Protein conformation of potato (*Solanum tuberosum*) lectin determined by circular dichroism

Gerrit-Jan van HOLST,* Stephen R. MARTIN,† Anthony K. ALLEN,‡§ David ASHFORD,‡|| Nila N. DESAI‡ and Albert NEUBERGER‡

*Plant Biology Program, Department of Biology, Washington University, St. Louis, MO 63130, U.S.A., and Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Victoria 3052, Australia, †Division of Physical Biochemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K., and ‡Department of Biochemistry, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, U.K.

The structure of potato (*Solanum tuberosum*) lectin, which is a hydroxyproline-rich glycoprotein, has been investigated by circular dichroism. The spectra of the native lectin, and of the oxidized, reduced and carboxymethylated and deglycosylated derivatives were examined, as was a hydroxyproline-rich glycopeptide and its deglycosylated derivative. It is concluded that the lectin contains about 35% polyproline II conformation, 34% type II β -turn and 31% irregular conformation. No indications were found for the presence of α -helix or β -sheet conformations. The polyproline II conformation is heat-stable, but is markedly destabilized by deglycosylation. The type II β -turn is destabilized by cleavage of disulphide bonds.

INTRODUCTION

The lectin from potato (*Solanum tuberosum*) tubers is a glycoprotein (monomer M_r 50000) of unusual composition, being particularly rich in hydroxyproline, cystine and glycine, and it contains 50% (w/w) of carbohydrate (Allen & Neuberger, 1973). The lectin consists of at least two dissimilar domains (Allen *et al.*, 1978), including a binding-site domain that depends on disulphide bridges for its stability (Ashford *et al.*, 1981), and a highly glycosylated domain with mono-, di-, tri- and tetra-saccharides of arabinofuranose linked to hydroxyproline and single galactopyranose residues linked to serine (Allen *et al.*, 1978; Ashford *et al.*, 1982).

Previous reports of c.d. (Van Driessche & Kanarek, 1975; Ashford, 1982) and o.r.d. (Allen *et al.*, 1978) were interpreted as showing the presence of polyproline II helix, but, in contrast, Matsumoto *et al.* (1983) concluded from an interpretation of similar c.d. spectra that the lectin contained 40% β -sheet and 60% irregular conformation. All other hydroxyproline-rich glycoproteins of plant origin studied to date show the presence of the polyproline II conformation (Homer & Roberts, 1979; Van Holst & Fincher, 1984; Van Holst & Varner, 1984). Van Holst & Varner (1984) showed that the plant cell-wall glycoprotein extensin, which, like potato lectin, is rich in arabinosylated hydroxyproline, is entirely in the polyproline II conformation and that it is stabilized by the oligosaccharide side chains. In the present paper we give evidence for the presence of this stabilized polyproline II conformation in potato lectin and, in addition, for a high content of type II β -turn conformation.

MATERIALS AND METHODS

Potato lectin was prepared as described previously (Desai & Allen, 1979; Desai *et al.*, 1983) from potato (var.

Ulster Sceptre) tubers (the composition is given in Table 1a). This preparation was used for the spectra shown in Fig. 2 (below). The lectin that was used in the experiments shown in Fig. 1 (below) was purchased from Sigma Chemical Co. (lot no. 72F-3872), and had been purified by the method of Owens & Northcote (1980). Its composition did not differ significantly from the composition given in Table 1(a), although different varieties of potatoes had been used for the preparations. Both samples gave single bands of M_r 95000 on SDS/10%-(w/v)-polyacrylamide-gel slabs when stained with Coomassie Blue. Performic acid oxidation of the lectin was by the method of Hirs (1967) (from the Sigma preparation), and reduction followed by *S*-carboxymethylation (from the var.-Ulster-Sceptre preparation) was performed as described previously (Allen *et al.*, 1978). These procedures gave complete conversion of cystine in to cysteic acid or SCMC respectively. Lectins were deglycosylated by the use of trifluoromethanesulphonic acid (Edge *et al.*, 1981; Desai *et al.*, 1983). This procedure removed more than 99% of the carbohydrate, as determined by g.l.c., but did not result in any loss of peptide material, as determined by amino acid analysis. The composition of the hydroxyproline-rich glycopeptide (M_r 32000–34000) that was derived from the *S*-carboxymethylated lectin by exhaustive Pronase digestion and the derivative that had been de-arabinosylated by acid treatment (M_r 9400) are given in Tables 1(b) and 1(c); details of the preparation and characterization were given previously (Allen *et al.*, 1978).

