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Some properties of the lectin from *Datura stramonium* (thorn-apple) and the nature of its glycoprotein linkages

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The lectin from *Datura stramonium* (thorn-apple; Solanaceae) has been purified by affinity chromatography and shown to be a glycoprotein containing about 40% (w/w) of carbohydrate. The most abundant amino acids are hydroxyproline, cystine, glycine and serine. Results obtained by gel filtration in 6M-guanidinium chloride on Sepharose 4B suggest that it has a subunit mol.wt. of about 30000 and that it probably associates into dimers. The lectin is inhibited specifically by chitin oligosaccharides and bacterial-cell-wall oligosaccharides, but only weakly by *N*-acetylglucosamine. Glycopeptides from soya-bean (*Glycine max*) lectin and fetuin are also strong inhibitors of *Datura* lectin, indicating that it interacts with internal *N*-acetylglucosamine residues. Its specificity is similar to, but not identical with, that of potato (*Solanum tuberosum*) lectin. After prolonged proteolytic digestion of reduced and *S*-carboxymethylated or *S*-aminoethylated derivatives of the lectin, glycopeptides of mol.wt. of about 18000 were isolated. The glycopeptides contained all the carbohydrate and hydroxyproline of the original glycoprotein, and lesser amounts of serine, *S*-carboxymethylcysteine and other amino acids. The arabinose residues of the glycoprotein are present as β-L-arabinofuranosides linked to the polypeptide chain through the hydroxyproline residues, and can be removed by mild acid treatment; the ratio of arabinose to hydroxyproline is 3.4:1. Some of the serine residues of the polypeptide chain are substituted with one or two α-galactopyranoside residues, most of which can be removed by the action of α-galactosidase. The galactose residues are more easily removed from the acid-treated glycopeptide (from which arabinose has been removed) than from the complete glycopeptide, indicating a steric hindrance of the galactosidase action by the adjacent chains of arabinosides. There is a slow release of galactose residues by a process of β-elimination in 0.5M-NaOH (pH 13.7) from the complete glycopeptide, and a fairly rapid release of galactose by this process from the acid-treated glycopeptide, which lacks arabinose. This is probably due to the inhibitory effect of the negative charge on the adjacent arabinofuranoside residues. The similarities and differences between the lectins from *Datura* and potato are discussed, as are their structural resemblance to glycopeptides that have been isolated from plant cell walls.

Lectins are cell-agglutinating proteins or glycoproteins with an enzyme-like specificity for particular mono- or oligo-saccharides [see reviews by Lis & Sharon (1973) and Goldstein & Hayes (1978)]. The presence of a lectin in extracts of seeds of *Datura stramonium* (thorn-apple or jimson weed) was first reported by von Eisler & von Portheim (1908), and further investigated by Boyd & Requera (1949), who showed that it agglutinated erythrocytes of humans irrespective of blood group, and of several other species.

It was later (Kilpatrick *et al.*, 1979) shown to be present in most parts of the plant as well as the seeds. Pardoe *et al.* (1970) showed that the lectin had a specificity directed towards oligosaccharides of chitin and similar structures containing β1,4-linked *N*-acetylglucosamine residues. Hořejší & Kocourek (1978) purified the lectin by affinity chromatography on *N*-acetylglucosamine-containing polysaccharides from the fungus *Aspergillus niger* and showed that the lectin was a glycoprotein containing hydroxyproline, with arabinose as the

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most abundant sugar. Kilpatrick & Yeoman (1978) also purified the lectin by affinity chromatography on immobilized fetuin. As was pointed out by Hořejší & Kocourek (1978), *Datura* lectin resembles the lectin from the potato (*Solanum tuberosum*) (Allen & Neuberger, 1973; Allen *et al.*, 1978; Muray & Northcote, 1978) in composition and specificity, and both plants are members of the family Solanaceae. We have previously isolated a glycopeptide from potato lectin (Allen *et al.*, 1978) and shown that it contains a core of hydroxyproline residues that are linked to β-L-arabinofuranosides, and serine residues linked to α-galactopyranosides, which is of particular interest, since very similar structures also occur in plant cell walls (Akiyama & Katō, 1977; O'Neill & Selvendran, 1980). We have now investigated *Datura* lectin and shown that it has a glycosylated region similar to, but not identical with, that in potato lectin, and that its sugar specificity also differs in detail from that of potato lectin. A preliminary communication on this subject has been published (Allen *et al.*, 1980).

