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Characterization of the hydroxyproline-rich protein core of an arabinogalactan-protein secreted from suspension-cultured *Lolium multiflorum* (Italian ryegrass) endosperm cells

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An arabinogalactan-protein (AGP) purified from the filtrate of liquid-suspension-cultured Italian-ryegrass (*Lolium multiflorum*) endosperm cells by affinity chromatography on myeloma protein J539–Sepharose was deglycosylated with trifluoromethanesulphonic acid to remove polysaccharide chains that are covalently associated with hydroxyproline residues in the peptide component of the proteoglycan. The protein core, which accounts for less than 10% (w/w) of the intact proteoglycan, was purified by h.p.l.c. It has an apparent M_r of 35000, but reacts very poorly with both Coomassie Brilliant Blue R and silver stains. Amino-acid-sequence analysis of the *N*-terminus of the h.p.l.c.-purified protein core and of tryptic peptides generated from the unpurified protein reveals a high content of hydroxyproline and alanine. These are sometimes arranged in short (Ala-Hyp) repeat sequences of up to six residues. Polyclonal antibodies raised against the protein core do not cross-react with native AGP, the synthetic peptide $(Ala\text{-}Hyp)_4$, poly-L-hydroxyproline or poly-L-proline. The results suggest that the polysaccharide chains in the native AGP render the protein core of the proteoglycan inaccessible to the antibodies and that the immunodominant epitopes include domains of the protein other than those rich in Ala-Hyp repeating units.

INTRODUCTION

Arabinogalactan-proteins (AGPs) are widely distributed plant proteoglycans containing high proportions of carbohydrate and usually less than 10% by weight of protein (Clarke *et al.*, 1979; Fincher *et al.*, 1983). The carbohydrate consists of polysaccharide chains in which a backbone of (1→3)-linked β-D-galactopyranose residues is branched through C(O)6 with (1→6)-linked β-D-galactopyranose side chains, which in turn are substituted with arabinofuranose and other less-abundant monosaccharides, often in terminal positions (Fincher *et al.*, 1983). There is evidence for the occurrence of regularly spaced periodate-oxidizable residues in the galactan framework, indicating a degree of structural regularity in the polysaccharide (Bacic *et al.*, 1987). In an AG-peptide from wheat (*Triticum aestivum*) endosperm (Fincher & Stone, 1974), the polysaccharide chains are attached to a protein core by β-D-galactose–hydroxyproline linkages (McNamara & Stone, 1981; Strahm *et al.*, 1981), and there is evidence that the same linkage group is present in the Italian ryegrass (*Lolium multiflorum*) AGP (Bacic *et al.*, 1987).

In contrast with the polysaccharide component, relatively little is known about the structure and organization of the protein core of AGPs, except that the protein is usually rich in alanine, hydroxyproline and serine (Fincher *et al.*, 1983). In the present work, the polysaccharide chains of an AGP secreted by suspension-cultured ryegrass endosperm cells were removed chemically and the residual protein core characterized. The ryegrass AGP is typical of other plant AGPs in that the carbohydrate component consists mainly of arabinose and galactose residues (Anderson *et al.*, 1977; Bacic *et al.*, 1987) and the protein, which represents 7% (w/w) of the AGP, contains 22.5% (mol/mol) alanine, 14.8% hydroxyproline and 9.8% serine (Anderson *et al.*, 1977). Levels of the hydroxyproline-rich cell-wall glycoproteins or 'extensins' are negligible in these cells (Anderson *et al.*, 1977), and confusion between the two classes of hydroxyproline-rich polymers is therefore avoided.