Carbohydrate derivatives

α - and β -methyl L-arabinofuranosides were prepared by the method of Augustad & Berner (1956) and were separated on Dowex-1 resin (OH⁻ form) (Neuberger & Wilson, 1971). α -Methyl D-galactopyranoside was prepared by the procedure of Neuberger & Wilson (1971).

Abbreviation used: SCMC, *S*-carboxymethylcysteine.

§ To whom correspondence and reprint requests should be addressed.

|| Present address: Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

Table 1. Amino acid analysis of potato lectin and derivatives

Results are expressed as mol of amino acid per 50 mol of hydroxyproline (Hyp) (equivalent to mol of amino acid/mol of protein). See the Materials and methods section for details of the sources and preparation of the derivatives. Results in (b) and (c) are from Allen *et al.* (1978).

Amino acid	Content (mol/50 mol of Hyp)		
	Native lectin (a)	Hyp-rich glycopeptide (b)	HCl-treated Hyp-rich glycopeptide (c)
Hyp	50	50	50
Asx	13	2.3	1.2
Thr	16	1.6	0.6
Ser	27	16.8	14.5
Glx	17	4.1	0.9
Pro	19	3.7	0.5
Gly	28	4.9	2.2
Ala	9	4.4	2.7
$\frac{1}{2}$ -Cys	28	0.0	0.0
Val	1	0.0	0.0
Met	1	0.0	0.0
Ile	3	0.5	< 0.2
Leu	3	3.0	2.1
Tyr	8	0.3	< 0.2
Phe	0	0.5	< 0.2
His	0	0.0	N.D.*
Orn	1	N.D.	N.D.
Lys	9	0.6	N.D.
Arg	2	0.6	N.D.
Trp	8	0.0	N.D.
Total amino acids	243	94	75
Ara	169	172	0
Gal	14	9	9

* N.D., not determined.

Mixed hydroxyproline-arabinosides were isolated from potato lectin by alkaline hydrolysis as previously described (Allen *et al.*, 1978).

Amino acid and carbohydrate analysis

Proteins were hydrolysed in either 3 M-toluene-4-sulphonic acid or in constant-boiling HCl containing 0.05% (w/v) 2-mercaptoethanol under N₂ for 24 h at 110 °C. The amino acids were measured on an automatic amino acid analyser by reaction with ninhydrin. Sugars were determined by g.l.c. after methanolysis and trimethylsilylation [see Allen *et al.* (1978) for further details].

Circular dichrometry

The c.d. spectra presented in Fig. 1 (below) were measured with a Jasco-Durrum J-20 spectropolarimeter under constant nitrogen flush. It was calibrated at three wavelengths. A (+)-10-camphorsulphonic acid (Eastman-Kodak) solution in water at 25 °C {[θ]₂₉₀^{max.} = 7.80 × 10³ degrees·cm²·dmol⁻¹ (Chen & Yang, 1977)} was used as the primary standard. With this calibration the (+)-10-camphorsulphonic acid solution gave [θ]₁₉₂^{min.} = -16 × 10³ degree·cm²·dmol⁻¹ (Chen & Yang, 1977). D-Pantolactone (D-2-hydroxy-3,3-dimethylbutyrolactone) (Fluka) in methanol at 25 °C gave [θ]₂₂₂^{min.}

= -11.7 × 10³ degrees·cm²·dmol⁻¹. At 20 °C the [θ]_{290 nm}^{max.} of (+)-10-camphorsulphonic acid in water was the same as at 25 °C; at 75 °C the ellipticity ([θ]₂₉₀^{max.}) was 7.33 × 10⁻³ degrees·cm²·dmol⁻¹.