Experimental

Materials

Seeds of *Datura stramonium* were bought from Thompson and Morgan Ltd., Ipswich, Suffolk, U.K., or were collected from plants that had been grown from these seeds. Potato lectin was prepared by the method of Desai & Allen (1979). Hen ovomucoid was a product of Worthington Biochemical Corp., Freehold, NJ, U.S.A. The sources of the other proteins and the saccharides have been given elsewhere (Allen *et al.*, 1976; Allen, 1979). *N*-(Iodoethyl)trifluoroacetamide (sold under the name of Aminoethyl-8) was bought from Pierce Chemical Co., Rockford, IL, U.S.A. Other chemicals used were BDH AnalaR grade or of the highest purity available.

Methods

Analysis of amino acids. Amino acids were measured on a Locarte amino acid analyser after hydrolysis of the glycoprotein or glycopeptide in 3 M-toluene-*p*-sulphonic acid or constant-boiling HCl under N_2 at 100°C for 24h. The elution systems, correction factors for serine and threonine, methods for measuring cystine content and the spectrophotometric measurement of tryptophan were those used previously (Allen & Neuberger, 1973; Allen *et al.*, 1976, 1978).

Sugar analysis. Neutral sugars were measured by g.l.c. after methanolysis and trimethylsilylation of the glycoprotein (Chambers & Clamp, 1971), with Perkin–Elmer F.11 or F.33 gas chromatographs fitted with dual columns and flame-ionization detection. Sugar analyses were related to the amino acid analyses by adding internal standards (mannitol for neutral sugars and *p*-fluorophenylalanine for amino acids) to samples taken from the same stock solution of glycoprotein or glycopeptide.

Polarimetry. Optical rotations were determined on a Perkin–Elmer model 141 polarimeter at 25°C in a 10cm-pathlength cell.

Preparation of glycopeptides from Datura lectin

Glycopeptides were prepared by enzymic digestion of the lectin which had been reduced and then either *S*-carboxymethylated (*S*-carboxymethylcysteinyl-lectin) or *S*-aminoethylated (*S*-aminoethylcysteinyl-lectin). *S*-Carboxymethylation of 10mg of *Datura* lectin was performed by the method of Konigsberg (1972), as previously described for the production of a glycopeptide from potato lectin (Allen *et al.*, 1978). *S*-Aminoethylation of 8.5mg of *Datura* lectin was performed by using Aminoethyl-8 according to the suppliers' instructions.

The *S*-carboxymethylcysteinyl-lectin and the *S*-aminoethylcysteinyl-lectin were digested with Pronase under the conditions used by Spiro & Bhoyroo (1974) for the digestion of fetuin. The glycopeptides were isolated by chromatography on Bio-Gel P-2 and analysed under the same conditions as were used for the investigation of a glycopeptide from potato lectin (Allen *et al.*, 1978).

Partial acid hydrolysis of the glycopeptides

The peptides were hydrolysed at 30°C in 1 M-HCl, and the rate of reaction monitored by the rate of change of optical rotation. From these readings, half-lives for the compounds were derived. The products of hydrolysis were purified by chromatography on a column (60cm × 2cm) of Bio-Gel P-2 eluted with water (see Allen *et al.*, 1978).

Alkaline treatment of the glycopeptides

Both the *S*-carboxymethylcysteinyl-glycopeptide and the acid-treated *S*-carboxymethylcysteinyl-glycopeptide were incubated at 30°C in 0.5 M-NaOH; samples were taken after 1 and 7 days and analysed for their amino acid and sugar content.

α-Galactosidase treatment of glycopeptides

Glycopeptide (100μg) was dissolved in 200μl of 0.05 M-sodium citrate/sodium phosphate buffer, pH6.0, and incubated with 40μl (2 units) of coffee-bean (*Coffea*) α-galactosidase at 37°C for 7 days in the presence of toluene (one unit will hydrolyse 1.0μmol of *p*-nitrophenyl α-D-galactoside/min at 25°C). The incubation mixture was then dialysed against water and analysed.

Assay of agglutination

The lectin was assayed by observing its agglu-

tinating effect on a 1% suspension of rabbit erythrocytes in phosphate-buffered saline (7.20g of NaCl, 1.48g of Na_2HPO_4, 0.43g of KH_2PO_4/litre; final pH7.2). Details of this procedure are given elsewhere (Allen & Neuberger, 1973; Allen *et al.*, 1976).