MATERIALS AND METHODS

Purification of AGP

Liquid suspension-cultures of ryegrass endosperm cells were maintained in the dark at 28 °C on a White's medium containing 4% (w/v) sucrose (Smith & Stone, 1973). The AGP was isolated from the culture filtrate of 7-day cultures (mid-exponential phase) by affinity chromatography on myeloma protein J539–Sepharose (Andrew & Stone, 1983; Bacic *et al.*, 1987). The AGP isolated by this method has been shown to have monosaccharide and amino acid compositions identical with those of preparations isolated previously (Anderson *et al.*, 1977; van Holst & Fincher, 1984; Bacic *et al.*, 1987). The yield of AGP was approx. 12 mg per 200 ml of culture medium.

Metabolic labelling of AGP

Ryegrass cells were metabolically labelled with [^{14}C]-proline (Pollard & Fincher, 1981). When cells were

Abbreviations used: AGP, arabinogalactan-protein; AG-peptide, arabinogalactan-peptide; TFA, trifluoroacetic acid.

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subcultured, 5 μCi of L-[U-^{14}C]proline (280 mCi/mmol; Amersham Australia Pty. Ltd., Melbourne, Australia) was added to 130 ml of cell suspension, consisting of 100 ml of fresh medium and 30 ml of stationary-phase ryegrass-cell suspension. After 7 days culture, cells were removed by filtration and centrifuged at 10000 *g* for 30 min. The ^{14}C-labelled AGP was isolated from the culture medium by affinity chromatography (Bacic *et al.*, 1987). The yield of [^{14}C]AGP was 33000 c.p.m. from 300 ml of culture medium (approx. 1800 c.p.m./mg of AGP).

Deglycosylation

The purified AGP was deglycosylated with trifluoromethanesulphonic acid (Fluka) (Edge *et al.*, 1981). Typically, 15 mg of purified AGP, dried *in vacuo* over P_2O_5, was dissolved in 2 ml of cold anisole/trifluoromethanesulphonic acid (1:2, v/v) mixture in a glass vial with a Teflon-lined screw cap, the sample flushed with nitrogen and stirred at 0 °C for 3 h. The reaction mixture was transferred to a larger tube and 4 ml of diethyl ether (precooled in solid CO_2/ethanol) was added, followed by 6 ml of ice-cold aq. 50% (v/v) pyridine. The precipitate which formed was redissolved with vigorous shaking, and the diethyl ether phase was removed. After re-extraction with diethyl ether, the protein was recovered from the aqueous phase either by gel-filtration chromatography on Bio-Gel P2 (Bio-Rad Laboratories, Richmond, CA, U.S.A.) in 25 mM-pyridine acetate or by ultrafiltration through an Amicon YM2 (Amicon Corp., Danvers, MA, U.S.A.) membrane. The sample was freeze-dried. Dialysis was avoided to prevent the loss of small peptides. Protein was detected by assaying for hydroxyproline (Drózdz *et al.*, 1976) after hydrolysis of the sample in constant-boiling-point HCl under N_2 at 110 °C for 20 h. Total neutral sugars were determined colorimetrically by the phenol/H_2SO_4 assay (Dubois *et al.*, 1956), with galactose as standard. Monosaccharide composition was determined by g.l.c. of alditol acetates (Blakeney *et al.*, 1983). Galactose content of acid hydrolysates was also estimated by the galactose dehydrogenase assay (Finch *et al.*, 1969).

H.p.l.c. purification of deglycosylated AGP

The deglycosylated AGP was purified by reversed-phase h.p.l.c. on a Bondapak C_{18} column (Waters) using a flow rate of 1 ml/min and a linear gradient of 0 to 50% solvent B, where the starting solvent A was water/0.1% trifluoroacetic acid (TFA) and solvent B was acetonitrile/0.1% TFA.

Preparation of antisera

Antiserum to the deglycosylated AGP was raised in a New Zealand White rabbit by subcutaneous injection of 300 μg of deglycosylated AGP in an equal volume of Freund's complete adjuvant, followed by 150 μg of antigen in Freund's incomplete adjuvant 28 days later. Serum was collected 6 and 9 days after the second injection.