The cells of 0.5 and 0.1 cm path length were calibrated with (+)-10-camphorsulphonic acid at 290 nm. A water-jacketted copper block attached to a water bath served as cell holder and allowed the temperature to be regulated. The cell temperature was measured with an electronic probe.

The amino acid concentration in the samples (about 0.13 mg/ml in water) was calculated from the hydroxyproline concentration (Drozd *et al.*, 1976) after acid hydrolyses. C.d. data are expressed in terms of mean amino acid residue ellipticity, [θ], in degrees·cm²·dmol⁻¹.

C.d. spectra presented in Fig. 2 (below) were recorded with a Jasco J41C spectropolarimeter equipped with a model J-DPY data processor. This instrument was also calibrated with (+)-10-camphorsulphonic acid at 290 nm as described above.

The content of polyproline II conformation of the peptide was calculated from the minimum (at about 202 nm) and the maximum (at 225 nm) independently, the values for polyhydroxyproline (Van Holst & Varner, 1984) and for denatured collagen in 0.15 M-KF (Piez & Sherman, 1970) being used as standards. Polyhydroxyproline was assumed to have 100% of its residues in the polyproline II conformation, whereas denatured rat skin collagen (gelatin) was assumed to have none (0%) of its residues in the polyproline II conformation at 44 °C and to be a standard for the irregular conformation (I.C.). The percentages of polyproline II conformation (P.II.C.) in the lectin derivatives was then assigned according to the formula:

$$\text{P.II.C. (\%)} = \frac{[\theta] \text{ lectin derivative} - [\theta] \text{ I.C.}}{[\theta] \text{ P.II.C.} - [\theta] \text{ I.C.}}$$

For the native lectin at 20/22 °C the percentage of polyproline II conformation was calculated from the mean of the values for the S-carboxymethylated and performic acid-oxidized lectins. For the deglycosylated lectin at 22 °C the value for the performic acid-oxidized lectin was taken.

For the calculation of the percentage of type II β -turns, the contribution that would have been expected from the polyproline II and irregular conformations was deducted from the observed values at 202 or 204 nm, and the resultant positive value was then assigned to the differences between type II β -turn and irregular conformation. The value obtained by Crisma *et al.* (1984) for pivaloyl-L-prolyl-D-alanylmethylamine (in methanol/water, 1:9, v/v) was used as a standard for the type II β -turn. The formulae used were:

Type II β -turn in lectin (%) =

$$\frac{[\theta] \text{ lectin} - [\theta] 35\% \text{ P.II.C.} + 65\% \text{ I.C.}}{[\theta] \text{ type II } \beta\text{-turn} - [\theta] \text{ I.C.}}$$

Type II β -turn in deglycosylated lectin (%) =

$$\frac{[\theta] \text{ deglycosylated lectin} - [\theta] 7\% \text{ P.II.C.} + 93\% \text{ I.C.}}{[\theta] \text{ type II } \beta\text{-turn} - [\theta] \text{ I.C.}}$$

The absence of α -helix and β -sheet was determined by comparison of the spectra with published examples (Fig. 2 of Brahms & Brahms, 1980).

Fig. 1. C.d. spectra of (a) native lectin, (b) performic acid-oxidized lectin and (c) deglycosylated performic acid-oxidized lectin in water at 20 °C (—) and at 75 °C (....)

RESULTS

The c.d. spectra of native potato lectin at 20 °C or 22 °C in the range 195–250 nm are shown in Fig. 1(a). The lectins from the two sources gave identical spectra characterized by a trough at 202 nm and a peak at 224 nm. Although this spectrum gives a possible indication of a polyproline II structure, it is difficult to interpret, owing in particular to the contributions of disulphide groups. Derivatives of the lectin in which the cystine residues had been converted into cysteic acid by performic acid oxidation or into SCMC by reduction and alkylation were therefore prepared, since these groups do not contribute to the c.d. spectrum.