Preparation of ovomucoid–Sepharose conjugate

Sepharose 4B was activated with CNBr by the method of Porath *et al.* (1967). The following procedure, which we used for coupling ovomucoid to the activated gel, was similar to that used by Porath *et al.* (1967) for the coupling of chymotrypsin. Activated Sepharose 4B (10ml settled volume) was added to a solution of 125mg of ovomucoid dissolved in 10ml of 0.1M-$NaHCO_3$, pH8.5, at 2°C and gently mixed overnight. Ethanolamine hydrochloride (0.1ml of a 1M solution, pH8) was added to block any remaining active groups and the agitation was continued for a further 2h. The slurry was then washed successively with 0.1M-$NaHCO_3$, 1M-NaCl, water and 1M-NaCl. Storage was in 1M-NaCl and 0.025% NaN_3.

Purification of Datura stramonium lectin (Table 1)

Seeds of *Datura stramonium* (100g) were ground in a hand-driven coffee mill (Spong and Co., Basildon, Essex, U.K.) at room temperature, but the subsequent extraction procedure was performed at 4°C. The resulting meal was stirred overnight with 1000ml of phosphate-buffered saline and the suspension was then centrifuged (17000*g* for 30min) with the supernatant being retained. The deposit was resuspended in phosphate-buffered saline, the procedure repeated, and the combined supernatants were used for the next step. Solid $(NH_4)_2SO_4$ (39.8g/100ml) was added to the combined supernatants to give 65% saturation at 4°C. The precipitate was allowed to settle overnight and then collected by centrifugation at 9000*g* for 20min. The precipitate was suspended in a minimum volume of phosphate-buffered saline and then dialysed first against the same buffer and then extensively against water. The resulting suspension was centrifuged at 9000*g* for 20min, and the supernatant was freeze-dried (yield 2.2g).

The freeze-dried material was dissolved in 2ml of phosphate-buffered saline and applied at room temperature to the ovomucoid–Sepharose 4B column (1.7cm × 6cm), which was in equilibrium with the same buffer. The column was eluted with phosphate-buffered saline. Fractions (10ml) were collected and assayed for agglutinating activity and for protein by u.v. absorption. Although 97% of the total protein was eluted with this buffer, only traces of the agglutinating material were in this fraction. The column was then sequentially eluted with 1M-NaCl (1000ml), 0.1M-sodium acetate buffer, pH4.5 (250ml), and water (250ml). The lectin was finally eluted from the column with 0.1M-acetic acid and 5ml fractions were collected. Those fractions that contained agglutinating activity were pooled, dialysed against water and freeze-dried. The yield was 48mg of lectin/100g of seeds.

Results and discussion

Properties of Datura lectin

Molecular-weight determinations. Hořejší & Kocourek (1978) showed that *Datura* lectin has a sedimentation coefficient of 3.8S, which compares with 3.18S for potato lectin (Allen *et al.*, 1978). We have mainly depended on the method of Mann & Fish (1972) for molecular-weight determination, which involves gel filtration in 6M-guanidinium chloride on Sepharose 4B. The column was equilibrated at room temperature and calibrated with either native or *S*-carboxymethylated derivatives respectively of cytochrome *c*, α-chymotrypsinogen, ovalbumin, bovine serum albumin, immunoglobulin G, human Tamm–Horsfall glycoprotein and potato lectin. When plotted, both curves showed a linear relationship between log(molecular weight) and elution volume (Allen & Neuberger, 1973). Under these conditions the native lectin gave two peaks equivalent to mol.wts. of 67000 (±7000) and 28000 (±3500), whereas the *S*-carboxymethylated lectin gave a single peak equivalent to a mol.wt. of 32000 (±4000).

Our interpretation of these results is that the native lectin consists of two similar subunits of about 30000mol.wt., which will partially dissociate in the

Table 1. *Purification of Datura lectin*

This procedure started with 100g of seeds. Owing to the inaccuracy of the haemagglutination assay, the activities are liable to an error of ±20%. In the first step, protein was determined by the method of Warburg & Christian (1941), and in the second and third the freeze-dried weights were used.