The synthetic peptide (Ala-Hyp)$_4$-Ala-Cys was conjugated to *Limulus polyphemus* (King crab) haemocyanin (Sigma Chemical Co., St Louis, MO, U.S.A.) with glutaraldehyde (Reichlin, 1980). Antiserum was prepared as described above, with 500 μg of conjugate for the primary inoculum and 100 μg for the secondary inoculum.

E.l.i.s.a.

E.l.i.s.a. was performed in 96-well polyvinyl plates coated with 50 μl of antigen in phosphate-buffered saline (10 mM-sodium phosphate/150 mM-NaCl), pH 7.4, for 16 h at 4 °C. Excess binding sites were blocked with 1% bovine serum albumin in buffer A (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 80 and 0.05% NaN_3) for 2 h at room temperature. After washing in buffer A, plates were incubated with antisera (50 μl/well, diluted 2-fold in buffer A containing 0.25% ovalbumin) for 2 h at room temperature. The plates were washed twice with buffer A and twice with phosphate-buffered saline, pH 7.4, and incubated with sheep anti-rabbit antibodies conjugated with urease (Commonwealth Serum Laboratories, Melbourne, Australia) (50 μl/well after 200-fold dilution of the supplied conjugate with buffer A containing 0.25% ovalbumin) for 2 h at room temperature. Plates were washed twice with buffer A, twice with phosphate-buffered saline, pH 7.4, four times with distilled water and the plates incubated at 37 °C for 30–60 min with urease substrate (50 μl of 16.6 mM-urea/0.2 mM-EDTA, pH 4.5, per well) for colour development.

Polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed using the buffer system of Laemmli (1970). Gels were stained for protein with Coomassie Blue R250 or with silver stain (Wray *et al.*, 1981).

After electrophoresis, gels containing ^{14}C-labelled proteins were treated with Amplify fluorographic enhancer (Amersham) according to the manufacturer's recommendations. Gels were dried and fluorographs obtained using Kodak X-Omat AR film. ^{14}C-labelled molecular-mass standards were from Amersham.

For immunoblots, 12.5% (w/v) polyacrylamide gels were run under reducing conditions. After electrotransfer to nitrocellulose, the filter was quenched with 3% (w/v) bovine serum albumin and incubated with antiserum raised against deglycosylated AGP or with pre-immune serum [each diluted 250-fold in 10 mM-Tris/HCl, pH 7.4, containing 0.9% NaCl and 3% (w/v) bovine serum albumin]. After incubation in the same buffer containing ^{125}I-protein A (10^6 c.p.m./ml; 25 μCi/μg; generously provided by Dr. Claude Bernard), the filters were washed as described above before autoradiography.

Amino acid analysis

Proteins were hydrolysed with constant-boiling HCl, under N_2, at 110 °C for 24 h. Amino acids (approx. 200 pmol total) were analysed on a Beckman System 6300 amino acid analyser with ninhydrin detection. Values for serine and threonine were corrected for destruction during acid hydrolysis at a rate of 10% and 5% respectively.

Amino acid sequence

Automated amino acid sequence analysis was performed in an Applied Biosystems model 470A gas–liquid phase sequencer (Wettenhall *et al.*, 1987) using trifluoroacetic acid conversion chemistry (Hewick *et al.*, 1981). Phenylthiohydantoin derivates of amino acids were analysed by h.p.l.c. on a Zorbax C_8 reverse-phase column (du Pont, Wilmington, DE, U.S.A.) eluted with a discontinuous gradient of acetonitrile in aq. 10–20 mM-

sodium acetate buffer, pH 5.0 (Zimmerman *et al.*, 1977). Tryptic peptides were generated by hydrolysing deglycosylated AGP fractions with 20 μg/ml tosylphenylalanylchoromethane ('TPCK')-treated trypsin (Cappell Worthington Biochemicals, Malvern, PA, U.S.A.) in 10 mM-Tris/HCl, pH 8.0 for 1 h and 24 h at room temperature. The reaction was stopped by addition of trifluoroacetic acid to 0.2% (v/v). Tryptic peptides were fractionated by reversed-phase h.p.l.c. on a Vydac C_{18} column using a 0–50% (v/v) acetonitrile gradient in aq. 0.1% (v/v) trifluoroacetic acid. H.p.l.c. fractions were dried and peptides dissolved in 50% (v/v) trifluoroacetic acid for sequencing.