The c.d. spectrum of performic acid-oxidized lectin is shown in Fig. 1(b) and that of the *S*-carboxymethylated lectin in Fig. 2(b). The derivatives give similar spectra, and in both the ellipticity of the trough and the peak are decreased (i.e. more negative) in comparison with results with untreated potato lectin. The shapes of the curves are characteristic of the polyproline II conformation, but the

positions of the minima are at lower wavelengths than that of polyproline or polyhydroxyproline. This is probably due to the lower wavelength for the absorption of peptide linkages formed by the intercalated primary amino than by imino groups and is generally observed in those plant glycoproteins that contain polyproline II conformation (Homer & Roberts, 1979; Van Holst & Varner, 1984; Van Holst & Fincher, 1984). The calculations from the data given in Table 2 indicate that the content of polyproline II conformation is about 35% for both derivatives. In addition, the c.d. spectrum of the

Fig. 2. C.d. spectra of (a) deglycosylated lectin, (b) carboxymethylated lectin, (c) hydroxyproline-rich glycopeptide and (d) HCl-treated hydroxyproline-rich glycopeptide in water at 22 °C

Table 2. Calculations of the secondary conformation from c.d.

The contributions of polyproline II, type II β -turn and irregular conformation were calculated from the ellipticity of the extrema (or shoulder) by using the ellipticity of the extrema (or shoulder) of the standard reference spectra of respectively polyhydroxyproline, pivaloyl-L-prolyl-D-alanylmethylamine and heated rat skin collagen. The contributions of polyproline II, and type II β -turn conformations are tabulated; the remainder is assigned to the irregular conformation. All samples were dissolved in water unless stated otherwise.

Material	Temperature (°C)	Extrema or shoulder*		Polyproline II (%)	Type II β -turn (%)
		λ (nm)	$10^{-3} \times [\theta]$ (degrees $\cdot$ cm ² $\cdot$ dmol ⁻¹)		
Native lectin	20/22	224	9.8	IDS	IDS
		202	-16.0	35	34
Deglycosylated lectin	22	226	2.1	IDS	IDS
		204	-7.2	7	30
Carboxymethylated lectin	22	227	1.6	32	0
		202	-25.0	40	0
Performic acid-oxidized lectin	20	225	1.9	36	0
		202	-22.0	31	0
Deglycosylated performic acid-oxidized lectin	20	(225)*	(-0.5)*	8	0
		200	-13.2	6	0
Hydroxyproline-rich glycopeptide	22	225	7.6	101	0
		202	-27.5	47	0
HCl-treated hydroxyproline-rich glycopeptide	22	227	0.1	0	0
		211-197	-1.8	0	0
Native lectin	75	(225)	(-0.8)	IDS	IDS
		202	-20.8	28	0
Performic acid-oxidized lectin	75	(225)	(0)	14	0
		201	-18.4	21	0
Deglycosylated performic acid-oxidized lectin	75	(225)	(-1.2)	0	0
		200	-11.6	1	0
Polyhydroxyproline†	20	226	7.5	100	0
		206	-45.5	100	0
Piv-L-Pro-D-Ala-NHMe‡	22	230	0.9	0	100
		200	10.1	0	100
Denatured collagen§	44	(225)	(-1.2)	0	0
		199	-11.3	0	0

* The values for shoulders are shown in parentheses.

† Used as standard reference spectrum; results for polyhydroxyproline in water are from Van Holst & Varner (1984).

‡ Results for pivaloyl-L-prolyl-D-alanylmethylamine (Piv-L-Pro-D-Ala-NHMe) in water/methanol (9:1, v/v) are from Crisma *et al.* (1984), after correction for differences in instrument calibration.

§ Results for denatured rat skin collagen in 0.15M-KF are from Piez & Sherman (1970).

|| Abbreviation used: IDS, interference of disulphide bonds.

hydroxyproline-rich glycopeptide (Fig. 2c), in which 53% of the residues are hydroxyproline, shows a high proportion of polyproline II conformation.