Procedure	10^3 × Total activity (agglutinating units)	Total protein (g)	Specific activity (units/mg)	Overall yield (%)
Extraction with phosphate-buffered saline	580	19.56	29.6	100
Supernatant after $(NH_4)_2SO_4$ pptn. and dialysis	400	3.02	132.4	70
Active fraction off affinity column	120	0.048	2500	30

presence of 6M-guanidinium chloride, giving the pattern of two peaks on Sepharose 4B that was observed for the native protein. After reduction and *S*-carboxymethylation the subunits do not remain associated in the presence of 6M-guanidinium chloride and are therefore eluted as a single peak corresponding to a mol.wt. of about 32000. We have taken the average (30000) of the estimations of native and *S*-carboxymethylcysteinyl-lectins as the basis for our calculations for amino acid and carbohydrate content.

Polyacrylamide-gel electrophoresis. Polyacrylamide-gel electrophoresis in the presence of a reducing agent (Weber *et al.*, 1972) and subsequent staining with Coomassie Blue or the periodate/Schiff reagent showed three main bands, as was reported by Hořejší & Kocourek (1978). The proteins of these bands corresponded to mol.wts. of about 68000, 60000 and 49000, and it was suggested by Hořejší & Kocourek (1978) that they could represent an intact subunit and two fragments of that subunit; with such a high content of carbohydrate, an overestimate of the molecular weight of these components is to be expected.

Amino acid analysis. The analysis of the whole lectin is given in Table 2; the values are calculated as molar proportions assuming that the molecular weight of the monomer is 30000. The analysis is mostly in reasonable agreement with that reported by Hořejší & Kocourek (1978), except that the proportion of hydroxyproline is higher in our preparation. We conclude that the proportion of carbohydrate in the lectin is 40%. As noted by Hořejší & Kocourek (1978), the *Datura* lectin shows a general resemblance to potato lectin (Allen & Neuberger, 1973; Allen *et al.*, 1978), as it contains both hydroxyproline and arabinose, as well as high contents of cystine, glycine and serine. We did not find ornithine to be present, although this amino acid is present in potato lectin (Allen & Neuberger, 1973; Muray & Northcote, 1978).

Isolation of glycopeptides from Datura lectin. After digestion of both the *S*-carboxymethylcysteinyl-lectin and the *S*-aminoethylcysteinyl-lectin with Pronase, glycopeptides of high molecular weight were separated by chromatography on Bio-Gel P-2. The analyses of these glycopeptides are given in Table 3. They contained all the hydroxyproline, arabinose and most of the galactose of the original molecule, together with serine and other amino acids. The fractions that contained the free amino acids released by the action of Pronase lacked hydroxyproline and arabinose. It is noteworthy that whereas the glycopeptide that was derived from the *S*-aminoethylcysteinyl-lectin contained no *S*-aminoethylcysteine, the glycopeptide that was derived from the *S*-carboxymethylcysteinyl-lectin still contained nine residues of *S*-carboxymethylcysteine.

Table 2. *Amino acid composition of Datura stramonium lectin*

Results are expressed as molar ratios relative to 24 hydroxyproline residues (total mol.wt. 30400). The values for each amino acid are the averages for at least three determinations and expressed to the nearest whole integer. For further details, see the text.

Amino acid	Molar ratio
Hyp	24
Asx	12
Thr	10*
Ser	23*
Glx	12
Pro	11
Gly	23
Ala	7
½-Cys	22†
Val	10
Met	1
Ile	2
Leu	3
Tyr	4
Phe	2
His	1
Lys	3
Orn	0
Arg	5
Trp	3
Total residues	170
Ara	76
Gal	14

* Corrected for destruction during hydrolysis.

† Determined separately as cysteic acid after hydrolysis of a performic acid-oxidized sample (Hirs, 1956).

From this observation it seems that bonds involving *S*-aminoethylcysteine, which are known to be susceptible to cleavage by trypsin (Lindley, 1956), are much more readily cleaved by Pronase than are bonds involving *S*-carboxymethylcysteine.

The glycopeptides did not pass through Visking 8/32 dialysis tubing when dialysed at 4°C in water for 24h, and from this it seemed likely that they had a molecular weight greater than 10000 and might be similar to the glycopeptide with a mol.wt. of 34000 that we isolated from potato lectin (Allen *et al.*, 1978). If one assumes that all 24 hydroxyproline residues of the original glycoprotein are in one peptide chain, the molecular weight of the *S*-carboxymethylcysteinyl-glycopeptide is 18700, and of the *S*-aminoethylcysteinyl-glycopeptide, 16900.