RESULTS

Deglycosylation of the AGP

When purified AGP, containing 93% (w/w) carbohydrate and 7% protein, was treated with trifluoromethanesulphonic acid, a product containing less than 10% carbohydrate and more than 90% protein was obtained, indicating that more than 99% of the original carbohydrate was removed. The remaining carbohydrate consisted entirely of galactose residues, as determined by g.l.c. analysis of alditol acetate derivatives and by the galactose dehydrogenase assay.

When the deglycosylated AGP preparation was analysed by SDS/polyacrylamide-gel electrophoresis at loadings of up to 2 μg, the protein could not be detected readily with either Coomassie Blue or the silver stain. This probably results from the low content of basic amino acids in the polypeptide (Wray *et al.*, 1981). To locate the deglycosylated AGP on gels, ^{14}C-labelled AGP was isolated, deglycosylated and the residue detected by autoradiography after electrophoresis. A single major band of apparent M_r 35000 was observed (Fig. 1*a*).

Antiserum to the deglycosylated AGP

Polyclonal antibodies against the deglycosylated AGP were raised in rabbits and were shown to recognize the major polypeptide of M_r 35000 in immunoblot analyses of the deglycosylated AGP (Fig. 1*b*). Antibody specificity was examined by using e.l.i.s.a. (Table 1); the antibodies specifically bind to the deglycosylated AGP. The absence of binding to native AGP or to a larch (1→3,1→6)-β-galactan of similar structure to the ryegrass AGP, together with the observation that 200 mM-galactose did not inhibit the binding with deglycosylated AGP (Table 1), strongly suggest that the specificity of the antiserum is directed towards the protein core of the AGP.

H.p.l.c. purification of the deglycosylated AGP

Initial attempts to obtain *N*-terminal sequence information indicated that some residual free sugars and their acid-degradation products remained in the sample. In addition, multiple *N*-terminal sequences were detected. The preparation was therefore purified further by h.p.l.c. and the fractions containing deglycosylated AGP identified by ^{14}C-labelling, by reaction with antiserum to the deglycosylated AGP in an e.l.i.s.a. (Fig. 2) and by amino acid analysis of fractions. Amino acid analyses indicated that hydroxyproline-containing proteins were associated predominantly with the asymmetrical A_{215} peak that was eluted at 34–35 ml (Fig. 2). The h.p.l.c. analyses of deglycosylated AGP from ryegrass cells cultured in [^{14}C]proline also showed that the major peak

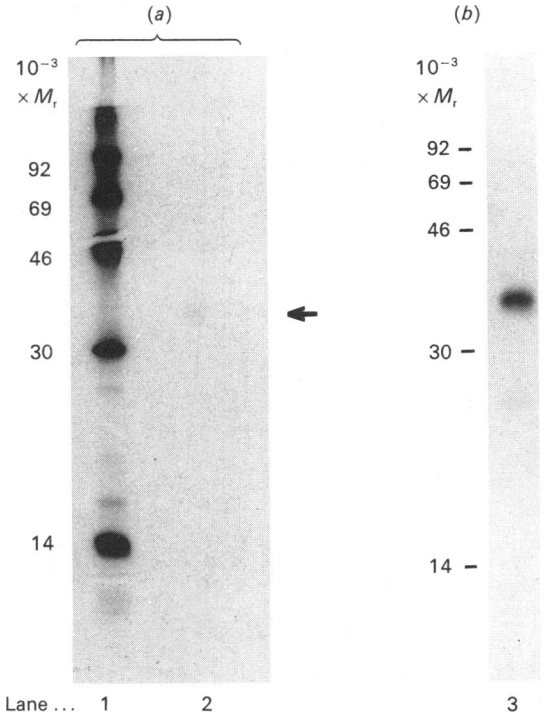

Fig. 1.(*a*) Autoradiogram of the protein core released by deglycosylation of ^{14}C-labelled ryegrass AGP (lane 1, ^{14}C-labelled molecular-size markers; lane 2, deglycosylated AGP) and (*b*) immunoblot analysis of deglycosylated ryegrass AGP probed with polyclonal antibodies and ^{125}I-protein A

