The properties of potato (*Solanum tuberosum*) lectin after deglycosylation by trifluoromethanesulphonic acid

Nila N. DESAI, Anthony K. ALLEN and Albert NEUBERGER*

Department of Biochemistry, Charing Cross Hospital Medical School, Fulham Palace Road, London W6 8RF, U.K.

(Received 6 January 1983/Accepted 21 January 1983)

Potato (*Solanum tuberosum*) lectin, which is a very highly glycosylated glycoprotein, has been completely deglycosylated by use of the trifluoromethanesulphonic acid reagent described by Edge, Faltnek, Hof, Reichert & Weber [(1981) *Analyt. Biochem.* **118**, 131–137]. This shows that both hydroxyproline–arabinofuranoside and serine–galactopyranoside linkages are hydrolysed. The deglycosylated lectin is still functional and cross-reacts with one component of an anti-(potato lectin) antiserum.

Potato (*Solanum tuberosum*) lectin is an unusual glycoprotein with 50% (w/w) carbohydrate and is thus one of the most highly glycosylated of the lectins (Allen & Neuberger, 1973). The glycosylated amino acids are localized in one region of the polypeptide chain (Allen *et al.*, 1978), and these are hydroxyproline residues that are mainly linked *O*-glycosidically to tri- and tetraarabinofuranosides and serine residues linked *O*-glycosidically to monogalactopyranoside (Allen *et al.*, 1978; Ashford *et al.*, 1982a). Since similar structures are found in plant cell walls (Lamport, 1977, 1980) with identical glycosylations (Akiyama *et al.*, 1980), potato lectin, which is soluble, can be regarded as a useful model for biosynthetic studies of the insoluble plant-cell-wall glycoproteins [see Ashford & Neuberger (1980) for discussion].

Deglycosylation of potato lectin is desirable for a number of reasons; the non-involvement of covalently bound carbohydrate in the lectin binding site has been assumed but not proved. On the other hand, the involvement of covalently bound carbohydrate in the association of monomers, which is a prerequisite for agglutination, is possible. The use of potato lectin as a model for biosynthetic studies would also be enhanced by full deglycosylation. Previous attempts in this laboratory (Ashford, 1982) at deglycosylation of the lectin with dilute HCl also resulted in loss of part of the polypeptide chain and reduction in binding activity. Edge *et al.* (1981) have fairly recently demonstrated that trifluoromethanesulphonic acid is an effective reagent for deglyco-

syating glycoproteins that contain serine or threonine bound to *N*-acetylgalactosamine, or serine–xylose linkages, as well as the *O*-linkages between sugars. In the present paper we demonstrate that the hydroxyproline–arabinose and serine–galactose linkages are also split by this reagent without producing any hydrolysis of the polypeptide chain, and that the binding and agglutinating activity of the lectin is retained.

Experimental

Materials

Potato (*Solanum tuberosum* var. Ulster Sceptre) lectin was prepared by affinity chromatography as described previously (Desai & Allen, 1979), except that the lectin was eluted from the column with 2 mM-diethylamine rather than 0.1 M-acetic acid. Trifluoromethanesulphonic acid and anisole were obtained from Aldrich Chemical Co.

Methods

Analysis of amino acids and sugars. Amino acids were measured on a Locarte Mini amino acid analyser by reaction with ninhydrin; details of the elution programs which were used were given previously (Allen *et al.*, 1978; Ashford *et al.*, 1982a). Tryptophan was measured in the deglycosylated lectin after hydrolysis in 4 M-methanesulphonic acid (Simpson *et al.*, 1976), with hen's-egg-white lysozyme as a standard for tryptophan destruction. Sugars were measured by g.l.c. after methanolysis and trimethylsilylation as described previously (Ashford *et al.*, 1982a). The two sets of analyses were related by means of internal standards.

Abbreviation used: SDS, sodium dodecyl sulphate.

* To whom correspondence and requests for reprints should be sent.

Polarimetry. Optical rotation was measured on a Perkin-Elmer model 141 polarimeter in a 10 cm cell at room temperature at fixed points over the range 365–589 nm.

Polyacrylamide-gel electrophoresis. The method of Weber *et al.* (1972) was used. Further details are given by Allen (1979). Gels were stained for protein with Coomassie Blue and for carbohydrate by the thymol/ H_2SO_4 reagent described by Racusen (1979).

Haemagglutinating activity. This was measured on rabbit erythrocytes in 1% suspension in plastic microtitration cells (Ashford *et al.*, 1981).

Immunodiffusion tests. Double immunodiffusion was carried out in 1% (w/v) agarose gel with a rabbit antiserum to potato lectin (Ashford *et al.*, 1982b).