The o.r.d. (optical rotatory dispersion) of the *S*-carboxymethylcysteinyl-glycopeptides is shown in Fig. 1, the $[\alpha]_D$ of the *S*-carboxymethylcysteinyl-glycopeptide is +32°, and that of the *S*-aminoethylcysteinyl-glycopeptide, which is not shown, is +62°. The values for the rotations are shown for the

Table 3. *Amino acid composition of Datura stramonium lectin compared with glycopeptides obtained after Pronase digestion of S-carboxymethylcysteinyl and S-aminoethylcysteinyl derivatives of the lectins and with these glycopeptides after mild acid hydrolysis*

The values for the native lectin are to the nearest whole residue, those for the glycopeptides to the nearest tenth of a residue. Residues which are present at less than 0.2 mol/mol in the glycopeptides are not included. For further details, see the text and the footnotes.

Amino acid	(*a*) Native lectin	(*b*) *S*-carboxymethyl-cysteinyl-glyco-peptide after Pronase digestion	(*c*) *S*-carboxymethyl-cysteinyl-glyco-peptide after Pronase digestion and 1 M-HCl treatment	(*d*) *S*-aminoethyl-cysteinyl-glyco-peptide after Pronase digestion	(*e*) *S*-aminoethyl-cysteinyl-glyco-peptide after Pronase digestion and 1 M-HCl treatment
Hyp	24	24	24	24	24
Asx	12	3.8	2.5	1.6	1.7
Thr	10*	1.6*	1.0*	0.5*	0.4*
Ser	23*	9.5*	8.3*	9.5*	9.2*
Glx	12	3.0	2.4	3.1	2.9
Pro	11	4.6	3.9	2.6	2.6
Gly	23	3.1	1.8	1.7	1.3
Ala	7	1.8	1.1	1.6	0.6
½-Cys	22†	9.1‡	9.1‡	0.0§	0.0§
Met	1	0.0	0.0	0.5	0.3
Leu	3	1.1	0.8	1.3	1.2
Tyr	4	0.5	0.7	1.2	1.2
Total residues	(170)	62	56	48	45
Ara	76	81	8	80	10
Gal	14	10	9	8	8
Total mol.wt.	30400	18700	8400	16900	7500

* Corrected for destruction during hydrolysis.
† Determined as cysteic acid.
‡ Determined as *S*-carboxymethylcysteine.
§ Determined as *S*-aminoethylcysteine.

glycopeptide (10 mg/ml) in water in a 10 cm-path-length cell. The similar *S*-carboxymethylcysteinyl-potato-lectin glycopeptide had an $[\alpha]_D$ of +59° (Allen *et al.*, 1978).

Partial hydrolysis of the glycopeptides. The glycopeptides were hydrolysed in 1 M-HCl at 30°C for 8 days, and the course of the reaction was monitored by the rate of change of optical rotation. The products of hydrolysis were chromatographed on Bio-Gel P-2 and it was found that almost 90% of the arabinose had been released from the glycopeptides. This sugar had an $[\alpha]_D$ of +102° at equilibrium, showing that it was the natural L-stereoisomer (literature value +104.5°).

The acid-treated glycopeptides contained almost all the amino acid residues as well as the galactose of the original glycopeptides. The o.r.d. of the acid-treated *S*-carboxymethylcysteinyl-glycopeptide is shown in Fig. 1, as are the o.r.d. curves for the untreated glycopeptide, poly-L-hydroxyproline and free hydroxyproline which are adjusted to the same value for hydroxyproline content. Similar observations were reported for potato lectin glycopeptides (Allen *et al.*, 1978). The optical rotation of the acid-treated glycopeptide is much greater (about three to four times) than would be expected (in terms of the rotation of free hydroxyproline) from its hydroxyproline content (Fig. 1). The acid-treated *S*-carboxymethylcysteinyl-glycopeptide is more laevorotatory than expected, and this may be due to the effect of *S*-carboxymethylcysteine residues, which have strong negative rotation. It is, however, slightly more laevorotatory than an equivalent quantity of polyhydroxyproline. This indicates that (like potato-lectin glycopeptide) the hydroxyproline is present in the glycopeptide as contiguous units of three or more residues that could form an open rigid left-handed helix in the conformation of polyproline (form II).