Table 1. Specificity of antisera to deglycosylated ryegrass AGP

	Amount (ng)	Titre
Native AGP	5000	< 50
Deglycosylated AGP	50	1600
Deglycosylated AGP + 200 mM-galactose	50	1600
Oxalic acid-treated larch galactan*	5000	< 50
(Ala-Hyp)$_4$–haemocyanin	500	< 50
Poly-L-Pro	500	< 50
Poly-L-Hyp	500	< 50

* Oxalic acid treatment removed arabinofuranose residues from the larch arabinogalactan to expose the (1→3,1→6)-β-galactan framework, which is similar in structure to the galactan framework of the ryegrass AGP (Fincher *et al.*, 1983).

of newly synthesized protein containing proline and hydroxyproline coincided with the A_{215} peak that was eluted at 34–35 ml (Fig. 2). The same peak included the only h.p.l.c. fractions containing immunoreactive material, as measured by e.l.i.s.a. with antisera raised against the deglycosylated AGP (Fig. 2). A second region of ^{14}C-labelled protein, which eluted as a shoulder on the trailing edge of the main peak (peak II, Fig. 2), appeared to be a distinct hydroxyproline-containing species, on the basis of amino acid composition (Table 2) and its lack of immunoreactivity towards deglycosylated AGP antiserum (Fig. 2). Levels of hydroxyproline corresponding to approx. 20–25% of those associated with the first A_{215}

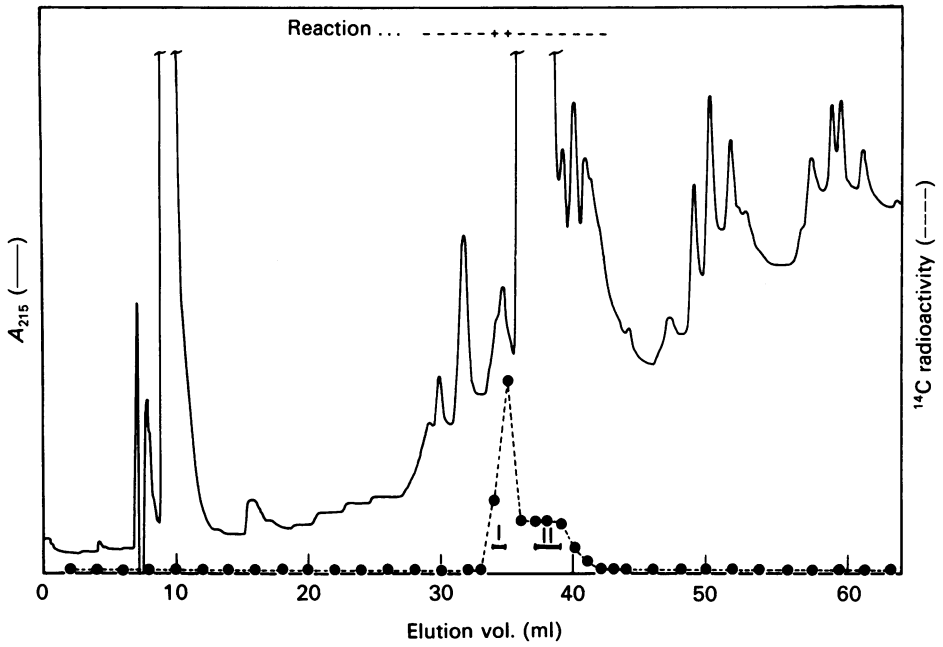

Fig. 2. H.p.l.c. purification of deglycosylated ryegrass AGP

Fractions I and II were pooled as indicated for further analysis. Fractions reacting positively with antibodies against the deglycosylated AGP in an e.l.i.s.a. are indicated by '+', fractions not reacting are indicated by '−'. Peaks containing no ^{14}C label represent released monosaccharides, oligosaccharides and carbohydrate degradation products.