Deglycosylation of potato lectin. This was done by the method of Edge *et al.* (1981). Anisole (2 ml) and trifluoromethanesulphonic acid (4 ml) were flushed with N_2 and mixed in a glass vial with a Teflon-lined screw cap and cooled in ice. A freeze-dried sample of potato lectin (50 mg) which had been dried overnight *in vacuo* over P_2O_5 was added to 5 ml of the mixture and the mixture stirred in an iced-water bath for 3 h. The protein was then freed of reagents and sugars by method A of Edge *et al.* (1981), dialysed extensively against 1 M-NaCl, followed by water, and freeze-dried. The yield (21 mg) was almost that obtainable theoretically.

Results

Polyacrylamide-gel electrophoresis

Both native and deglycosylated potato lectin were subjected to SDS/polyacrylamide-gel electrophoresis after treatment with mercaptoethanol. Both proteins gave single bands which stained equally well with Coomassie Blue. The apparent molecular weights in comparison with marker proteins were 95000 for the native lectin and 71000 for the deglycosylated lectin. On treatment with the thymol/ H_2SO_4 reagent described by Racusen (1979), the native lectin stained strongly for carbohydrate, whereas the deglycosylated lectin did not react, nor was there any reaction in the position of native lectin on this gel.

Polarimetry

We had previously (Allen *et al.*, 1978) compared the optical-rotatory-dispersion spectra of glycosylated and deglycosylated glycopeptides of potato lectin at wavelengths from 365 to 589 nm. This had shown a positive rotation for the glycosylated glycopeptide and a highly negative rotation for the deglycosylated glycopeptide. This change was found to be due to the loss of the highly dextrorotatory β -arabinofuranosides from the glyco-

Fig. 1. Optical-rotatory-dispersion curve for native potato lectin and deglycosylated potato lectin

○, Native potato lectin (2 mg/ml in water); ●, deglycosylated potato lectin (2 mg/ml in water).

peptide. A comparison of equimolar amounts of native potato lectin ($\alpha_D = +42^\circ$) and deglycosylated lectin (equivalent $\alpha_D = -45^\circ$) showed very similar changes of optical rotatory dispersion from positive to negative (see Fig. 1). The observed difference of 87° was in reasonable agreement with the calculated contribution for the rotation of sugars of $+79^\circ$.

Amino acid and sugar analyses

The analyses of native and deglycosylated potato lectin are shown in Table 1. They are given as mol/mol of monomer on the assumption that the molecular weight of the monomer of the native lectin is 50000 (Allen *et al.*, 1978) and that the hydroxyproline content is 50 mol/mol. No sugars were detected by g.l.c. in the deglycosylated lectin. Differences between the two compositions would indicate that there has not been a significant loss of amino acids on deglycosylation.

Haemagglutinating and binding activity

When native lectin and deglycosylated lectin were compared for their haemagglutinating activity they gave an equal titre, based on equivalent weights of protein. Although this would indicate that the deglycosylated lectin is only half as active on a molar basis, a difference of one dilution in a haemagglutination assay is not regarded as representing substantial inactivation (see Ashford *et al.*, 1981).

A sample (1 mg) of the deglycosylated lectin was applied in phosphate-buffered saline (7.20 g of NaCl, 1.48 g of Na_2HPO_4 and 0.43 g of KH_2PO_4 /litre), pH 7.2, to the affinity column of $\text{NN}'\text{N}''$ -

Table 1. *Amino acid and carbohydrate composition of native potato lectin and deglycosylated potato lectin*
Values given were obtained from 24 h hydrolysates. The values for the native lectin and deglycosylated lectin are to the nearest whole residue. For further details, see the text. Abbreviation used: ND, not determined.

Amino acid	Composition [relative to hydroxyproline (= 50 mol/mol of monomer)]	
	(a) Native potato lectin	(b) Deglycosylated potato lectin
Hyp	50	50
Asp	13	12
Thr	16*	17
Ser	27*	29*
Glu	17	19
Pro	19	20
Gly	28	28
Ala	9	11
½-Cys	28†	32†
Val	1	ND
Met	1	1
Ile	3	4
Leu	3	4
Tyr	8	8
Phe	<0.5	0.3
His	ND	1.0
Lys	9	10
Orn	ND	ND
Arg	2	2
Trp	8	6.5
Ara	169	0
Gal	14	0

* Corrected for destruction during hydrolysis.

† Determined as cysteic acid.

Fig. 2. *Immunodiffusion of native potato lectin and deglycosylated potato lectin against anti-(potato lectin) serum*

Centre well (C), rabbit serum (20 µl); well 1, potato lectin (1 µg); well 2, deglycosylated potato lectin (10 µg).

precipitation line, which fuses to give a line of identity with the potato-lectin line, which is considered to react with the non-glycosylated regions.