Deglycosylation with trifluoromethanesulphonic acid removes all the carbohydrate from the hydroxyproline-rich domain, but leaves the peptide bonds intact. The c.d. spectrum of the deglycosylated native lectin, which retains its lectin activity (Desai *et al.*, 1983), shows (Fig. 2a) a lower ellipticity at 226 nm and a higher ellipticity at 204 nm compared with the native lectin. Similarly, the c.d. spectrum of the deglycosylated performic acid-oxidized potato lectin (Fig. 1c) at 20 °C shows a shallow trough at 200 nm and no peak in the region of 225 nm. Both these spectra can be interpreted in terms of a loss of polyproline II conformation. The spectrum of the deglycosylated performic acid-oxidized lectin shows characteristics of an irregular conformation, and we calculate that about 7% polyproline II conformation could be present (Table 2). At 75 °C the deglycosylated performic acid-oxidized potato lectin (in contrast with the

glycosylated derivatives) showed none of the characteristics of the polyproline II conformation (Table 2 and Fig. 1c). The deglycosylated hydroxyproline-rich glycopeptide that lacks arabinose but has 67% of hydroxyproline residues shows minimal c.d. (Table 2 and Fig. 2d) and apparently lacks the polyproline II conformation.

The differences in the spectra before and after deglycosylation of the native lectin and the performic acid-oxidized lectin are not due to the c.d. spectra of the carbohydrate moiety itself, as the c.d. spectra of the α - and β -methyl L-arabinofuranosides, α -methyl D-galactopyranoside and mixed hydroxyproline-arabinosides show negligible contributions on a molar basis at wavelengths above 195 nm (results not shown).

DISCUSSION

The c.d. spectrum of potato lectin can be interpreted by considering its two dissimilar domains (Allen *et al.*, 1978); one domain contains the glycosylated amino acids

and is hydroxyproline-rich and the other is the binding-site domain, which is rich in cystine and glycine and depends on disulphide bridges for its stability (Ashford *et al.*, 1981).

Reduction followed by carboxymethylation, or performic acid oxidation, breaks the disulphide bridges and eliminates their c.d. contribution. The c.d. spectra of performic acid-oxidized lectin and carboxymethylated lectin both show the presence of about 35% polyproline II conformation (Table 2). This corresponds well with 38% of the peptide being in the extensin-like hydroxyproline-rich domain (Allen *et al.*, 1978), as extensin is in the polyproline II conformation (Van Holst & Varner, 1984). The c.d. spectrum of the isolated hydroxyproline-rich glycopeptide also shows the presence of a high percentage of polyproline II.

The polyproline II helix in the performic acid oxidized lectin is similar to that of extensin in terms of its heat stability (Van Holst & Varner, 1984), with half of the polyproline II conformation still present at 75 °C. After deglycosylation of either the native or the performic acid-oxidized lectin, the content of polyproline II conformation was dramatically reduced. This indicated that, as in extensin (Van Holst & Varner, 1984), the polyproline II conformation in potato lectin is stabilized by the oligosaccharide side chains of the glycoprotein, which are identical in structure with those of extensin [compare Ashford *et al.*, (1982) and Akiyama *et al.* (1980)]. The stabilization of the polyproline II conformation by the oligosaccharide side chains is apparently very strong, since heating to 75 °C does not destroy as much polyproline II conformation as does deglycosylation. Furthermore, deglycosylation of the hydroxyproline-rich glycopeptide produces a structure that is apparently completely devoid of polyproline II conformation from the c.d. spectrum. Although from previous o.r.d. data it had been concluded that some structure had been retained (Allen *et al.*, 1978), we have now re-interpreted the data and conclude that this is not the case. It is therefore clear that the saccharide chains have a stabilizing effect on the polyproline II conformation.