Table 2. Amino acid composition of ryegrass AGP and h.p.l.c.-purified deglycosylated AGP

Abbreviations: Cya, cysteic acid; ND, not determined.

	Composition (mol/100 mol)		
		H.p.l.c.-purified* deglycosylated-AGP	
Amino acid	Native ryegrass AGP	I	II
Cya	ND	0.7	1.5
Hyp	14.8	20.4	19.6
Asx	5.5	2.3	3.7
Thr	6.5	8.2	5.9
Ser	9.8	8.6	8.6
Glx	4.9	5.3	6.1
Pro	5.6	3.6	4.7
Gly	6.7	7.8	14.6
Ala	22.5	27.8	19.8
Val	5.1	3.8	3.0
Met	1.6	0	0
Ile	1.3	0.4	1.7
Leu	5.0	3.0	3.2
Tyr	1.2	0.7	0.7
Phe	1.5	0	0
His	0.6	0.4	0.7
Lys	3.3	5.8	1.8
Arg	2.2	1.3	2.0
Trp	ND	ND	ND
Cys	1.0	ND	ND

* H.p.l.c. fractions I and II pooled as shown in Fig. 2.

peak were detected in the fractions up to 40 ml (Fig. 2); these levels decreased rapidly thereafter. A separate asparagine/aspartic acid/glutamine/glutamic acid-rich polypeptide was also associated with the large A_{215} peak eluted at 35–45 ml (Fig. 2) (results not shown). The origin of the material in this large peak is not clear, and although it contains some protein, other material (such as carbohydrate and acid-degradation products) may also be present. The amino acid analyses of fractions I and II, pooled as indicated in Fig. 2, are compared with that of native AGP in Table 2. Both fractions are characterized by high levels of alanine and hydroxyproline. However, some differences are observed between the two h.p.l.c. fractions, most notably the relatively lower levels of alanine and higher levels of glycine in fraction II (Table 2), and this suggests the presence of more than one protein in fraction II.

Amino acid sequence analysis of material derived from the deglycosylated AGP

In view of the concentration of radioactivity in fraction I, the presence in it of proteins which are recognized strongly by the antisera, and the clear separation of the peak from the large A_{215} peak (Fig. 2), we focused our attention on fraction I. Analysis of the h.p.l.c.-purified fraction I by *N*-terminal amino acid sequence analysis gave a single, unambiguous sequence of Ala-Glu-Ala-Hyp-Ala-Hyp-Ala-Hyp-Ala-Ser. Good recoveries of aminoacyl derivatives were reproducibly obtained for the first ten cycles of the Edman degradation procedure, but after the serine residue at cycle 10, the yield decreased sharply, and no further sequence could be unambiguously

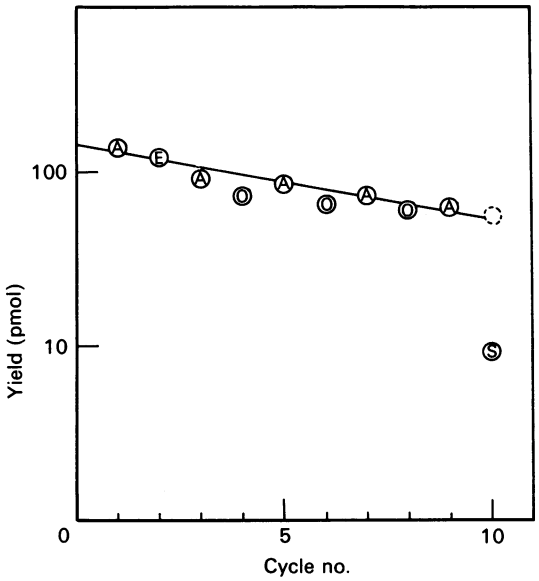

Fig. 3. *N*-Terminal amino acid sequence of deglycosylated ryegrass AGP purified by h.p.l.c. (fraction I; Fig. 2)