Discussion

The treatment of potato lectin with trifluoromethanesulphonic acid has produced a fully deglycosylated but otherwise apparently intact lectin. The compositional analyses show that, within the limits of accuracy of the methods, all the amino acids are still present in the molecule, but the sugars (both arabinofuranosides and galactopyranosides) have been completely removed. This is confirmed by the changes in optical-rotatory-dispersion pattern and in apparent molecular weight on SDS/polyacrylamide-gel electrophoresis. Although the monomeric molecular weight is considered to be about 50 000 (Allen *et al.*, 1978), rather than 95 000 obtained from SDS/polyacrylamide-gel electrophoresis, the difference in apparent molecular weight is in good agreement with that expected for the loss of all the carbohydrate from the lectin. The exposed polyhydroxyproline sequences would not bind SDS, since polyhydroxyproline is immobile under these conditions (Ashford, 1982), and would therefore not contribute to the mobility of the deglycosylated

triacylchitotriose-Sepharose, which had been used to purify the native lectin. The column was then washed with phosphate-buffered saline, but no lectin was eluted. The lectin was eluted with the usual eluent, 2 mM-diethylamine.

Immunodiffusion studies

The immunodiffusion of potato lectin and deglycosylated potato lectin against rabbit anti-(potato lectin) serum is shown in Fig. 2. As has been shown previously (Ashford *et al.*, 1982b), the native lectin gives two precipitation lines with the antiserum, and it was suggested that there are two populations of antibodies with different specificities. The line which is furthest away from the centre well has been shown to be due to the population of antibodies that react with the arabinofuranosides (Ashford *et al.*, 1982b), and the other weaker line to be against the non-glycosylated regions of the molecule. The deglycosylated potato lectin gives only one pre-

lectin. It is noteworthy that the deglycosylated lectin gives a distinct band, with no evidence of fragmentation. The haemagglutinating activity of the deglycosylated lectin is almost equivalent to that of the native lectin, and they both bind to the strong inhibitor *NN'N''*-tri-*N*-acetylchitotriose, which is attached to Sepharose in the affinity-column matrix. This shows that the protein does not require its carbohydrate moiety in order to function as a lectin. The slight decrease in haemagglutinating activity observed on deglycosylation could be due to conformational changes affecting either the binding site or the process of dimerization.

The results reported in the present paper also demonstrate that the *O*-hydroxypropyl glycosides are hydrolysed by trifluoromethanesulphonic acid at least as effectively as are seryl or threonyl glycosides. This reagent has considerable potential use for studies of plant-cell-wall glycoproteins. The complete cleavage of the galactose-serine linkages in 3 h at 0°C is in contrast with the much slower (33% in 2.5 h at 0°C) cleavage of the linkage of *N*-acetyl-galactosamine to serine and threonine reported by Edge *et al.* (1981). This differential resistance could be explained by an inhibitory effect of the dipole of the acetamide group on hydrolysis.

We thank The Nuffield Foundation for financial support, and Miss Rosalind Rossell for excellent technical assistance.

References

- Akiyama, Y., Mori, M. & Katō, K. (1980) *Agric. Biol. Chem.* **44**, 2487–2489
- Allen, A. K. (1979) *Biochem. J.* **183**, 133–137
- Allen, A. K. & Neuberger, A. (1973) *Biochem. J.* **135**, 307–314
- Allen, A. K., Desai, N. N., Neuberger, A. & Creeth, J. M. (1978) *Biochem. J.* **171**, 665–674
- Ashford, D. (1982) Ph.D. Thesis, University of London
- Ashford, D. & Neuberger, A. (1980) *Trends Biochem. Sci.* **5**, 245–248
- Ashford, D., Menon, R., Allen, A. K. & Neuberger, A. (1981) *Biochem. J.* **199**, 399–408
- Ashford, D., Desai, N. N., Allen, A. K., Neuberger, A., O'Neill, M. A. & Selvendran, R. R. (1982a) *Biochem. J.* **201**, 199–208
- Ashford, D., Allen, A. K. & Neuberger, A. (1982b) *Biochem. J.* **201**, 641–645
- Desai, N. N. & Allen, A. K. (1979) *Anal. Biochem.* **93**, 88–90
- Edge, A. S. B., Faltnek, C. R., Hof, L., Reichert, L. E. Jr., & Weber, P. (1981) *Anal. Biochem.* **118**, 131–137
- Lamport, D. T. A. (1977) *Recent Adv. Phytochem.* **11**, 79–115
- Lamport, D. T. A. (1980) in *Biochemistry of Plants: A Comprehensive Treatise* (Stumpf, P. K. & Conn, E. E., series eds.), vol. 3 (Preiss, J., ed.), pp. 501–541, Academic Press, New York
- Racusen, D. (1979) *Anal. Biochem.* **99**, 474–476
- Simpson, R. J., Neuberger, M. R. & Liv, T. Y. (1976) *J. Biol. Chem.* **251**, 1936–1940
- Weber, K., Pringle, J. R. & Osborn, A. (1972) *Methods Enzymol.* **26**, 3–27