The optical rotation of the glycopeptide changed from positive to negative on removal of the arabinose by acid treatment (Fig. 1). The value for the specific molar rotation of the arabinose residues was calculated from this difference by using analytical values for the glycopeptides obtained by amino acid analysis and g.l.c. The value of +198° was obtained for the molar rotation of the arabinose in the glycopeptide, which is close to that for the β-methyl arabinofuranoside (+194°), and also agrees with the value obtained for arabinose in the potato-lectin glycopeptide (Allen *et al.*, 1978). Therefore we can say that the arabinose residues are

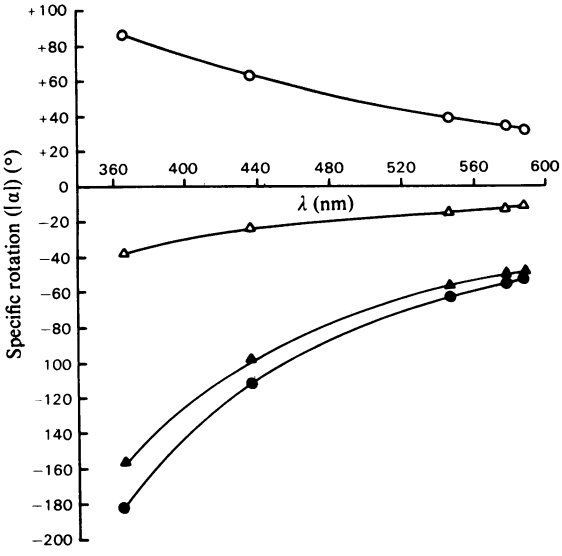

Fig. 1. *Optical-rotatory-dispersion curves for Datura-lectin glycopeptides and hydroxyproline*

O, Complete arabinoglycopeptide from *S*-carboxymethylcysteinyl-*Datura* lectin; ●, the glycopeptide from which arabinose has been removed by mild acid hydrolysis; △, free hydroxy-L-proline; ▲, polyhydroxy-L-proline. The curve for the specific rotation of the complete arabinoglycopeptide is shown; the values for the other compounds are adjusted to a concentration of hydroxyproline that is equimolar with that of the complete glycopeptide. The composition of the glycopeptides is given in Table 3. For further details, see the text.

present as β-furanosides. The furanoside nature of the linkages was further confirmed by their acid-lability. The half-life of the arabinose in the glycopeptide is 2.1 days, compared with 2.0 days for potato-lectin glycopeptide. The *S*-aminoethylcysteinyl-glycopeptide showed a similar change in rotation on treatment with acid.

Effect of alkaline treatments on whole glycopeptide and acid-treated glycopeptide. Since galactose still remained attached to the peptide chain after mild acid hydrolysis (Table 3), it was concluded that it was present as a pyranoside and was linked directly to the hydroxy group of an amino (or imino) acid of the peptide chain, presumably by a glycosidic linkage to hydroxyproline or serine. The alkali-stability of hydroxyproline glycosides (Fincher *et al.*, 1974; Allen *et al.*, 1978), and the alkali-lability of the serine or threonine glycosides (Marshall & Neuberger, 1970) make it easy to distinguish the amino (or imino) acid involved in the linkage.

Treatment with 0.5M-NaOH. Both complete *S*-carboxymethylcysteinyl-glycopeptide (with arabinose still attached) and acid-treated *S*-carboxymethylcysteinyl-glycopeptide (almost all arabinose removed) were treated with 0.5M-NaOH at 30°C for 192h. After this time, in total, 3.8 residues of serine and 5.1 residues of galactose were eliminated from the whole glycopeptide, whereas 4.2 residues of serine and 7.4 residues of galactose were lost from the acid-treated glycopeptide (Table 4). This showed that the serine residues could be substituted with one or two galactose residues. Recent investigations of this lectin (M. A. O'Neill & R. R. Selvendran, unpublished work) by methylation and mass spectrometry have indicated that disaccharides of galactose are present in native *Datura* lectin. Cho & Chrispeels (1976) have also suggested the possibility of having serine residues substituted with one or two galactose residues in the glycopeptides obtained from carrot (*Daucus carota*) cell walls. In contrast, in potato-lectin glycopeptide, we (Allen *et al.*, 1978) have shown that the serine residues were substituted with only single galactose residues.