Performic acid oxidation or reduction and carboxymethylation destroys the structure of the cystine-rich binding domain (Ashford *et al.*, 1981). From a comparison of the c.d. spectra of these derivatives and the native lectin and the above derivatives it is apparent that the structure that was destroyed must have made a marked positive contribution to the ellipticity of the native lectin over the range 195–245 nm, but the relative contributions of the peptide chain and the disulphide bridges are unknown. Heating the native lectin also disrupts this protein conformation, since only polyproline II conformation is left at 75 °C (note that the spectra of the native lectin and the performic acid-treated lectin at 75 °C are similar). Here again, the conformation destroyed by heating must have made a positive contribution to the ellipticity over the range 195–245 nm. Although in this case the disulphides remain, the c.d. of the disulphide bridges can also change owing to angular rotation of the disulphide bonds. On examination of published c.d. spectra, only type II β -turns (Crisma *et al.*, 1984) have a positive ellipticity over the studied range. As the contribution of disulphide bonds is relatively greatest at the longer wavelength, we calculated the type II β -turn content from the ellipticity at the minimum from reference spectra (see the Materials and methods section

and Table 2). We found that about 34% of the protein could be in the type II β -turn conformation in the native lectin and 30% in the deglycosylated lectin (Table 2). In β -turns the first residue is termed *i* and the succeeding residues *i* + 1, *i* + 2 and *i* + 3. In the type II β -turn there is potential steric hindrance between the carboxy-group oxygen of residue *i* + 1 and the side chain of the residue *i* + 2, so that in proteins residue *i* + 2 can only be glycine (Venkatchalam, 1968). For 34% type II β -turn conformation the protein must therefore have at least 11.3% glycine content. The potato lectin fulfils this requirement (Table 1) with almost all the glycine residues being present in the non-glycosylated domain (Allen *et al.*, 1978). We therefore conclude that the type II β -turn conformation is confined to this domain.

General conclusions

The c.d. spectrum reported by Matsumoto *et al.* (1983) was qualitatively similar to that which we report here, but the ellipticity was only half the value that we have quoted. The explanation of this would seem to be that they did not take into account the fact that half the weight of the lectin is carbohydrate, which does not make a significant contribution towards the c.d. Matsumoto *et al.* (1983) concluded that potato lectin contained 40% β -form and 60% unordered form. This conclusion seems to be based on the $[\theta]$ values at 195 nm, but is not able to account for the observed positive ellipticity at 224 nm. An analysis by Chang *et al.* (1978) shows that a 40%/60% distribution of β - and unordered forms would be expected to show a negative ellipticity at 222 nm, and inspection of Fig. 2 of Brahms & Brahms (1980) shows that any protein with a significant α -helix or β -sheet content would show negative ellipticity at 222 nm, which is the reverse of the situation with potato lectin.

From our studies of the c.d. of potato lectin and its derivatives and from earlier studies based on partial degradation of the lectin (Allen *et al.*, 1978; Ashford *et al.*, 1982), we are able to conclude that there are domains of potato lectin differing in their structures. The glycosylated hydroxyproline-rich region is in a polyproline II conformation and accounts for 35% of the polypeptide chain. This conformation is largely lost when the carbohydrate is removed and must depend on the sugar side chains for its stability.

The type II β -turns are located in the non-glycosylated region where the saccharides are bound and the disulphide bridges are located (Allen *et al.*, 1978; Ashford *et al.*, 1982). This conclusion is reached because the glycosylated domain has insufficient glycine to permit a high proportion of type II β -turns, and these β -turns are lost on oxidation or reduction of disulphide bridges. This type II β -turn component accounts for about 34% of polypeptide chain, the remaining 31% being assigned to an irregular conformation.

G. J. Van H. thanks Professor J. E. Varner for scientific and other support. Part of this work was supported by the National Science Foundation (U.S.A.) (grant PCM 8104516 to J. E. V.) and by a Melbourne University Fellowship (to G. J. Van H.). Part of this work was supported by the Nuffield Foundation and the Agricultural Research Council.

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Received 7 May 1985/19 September 1985; accepted 3 October 1985