Good recoveries of phenylthiohydantoin derivatives of amino acids were obtained until cycle 10, after which no further sequence was obtained. The letters A, E and S are the usual one-letter symbols for alanine, glutamic acid and serine; in addition O (a non-recognized symbol) represents hydroxyproline.

Table 3. Sequences of tryptic peptides isolated from deglycosylated AGP

Both residues of ambiguous assignments are shown; uncertain residues are in parentheses.

Peptide	Sequence
1	Lys-Ala-Ala-Ala-Ser-Hyp-Hyp-Ala-Hyp-Ala-Hyp-Lys
2	Ala-Hyp-Ala-Hyp-Ala-Hyp-$\frac{\text{Val}}{\text{His}}$-Hyp-Glu-Ala
3	$\frac{\text{Ser}}{\text{Leu}}$-Thr-Ala-Hyp-Val-Ala-Ala-Hyp-Thr-Thr-(Xaa)-Hyp
4	Ser-Hyp-Pro-Ala-Hyp-Ala
5	Ala-Ala-Ala-(Ser)-Leu-(Lys)

determined (Fig. 3); a background sequence was detected after cycle 10. The reason for the termination of the sequence is not yet clear. Because of difficulties in obtaining sufficient material from fraction I for tryptic peptide analyses, additional amino acid sequence was obtained from h.p.l.c.-purified tryptic peptides generated from unfractionated, deglycosylated AGP (profile not shown). Sequences of selected tryptic peptides are shown in Table 3. Tryptic peptide 1 (Table 3) appears to be derived from incomplete tryptic digestion at two adjacent basic residues (Lys-Lys or Arg-Lys).

DISCUSSION

To facilitate the characterization of the protein core of the ryegrass AGP, arabinogalactan chains were first stripped from the proteoglycan with trifluoromethanesulphonic acid. More than 99% of the carbohydrate was removed by this treatment, which yielded a polypeptide band of apparent M_r 35000 on SDS/polyacrylamide gels. Preferential hydrolysis of *O*-glycosidic linkages with trifluoromethanesulphonic acid (Edge *et al.*, 1981) has been used successfully to remove the carbohydrate components of other glycoproteins without any apparent cleavage of peptide bonds (Desai *et al.*, 1983). Although the deglycosylated AGP migrated as a single major protein component on SDS/polyacrylamide-gel electrophoresis, the protein band was diffuse rather than sharp (Figs. 1*a* and 1*b*). The relatively narrow size distribution of the band suggests that extensive acid degradation of the protein had not occurred, but we are unable to conclude whether the apparent heterogeneity results from the non-uniform distribution of the residual carbohydrate on a single protein or to a polydisperse population of protein chains. However, the resolution of two, or possibly more, distinct hydroxyproline-rich polypeptides during the h.p.l.c. purification of the deglycosylated AGP (Table 2 and Fig. 2) indicates that the protein core of the AGP preparation is indeed heterogeneous. Charge and size heterogeneity of AGP preparations have been reported during flower development of a tobacco species *Nicotiana alata* (Gel *et al.*, 1986) and in different tissues of a tomato species, *Lycopersicon peruvianum* (van Holst & Clarke, 1986), although the nature of the charged residues is not known, and heterogeneity in *N*-terminal amino acid sequences of carrot (*Daucus carota*) AGPs has been attributed to a heterogeneous population of protein cores in the AGP (Jermyn & Guthrie, 1985).