The galactose residues were removed from the serine residues of the glycopeptide by a process of β-elimination. However, the reaction took place more slowly in the intact glycopeptide than in the acid-treated glycopeptide. By analogy with potato lectin, this difference could be due to the inhibitory effect of the arabinofuranoside residues on the β-elimination reaction, which is probably caused by a negative charge on their hydroxy groups at the pH used. In addition, the negative charge on the carboxy groups of *S*-carboxymethylcysteine might also exert an inhibitory effect on β-elimination.

Table 4. *Treatment of glycopeptides with 0.5M-NaOH*

The effects of treatment with 0.5M-NaOH on the amino acid and carbohydrate composition of (*a*) the glycopeptide derived from *Datura* lectin by Pronase digestion (contains arabinose) and (*b*) the same glycopeptide after treatment with 1M-HCl at 30°C for 10 days (contains small amounts of arabinose) are shown. Results are expressed as molar ratios relative to hydroxyproline (= 24). For further details, see the text.

	Composition			
	(*a*)		(*b*)	
Amino acid or carbohydrate	Untreated	192h at 30°C	Untreated	192h at 30°C
Hyp	24	24	24	24
Ser*	9.5	5.7	8.3	4.1
Ara	81	81	7.5	7.0
Gal	10.0	4.9	9.3	1.9

* Corrected for destruction during hydrolysis.

Galactosidase treatment. The whole glycopeptide and the acid-treated glycopeptide were incubated with α-galactosidase (see under 'Methods') for 72h, dialysed and then analysed for carbohydrate content. α-Galactosidase removed half the galactose from the acid-treated glycopeptide, but under the same conditions removed only a quarter of the galactose from the native glycopeptide (Table 5). It is therefore concluded that at least five of the galactose residues of the glycoprotein are α-linked to serine, and the presence of arabinose in the glycopeptide partially inhibits the action of the galactosidase, presumably by steric hindrance.

Specificity of Datura lectin and potato lectin

A comparison of the inhibitory activity of various saccharides on *Datura* lectin and potato lectin (see also Allen & Neuberger, 1973) is shown in Table 6. Our inhibition experiments confirm the observations of Pardoe *et al.*, (1970), Hořejší & Kocourek (1978) and Kilpatrick & Yeoman (1978) that both the lectins have similar carbohydrate specificity, although there are some marked differences in their binding to various saccharides (Table 6). The agglutinating activity of *Datura* lectin was not inhibited by the monosaccharides D-glucose, D-galactose, D-mannose, L-fucose, *N*-acetyl-D-galactosamine and *N*-acetyl-D-mannosamine, even at a final concentration of 500mM. *N*-Acetyl-D-glucosamine (GlcNAc) and its methyl glycosides are weak inhibitors of both lectins, although the β-methyl glycoside is definitely more effective than the α-glycoside.

Both lectins are specifically inhibited by β-1,4-linked oligosaccharides containing GlcNAc, and the inhibitory power increases with chain length up to the tetrasaccharide. For *Datura* lectin, the bacterial-cell-wall disaccharide GlcNAc-MurNAc is as inhibitory as the disaccharide $(GlcNAc)_2$, but $(GlcNAc\text{-}MurNAc)_2$ is 100 times more effective than $(GlcNAc)_4$. Thus for binding to take place, a free 3-hydroxy group in alternate residues is not required, and 3-*O*-lactyl substitution may actually enhance the binding to the lectin. In contrast, for potato lectin, the bacterial-cell-wall saccharides are both weaker inhibitors than oligosaccharides derived from chitin.

Datura lectin and potato lectin are equally inhibited by the fetuin and soya-bean-agglutinin glycopeptides (Table 6). Both these glycopeptides

Table 5. *Galactosidase treatment of glycopeptides*

The effect of treatment with α-galactosidase on the carbohydrate composition of (*a*) the whole glycopeptide and (*b*) the same glycopeptide after treatment with 1M-HCl at 30°C are shown. Results are expressed as molar ratios relative to hydroxyproline (= 24).

