen *et al.*, 1973). It is noteworthy that the bacterial cell wall saccharides $(GlcNAc\text{-}MurNAc)_n$ are weaker inhibitors than the oligosaccharides derived from chitin $(GlcNAc)_n$, and that cellobiose (Glc-Glc) and *N*-acetyl lactosamine (Gal-GlcNAc) are not inhibitors. We have also found that cellobiose is not an inhibitor of wheat-germ agglutinin, and in this respect the lectins from potato and wheat germ differ from the blood-group-O-specific lectins found in *Cytissus sessilifolius* and *Laburnum alpinum* which are inhibited by both chitobiose and cellobiose (Watkins & Morgan, 1962).

Table 3. *Inhibitory effect of various sugars on the agglutinating activity of potato lectin*

For abbreviations see the text and for conditions of assay see 'Methods'. All compounds were pyranosides and all oligosaccharides contained β-(1→4)-linkages.

Compound	Concn. needed for 50% inhibition (mM)
GlcNAc	(0% at 200mM)
Methyl α-D-GlcNAc	(0% at 200mM)
Methyl β-D-GlcNAc	(0% at 200mM)
Benzyl α-D-GlcNAc	40
$(GlcNAc)_2$	0.1
$(GlcNAc)_3$	0.05
$(GlcNAc)_4$	0.005
$(GlcNAc)_5$	0.002
GlcNAc-MurNAc	8
$(GlcNAc\text{-}MurNAc)_2$	0.1
Gal-GlcNAc	(0% at 3mM)
Glc-Glc	(0% at 200mM)

Discussion

The glycoprotein that we isolated gave a single band on electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulphate and mercaptoethanol, showed no heterogeneity on ultracentrifugation and was eluted as a single symmetrical peak from SP-Sephadex. In addition, both the native and *S*-carboxymethylated proteins were eluted as single symmetrical peaks from Sephadex G-150 in aqueous buffer and from Sepharose 4B in 6M-guanidine–HCl. There was no sign of multiple molecular forms (isolectins) such as have been found for wheat-germ agglutinin (Allen *et al.*, 1973) and other lectins (Sharon & Lis, 1972).

The most reliable estimates of molecular weight should be obtained from gel-filtration in 6M-guanidine–HCl rather than by the use of polyacrylamide gel runs in sodium dodecyl sulphate, or by gel filtration in aqueous buffers. This is because estimations of molecular weight with sodium dodecyl sulphate are likely to be too high owing to the presence of carbohydrate and hydroxyproline, which are liable to interfere with the binding of this detergent (Reid *et al.*, 1972). Values obtained for the molecular weight of glycoproteins by gel filtration in aqueous buffers have been found to be less reliable than those for other proteins (Andrews, 1965). However, the value of 120000 obtained for the native lectin by gel filtration can still be regarded as a useful indication that there is some degree of aggregation of subunits. Gel filtration in 6M-guanidine–HCl gave the correct values for the molecular weights of the subunit of human Tamm–Horsfall urinary glycoprotein and for ovomucoid as well as for a range of non-glycoproteins. We therefore conclude that the value of 46000

obtained for the molecular weight of both the native lectin and its reduced and *S*-carboxymethylated derivative is likely to be a reliable estimate. The true molecular weight of the potato lectin in aqueous buffer is probably between 85000 and 100000. We suggest that the glycoprotein is composed of two (or possibly more) identical subunits held together by non-covalent bonds and which therefore dissociate in the presence of guanidine–HCl. This result is not unexpected as, with the exception of wheat-germ agglutinin, all the lectins so far investigated have been shown to be aggregates of two or more subunits.

The amino acid composition is unusual for a lectin. One feature is that 11.5% of residues are half-cystine; the only other lectins reported to contain large amounts of cystine are wheat-germ agglutinin (20% half-cystine) and the pokeweed mitogen (21% half-cystine) (Reisfeld *et al.*, 1967). The other lectins that have been purified contain little or no cystine (Sharon & Lis, 1972). Hydroxyproline is the most abundant amino acid in potato lectin and this is the first report of a lectin containing this amino acid.

Potato lectin is also unusual in that arabinose is the most abundant sugar residue. In this respect, as well as in its high hydroxyproline content, the lectin resembles glycoproteins that have been isolated from the cell walls of many plants, including the potato (Lamport, 1969; Heath & Northcote, 1971). Similar glycoproteins are common constituents of the cell sap (Pusztai & Watt, 1969). It has been shown that the carbohydrate is attached to the protein by means of an arabinose to hydroxyproline linkage in tissue-culture cells derived from tomato (Lamport, 1969) and sycamore (Heath & Northcote, 1971).

Marshall (1972) has reviewed the glycoprotein linkages, and observed that of the protein amino acids, only asparagine, serine, threonine, cysteine, hydroxylysine and hydroxyproline are involved in linkage compounds. Of these possibilities, hydroxylysine is not present in the potato lectin, and cysteine cannot be involved, as all the cysteine residues are alkylated after the protein has been reduced, and the resultant *S*-carboxymethylated potato lectin still contains carbohydrate. *O*-Glycosidic linkages that involve the hydroxyl groups of serine and threonine are known to be generally alkali-labile by a process of β-elimination (see review by Marshall & Neuberger, 1970). The conditions which we used for alkaline treatment (see 'Methods') would have caused the hydrolysis of any such bonds; since there was no decrease in the carbohydrate content of the lectin after this treatment, it is unlikely that serine or threonine are involved in glycosidic linkages. Asparagine has only been found linked to *N*-acetylglucosamine and we have found one glucosamine residue (presumably *N*-acetylated) per molecule of subunit. It is possible that it is involved in a linkage compound, but a linkage involving *N*-acetylglucosamine to which all 164 carbohydrate residues are attached seems unlikely.

The most likely linkage for the attachment of carbohydrate to the protein moiety of this lectin is that between hydroxyproline and arabinose. This linkage is known to be stable to alkali (Lamport, 1969) and would involve the most abundant amino acid and the most abundant sugar. It is also a linkage which has been found in plant proteins containing arabinose and hydroxyproline. However, an unequivocal answer to the question of the linkage must await the isolation of the relevant glycopeptides from a digest of potato lectin.

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