On the basis of an M_r of 200000 and a protein content of 7% (w/w) (Anderson *et al.*, 1977; Bacic *et al.*, 1987), one would expect a single-chain polypeptide core of the ryegrass AGP to have an M_r of approx. 15000. However the observed molecular weight for the core protein was 35000 (Fig. 1). The discrepancy might be explained by problems associated with accurate molecular weight determination of the native AGP (Anderson *et al.*, 1977; Bacic *et al.*, 1987), by the effect of residual carbohydrate on the deglycosylated AGP residue or by an anomalous mobility of the protein which results from its unusual amino acid composition. Polyhydroxyproline has been reported not to bind to SDS (Desai *et al.*, 1983), and the high hydroxyproline content of the protein of the ryegrass AGP may lead to an electrophoretic mobility slower than that expected for its size. The aberrant mobility of other hydroxyproline-rich molecules during electrophoresis has been noted. The deglycosylated potato (*Solanum tuberosum*) lectin, which has a hydroxyproline content of 50% and an M_r of 25000, exhibits an apparent M_r of 71000 by SDS/polyacrylamide-gel electrophoresis (Desai *et al.*, 1983).

Amino-acid-sequence analysis of the deglycosylated ryegrass AGP revealed a novel repeating dipeptide (Ala-Hyp) at the *N*-terminus (Fig. 3) and in a number of tryptic peptides generated from the unfractionated deglycosylated AGP (Table 3). This structure is in keeping with the high levels of alanine and hydroxyproline in the protein core of the ryegrass AGP (Table 2; Anderson *et al.*, 1977; Bacic *et al.*, 1987) and has been noted in carrot AGPs (Jermyn & Guthrie, 1985). However, in the peptides we have analysed so far, the Ala-Hyp dipeptide is not repeated over long distances; three adjacent Ala-Hyp dipeptides is the longest repeating sequence observed

(Fig. 3; Table 3). The tryptic peptides derived from the unfractionated deglycosylated AGP also include clusters of adjacent alanine residues, for example Ala-Ala-Ala-Ser (Table 3). Nevertheless, independent evidence from c.d. spectra indicates that the protein moiety of the intact AGP has a significant degree of structural regularity (van Holst & Fincher, 1984). The Ala-Hyp of the ryegrass AGP sequence is clearly distinguished from the $(Ser\text{-}Hyp)_4$ and other repeated sequences of the hydroxproline-rich cell-wall glycoproteins (Cassab & Varner, 1988). The hydroxyproline residues of AGPs and hydroxproline-rich cell-wall glycoproteins are glycosylated with galactose (Fincher *et al.*, 1983) and arabinose (Lamport, 1977) respectively. It is likely that the distinctive amino acid sequences adjacent to the hydroxyproline residues account for the different glycosylation patterns in these two classes of plant glycoproteins. However, the variety of sequences around hydroxyproline residues in the ryegrass AGP (Table 3) raises the possibility that enzymes responsible for glycosylation of the AGP protein core recognize several sequences in the polypeptide chain.

Polyclonal antibodies raised against the deglycosylated ryegrass AGP did not recognize native AGP (Table 1), and AGPs from a number of sources are resistant to the action of certain endopeptidases (Fincher *et al.*, 1974; Jermyn & Yeow, 1975). The most likely explanation for these observations is that the protein component of the AGP constitutes a molecular core which functions as a support for arabinogalactan chains attached to hydroxyproline residues (Fincher *et al.*, 1983) and which is rendered inaccessible to immunoglobulins and peptidases by the multiple highly-branched polysaccharide chains which surround it. Steric shielding of epitopes by low levels of residual carbohydrate in the deglycoslated AGP might explain why some hydroxyproline-rich fractions in the h.p.l.c. separation of the deglycosylated AGP do not react with the antisera (Fig. 2). The lack of cross-reactivity of antibodies with either the synthetic peptide $(Ala\text{-}Hyp)_4$ or polyhydroxyproline (Table 1) suggests that the immunodominant epitopes of the protein core do not include the hydroxyproline-rich domains.

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