	Composition			
	(*a*)		(*b*)	
Carbohydrate	Untreated	α-Galactosidase	Untreated	α-Galactosidase
Ara	81	81	7.9	7.9
Gal	10.0	7.6	9.3	4.7

Table 6. *Comparison of the inhibitory effect of various saccharides on the agglutinating activity of Datura lectin and potato lectin*

For conditions of the assay, see under 'Methods'. Oligosaccharides contained β-1,4 linkages. The molarity of the glycopeptides is expressed in terms of core GlcNAcβ1→4GlcNAc content.

	Concn. needed for 50% inhibition (mM)	
Compound	*Datura* lectin	Potato lectin
GlcNAc	375	185
Methyl-α-D-GlcNAc	500	250
Methyl-β-D-GlcNAc	185	62
$(GlcNAc)_2$	4	0.06
$(GlcNAc)_3$	2	0.03
$(GlcNAc)_4$	1	0.008
GlcNAc-MurNAc	4	8
$(GlcNAc\text{-}MurNAc)_2$	0.008	0.1
Fetuin glycopeptide	0.24	0.24
Soya-bean-agglutinin glycopeptide	0.24	0.24

have been shown to have a core of Manβ1→4GlcNAcβ1→4GlcNAcβ→Asn (Spiro, 1973; Lis & Sharon, 1978), in neither glycopeptide are GlcNAc residues terminal, and in soya-bean agglutinin GlcNAc is confined to the core disaccharide. We therefore conclude that, in common with wheat-germ agglutinin (Allen *et al.*, 1973; Goldstein *et al.*, 1975) and marrow lectin (Allen, 1979), both *Datura* and potato lectins have extended binding sites that are capable of binding oligosaccharides, including the internal GlcNAc residues of glycopeptides. It is noteworthy that the glycopeptides are stronger inhibitors of *Datura* lectin than are the oligosaccharides of chitin; this may be due to interaction with the β-linked mannose or the asparagine, or perhaps, in the case of fetuin, with sialic acids. Potato lectin binds most strongly to a long chain of β-1,4-linked GlcNAc residues and will probably not interact so strongly with β-mannose. In this context it should be mentioned that although *Datura* lectin binds to a fetuin–Sepharose column (Kilpatrick & Yeoman, 1978), potato lectin does not (N. N. Desai & A. K. Allen, unpublished work). On the other hand, a $(GlcNAc)_3$–Sepharose column, which strongly binds wheat-germ agglutinin (Uy & Wold, 1977), marrow (*Cucurbita pepo*) lectin (Allen, 1979) and potato lectin (Desai & Allen, 1979), does not bind *Datura* lectin (N. N. Desai & A. K. Allen, unpublished work).

General discussion

The lectin from *Datura stramonium* is similar to, but by no means identical with, the lectin from the potato. Superficially they have the same specificities for chitin oligosaccharides, but, when examined in detail, *Datura* lectin shows its greatest affinity for glycopeptides and potato lectin for chitin oligosaccharides. Both lectins are glycoproteins, but the molecular size of the subunit differs, being about 30000 daltons for *Datura* lectin and 50000 daltons for potato lectin. Both lectins are divided into two or more very different domains, one of which contains many contiguous hydroxyproline residues, which are glycosylated, and the other, which lacks glycosylated residues but has very high proportion of cystine and probably contains the binding sites. In the case of the *Datura* it is obvious from the composition of the peptide produced by the action of Pronase on the *S*-carboxymethylcysteinyl-lectin that there is a cystine-rich region adjacent to the glycosylated region.

In both lectins the hydroxyproline residues are substituted with β-arabinofuranosides, and there are also serine residues in the glycosylated region that are substituted with α-galactopyranoside residues. In potato lectin there are only single galactose residues, but in *Datura* lectin some of the galactose may be present as a disaccharide. Further evidence for the similarity of the two lectins comes from work in this laboratory showing that *Datura* lectin gives an immunological cross-reaction with anti-(potato lectin) serum (Ashford *et al.*, 1980). In addition, Kilpatrick *et al.* (1980) have shown that anti-(*Datura* lectin) serum cross-reacts with extracts of potato tubers, fruit and seeds as well as extracts from fruits and seeds of a number of other members of the Solanaceae. As has been observed elsewhere (Allen *et al.*, 1978; O'Neill & Selvendran, 1980; Ashford & Neuberger, 1980), the structure of the glycosylated region of these lectins is very similar to that of the hydroxyproline-rich glycopeptides of plant cell walls, and these lectins could even be precursors of such material.

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